

# Wiadomości Lekarskie Medical Advances

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Memory of  
dr Władysław  
Biegański

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# Headache as a marker of psychological and somatic distress in children with non-psychotic mental disorders

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## ABSTRACT

**Aim:** To determine the role of headache as a marker of psychological and somatic distress in children with non-psychotic mental disorders (NPMD), with a focus on its relationship with conflicts in the family and school environment.

**Materials and Methods:** A retrospective analysis was performed on medical records of 168 children aged 8–10 years who were under observation by child psychiatrists. According to ICD-10 diagnostic criteria, the cohort was divided into four subgroups: neurotic disorders (ND;  $n = 42$ ), neurosis-like disorders (NSD;  $n = 46$ ), emotional and behavioural disorders (EBD;  $n = 45$ ), and developmental disorders of school skills (DSS;  $n = 35$ ). Clinical complaints including headache, anxiety, sleep disturbances, learning difficulties, behavioural dysregulation, and interpersonal conflicts were analysed. The associations between binary variables were assessed using the phi ( $\phi$ ) coefficient.

**Results:** Headache was reported by 41% of children with ND, 60% with NSD, 36% with EBD, and 29% with DSS. In ND and NSD, headache was combined with anxiety, sleep problems, and cognitive deficits, whereas in EBD and DSS, it was more closely associated with interpersonal conflicts and school maladaptation. We revealed moderate-to-relatively strong associations between headache and conflicts with parents ( $\phi = 0,28-0,48$ ), as well as with teachers ( $\phi = 0,33-0,46$ ), indicating that headache may reflect relational distress.

**Conclusions:** Headache in children with NPMD serves as a clinically relevant psychosomatic marker that integrates biological, emotional, and social dimensions of maladaptation. Recognition of headache as an indicator of psychological suffering highlights the importance of integrative, family-centred, and ethically sensitive approaches to diagnosis and treatment.

**KEY WORDS:** headache, children, mental disorders, psychological conflict

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## INTRODUCTION

Pediatric headache is highly prevalent, heterogeneous in etiology, and associated with substantial functional, psychological, and sleep-related comorbidity. Recent narrative and systematic reviews emphasize the need for integrated assessment – encompassing neurological, psychological, sleep, and dental/orofacial domains – and for evidence-based, biopsychosocial interventions tailored to children and families [1–3].

Systematic reviews and meta-analyses report a high and increasing prevalence of primary headache in children and adolescents, with migraine prevalence estimated at approximately 10–15% in teenagers and tension-type headaches also common. Overall, headache represents one of the most frequent chronic pain complaints in youth and contributes substantially to school absence, reduced quality of life, and increased health care use [1, 2, 4].

Diagnostic classification in children follows the ICHD-3 (International Classification of Headache Disorders, 3rd edition) criteria but requires developmental sensitivity, as symptoms and phenotypes often differ from those in adults (shorter duration, bilateral pain, and more prominent autonomic and gastrointestinal features). Specific presentations of note include primary nighttime headaches and rarer sudden-onset headache syndromes that require prompt emergency department (ED) assessment to rule out secondary causes [5–7].

Large systematic reviews show that children and adolescents with migraine have higher rates of anxiety and depressive symptoms and disorders than their peers, suggesting the need for routine screening in both specialty and primary care. Alexithymia (difficulty identifying and describing feelings) has been associated with pediatric headache and may mediate poorer coping and higher disability. Psychological comorbidities

significantly increase overall burden and represent important treatment targets [8–10].

Structured approaches combining medical history, focused neurological examination, and selective imaging or laboratory tests reduce both missed secondary causes and unnecessary testing. Several reviews summarize triage and emergency department (ED) management strategies [7, 11].

Psychological therapies, including cognitive behavioral therapy (CBT) and other structured approaches, have demonstrated benefits for pediatric chronic and recurrent pain, including headache, reducing pain intensity and disability after treatment and, in some studies, at follow-up. Multimodal care addressing sleep, stress, school support, and family behavior is recommended as a first-line treatment or as an adjunct to pharmacotherapy [10, 12].

Accordingly, a retrospective analysis of headaches in children aged 8–10 years with non-psychotic mental disorders and school adaptation disorders who were treated by psychiatrists was conducted.

## AIM

To determine the role of headache as a marker of psychological and somatic distress in children with non-psychotic mental disorders and to assess its relationship with conflicts in the family and school environment.

## MATERIALS AND METHODS

The study included 168 children aged 8–10 years who were under observation by child psychiatrists and had diagnoses interpreted as borderline non-psychotic mental disorders according to ICD-10 (F category) [13]. A retrospective analysis of medical records was conducted with informed parental consent.

This study was conducted in accordance with the principles of bioethics and international legal standards, including the Declaration of Helsinki (World Medical Association, 2013), the Convention on Human Rights and Biomedicine (Oviedo Convention, 1997), and relevant Ukrainian legislation, such as the Law of Ukraine “On the Protection of Personal Data” (No. 2297-VI, 2010) and the Order of the Ministry of Health of Ukraine No. 690 (23.09.2009), which regulates ethical requirements for biomedical research involving human participants.

The research had a retrospective design, based on the analysis of medical records and psychological assessment results previously conducted as part of routine clinical practice. No experimental interventions were introduced, and the confidentiality of personal data

was strictly maintained. All children were examined and treated in compliance with current clinical and ethical standards. Parents or legal guardians provided informed consent for psychological assessment and medical treatment of their children. The study protocol complied with institutional ethical principles and posed no additional risk or burden to participants.

According to diagnostic criteria, the children were divided into four conditional subgroups. The first subgroup (predominantly neurotic disorders [PND]) consisted of 42 children. Psychogenic factors played a leading role in their etiopathogenesis, such as acute or chronic psychological trauma and emotional intrapsychic conflicts. The main diagnoses included childhood neurotic disorders (ICD-10: F95–F98) [13]. Comorbidities were also observed, including general underdevelopment of speech (level III) and, occasionally, impaired development of scholastic skills.

The second subgroup (neurosis-like disorders [NLD]) included 46 children. In their pathogenesis, central nervous system damage played a leading role. Their medical history revealed biological factors affecting the nervous system during early ontogenesis, such as pregnancy toxicosis, complicated childbirth, psychomotor developmental delays in the first year of life, and chronic somatic diseases. Neurological symptoms were also present, including nystagmus, synkinesias, instability in the Romberg posture, and other neurological signs. In addition to neurotic symptoms (ICD-10: F95–F98), children in this subgroup also had diagnoses from the F0 category, as confirmed by a neurologist.

The third subgroup (emotional and behavioral disorders [EBD]) consisted of 45 children. Their clinical picture was characterized by pronounced emotional and behavioral disturbances occurring at home and/or at school. The main diagnoses were “Hyperkinetic behavior disorder” (F90.1) and “Conduct disorders” (F91), particularly “Unsocialized conduct disorder” (F91.1). Occasionally, “Oppositional defiant disorder” (F91.3) and “Mixed disorders of conduct and emotions” (F92) were also diagnosed.

The fourth subgroup (specific developmental disorders of scholastic skills [SDD]) included 35 children. These were schoolchildren, whose main diagnoses involved speech disorders and delays in the acquisition of scholastic skills. Children in this subgroup most often underwent planned outpatient rehabilitation tailored to their individual neuropsychological and speech therapy needs, including work with speech therapists, special education teachers, and psychologists, as well as sensory integration classes. In the children’s hospital setting, patients with SDD were rarely encountered, most often in the context of psychological trauma or during initial psychiatric evaluations.



Clinical complaints were analyzed from medical records, including headache, increased anxiety, sleep disturbances, learning difficulties, attention deficits, behavioral problems, as well as conflicts with parents and teachers.

To assess interpersonal conflicts, the Etkind Color Association Test [14-15] was used. This nonverbal projective technique is based on the M. Lüscher Color Test. In the first stage, children were asked to associate colors with family members and other significant figures. In the second stage, colors were ranked by preference. Associations with colors placed in the 7th and 8th positions were interpreted as indicators of emotional conflict with the person assigned to that color. Long-term studies have confirmed the diagnostic validity of this method, which is effective in identifying unconscious psychological conflicts in both children and adults, particularly in family and school relationships.

We used IBM SPSS Statistics v. 27.0 (Armonk, NY: IBM Corp., USA) for statistical data analysis. Qualitative data were presented as relative frequency (%) and a standard error. To evaluate the associations between binary (dichotomous) variables, the phi ( $\phi$ ) coefficient was calculated based on 2x2 contingency tables. The strength of association was interpreted according to conventional thresholds: negligible ( $<0,1$ ), weak ( $0,1- <0,2$ ), moderate ( $0,2- <0,4$ ), relatively strong ( $0,4- <0,6$ ), strong ( $0,6- <0,8$ ), and very strong ( $\geq 0,8$ ).

## RESULTS

The analysis of symptom profiles across diagnostic subgroups revealed patterns of psychosomatic and behavioral manifestations, emphasizing the heterogeneity of headache expression within different categories of non-psychotic mental disorders in children. The results for each subgroup are presented below.

### PND SUBGROUP (N = 42)

For children in this subgroup, the most characteristic features were neurotic symptoms ( $81\% \pm 6\%$ ), with predominant manifestations of increased anxiety ( $64\% \pm 7\%$ ) and sleep disturbances ( $64\% \pm 7\%$ ). Somatized complaints were observed in the form of headache, which was reported in  $41\% \pm 8\%$  of cases. Thus, the PND subgroup was typified by a combination of inner tension and pronounced neurotic symptoms, generating significant distress in the interpersonal sphere. A considerable proportion of children experienced interpersonal difficulties, particularly conflicts within the family ( $50\% \pm 8\%$ ) and strained relationships with teachers ( $31\% \pm 7\%$ ).

### NLD SUBGROUP (N = 46)

This subgroup demonstrated an even more pronounced symptom complex, where leading features included neurotic manifestations ( $87\% \pm 5\%$ ) combined with learning difficulties ( $85\% \pm 6\%$ ). Sleep disturbances ( $72\% \pm 7\%$ ) and heightened anxiety ( $63\% \pm 7\%$ ) were also typical. Complaints of cognitive impairment were frequent, with memory decline ( $72\% \pm 7\%$ ) and attention deficits ( $67\% \pm 7\%$ ) being most common. Interpersonal conflicts occurred somewhat less often than in PND, but remained noticeable: with parents ( $37\% \pm 7\%$ ) and teachers ( $48\% \pm 7\%$ ). Headache was reported more often than in the PND subgroup –  $60\% \pm 7\%$ , underscoring the significant role of somatization. Overall, NLD was characterized by the combination of neurotic symptoms with cognitive and learning difficulties, substantially reducing children's adaptive capacity.

### EBD SUBGROUP (N = 45)

Children in this subgroup were distinguished by a predominance of attention deficits ( $87\% \pm 5\%$ ) and unbalanced behavior ( $87\% \pm 5\%$ ), often combined with learning difficulties ( $71\% \pm 7\%$ ). Unlike PND and NLD, in this group, behavioral and emotional-affective symptoms were more prominent, including phobic manifestations ( $62\% \pm 7\%$ ). Conflicts within the immediate environment were significant: with parents ( $64\% \pm 7\%$ ) and, to an even greater extent, with teachers ( $69\% \pm 7\%$ ). Somatic complaints in the form of headache were less frequent ( $36\% \pm 7\%$ ). Overall, EBD presented as a pattern of behavioral maladaptation with high conflict levels and a tendency toward emotional instability.

### SDD SUBGROUP (N = 35)

In this subgroup, leading features were learning difficulties ( $86\% \pm 6\%$ ), which were combined with attention deficits ( $71\% \pm 8\%$ ) and memory impairments ( $46\% \pm 8\%$ ). Unlike other groups, the academic domain served as the primary source of maladaptation, whereas marked neurotic or behavioral symptoms played a secondary role. Interpersonal problems were mainly reflected in conflicts with teachers ( $63\% \pm 8\%$ ), whereas conflicts with parents were less frequent ( $31\% \pm 8\%$ ). Headache had the lowest prevalence among all subgroups –  $29\% \pm 8\%$ . Thus, subgroup SDD was characterized by predominant cognitive and scholastic difficulties with relatively limited involvement of the emotional and behavioral domains.

Importantly, the symptom manifestations differed across diagnostic subgroups. In the ND subgroup, headache was predominantly associated with family-related

**Table 1.** The associations between headache and conflicts in different subgroups

| N | Subgroup   | Headache ↔ Parental<br>conflicts (φ) | Headache ↔ Teacher<br>conflicts (φ) | Interpretation                                                                        |
|---|------------|--------------------------------------|-------------------------------------|---------------------------------------------------------------------------------------|
| 1 | ND (n=42)  | 0.42                                 | 0.33                                | Somatization of emotional distress, more strongly<br>linked to the family environment |
| 2 | NSD (n=46) | 0.28                                 | 0.40                                | Cognitive and academic difficulties reflected in<br>school conflicts                  |
| 3 | EBD (n=45) | 0.48                                 | 0.46                                | High conflict proneness in both family and school<br>settings                         |
| 4 | SDL (n=35) | 0.37                                 | 0.43                                | Headache accompanies academic frustration and<br>school stigmatization                |

Source: compiled by the authors of this study

emotional distress, anxiety, and sleep disturbances. In the NSD subgroup, headache was more prevalent and closely linked to cognitive difficulties and academic challenges. The EBD subgroup presented with attentional deficits, impulsivity, and heightened interpersonal conflict, with headache appearing as a secondary but clinically meaningful symptom. In the SDD subgroup, academic difficulties were the most pronounced, while headache, when present, tended to accompany emotional tension in peer and teacher relationships.

At the next stage, we revealed significant associations between headache and interpersonal conflicts both within the family and at school across all subgroups of children with non-psychotic mental disorders (Table 1).

In the ND subgroup, headache showed a relatively strong association with conflicts with parents ( $\phi=0,42$ ) and a moderate association with conflicts with teachers ( $\phi=0,33$ ). In the NSD subgroup, the association with family conflicts was moderate ( $\phi=0,28$ ) but more pronounced with school conflicts ( $\phi=0,40$ ). Among children in the EBD subgroup, the strongest associations were found: headache was associated both with parental conflicts ( $\phi=0,48$ ) and teacher conflicts ( $\phi=0,46$ ). Finally, in the SDD subgroup, headache showed a moderate association with family conflicts ( $\phi=0,37$ ) and a somewhat stronger association with school conflicts ( $\phi=0,43$ ).

Thus, the analysis revealed a significant association between headache complaints and relational conflicts across both family and school contexts, highlighting its role as an indicator of systemic maladaptation. Family dynamics emerged as particularly influential: conflictual, overcritical, or emotionally detached parenting styles were associated with higher headache prevalence, and relational tensions at home frequently extended into the school environment, reinforcing maladaptive behavioral patterns.

The observed associations between headache and interpersonal conflicts—especially within the family—highlight the pivotal role of family dynamics in chil-

dren’s psychosocial adaptation and school adjustment. These findings demonstrate that headache functions as a distinct psychosomatic marker in children with non-psychotic mental disorders, reflecting the complex interplay of emotional distress, cognitive impairments, and maladaptive behavioral patterns.

The obtained results underscore the necessity of an integrated, multidisciplinary approach to management, combining medical, psychological, family-oriented, and school-centered interventions.

**DISCUSSION**

Headache is among the most prevalent somatic complaints in children and adolescents, and its clinical significance becomes particularly evident in those with non-psychotic mental disorders [1, 4, 5]. In pediatric psychiatry and clinical psychology, headache is increasingly viewed not as an isolated neurological symptom but as a psychosomatic marker reflecting underlying emotional distress and maladaptation [2, 3, 8, 9].

Epidemiological studies estimate its prevalence at over 30% in school-age populations [1, 4], with even higher rates among children with psychiatric comorbidities [8, 10]. In line with our findings, headache frequently co-occurs with anxiety, sleep disturbances, cognitive impairments, learning difficulties, and behavioral problems [2, 8, 9, 12, 16], thereby representing a multidimensional indicator of distress that encompasses the emotional, cognitive, and behavioral domains of functioning [5, 9, 10].

The etiology of headache in childhood is multifactorial. Biological mechanisms—including genetic predisposition, neurochemical imbalances, somatic illnesses, and sleep disorders—play an important role [6, 7, 16]. However, growing evidence highlights the influence of psychosocial determinants such as emotional stress, school maladaptation, insufficient social support, and family conflict [8, 9, 10, 17]. These factors have been shown to contribute not only to the onset but also to

the chronicity and treatment resistance of pediatric headache [10, 12, 16, 17].

The role of family relationships deserves special emphasis. Parenting style and the quality of emotional contact between parents and children critically shape psychosomatic well-being [9, 10, 12]. Authoritarian practices, excessive criticism, emotional detachment, and lack of support have been linked to higher rates of somatization, often manifesting as recurrent headaches [9, 10]. Conversely, democratic parenting, openness, and acceptance serve as protective factors that buffer stress and reduce the likelihood of somatic symptom formation [12, 15]. In our study, conflicts with parents were significantly associated with headache complaints, suggesting that intrafamilial stress may act as a core driver of psychosomatic manifestations in this population.

Importantly, the family environment appears to extend its influence into the school context. Conflicts with teachers were also associated with headache prevalence, indicating that maladaptive interaction patterns established within the family may be projected onto other authority figures [9, 10, 15]. This observation is consistent with theories of transference and relational patterns in child development, which posit that unresolved family conflicts often generalize to broader social contexts [9, 12]. Thus, headache in children with non-psychotic mental disorders may be conceptualized as a clinical marker of systemic maladaptation that bridges somatic, emotional, and relational dimensions [2, 5, 10].

From a clinical perspective, the analysis of headache as a psychosomatic symptom opens avenues for integrative diagnostic and therapeutic strategies [11,

12, 16]. A narrow biomedical approach may overlook the psychosocial context in which headache develops and persists. Conversely, incorporating psychological and family-centered interventions—such as family therapy, parent training, and school-based support—may enhance treatment outcomes [11, 12]. Moreover, understanding headache as a psychosomatic indicator provides clinicians with an accessible and observable symptom through which underlying distress and family dysfunction can be identified early [5, 8, 12].

Finally, these findings highlight important bioethical considerations. Integrating biological, psychological, and social dimensions of care aligns with the principles of holistic, patient-centered, and ethically responsible practice in child treatments [10, 12, 13].

## CONCLUSIONS

Headache represents a key psychosomatic marker in children with non-psychotic mental disorders, reflecting the interaction of emotional distress, cognitive dysfunction, and maladaptive behavior. Symptom patterns varied across diagnostic subgroups, with neurotic and neurosis-like disorders showing the strongest links between headache, anxiety, and family conflict, whereas emotional-behavioral and developmental learning disorders were characterized by interpersonal and academic maladaptation.

From a bioethical standpoint, headache in children should be regarded not merely as a clinical symptom but as an expression of psychological suffering—requiring a patient-centered, non-stigmatizing approach aimed at fostering trust, supporting emotional well-being, and ensuring effective psychiatric care.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Interleukins (IL-1 $\beta$ , IL-4, IL-6, and IL-8) in aqueous humor as potential biomarkers of diabetic retinopathy severity

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## ABSTRACT

**Aims:** To determine the concentrations of interleukins (IL1 $\beta$ , IL4, IL6, IL8) in the aqueous humor and their association with diabetic retinopathy severity.

**Materials and Methods:** Singlecenter crosssectional study with prospective sampling of 110 adults with type 2 diabetes mellitus spanning five categories of diabetic retinopathy and 25 nondiabetic controls. Aqueous humor (0.1 mL) obtained during phacoemulsification was analyzed by ELISA. Nonparametric tests assessed group differences; optimal thresholds were derived by multiclass onevsall ROC-analysis, followed by clinically relevant binary stratification (mild/moderate vs severe DR). Spearman rank correlations evaluated relationships with age, diabetes duration, fasting glucose, HbA1c, and Cpeptide.

**Results:** All interleukins differed between groups ( $p < 0.001$ ). IL1 $\beta$  and IL6 increased with stage; IL8 peaked at severe nonproliferative disease and declined in proliferative disease; IL4 decreased. Multiclass accuracies were modest: 56%, 58%, 53%, and 44% for IL-6, IL8, IL1 $\beta$ , and IL4. In the binary model, IL6 achieved 72.6% accuracy (95% CI 64.3–79.9), with cutoffs of 34.4–86.2 and  $>86.2$  pg/mL for mild/moderate and severe disease. IL8 was supportive (62.2%; severe  $>216.9$  pg/mL). IL1 $\beta$  and IL4 each yielded about 55.6%. Correlations were weak to moderate: IL6 and IL8 tracked diabetes duration and glycemia, while IL4 inversely tracked Cpeptide.

**Conclusions:** IL6 is the most informative single local biomarker for binary severity stratification of diabetic retinopathy, with IL8 as an adjunct and IL1 $\beta$ /IL4 of limited standalone value. IL6 thresholds may guide riskadapted followup and monitoring with optical coherence tomography and optical coherence tomography angiography. External validation, preanalytical standardization, and prospective outcome studies are needed.

**KEY WORDS:** diabetic retinopathy, diabetes mellitus type 2, aqueous humor, interleukins, interleukin-1 $\beta$ , interleukin-4, interleukin-6, interleukin-8, biomarkers, disease severity

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## INTRODUCTION

Diabetic retinopathy (DR) remains one of the leading causes of vision loss in the workingage population, and its burden is rising in parallel with the global epidemic of type 2 diabetes mellitus (T2DM) [1]. According to the 11th edition of the International Diabetes Federation (IDF) Atlas, 588.7 million adults (20–79 years; prevalence 11.1%) were living with diabetes in 2024, with numbers projected to reach 852.5 million (13.0%) by 2050 – trends that will inevitably amplify the incidence of DR [1]. In the global landscape of vision loss, DR consistently ranks among the principal causes of blindness and moderate-to-severe visual impairment in individuals aged  $\geq 50$  years, as documented by the GBD/VLEG consortium analysis of 1990–2020 data [2]. In clinical practice, disease severity is graded using the International Clinical Diabetic Retinopathy (ICDR, 2003) scale, which distinguishes DR0, mild/moderate/severe nonproliferative DR (NPDR), and proliferative DR (PDR), whereas diabetic macular edema

(DME) is evaluated separately [3]. However, this discrete, structurebased grading does not fully capture early ischemic and neuroinflammatory processes that precede overt microvascular signs [4, 5].

Optical coherence tomography angiography (OCTA) has enabled the visualization of retinal perfusion defects – particularly within the deep capillary plexus (DCP) – that are associated with downstream complications as early as moderate NPDR and even in individuals with T2DM without clinically apparent DR [4, 5]. This mismatch between the continuous pathophysiology of DR progression and categorical clinical grading underscores the need for local biomarkers capable of biochemically profiling the retinal microenvironment and complementing imaging data to refine risk stratification for the development and progression of DR.

Chronic lowgrade inflammation is recognized as a principal driver of DR pathogenesis: hyperglycemia activates proinflammatory cells, augments oxidative stress,

and impairs endothelial function, thereby triggering a cascade of mediators that damage the retinal neurovascular unit [6, 7]. A central role in this cascade is played by the NLRP3 inflammasome (NODlike receptor family, pyrin domaincontaining 3): its activation induces secretion of IL1 $\beta$ /IL18 and pyroptotic cell death, amplifying inflammation and microvascular dysfunction [8]. Against this background, interleukins – as universal regulators of innate and adaptive immunity – form inflammatory “signatures” that can be locally monitored in DR, given their amenability to qualitative and quantitative assessment in ocular tissues.

IL1 $\beta$  is a classical proinflammatory mediator that injures the retinal capillary endothelium via NF $\kappa$ B (nuclear factor kappa-light-chain-enhancer of activated B cells) activation, increased oxidative stress, and mitochondrial damage [9]; elevated levels have been reported in experimental models and in intraocular fluids of patients with PDR [10]. IL4, a key cytokine of the Thelper type 2 (Th2) response, antagonizes several proinflammatory pathways [11] and is considered a potential immunomodulatory therapeutic target beyond ophthalmology, although its role in DR remains contextdependent. IL6 is a pleiotropic cytokine with both pro and antiinflammatory actions, serving as a marker of neuroinflammation and endothelial dysfunction [12]. In DR, higher aqueous humor (AH) levels of IL6 are associated with the presence/severity of DME and overall disease activity and, in some reports, with poorer functional outcomes of therapy [12–14]. Finally, IL8 (CXC motif chemokine ligand 8, CXCL8) is a potent chemoattractant with proangiogenic activity; its concentrations are elevated in serum, AH, and vitreous in PDR/DME and correlate with retinal edema and vascular remodeling [15, 16]. Taken together, these data support the concept of a cytokine imprint of the ocular microenvironment that reflects the activity of proinflammatory and proangiogenic processes across DR phenotypes.

Local intraocular biofluids are indispensable matrices for quantifying these markers. Among them, the AH is the most readily accessible medium proximal to the posterior segment; its composition reflects tissue and celllevel processes within the retina and choroid [17]. Multiomics investigations of AH in DR/DME have demonstrated shifts in metabolic profiles and immunoinflammatory pathways, including elevations in multiple cytokines and extracellularmatrix remodeling factors [18]. Proteomic studies have identified dozens of candidate proteins that correlate with DR severity and its complications [19, 20]. The concept of an ocular “liquid biopsy” has been convincingly validated in retinoblastoma, where AH outperformed blood with respect to tumor-derived DNA and protein biomarkers [21].

In view of the above, we selected interleukins as local biochemical indicators of DR progression. Published evidence indicates that IL6 and IL8 in AH most consistently associate with the presence of DR/DME; however, robust stagespecific thresholds have not yet been established [16, 22]. Against this background, AH may be considered a local molecular mirror of hypoxicinflammatory processes in the posterior segment – particularly the retina – in DR, complementing OCT-derived perfusion metrics in the DCP and other retinal layers to enable integrated risk stratification [4, 5].

## AIM

Objective – to determine the concentrations of interleukins (IL1 $\beta$ , IL4, IL6, IL8) in the aqueous humor (AH) and their association with diabetic retinopathy (DR) severity.

## MATERIALS AND METHODS

All procedures conformed to the Council of Europe Convention on Human Rights and Biomedicine and to the principles of the Declaration of Helsinki (1964, with subsequent amendments including the 2000 revision) and complied with Ukrainian legislation. The study protocol was approved by the Bioethics and Academic Integrity Committee of Bogomolets National Medical University (Protocol No. 196, 23 June 2025). The study was a singlecenter, crosssectional analysis with prospective biospecimen collection.

We enrolled 110 patients with T2DM and DR; analyses were based on the worstaffected eye (110 eyes). According to the ICDR severity scale [3], participants were allocated to five groups: (1) no retinopathy (DR0),  $n=15$ ; (2) mild NPDR,  $n=40$ ; (3) moderate NPDR,  $n=25$ ; (4) severe NPDR,  $n=12$ ; and (5) PDR,  $n=18$ .

Participants were 50–76 years old (median 62.5 [51.3–69] years); men: 92 (83.6%), women: 18 (16.4%), with no between-group sex differences ( $p=0.970$ ). DME was present in 12/110 (10.9%) patients, of whom 10 (83.3%) belonged to groups 4 and 5. A control cohort of 25 age and sexcomparable individuals without diabetes or DR, undergoing surgery for age-related cataract, was also included.

All participants underwent standardized ophthalmic evaluation, including distance visual acuity testing with a premium test chart projector (C.S.O. srl., USA) using Optiek XL trial lenses (USA) and a Takagi VT5 phoropter (Takagi Seiko Co., Ltd., Japan); autorefractometry (TOPCON KR7000P; TOPCON Corporation, Japan); noncontact tonometry (Huvitz HNT7000) and keratopachymetry (HNT1P; Huvitz, Korea); slitlamp biomicroscopy (CSO SL9900 with LED 5 $\times$  video system; Italy) and binocular



**Table 1.** Aqueous humor interleukin concentrations by study group (Me; Q1–Q3)

| Analyte                 | Study group                              |                                        |                                        |                                           |                                           |                                           | P      |
|-------------------------|------------------------------------------|----------------------------------------|----------------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------|--------|
|                         | Control                                  | 1st (DR0)                              | 2nd (NPDR1)                            | 3rd (NPDR2)                               | 4th (NPDR3)                               | 5th (PDR)                                 |        |
| IL-1 $\beta$ ,<br>pg/mL | 2,12 <sup>abcde</sup><br>(0,89–<br>2,49) | 3,99 <sup>ode</sup><br>(3,16–<br>5,21) | 4,04 <sup>ode</sup><br>(2,77–<br>5,68) | 5,46 <sup>oe</sup><br>(40,3–<br>6,32)     | 9,05 <sup>0ab</sup><br>(7,59–<br>11,04)   | 21,86 <sup>0abc</sup><br>(17,2–<br>28,33) | <0,001 |
| IL-4,<br>pg/mL          | 9,22 <sup>bcd</sup><br>(6,89–<br>10,52)  | 5,24 <sup>cde</sup><br>(4,68–<br>7,47) | 4,64 <sup>oce</sup><br>(3,75–<br>5,74) | 2,67 <sup>0ab</sup><br>(2,12–<br>3,96)    | 2,41 <sup>0a</sup><br>(1,44–<br>3,56)     | 1,1 <sup>0ab</sup><br>(0,64–<br>1,43)     | <0,001 |
| IL-6,<br>pg/mL          | 15,7 $\pm$<br>7,7 <sup>bcd</sup>         | 29,1 $\pm$<br>11,8 <sup>de</sup>       | 38,5 $\pm$<br>13,7 <sup>ode</sup>      | 47,2 $\pm$<br>14,7 <sup>ode</sup>         | 129,2 $\pm$<br>78,7 <sup>0abce</sup>      | 314,3 $\pm$<br>68,9 <sup>0abcd</sup>      | <0,001 |
| IL-8,<br>pg/mL          | 34,8 <sup>cde</sup><br>(27,2–<br>41,6)   | 45,2 <sup>cde</sup><br>(27,8–<br>54,3) | 57,4 <sup>cde</sup><br>(37,6–<br>74,9) | 143,7 <sup>0ab</sup><br>(113,1–<br>182,9) | 350,1 <sup>0ab</sup><br>(309,7–<br>369,8) | 269,4 <sup>0ab</sup><br>(226,6–<br>301,9) | <0,001 |

Notes: Between-group comparisons used ANOVA (for normally distributed data) or the Kruskal–Wallis test (for nonnormal data); posthoc testing employed Tukey–Kramer or Dunn's tests, respectively:

<sup>0</sup> – statistically significant differences versus control group,  $p < 0,05$ ;

<sup>a</sup> – statistically significant differences versus 1st group,  $p < 0,05$ ;

<sup>b</sup> – statistically significant differences versus 2nd group,  $p < 0,05$ ;

<sup>c</sup> – statistically significant differences versus 3rd group,  $p < 0,05$ ;

<sup>d</sup> – statistically significant differences versus 4th group,  $p < 0,05$ ;

<sup>e</sup> – statistically significant differences versus 5th group,  $p < 0,05$ .

indirect ophthalmoscopy (Heine OMEGA 600 Traveler Set; HEINE, Germany); gonioscopy using a contact threemirror Optiek XL lens (USA); ophthalmoscopy with Volk Digital Wide Field lenses (Germany) and a Goldmann threemirror lens; and optical coherence tomography with fundus camera and angiography (HOCT1F, Huvitz Co. Ltd., Korea).

At the start of phacoemulsification, 0.1 mL of AH was obtained via anterior chamber paracentesis. Concentrations of IL1 $\beta$ , IL4, IL6, and IL8 (pg/mL) were measured by solidphase ELISA using Invitrogen (Thermo Fisher Scientific, USA) kits.

Statistical analyses were performed in EZR v.1.54 (graphical interface to R v.4.0.3; R Foundation for Statistical Computing, Austria) [23]. Because data deviated from normality, results are reported as medians (Me) with interquartile ranges (Q1–Q3). Group comparisons used the Kruskal–Wallis test with Dunn's post hoc procedure;  $\alpha = 0.05$  [24]. Diagnostic cutoffs for interleukin concentrations were derived using multiclass classification (onevsall approach) with ROCbased performance assessment [25, 26].

## ETHICS STATEMENT

This study involved human participants and was approved by the local bioethics committee. Written informed consent was obtained from all participants. The research was conducted in accordance with the

Declaration of Helsinki. No animal experiments were performed.

## RESULTS

Analyses were performed across five clinical groups of patients with T2DM who either had no DR or exhibited DR at various stages (mild, moderate, severe NPDR, or PDR). Sex distribution was balanced across groups (women: 18 (16.4%); men: 92 (83.6%);  $p = 0.9703$ ), minimizing sex as a potential confounder. Age was comparable between groups (global  $p = 0.108$ ), whereas diabetes duration increased in a stepwise fashion with DR stage: DR0 – 5.0 (3.3–7.5) years; NPDR1 – 10.0 (5.0–14.5); NPDR2 – 14.0 (10.0–18.5); NPDR3 – 16.5 (10.0–21.0); PDR – 15.5 (15.0–25.0);  $p < 0.001$ .

At enrollment, indices of carbohydrate metabolism (fasting plasma glucose, HbA1c) did not differ significantly across groups ( $p = 0.176$  and  $p = 0.101$ , respectively), whereas Cpeptide showed a downward trend in more severe phenotypes without reaching statistical significance ( $p = 0.108$ ). Thus, the clinical groups were comparable for age, sex, and glycemic control and differed primarily by diabetes duration – parameters that define the baseline context for subsequent analyses of AH interleukin levels.

Table 1 summarizes the concentrations of IL1 $\beta$ , IL4, IL6, and IL8 in AH by group. Global between-group differences were statistically significant for all markers (all  $p < 0.001$ ) and were corroborated by appropriate



**Table 2.** Analytical performance of stage prediction according to aqueous humor interleukin concentrations at different stages of diabetic retinopathy

| Metric              | Study group        |           |            |             |             |        |
|---------------------|--------------------|-----------|------------|-------------|-------------|--------|
|                     | Control            | DR0       | NPDR1      | NPDR2       | NPDR3       | PDR    |
| IL-1β, pg/mL        |                    |           |            |             |             |        |
| Cutoff              | <2.98              | 2.98-6.21 | 6.22-7.87  | 7.88-8.35   | 8.36-13.68  | >13.68 |
| Sensitivity, %      | 100                | 73.3      | 17.5       | 4.0         | 75.0        | 100    |
| Specificity, %      | 84.5               | 77.5      | 91.6       | 100         | 100         | 99.1   |
| Overall accuracy, % | 53% (CI 44% – 62%) |           |            |             |             |        |
| IL-4, pg/mL         |                    |           |            |             |             |        |
| Cutoff              | >5.98              | 5.98-4.20 | 4.19-3.10  | 3.11-1.84   | 1.83-0.65   | <0.65  |
| Sensitivity, %      | 96.0               | 46.7      | 20.0       | 40.0        | 41.7        | 27.8   |
| Specificity, %      | 83.6               | 90.0      | 92.6       | 93.6        | 86.2        | 96.6   |
| Overall accuracy, % | 44% (CI 35% – 53%) |           |            |             |             |        |
| IL-6, pg/mL         |                    |           |            |             |             |        |
| Cutoff              | <23.8              | 23.8-34.3 | 34.4-67.0  | 67.1-86.2   | 86.3-288.5  | >288.5 |
| Sensitivity, %      | 92.0               | 46.7      | 62.5       | 0           | 58.3        | 72.2   |
| Specificity, %      | 90.0               | 90.0      | 69.5       | 100         | 95.9        | 100    |
| Overall accuracy, % | 56% (CI 47% – 64%) |           |            |             |             |        |
| IL-8 pg/mL          |                    |           |            |             |             |        |
| Cutoff              | <54.7              | 54.7-70.3 | 70.4-113.8 | 113.9-256.5 | 256.6-351.2 | >351.2 |
| Sensitivity, %      | 100                | 26.7      | 32.5       | 76.0        | 50.0        | 66.7   |
| Specificity, %      | 70.2               | 94.7      | 94.4       | 96.2        | 100         | 96.6   |
| Overall accuracy, % | 58% (CI 49% – 67%) |           |            |             |             |        |

post hoc testing (Tukey-Kramer or Dunn’s, according to distributional assumptions).

For IL1β, a monotonic increase was observed from control to PDR: control, median 2.12 pg/mL (0.89-2.49); PDR, 21.86 pg/mL (17.2-28.33), with sequential stepups between intermediate classes.

IL4 decreased with increasing DR severity: control, 9.22 pg/mL (6.89-10.52) to PDR, 1.10 pg/mL (0.64-1.43), a pattern compatible with a shift away from a Th2type immune profile as DR progresses.

IL6 exhibited the largest dynamic range (mean±SD), rising from 15.7±7.7 pg/mL in controls to 314.3±68.9 pg/mL in PDR, with a pronounced surge at NPDR3 (129.2±78.7 pg/mL).

For IL8, a characteristic “peaked” pattern emerged: values increased from control 34.8 pg/mL (27.2-41.6) to NPDR3 350.1 pg/mL (309.7-369.8), followed by a partial decline in PDR to 269.4 pg/mL (226.6-301.9), consistent with a chemoattractant/proangiogenic axis predominating at nonproliferative stages and transitioning into a remodeling phase thereafter [27].

To analyze AH interleukin levels across DR stages and to evaluate their association with stagewise progression, we selected optimal thresholds using a OnevsAll multiclass classification approach [25].

The visualization in Figure 1 revealed orderly yet distinct gradients: IL1β increased monotonically from control to PDR; IL4 declined with advancing severity; IL6 showed the largest dynamic range with a marked surge at NPDR3; and IL8 exhibited a “peaked” profile with a maximum at NPDR3 followed by a relative decrease in PDR.

These visual trends informed a sixclass classification scheme (control, DR0, NPDR1, NPDR2, NPDR3, PDR), for which Table 2 provides cutoff intervals and classspecific metrics (sensitivity/specificity) together with overall model accuracy.

IL1β cutoffs: <2.98 (control), 2.99-6.21 (DR0), 6.22-7.87 (NPDR1), 7.88-8.35 (NPDR2), 8.36-13.68 (NPDR3), >13.68 pg/mL (PDR). Very high specificity was observed at the extremes (notably PDR), but overlap between adjacent NPDR classes yielded an overall accuracy of ≈ 53% (95% CI: 44-62%).

IL4 cutoffs shifted downward from higher values in controls to lower values in PDR (from >5.98 to <0.65 pg/mL), with overall accuracy ≈ 44% (35-53%).

IL6 cutoffs: <23.8 (control), 23.8-34.3 (DR0), 34.4-67.0 (NPDR1), 67.1-86.2 (NPDR2), 86.3-288.5 (NPDR3), >288.5 pg/mL (PDR), with overall accuracy ≈ 56% (47-64%).

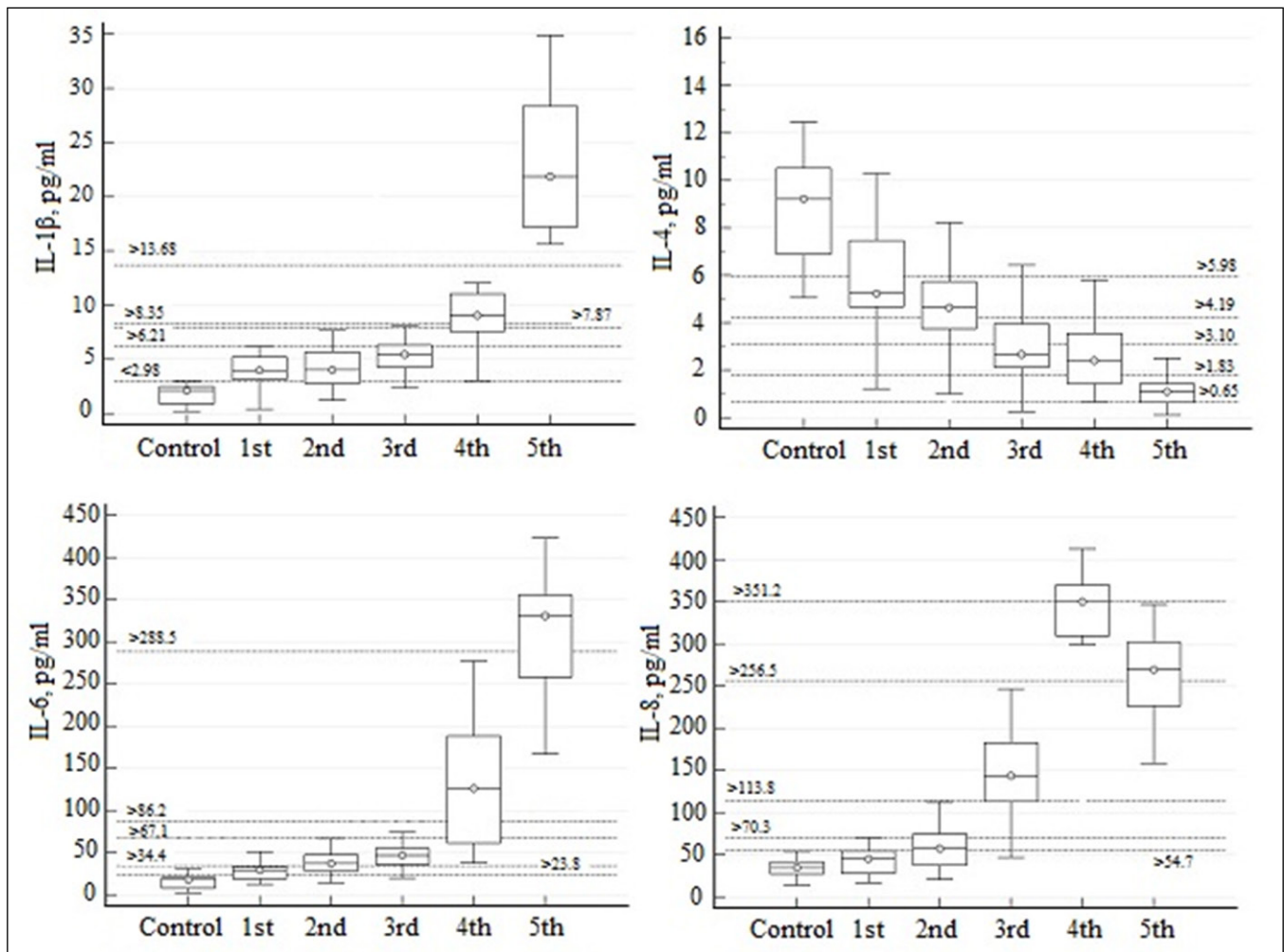


**Figure 1.** Aqueous humor interleukin concentrations by group; yaxis: interleukin concentration (pg/mL). Cutoff values (pg/mL) used for DR stage prediction are indicated on the plots.

IL8 cutoffs respectively: <54.7, 54.3-70.3, 70.4-113.8, 113.9-256.5, 256.6-351.2, >351.2 pg/mL, with overall accuracy  $\approx$  58% (49–67%).

Thus, in the sixclass formulation, all four interleukins provided better metrics at the extremes (control, PDR) due to high specificity, with expected distributional overlap among adjacent NPDR categories that limited classbyclass discrimination (Figure 1, Table 2).

To account for the continuous biology of DR progression and the discrete nature of the ICDR clinical scale - and to improve analytical performance - we applied clinically relevant binarization of phenotypes into two integrated groups: mild/moderate DR (NPDR1 + NPDR2) and severe DR (NPDR3 + PDR) (Figure 2).

On Figure 2 the binary thresholds are summarized, while Table 3 reports their analytical characteristics (sensitivity, specificity, accuracy). Among the markers, IL6 performed best: 34.4–86.2 pg/mL corresponded to mild/moderate DR, whereas > 86.2 pg/mL indicated severe DR; overall accuracy was 72.6% (95% CI 64.3–79.9)

with an acceptable balance of sensitivity and specificity.

For IL8, the corresponding thresholds 70.4–216.9 and > 216.9 pg/mL yielded 62.2% (95% CI 53.2–70.7) accuracy, supporting its role as an auxiliary marker in the binary model. By contrast, IL1 $\beta$  (6.22–8.24 and > 8.24 pg/mL) and IL4 (1.83–4.18 and < 1.83 pg/mL) each achieved  $\approx$  55.6% accuracy, indicating limited practical utility for distinguishing mild/moderate from severe DR.

Taken together, Figure 2 and Table 3 illustrate the translation of continuous interleukin changes into a parsimonious prognostic scale, with IL6 emerging as the primary single local biomarker for rapid risk stratification.

To align the biochemical profile with the clinical/metabolic context, we computed Spearman rank correlations between AH interleukin levels and age, diabetes duration, fasting glucose, HbA1c, and Cpeptide (Figure 3).

The expected pattern of weak/moderate correlations emerged: positive associations for inflammatory/angiogenic markers – particularly IL6 and IL8 – with diabetes

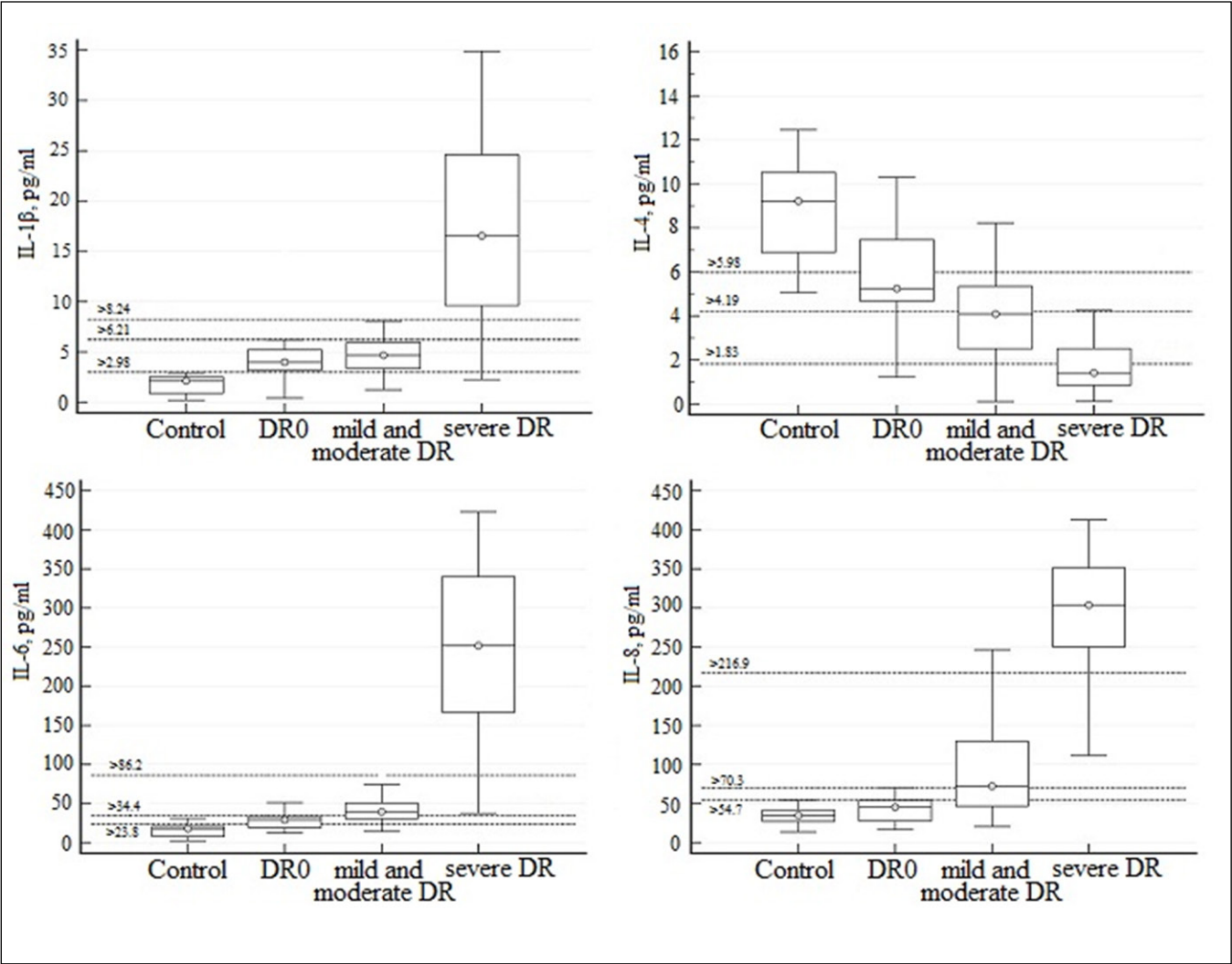


**Figure 2.** Threshold interleukin levels in aqueous humor (integrated groups); yaxis: interleukin concentration (pg/mL). Cutoff values (pg/mL) are shown for predicting mild and moderate DR (NPDR2 and NPDR3 groups) and severe DR (NPDR3 and PDR groups).

duration and selected indices of carbohydrate metabolism, and negative associations for IL4 and Cpeptide (as an indicator of  $\beta$ cell reserve). Several pairs reached  $p<0.05$ ; however, the absolute magnitudes remained within the weak/moderate range, consistent with the local (intraocular) nature of the markers compared with systemic metrics. This correlation profile strengthens the biologic plausibility of the proposed thresholds: as diabetes duration increases and/or glycemic control deteriorates, neuroinflammatory/angiogenic indicators shift toward higher ranges, whereas the regulatory IL4 decreases; at the same time, a substantial portion of variance is extra-systemic, i.e., driven by local ocular processes, further justifying AH as a target matrix for biomarker analysis.

DISCUSSION

Our findings confirm that the AH exhibits a clear proinflammatory/proangiogenic gradient across analytes

with advancing DR stage. In a clinically relevant binary framework (mild/moderate vs severe DR), IL6 showed the highest discriminative performance (overall accuracy 72.6%), whereas IL1 $\beta$  and IL4 were weak classifiers in our cohort, and IL8 functioned as an auxiliary marker with better specificity at the extremes. This hierarchy accords with contemporary concepts on the central role of IL6 in retinal microvascular dysfunction – including transsignaling via sIL6R (soluble interleukin 6 receptor)/gp130 (glycoprotein 130) – which integrates neuroinflammation, endothelial hyperpermeability, and hypoxiadriven cascades [28-31]. The biological rationale for this hierarchy is well explained by current insights into NLRP3mediated inflammation, IL6 transsignaling, and neutrophil chemoattraction [32]. Hyperglycemia activates the NLRP3 inflammasome, triggering IL1 $\beta$  and IL18 release, pyroptotic death within the retinal neurovascular unit, and disruption of the blood-retinal barrier (BRB) [32]. These

**Table 3.** Analytical performance of predictions based on aqueous humor interleukin concentrations (integrated groups)

| Metric              | DR stage                 |           |                    |           |
|---------------------|--------------------------|-----------|--------------------|-----------|
|                     | Control                  | DR0       | Mild & Moderate DR | Severe DR |
| IL-1β, pg/mL        |                          |           |                    |           |
| Cutoff              | <2.98                    | 2.98-6.21 | 6.22-8.24          | >8.24     |
| Sensitivity, %      | 88.0                     | 73.3      | 23.1               | 90.0      |
| Specificity, %      | 84.5                     | 90.0      | 98.6               | 96.2      |
| Overall accuracy, % | 55.6% (CI 46.8% – 64.1%) |           |                    |           |
| IL-4, pg/mL         |                          |           |                    |           |
| Cutoff              | >5.98                    | 4.19-5.98 | 1.83-4.18          | <1.83     |
| Sensitivity, %      | 96.0                     | 46.7      | 36.9               | 66.0      |
| Specificity, %      | 83.6                     | 91.7      | 88.6               | 89.5      |
| Overall accuracy, % | 55.6% (CI 46.8% – 64.1%) |           |                    |           |
| IL-6, pg/mL         |                          |           |                    |           |
| Cutoff              | <23.8                    | 23.8-34.3 | 34.4-86.2          | >86.2     |
| Sensitivity, %      | 80.0                     | 46.7      | 70.8               | 83.3      |
| Specificity, %      | 90.0                     | 92.5      | 84.3               | 100       |
| Overall accuracy, % | 72.6% (CI 64.3% – 79.9%) |           |                    |           |
| IL-8, pg/mL         |                          |           |                    |           |
| Cutoff              | <54.7                    | 54.8-70.3 | 70.4-216.9         | >216.9    |
| Sensitivity, %      | 95.7                     | 26.7      | 50.8               | 83.3      |
| Specificity, %      | 70.2                     | 96.4      | 91.9               | 98.1      |
| Overall accuracy, % | 62.2% (CI 53.2% – 70.7%) |           |                    |           |

pathways – corroborated by experimental data, clinical specimens, and systematic reviews [32-34] – provide the substrate for the pleiotropic actions of IL6 and the proangiogenic/chemokine effects of IL8.

In the literature, IL1 $\beta$  is consistently linked to BRB dysfunction (NF $\kappa$ B activation, oxidative stress, mitochondrial injury) and pyroptosis [35], as well as to pericyte loss [36], a classic early hallmark of diabetic microangiopathy [37]. Although elevated IL1 $\beta$  has been repeatedly documented in retinal tissue and intraocular fluids in experimental diabetes and in patients with PDR, our classification analyses – unsurprisingly – revealed overlap of IL1 $\beta$  concentrations between adjacent NPDR stages, leading to low overall accuracy despite high specificity at the extremes (DR0, PDR). Thus, IL1 $\beta$  emerges as a sensitive indicator of retinal inflammatory activity but not a reliable stratifier of DR severity.

A similar rationale applies to IL4. As a Th2 associated immunomodulator, IL4 has been shown to protect pericytes, reduce endothelial permeability, and modulate the microglial response via signal transducer and activator of transcription6 (STAT6)-dependent mechanisms [38]. However, in realworld clinical settings – amid variability in glycemia, diabetes duration, and other systemic parameters – the antiinflammatory axis of IL4

manifests as a general downward trend without a clear demarcation between adjacent stages. Consequently, within a binary severity framework, IL4 offers limited value as a “rulein/ruleout” marker despite its evident biological relevance.

The literature contains substantial evidence linking intraocular IL6 with DR/DME phenotypes, reinforcing the external plausibility of our conclusion that IL6 is the priority biomarker for binary stratification. Recent reviews consistently demonstrate associations between elevated IL6 in aqueous/vitreous humor and retinal edema, microvascular disorganization, and concordant imaging changes, underscoring the conceptual nodal role of IL6 along the DR-DME pathobiologic axis [39, 40]. In addition, multiomics studies of AH delineate protein networks in which IL6 occupies a central position among inflammatory and extracellularmatrix remodeling mediators, aligning with our empirical identification of IL6 as the most informative single local indicator of DR severity for a clinically relevant twoclass model [31,42]. Collectively, these data support the use of IL6 threshold intervals as a practical instrument for risk stratification in routine clinical scenarios [40].

The stagedependent behavior of IL8 in our cohort – marked elevation through NPDR3 with a relative decrease



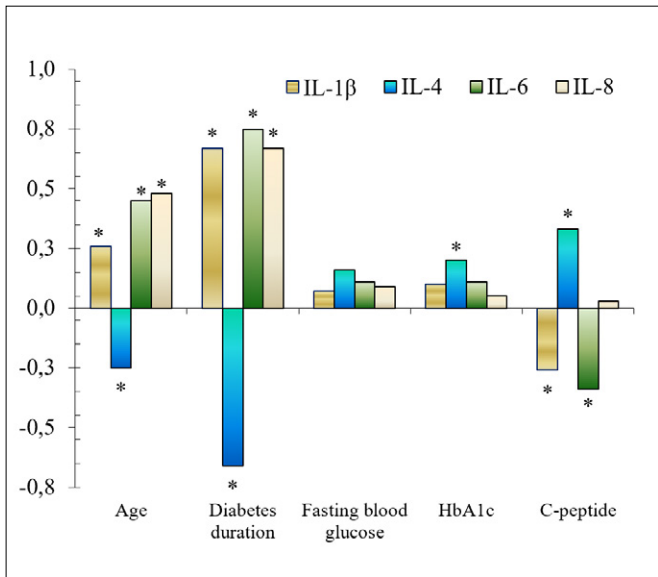


**Figure 3.** Spearman rank correlation coefficients for AH interleukins versus age, diabetes duration, fasting blood glucose, HbA1c, and Cpeptide; yaxis: correlation coefficient. \* indicates  $p < 0.05$ .

in PDR – explains why it underperforms IL6 in binary metrics despite strong specificity at the extremes. Broader transitional ranges between adjacent classes produce overlap and reduce overall accuracy. This pattern accords with aggregated clinicallaboratory observations in which IL8 consistently associates with inflammatory activity, edema severity, and microvascular remodeling, more reliably flagging phases of maximal activity than faithfully mirroring gradations of process severity across DR [22, 43].

The conceptual framework for interpreting our molecular thresholds is their concordance with quantitative imaging of retinal microperfusion. OCTA data indicate that the earliest alterations are localized to the DCP, characterized by perivenular capillary rarefaction and an expansion of nonperfusion, which correlate with the occurrence of complications and with DR progression [44]. Moreover, in a subset of patients, applied OCTA metrics – such as the algorithmic diabetic macular ischemia (DMI) index, branching/fragmentation indices, and foveal avascular zone (FAZ)derived parameters – carry prognostic value [45-49].

Against this backdrop, IL6 threshold intervals can serve as a molecular complement to the structural-perfusion signal: OCTA tracks the consequences of microvascular dysfunction, whereas the AH composition captures the active biochemical processes sustaining it. Integrating these domains thus provides the basis for individualized risk assessment of DR progression within a unified clinical strategy [50].

The correlation analysis merits separate consideration as a tool for aligning local AH interleukin dynamics with the systemic background of DR (age, diabetes duration,

fasting glucose, HbA1c, Cpeptide). The observed weakto-moderate correlations are methodologically expected: in biomedical datasets, correlation coefficients are seldom large and should be interpreted not solely by  $\rho$ , but primarily through effect size and clinical significance [51, 52]. More broadly, published correlational effect sizes in clinical sciences tend to cluster within the small/moderate range [53], underscoring the value of combining molecular and imaging indicators to enhance prognostic sensitivity.

Substantively, the positive correlations of IL6/IL8 with diabetes duration and with selected glycemic indices mirror established epidemiological patterns: disease duration and metabolic milieu (including HbA1c variability) are associated with DR risk/progression, although their direct influence on local intraocular mediators remains incompletely defined [54-56]. Conversely, the inverse correlations with Cpeptide – characteristic of regulatory parameters in our sample – are consistent with reports on the prognostic relevance of postprandial Cpeptide levels and Cpeptide-to-glucose ratios for DR risk stratification in type 2 diabetes [57, 58]. Importantly, these systemic factors explain only a limited proportion of the variability in intraocular interleukin levels; the major contribution arises from local ocular processes, as supported by contemporary profiling studies [42].

A key methodological anchor of our approach is its continuity with previously validated local biomarkers in aqueous humor within the same disease model, particularly HIF1 $\alpha$  as an indicator of hypoxic load: an analogous thresholdbased and binary framework provided practical utility for severity stratification in our prior work [17]. This concordance strengthens the credibility of interpreting local threshold readouts and furnishes a rationale for their subsequent integration into a composite diagnostic panel.

Practical implications for clinical management can be summarized as follows. First, IL6 should be considered a primary local risk indicator in patients with DR: values above the upper bound of the mild/moderate interval in our dataset ( $>86.2$  pg/mL) support classification into the severe DR group and justify shorter followup intervals with targeted OCT/OCTA surveillance, whereas values within the mild/moderate interval may support standard visit frequency with emphasis on optimizing systemic metabolic risk factors for DR.

Second, IL8 may be used as an adjunct to corroborate polar phenotypes; however, midspectrum interpretation warrants caution because of broader interclass overlap.

Third, IL1 $\beta$  and IL4 do not provide decisive standalone information in the binary framework, limiting their use as solitary cutoffs; their application appears more

promising within combined panels subject to external validation [47,50].

Strengths of the study include a single-cohort design with consistent AH sampling, centralized and standardized preanalytics within one center, and a harmonized statistical framework (threshold intervals; multiclass and binary models with estimates of sensitivity, specificity, and overall accuracy), all of which ensured internal coherence of conclusions and reproducibility of the interpretive logic. Corroboration by independent sources (reviews/analyses on interleukins, AH proteomics, and OCTA metrics) enhances the external validity of the key conclusion prioritizing IL6 in binary stratification [39, 41, 45].

Limitations include the single-center, cross-sectional design without evaluation of downstream clinical outcomes, which constrains causal inference and limits generalizability of numeric thresholds. Class imbalance across stages (uneven group representation) may influence the stability of certain metrics – particularly accuracy – in external datasets, despite the invariance of ROC curves to class prevalence; hence the need for external testing in representative cohorts [59, 60].

Results may also be affected by preanalytical factors (storage duration/conditions, freeze–thaw cycles) and crossplatform differences among immunoassay methods (ELISA, multiplex systems). Although unified procedures were followed, contemporary guidance recommends protocol standardization and interlaboratory comparison prior to routine implementation of quantitative cutoffs [61–63]. Finally, the absence of long-term follow-up precludes assessment of the prognostic value of the proposed thresholds for clinically meaningful endpoints (progression to NPDR3/PDR, development of DME), defining priorities for future multicenter prospective studies with independent external validation [64].

## CONCLUSIONS

1. We observed significant between-group differences in AH interleukin levels (all  $p < 0.001$ ): IL1 $\beta$  and IL6 increased with advancing stage; IL8 peaked at NPDR3 with a relative decline in PDR; IL4 progressively decreased as DR progressed.
2. In the multiclass model, single-analyte performance yielded only moderate overall accuracy (IL6  $\approx 56\%$ , IL8  $\approx 58\%$ , IL1 $\beta$   $\approx 53\%$ , IL4  $\approx 44\%$ ), with the best discrimination at the extremes – findings that supported a shift to binary stratification.
3. In the binary model (mild/moderate vs severe DR), IL6 demonstrated the highest informativeness (accuracy 72.6%, 95% CI 64.3–79.9), IL8 provided auxiliary value (62.2%), whereas IL1 $\beta$  and IL4 were of limited practical utility for classification.
4. Operational cutoffs for clinical management were established: for IL6 – 34.4–86.2 pg/mL (mild/moderate DR) and  $>86.2$  pg/mL (severe DR); for IL8 –  $>216.9$  pg/mL as a supportive indicator of severe DR.
5. Correlation analysis confirmed weak-to-moderate positive associations of IL6/IL8 with diabetes duration and selected glycemic indices, and inverse correlations with C-peptide, characteristic of regulatory parameters. This profile reflects the local nature of AH mediators and supports the biological plausibility of the proposed thresholds.
6. IL6 can serve as a primary local risk marker to identify patients with a high likelihood of severe DR who warrant shortened follow-up intervals and intensified structural-functional surveillance using OCTA. Next steps include external validation of numeric cutoffs, preanalytical standardization, and prospective studies evaluating clinically meaningful endpoints.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# The role of MAOA gene expression in human aggressive behaviors

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## ABSTRACT

**Aim:** To identify whether the association between MAOA gene expression and exposure to antisocial outcomes.

**Materials and Methods:** A hundred and eighty samples (100 depressed patients going to psychiatric clinics and 80 healthy) of males and females were involved in the present study. Gene expression was performed for patients and controls using the real-time PCR technique to compare the expression of the MAOA gene when exposed to different types and levels of trauma.

**Results:** The results show a decrease in the expression activity of the MAOA gene in most patients. The data revealed that males have a lower expression of the MAOA gene than females. There were no significant differences down-regulated in the folding expression  $2^{-\Delta\Delta Ct}$  of MAOA gene in patients who were prone to trauma exposure (missing someone, domestic violence, exposure to community violence, alcohol abuse, physical abuse, and sexual abuse). In terms of marital status, there was no significant upregulation of fold changes in the expression of the MAOA gene recorded in the patient group ( $2^{-\Delta\Delta Ct} = 1.3, 1.3, \text{ and } 1.5$ ) when compared with the healthy group ( $2^{-\Delta\Delta Ct} = 1$ ).

**Conclusions:** Our results suggest that the MAOA gene controls aggression and its effect on trauma history and levels.

**KEY WORDS:** MAOA gene, antisocial behavior, aggression, gene expression, RTPCR

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## ABBREVIATIONS

MAOA: Monoamine oxidase A

TTEs total: trauma exposure score

qRT-PCR: Quantitative Real-Time PCR

RNA: Ribonucleic acid

DNA: deoxyribonucleic acid

cDNA: complementary DNA

## INTRODUCTION

Monoamine oxidase-A (MAOA) is an enzyme that preferentially breaks down the catecholamine neurotransmitters (norepinephrine, serotonin, and dopamine) and is produced in the mitochondrion [1]. The level of this enzyme was affected directly by the MAOA gene [2]. The MAOA gene is located on the X-chromosome (Xp11.23) and has become known as a significant genetic factor associated with mental disorders [3]. Notably, MAOA gene activity is regulated by the essential region of its promoter, which spans between -71 and -40 bp, and deletion affects the activity of the MAOA gene and reduces it by 64–83% in different human

cell lines [4]. Importantly, when the MAOA gene is normally expressed, it was found that the level of the enzyme is in the normal range [5], but if the gene expression were decreased, the activity of the enzyme would affect human behavior, increasing tension, aggression, and anti-society [6]. Theoretically, the MAOA gene effects human depression and behaviors when the expression is too high or too low, and negative effects will be obtained in both situations [7]. Only a few studies in the past have mentioned and associated aggression with the genetic effect [8]. Studies reported that human behavior is affected by many environmental, external, and genetic factors; these factors interacted with each other to produce human behavior of human [9]. Despite the interaction and overlap of these factors, it is possible to distinguish the role of each factor separately and study their impact. Many evidences refer to the possible link between violent and criminal human behavior and genetics [11]. Although studies indicate that this link is very strongly related to the MAOA gene and especially its protein products, which are shaped and characteristic in a special way to form some hormones [12].

**Table 1.** Score of trauma history exposure in patients and control

| Trauma history Exposure               | Male control (40) |    |   | Female control (40) |    |   | Male patients (44) |    |    | Female patients (56) |    |    |
|---------------------------------------|-------------------|----|---|---------------------|----|---|--------------------|----|----|----------------------|----|----|
| Scale                                 | 0                 | 1  | 2 | 0                   | 1  | 2 | 0                  | 1  | 2  | 0                    | 1  | 2  |
| Physical Abuse                        | 30                | 8  | 2 | 34                  | 4  | 2 | 22                 | 14 | 8  | 20                   | 24 | 12 |
| Sexual Abuse                          | 38                | 2  | 0 | 38                  | 2  | 0 | 40                 | 6  | 0  | 42                   | 12 | 2  |
| Domestic Violence                     | 32                | 8  | 0 | 33                  | 6  | 0 | 20                 | 18 | 8  | 28                   | 22 | 6  |
| Exposure to Community Violence        | 20                | 18 | 2 | 26                  | 14 | 0 | 22                 | 14 | 10 | 26                   | 22 | 8  |
| Missing some body Bad love experience | 30                | 6  | 4 | 30                  | 10 | 0 | 32                 | 10 | 2  | 36                   | 16 | 4  |

Scoring criteria: 0 - means No exposure

**Table 2.** Age variable in both males and females of the studied groups

| Groups   | Age (mean± SD) |           | p-value |
|----------|----------------|-----------|---------|
|          | Males          | Females   |         |
| Patients | 38.5±10.8      | 34.2±13.5 | 0.221   |
| Control  | 40.7±11.04     | 39.3±12.2 | 0.68    |
| p-value  | 0.43           | 0.185     |         |

Data was presented as mean± SD according to student t-test

AIM

The aim of this work was to identify whether the association between MAOA gene expression and exposure to antisocial outcomes.

MATERIALS AND METHODS

SUBJECTS OF THE STUDY

One hundred and eighty males and females (ages 17–50) were involved in this study. One hundred samples comprised aggression and depressed patients who attended a psychiatric clinic, with an age range of males 38.5±10.8 and females 34.2±13.5. In addition, eighty healthy volunteers with an age range of males 40.7±11.04 and females 39.3±12.2 were included in the current study.

ETHICAL APPROVEMENT

The agreement of the Ethics Committees was approved by the Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq, with reference numbers M.B.8 / 2.1. 23). Informed consent was given by all the participants.

DATA AVAILABILITY STATEMENT

The corresponding author can provide the datasets used in and/or analyzed during the current work upon justifiable request.

DETERMINE THE TOTAL TRAUMA EXPOSURE SCORE (TTES)

Several source and data information were taken to detect trauma exposure history in patients and control. In this study, a form of trauma was evaluated involve (Missing some body, marital status, Domestic Violence, Exposure to Community Violence, Alcohol abuse, Physical abuse and Sexual abuse in different scales from 0 to 2 (Table 1).

Physical abuse rating:

- 1 - excessive physical discipline (e.g., hit with object, no bruising)
- 2 - physical abuse with bruising or more serious injury

Sexual abuse:

- 1 - exposure to pornography or peer-related sexual misconduct
- 2 - sexual abuse with an adult of sufficient severity to warrant criminal or protective services intervention

Domestic violence:

- 1 - frequent intense verbal arguments, throw objects at one another
- 2 - more serious domestic disputes (e.g, hit other, use weapons, injury)

Community violence:

- 1 - gunshots heard in neighborhood, witness serious physical fight
- 2 - witness shooting, death, or serious injury of another.

Missing some body or Bad love experience:

- 1-struggling with upsetting emotion and memories
- 2- effect on all life activities



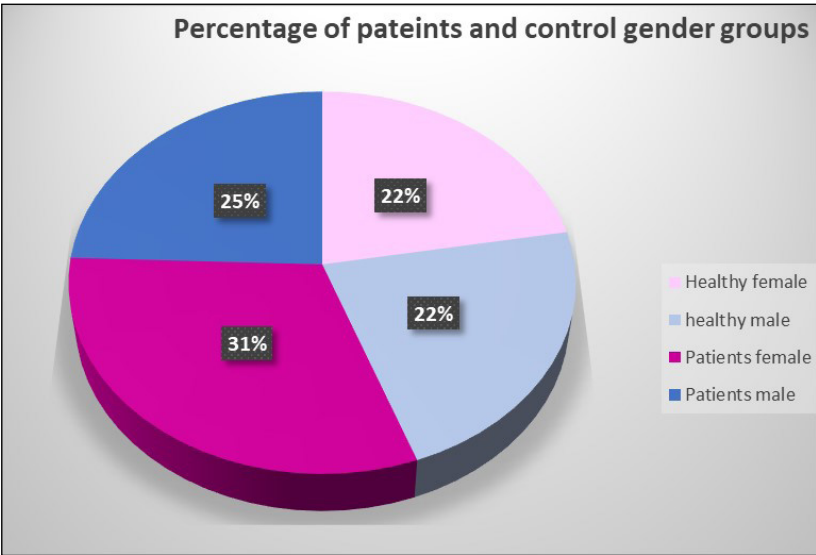


**Fig. 1.** Percentage of gender for patient and control groups  
*Source: Own materials*

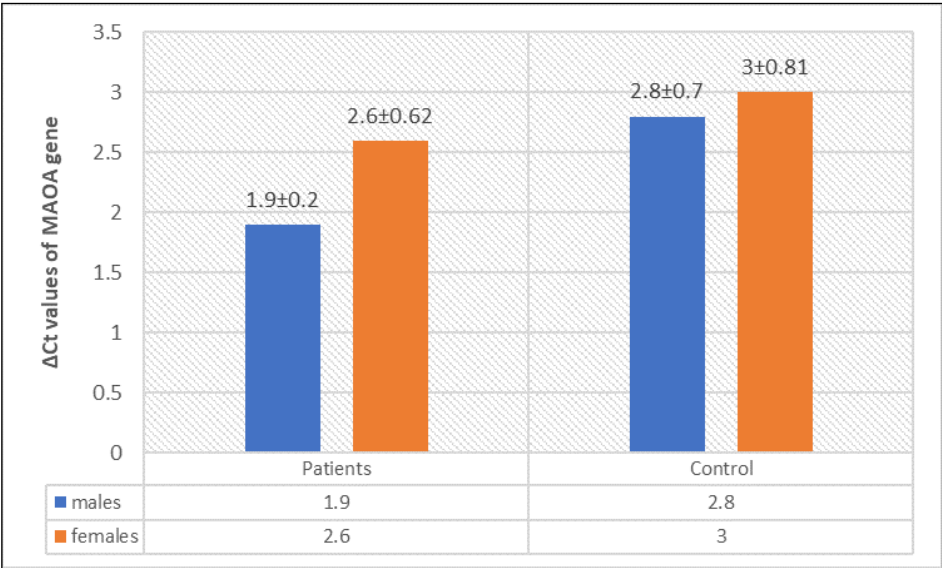


**Fig. 2.**  $\Delta$ Ct value of MAOA gene in both males and females of patients and control group  
Data was presented as mean  $\pm$  SD according to student t-test  
*Source: Own materials*

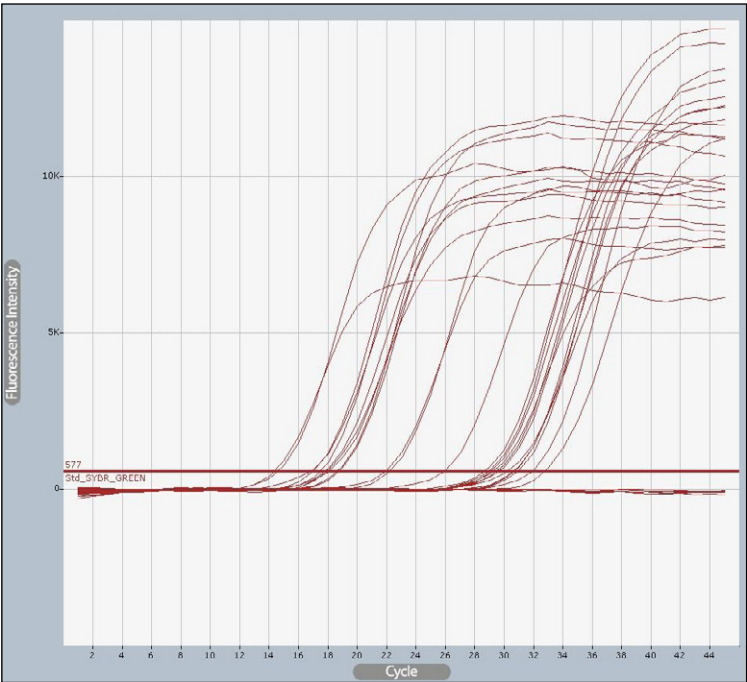


**Fig 3.** Amplification plots of MAOA gene done by qPCR  
*Source: Own materials*



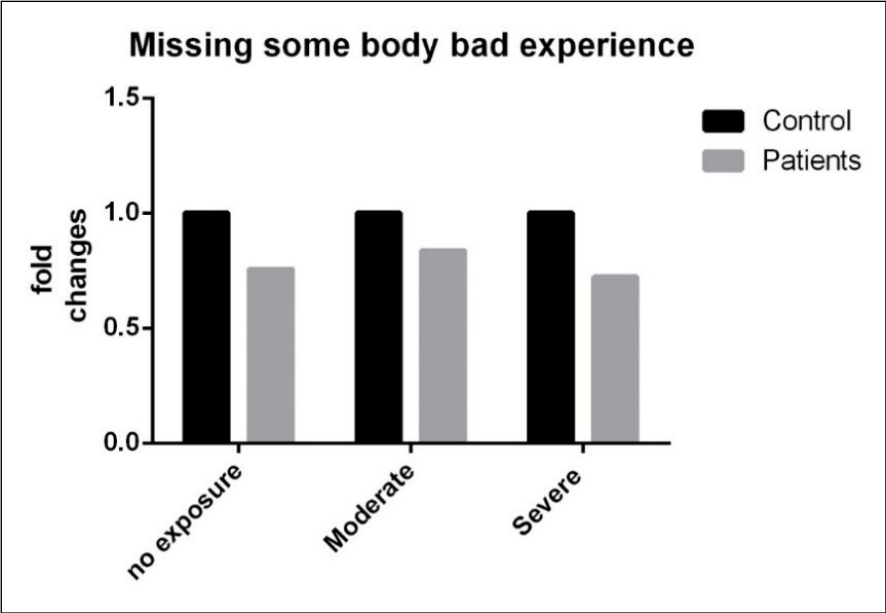


**Fig 4.** Comparison the effect of the loss of a person or bad love experience in relative fold changes of studied groups  
*Source: Own materials*

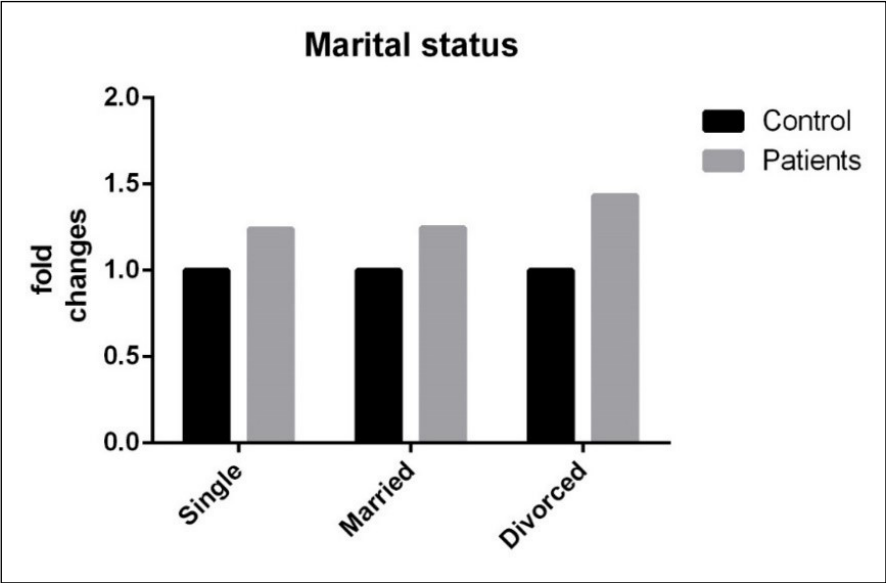


**Fig 5.** Comparison the effect of Marital status in fold changes for healthy and patients' group  
*Source: Own materials*

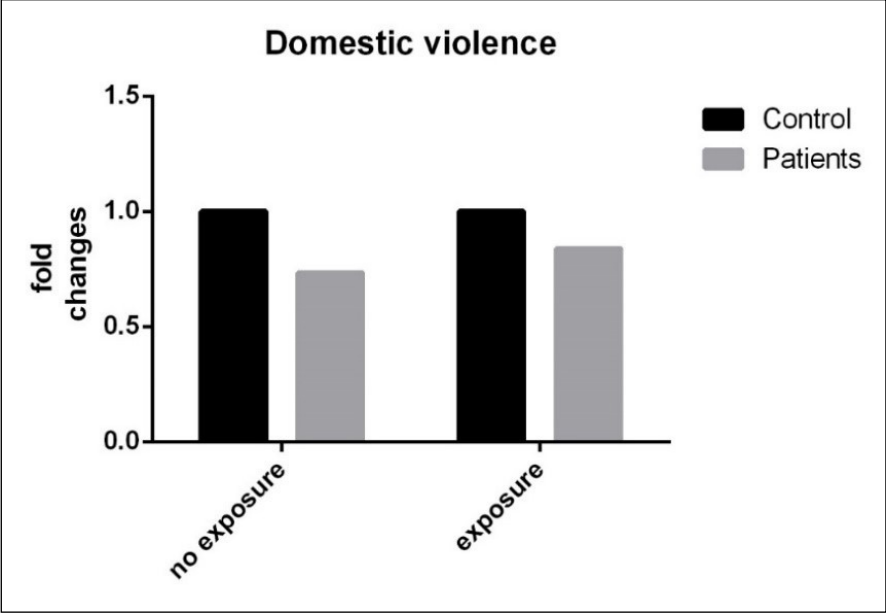


**Fig 6.** Comparison the effect of Domestic violence in fold changes for healthy and the patients group  
*Source: Own materials*

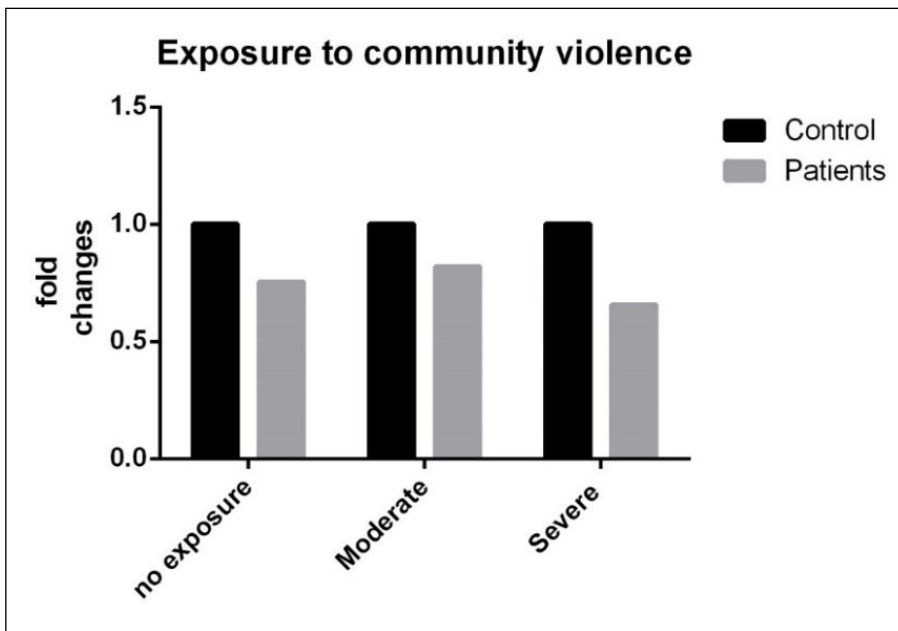


**Fig. 7.** Comparison the effect of exposure to community violence in fold changes for healthy and the patients group  
Source: Own materials

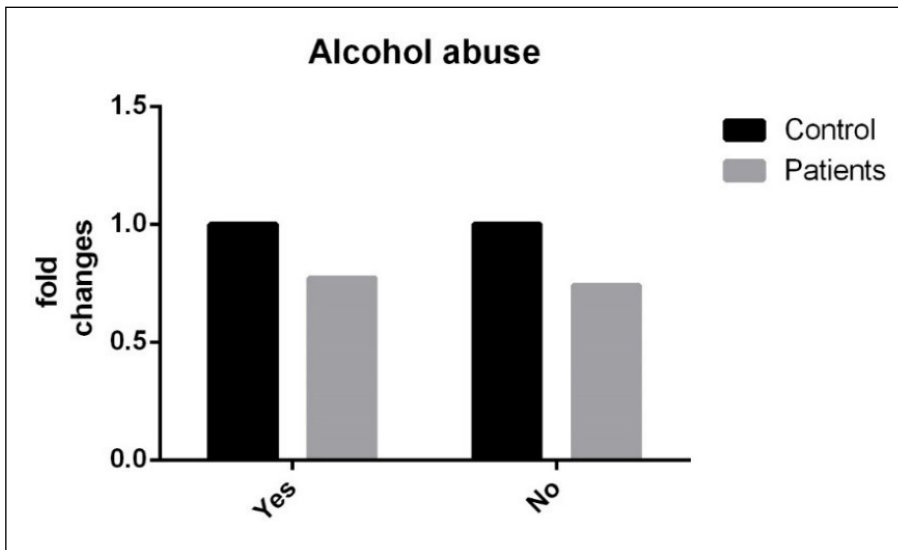


**Fig. 8.** Comparison the effect of alcohol abuse in fold changes for healthy and the patients group  
Source: Own materials

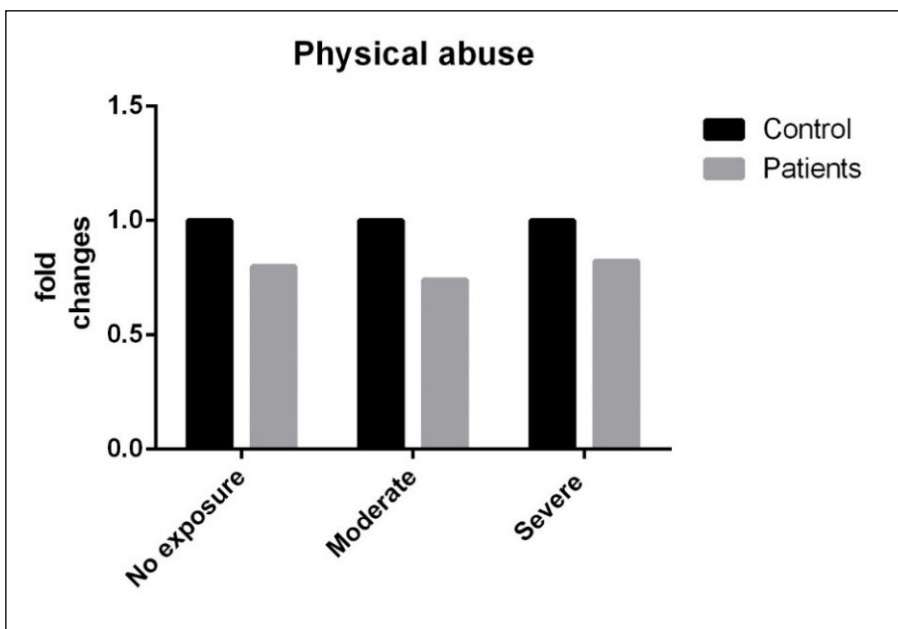


**Fig. 9.** Comparison the effect of physical abuse in fold changes for healthy and the patients group  
Source: Own materials

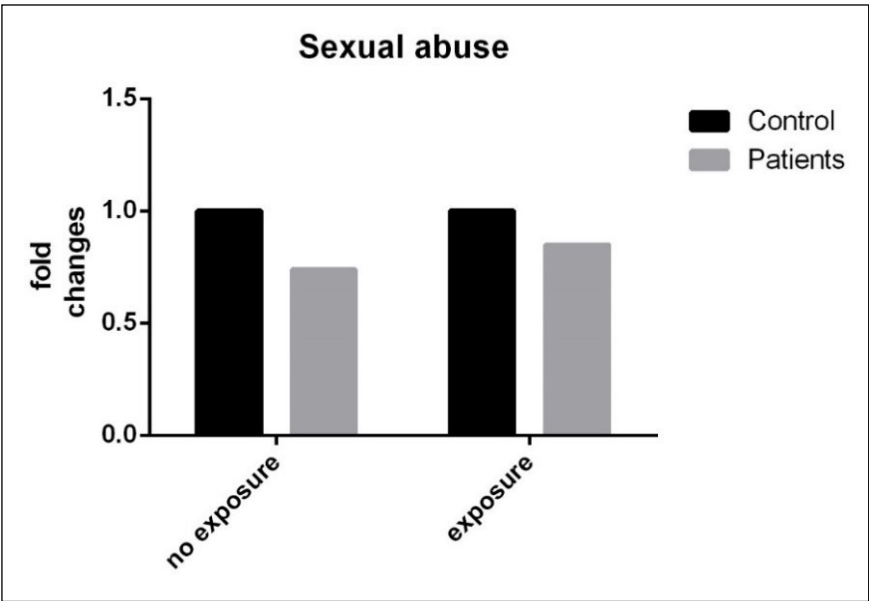


**Fig 10.** Comparison the effect of sexual abuse in fold changes for healthy and the patient groups  
*Source: Own materials*

**RNA EXTRACTION**

The quantification of gene expression of the MAOA gene was identified by qRT-PCR. Five ml of blood were collected from the patients and healthy subjects, and then the total RNA was isolated from the peripheral blood samples using TRIzol® LS Reagent according to manufacturer protocol (Invitrogen™). In brief, TRIzol™ Reagent was added, followed by 1 ml of chloroform for lysis, then thoroughly mixed by shaking, and incubated for 2–3 minutes. The mixture was centrifuged for 15 minutes at 12,000 × g at 4°C, then the mixture separated into a lower phenol-chloroform, an interphase, and a colorless upper aqueous phase and transferred the aqueous phase containing the RNA to a new tube. 0.5 ml of isopropanol was added to the aqueous phase, incubated for 10 minutes at 4°C, and centrifuged for 10 minutes at 12,000 × g at 4°C. The supernatant was discarded. Washed the RNA using ethanol, then solubilized the RNA using RNase-free water, then stored at –20°C until further processing.

**CDNA SYNTHESIS AND GENE EXPRESSION USING QUANTITATIVE REAL-TIME PCR (QRT-PCR)**

The gene expression process includes two steps. In the first step, RNA was converted to cDNA to be utilized in the real-time PCR reaction using a cDNA synthesis kit (Geneaid kit, Korea). Secondly, the gene expression of the MAOA gene was assessed by qRT-PCR and (SYBR)™ qPCR Kits using two primers forward and reverse (F-CTGCCATCATGGGCTT and R-TTGCTGATCCACA) at 154 bp [13]. Another set of primers was used to amplify the housekeeping gene GAPDH F-GAAATCCCATCACATCTTCCAGG-3 and R-GAGCCCCAGCCTTCTCCATG-3

as a reference gene for normalizing the level of gene expression.

The program of thermal reaction was: pre-denaturation or enzyme activation at 95°C for 10 minutes of 1 cycle, followed by denaturation at 95°C for 10 seconds of 35 cycles, annealing at 53°C for 30 seconds, and extension for 30 seconds at 60°C. The level of gene expression was determined by the folding expression (2-ΔΔCt) method [14].

**STATISTICAL ANALYSIS**

GraphPad Prism software was used for comparison of the folding expression (2-ΔΔCt). A student t-test was performed to compare the mean values of age and ΔCt. The scoring criteria for trauma exposure were presented as a Z-score (0 = no exposure, 1 = moderate exposure, 2 = severe exposure)

**RESULTS**

In our study, we measure and determine the mean level of expression of MAOA gene in the males and females of the studied groups (Fig. 1). The number of patients include males 44 (25%), females 56 (31%) and 40 (22%) of both males and females as control group. In the Table 2, no significant association was noted in the age between the study groups in both genders (p>0.05).

In the design used in the current study and according to the range of ΔCt values of the MAOA gene (Fig. 2) in healthy people and patients, we found that the level of gene expression is lower in patients than in healthy people, and the patients with lower expression have more antisocial behavior and aggression than the others (Fig. 3).

The results indicate that the level of MAOA gene expression is higher in females than males. It was demonstrated that male patients are more frequently attending psychiatric clinics than female patients. A significant interaction between exposure to trauma levels and the activity of MAOA gene expression shows a relationship with the risk of aggression.

Some influencing factors were studied and their effects on patients and healthy participants analyzed. In respect to the factors of our study, they include Missing someone, bad love experience, marital status, domestic violence, exposure to community violence, alcohol abuse, physical abuse, and sexual abuse. As the results show in the figures below, the relative fold changes alter with the level of trauma history or factor levels. Specifically, the findings of missing some body or bad love experience showed low activity or downregulation of MAOA gene expression  $2^{-\Delta\Delta Ct} < 1$  in the patient group (no exposure, moderate, and severe) when compared with the control group with no significant differences (Fig. 4).

In the marital status (single, married, and divorce), as shown in Figure 5, there was no significant upregulation of fold changes in the expression of the MAOA gene recorded in the patients' group ( $2^{-\Delta\Delta Ct} = 1.3, 1.3$ , and  $1.5$ , respectively) compared with the healthy group ( $2^{-\Delta\Delta Ct} = 1$ ).

Thus, the results of the expression level of the MAOA gene showed lower activity in people exposed to domestic violence ( $2^{-\Delta\Delta Ct}$  of no exposure =  $0.71$  and  $2^{-\Delta\Delta Ct}$  of exposure =  $0.86$ , respectively) than the control group ( $2^{-\Delta\Delta Ct} = 1$ ), and the findings had no significant differences (Fig. 6). In addition, the exposure to community violence observed a non-significant downregulation of the expression level of the MAOA gene in the patient group (no exposure, moderate, and severe) with a ( $2^{-\Delta\Delta Ct} < 1$ ) compared to the control group (Fig. 7).

Hence, alcohol abuse is another important factor affecting the gene expression of the MAOA gene. According to the data analysis, the results revealed a reduction in the activity of the relative expression  $2^{-\Delta\Delta Ct} < 1$  of the selected gene in the patients' group when compared to the control subjects. However, the reduction is not statistically significant (Fig. 8).

Regarding the physical abuse, the statistical comparison between the studied groups recorded a downregulation of MAOA gene expression ( $2^{-\Delta\Delta Ct} < 1$ ) in the patient group (no exposure, moderate, and severe) with the corresponding value of the control group ( $2^{-\Delta\Delta Ct} = 1$ ), and no significant differences were observed (Fig. 9).

Further data analysis of the MAOA gene expression with respect to sexual abuse revealed no significant decrease in the folding changes in the patient group

(no exposure, exposure) ( $2^{-\Delta\Delta Ct} < 1$ ) compared with control subjects (Fig. 10).

## DISCUSSION

Exposure to violence was linked to increased signs and symptoms of behavior disorder in young adulthood, which led to antisocial personality traits. This study was an assessment to explore the correlation between the expression level of the MAOA gene and the social disorder of Iraqi patients with aggressive behavior. The gene codes for the catabolic enzyme monoamine oxidase A (MAOA), which metabolizes several kinds of neurotransmitters in the brain. MAOA has been identified in catecholaminergic neurons in the human brain and metabolizes serotonin and norepinephrine more efficiently [15].

These neurotransmitters are affected by some hormonal disorders and external environmental factors. The MAOA gene influences aggressive behavior in the broader population [16].

Evidence of the current results indicates that the expression level of the MAOA gene was higher in females than males in both groups. The study by [17] found that antisocial behavior was more likely in males with the genotype for low MAOA activity who had been mistreated. Other studies found that the number of men who attend psychiatric clinics is greater than that of females, although it seems that the matter is due to the difference in culture and nature of societies. Furthermore, the previous work by [18] documented and showed the influence of MAOA on extraversion in males than in females, demonstrating a sex-specific link between the MAOA gene and personality traits. In contrast, [19] found in their studies the opposite effects of MAOA in women and mentioned that the high activity of MAOA gene expression is associated with an increased risk of aggression and depression. Other important findings by [20] suggested that the testosterone hormone can modify and alter the effect of the MAOA gene on the behavior of these women.

Our results observed a low expression form of MAOA gene  $2^{-\Delta\Delta Ct} < 1$  in all the study factors or trauma exposure, but only marital status has upregulated MAOA gene expression  $2^{-\Delta\Delta Ct} > 1$  when compared with the corresponding value of control  $2^{-\Delta\Delta Ct} = 1$ .

A team led by clinical psychologists in different colleges and countries found that exposure to physical or sexual abuse affects hormone status and some genetic products depending on the levels of abuse [21]. The deficiencies in MAOA gene expression have been associated with higher levels of aggression and violence [22].

A single nucleotide polymorphism SNP (missense mutation) of the MAOA gene was discovered in the Dutch family. Males who repeatedly engaged in violent criminal behavior over several generations exhibited an anomaly in MAOA expression, and this mutation prevented the synthesis of MAOA enzyme while the females were still unaffected [23]. Further investigation by [24] documented that the low-level expression of the MAOA-L gene estimates greater expressed happiness in females.

As noted earlier by [25, 26], the level of aggression is linked with the history of violence exposure in most cases. Moreover, studies by [27] suggested that divorce was significantly linked with aggression and made people more prone to antisocial behaviors. On the other hand, alcohol abuse causes damage to the brain by affecting the endings of nerve receptors, making it difficult for those nerve receptors to send important nerve signals. It also has an effect on hormone receptors and neurotransmitters [28]. An important study of 174 alcoholic violent offenders found that the MAOA-H genotype and childhood physical abuse before the age of 13 were linked to a higher risk of severe, impulsive, and aggressive recidivism [29].

Human experiments demonstrated for the MAOA-VNTR genotype that a low level of transcription activity was more prevalent in young men who had poor family relationships, suffered from sexual abuse or mistreatment in childhood, and reported a higher intake of alcohol [30]. Furthermore, a study published by Caspi and colleagues

in 2002 examined the effect of childhood mistreatment on the antisocial personality. An allele providing low activity of the monoamine oxidase A (MAOA) gene causes behavioral problems and violent crimes [16].

A previous study by [31] reported that the MAOA gene's tandem repeat (VNTR) variant affects the efficiency of gene expression. Carriers with alleles with 2 or 3 repeats have lower gene expression (MAOA-L) compared to those with 3.5 or 4 repeats (MAOA-H).

Another important result is that McDermott, et al. [32] discovered that individuals who carry the allele of low production (MAOA-L) had a more aggressive response when prone to provoke action compared to individuals who carry the allele of high production (MAOA-H) of the MAOA gene.

In general, managing the level of gene expression of the MAOA gene is a complex process that involves the interaction between genetic, epigenetic, and hormonal influences.

## CONCLUSIONS

In our study, the decreased expression level of the MAOA gene is relatively associated with trauma exposure based on the small sample size. The lower expression of the MAOA gene increases the risk of aggression. The males have slightly lower expression of the MAOA gene than the females. Therefore, it is essential for this study to be closely replicated in the future with a large sample size.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# The role of mine water pollution in increasing the risk of malignant neoplasms among the population of industrially affected region of Ukraine

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## ABSTRACT

**Aim:** To assess the potential impact of mine water contamination on the increased risk of malignant neoplasms among the population of an industrially affected region of Ukraine (Kryvyi Rih Iron Ore Basin).

**Materials and Methods:** Hygienic, epidemiological and statistical methods of research were used in the study. The study of mine water was conducted using samples obtained from 63 points representing the main mining horizons of the Kryvyi Rih Iron Ore Basin (Ukraine), using the following indicators: dissolved oxygen, biochemical oxygen demand over 5 days (BOD<sub>5</sub>), hydrogen index (pH), suspended solids, chemical oxygen demand (COD), dry residue, chlorides, sulfates, ammonium nitrogen, nitrites, nitrates, phosphates, oil products, total iron, and phenols.

**Results:** Laboratory analysis of mine water revealed that the concentrations of several substances exceeded permissible levels. The integral pollution coefficients suggested the water to be extremely polluted, and the ecological state of the environment as critical. The risk of malignant neoplasms in the Kryvyi Rih industrial region was 25,054 [95% CI 25,010–25,098]. Teenagers aged 15–17 had the highest risk of developing malignant neoplasms (OR 16,310 [95% CI 5,927–44,888]). Children under the age of 14 ranked second (OR 5,528 [95% CI 3,423–8,925]). Adults (≥18 years old) had a lower risk of malignant neoplasms (OR 2,461 [95% CI 2,309–2,622]). Children and adolescents were more affected than adults, i.e. the etiologic fraction of mine water pollution in the development of malignant neoplasms was 81,9% and 94,3%, respectively (vs. 59,6% in adults).

**Conclusions:** Considering the extremely polluted state of mine waters in the industrially affected region of Ukraine, and assessing the related malignant neoplasm risks in the context of public health, the development of innovative preventive strategies and methodologies is required, taking into account all relevant determinants, including environmental factors.

**KEY WORDS:** water pollution, malignant neoplasm, industrial regions

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## INTRODUCTION

Pollution of natural water sources is one of the main factors impacting the environment and public health [1, 2].

The multifactorial and multidirectional nature of the environment's impact on humans determines the procedural aspects of complex assessment of its quality and requires the creation of new approaches. Currently, the issues of quantitative assessment of the intensity of environmental factors' impact on public health are not sufficiently studied [3].

Industrial regions are characterized by a high concentration of enterprises, including mining and processing

industries, which generate varying amounts of wastewater as a result of their activities. The composition and quality of this wastewater primarily depend on the raw materials and the technological processes employed, while the volume often exceeds several times the amount of water actually used by the industrial enterprises themselves. The wastewater collected is subjected to varying degrees of treatment before being discharged into water bodies or reused for mining operations or other industrial sectors. The requirements for wastewater quality, both upon discharge into water bodies and during subsequent use, necessitate the

extensive application of diverse treatment methods and technologies [1, 4].

Malignant neoplasms, together with cardiovascular diseases, are key determinants of a nation's health status, as cancer ranks among the leading causes of mortality and disability. It accounts for approximately 12% of deaths (up to 15% according to some authors [5]) and 26% of disability cases, particularly affecting individuals of reproductive and working age. Among the working-age population, cancer-related mortality accounts for 14% of deaths in men and 29% in women [6–8].

## AIM

To assess the potential impact of mine water contamination on the increased risk of malignant neoplasms among the population of an industrially affected region of Ukraine (Kryvyi Rih Iron Ore Basin).

## MATERIALS AND METHODS

The hygienic, epidemiological, and statistical methods of research were used to achieve the study aim. The study of mine water was conducted using samples obtained from 63 points representing the main mining horizons of the Kryvyi Rih Iron Ore Basin (Ukraine), using the following indicators: dissolved oxygen, biochemical oxygen demand over 5 days ( $BOD_5$ ), hydrogen index (pH), suspended solids, chemical oxygen demand (COD), dry residue, chlorides, sulfates, ammonium nitrogen, nitrites, nitrates, phosphates, oil products, total iron, and phenols.

The laboratory analyses were carried out at the Kryvyi Rih District Laboratory of Research and at the University's scientific laboratory (Kryvyi Rih, Ukraine). The obtained results were compared with hygienic standards established for water bodies used for domestic and other public needs in Ukraine [9].

Certain integral coefficients were estimated in accordance with approved methodologies [9–11], namely the Water Pollution Index (WPI), the Conditional Complexity Coefficient (CCC), and the Complex Pollution Index (CPI).

The WPI (arbitrary units [a.u.]) was calculated based on the abovementioned 15 indicators according to the following formula:

$$WPI = \frac{1}{15} \sum_{i=1}^{15} \frac{C_i}{MAC_i}$$

where  $C_i$  is the arithmetic mean value of the  $i$ -th water quality parameter, and  $MAC_i$  is its corresponding maximum allowable concentration (MAC).

The WPI was interpreted according to the following classes:  $[\leq 0,30]$  – class I (very clear);  $[0,31 - 1,00]$  – class II (clear);  $[1,01 - 2,50]$  – class III (moderately polluted);  $[2,51 - 4,00]$  – class IV (polluted);  $[4,01 - 6,00]$  – class V (heavily polluted);  $[6,01 - 10,0]$  – class VI (severely polluted); and  $[>10,0]$  – class VII (extremely polluted).

The CCC (%) was calculated according to the following formula:

$$CCC = \frac{m'}{m} \times 100\%$$

where  $m'$  is the number of parameters with concentrations exceeding the MAC, and  $m$  is the total number of regulated parameters included in the study. When  $CCC < 10\%$ , an analysis of specific pollutants is carried out individually.

The CPI (a.u.) was calculated according to the following formula:

$$CPI = \sum_{i=1}^n \frac{C_i}{MAC_i}$$

where  $C_1, C_2, \dots, C_n$  are the concentration values of various pollutants in mine water ( $\text{mg}/\text{dm}^3$ ), and  $MAC_1, MAC_2, \dots, MAC_n$  are the corresponding MACs for these pollutants. The degree of groundwater pollution and the ecological condition of the natural environment were assessed based on the Table 1.

The level of contamination ( $K_i$ ) (a.u.) was calculated according to the following formula:

$$K_i = \frac{C_i}{MAC_i}$$

where  $C_i$  is the measured concentration of the  $i$ -th pollutant, and  $MAC_i$  is its MAC.

The level of pollution of water bodies was determined according to the following  $K_i$  rating:  $[0 \div 2]$  – low;  $[2 \div 10]$  – moderate;  $[10 \div 50]$  – high;  $[50 \div 100]$  – very high.

Our previous studies have revealed an association between the concentration of chemical substances in mine waters and the incidence of malignant neoplasms among the population of the Kryvyi Rih Iron Ore Region [10]. The analysis of the prevalence of oncological pathology among the residents of the Kryvyi Rih Iron Ore Basin provided the basis for identifying potential risk factors and their interrelations [3]. Importantly, mine water contamination represents one of the contributing factors.

To assess the prevalence of malignant neoplasms among the population, a cross-sectional epidemiological analysis was employed. The incidence of malignant

**Table 1.** Degree of groundwater pollution and ecological state of the natural environment

| Nº | Degree of groundwater pollution | CPI (a.u.) | Ecological state of the natural environment |
|----|---------------------------------|------------|---------------------------------------------|
| 1  | Conditionally clean             | 0 – 3      | Favorable                                   |
| 2  | Slightly polluted               | 3 – 10     | Satisfactory                                |
| 3  | Moderately polluted             | 10 – 20    | Strained                                    |
| 4  | Heavily polluted                | > 20       | Critical                                    |

Source: compiled by the authors of this study

**Table 2.** The chemical parameters of mine water and the level of its contamination,  $M \pm m$  (n=63)

| Water quality indicators | The current concentration (C), mg/dm <sup>3*</sup> | K, a.u. | Rating    |
|--------------------------|----------------------------------------------------|---------|-----------|
| Dissolved oxygen         | 7,68±0,04                                          | 1,92    | low       |
| BOD <sub>5</sub>         | 8,10±0,18**                                        | 2,66    | moderate  |
| pH                       | 7,74±0,17                                          | 0,87    | low       |
| Suspended solids         | 111,29±5,61**                                      | 240,13  | very high |
| COD                      | 318,95±12,09**                                     | 12,34   | high      |
| Dry residue              | 24172,57±1473,50**                                 | 19,3    | high      |
| Chlorides                | 11983,70±858,26**                                  | 40,66   | high      |
| Sulphates                | 936,84±24,14**                                     | 1,90    | low       |
| Ammonium nitrogen        | 0,89±0,07                                          | 0,21    | low       |
| Nitrites                 | 0,32±0,02                                          | 0,12    | low       |
| Nitrates                 | 9,70±0,24                                          | 0,19    | low       |
| Phosphates               | 0,063±0,001                                        | 3,17    | moderate  |
| Oil products             | 0,3±0,02                                           | 1,37    | low       |
| Total iron               | 0,3±0,01                                           | 5,73    | moderate  |
| Phenols                  | 0,001±0,0001                                       | 1,0     | low       |

Notes:  $M \pm m$  – mean  $\pm$  standard error; \* – except pH; \*\* – exceeded the MAC

Source: compiled by the authors of this study

neoplasms among the population was examined through a retrospective epidemiological analysis of statistical reports from healthcare institutions over a five-year period [1].

To assess the potential impact of mine water contamination on the increased risk of malignant neoplasms among the population of the Kryvyi Rih Iron Ore Basin, we applied risk assessment methodologies aimed at determining the etiologic fraction (EF) attributable to the harmful factor, namely contaminated mine water.

For this purpose, data on the prevalence of malignant neoplasms among the population of the city of Kryvyi Rih were used and compared with the corresponding data from conditionally «ecologically clean» regions of Ukraine (Ternopil and Chernivtsi regions), where there is no underground mining of mineral resources and, consequently, the population is not exposed to contaminated mine waters. In addition, the prevalence of oncological pathology was analyzed across age groups 0–14, 15–17, and  $\geq 18$  years old.

The risk assessment was carried out in accordance

with internationally accepted methods [11–13], with consideration of the following parameters: the probability of developing a malignant neoplasm (P), attributable risk (AR) (additional risk caused by water pollution), EF of water pollution in the incidence of malignant neoplasms, probability of exposure to a risk factor among residents (Pe), and odds ratio (OR) with a 95% confidence interval (CI).

## ETHICS

All the research methods used were considered by the local ethics committee, in accordance with the ethical principles of the Declaration of Helsinki, and approved for the use during the organization and conduct of research. No patients participated in the studies.

## FRAMEWORK

The study was conducted as a fragment of the scientific projects of the Separate Division “Scientific and Practical

**Table 3.** Mine water quality assessment according to the integral indices,  $M \pm m$  ( $n=63$ )

| Integral indices     | Value         | Rating                                                                |
|----------------------|---------------|-----------------------------------------------------------------------|
| CPI, a.u., $M \pm m$ | 331,57±100,24 | Heavily polluted, the ecological state of the environment is critical |
| WPI, a.u., $M \pm m$ | 22,10±5,12    | Extremely polluted<br>(class VII)                                     |
| CCC, %, $M \pm m$    | 67±7 %        | Over 10%, thus requiring the more detailed study                      |

Notes:  $M \pm m$  – mean ± standard error

Source: compiled by the authors of this study

**Table 4.** The risk of malignant neoplasms related to the mine water contamination in different age groups

| Parameters  | Age groups (years)     |                       |                        |
|-------------|------------------------|-----------------------|------------------------|
|             | 0-14                   | 15-17                 | ≥18                    |
| AR          | 0,000641               | 0,002273              | 0,010426               |
| EF          | 81,9 %                 | 94,3 %                | 59,6 %                 |
| $P_e$       | 0,001                  | 0,002                 | 0,018                  |
| OR (95% CI) | 5,528<br>(3,423–8,925) | 16,310 (5,927–44,888) | 2,461<br>(2,309–2,622) |

Source: compiled by the authors of this study

Medical Center of Occupational Health of Shupyk National Healthcare University of Ukraine” «Prevention of occupational diseases at employees of industrial enterprises» (state registration numbers 0124U003711; term: 2024–2025); and «Comprehensive assessment of the health state at employees of industrial enterprises by organizing and managing medical examinations» (state registration numbers 0124U003723); term: 2024–2025).

RESULTS

Mine waters are formed as a result of the infiltration of surface and groundwater into mining workings. As the water passes through these workings, it is exposed to varying levels of contamination; therefore, it cannot be discharged into natural water sources without treatment, nor used for technical purposes without appropriate processing [1].

According to the results of our own laboratory studies, the chemical composition of mine waters and the level of their contamination were examined (Table 2).

It has been revealed that the levels of chemical substances in the mine water (dissolved oxygen, pH, ammonium nitrogen, nitrites, nitrates, phosphates, oil products, total iron, phenols) are within acceptable limits (Table 2), and, if reused, will not have a negative impact on natural water bodies, the environment, or public health.

On the contrary, such parameters as BOD<sub>5</sub>, suspended solids, COD, dry residue, chlorides, and sulphates significantly exceed the MACs (Table 2) and pose an additional risk to the population when entering natural water bodies used for consumption and domestic purposes.

The impact of pollutants on human health may occur

in a combined manner – both directly and indirectly – through soil, food products, and other environmental pathways.

According to Table 2, mine water is severely polluted (class VI of the possible seven contamination classes). There are 11 substances consistently present in it: BOD<sub>5</sub>, dissolved oxygen, suspended solids, COD, dry residue, chlorides, sulphates, phosphates, oil products, and total iron. Moreover, BOD<sub>5</sub>, total iron, and phosphates have an average level of pollution, while COD, dry residue, and chlorides have a high level, and suspended solids have a very high level. Furthermore, the impact of such pollution on biological organisms corresponds to hazard class IV, the highest, for phenols, oil products, and total iron. These indicators require additional analysis.

The integral pollution coefficients were also calculated (Table 3).

The analysis of integral pollution coefficients indicated a tendency toward increasing pollution levels (Table 3).

According to the CPI value, the water is characterized as heavily polluted, and the ecological state of the natural environment as critical. WPI is characterized as extremely polluted (class VII). In addition, CCC is over 10% and requires more detailed study (Table 3).

Mine waters are drained into natural water bodies that are used for drinking and domestic purposes. The exceedance of MACs of chemicals in the mine water, along with the calculated integral coefficients of complex pollution, provided a basis for comparing malignant neoplasm incidence among the population of industrial regions with incidence among the population of «ecologically clean» regions.

Utilizing these data, the contribution of this water



pollution level to the incidence of environmentally related diseases in the population was quantified, and age-specific health risks were evaluated (Table 4).

It was established that the risk for developing malignant neoplasms in Kryvyi Rih industrial region was 25,054 (95 % CI 25,010–25,098) and depended on the age of the population. Teenagers aged 15–17 have the highest risk for developing malignant neoplasms (OR 16,310 [95% CI 5,927–44,888]). Children under the age of 14 hold the second place in this ranking (OR 5,528 [95% CI 3,423–8,925]). Adults ( $\geq 18$  years old) have a lower risk of malignant neoplasms (OR 2,461 [95% CI 2,309–2,622]) (Table 4).

The data shows that children and adolescents were more affected, than adults, i.e. the EF of mine water pollution in the development of malignant neoplasms is 81,9% and 94,3%, respectively (vs. 59,6% in adults) (Table 4).

Consequently, the discharge of untreated mine water represents a significant risk, potentially increasing the incidence of malignant neoplasms in the population [10].

## DISCUSSION

The environmental impact of mine water in the study area has gained increasing relevance and has been intensively investigated over the past 10 years. Numerous studies have addressed the formation and discharge of mine water across various basins both in Ukraine and throughout Europe [14, 15].

The incidence of malignant neoplasms shows an increasing trend. Globally, over 12,3 million new cases of malignant neoplasms are diagnosed each year, of which 7,6 million are fatal (according to the World Health Organization, 9,6 million deaths [16]). Worldwide, the annual incidence of cancer increases by 5–10% [5, 17]. In Ukraine, incidence has risen by 25% over the past ten years, with a steady annual increase of 2,6–3%, accompanied by a “younger” age profile of patients, reducing life expectancy by 3–3,5 years. This socio-economic phenomenon is further highlighted by the fact that 40% of patients are of working age [8, 17].

The rising global burden of oncological diseases is linked to water pollution, with mining activities playing a critical role in contaminating aquatic ecosystems with complex mixtures of established carcinogens. It contributes to the estimated 8–9% of general morbidity attributed cumulatively to environmental factors, such as air and water pollution. Studies from different continents confirm this severe health impact. For example, in Northern Chile, a population exposed to drinking water averaging 570  $\mu\text{g/L}$  of arsenic for over a decade

exhibited staggering excess bladder cancer mortality, with women recording an 820% increase and men a 600% increase compared to the expected rate. In China, population-level evidence from a cohort surrounding a metal-smelting area reported associations between long-term environmental cadmium and lead exposure and a significantly increased mortality rate from all cancers, indicating a link between metal contamination and adverse oncological outcomes [18].

Mine water, classified as “very polluted” (class VI) with persistent contamination, is discharged into surface water bodies. The primary pollutants include BOD<sub>5</sub>, dissolved oxygen, suspended solids, COD, total dissolved solids, chlorides, sulfates, phosphates, petroleum hydrocarbons, and total iron. The ecological status of the natural environment is characterized as critical [12].

Following settling and treatment, the water enters rivers utilized for economic activities. Nevertheless, the ecological status of the environment remains strained, and the population consumes “moderately polluted” water of class III quality, which inevitably impacts public health. These observations are supported by both domestic and international studies, underscoring the significance of the issue [14, 16, 19]. Large-scale mapping in China revealed that unacceptable carcinogenic risks are present in over half of mining areas, particularly for adults exposed to mining-affected surface or groundwater, especially where acid mine drainage contributes to high heavy metal burdens [20].

The data obtained regarding the etiological proportion of risk from environmental pollution in the development of malignant neoplasms in the population (EF), which ranges from 59,6% to 94,3%, confirm previous studies and emphasize the urgency and necessity of implementing environmental monitoring and preventive measures, as highlighted by international research [16, 19]. The greatest impact of pollution is observed among children and adolescents, with the highest risk of developing cancer among adolescents aged 15–17 years.

The results of malignant neoplasm analysis in the population may be considered baseline data and will allow monitoring of both the qualitative and quantitative state of mine waters and the health status of the population. These findings will form the basis for assessing the excess risk associated with the use of mine water and for determining the risk categories of carcinogenic and non-carcinogenic effects, as well as for the development of effective technologies for the treatment and demineralization of mine water.

## CONCLUSIONS

1. The analysis of laboratory data on mine water from

the industrially affected region of Ukraine (Kryvyi Rih Iron Ore Basin) revealed that the concentrations of substances exceed the MACs. According to CPI, the water is classified as heavily polluted, while the ecological state of the environment is assessed as critical.

2. The highest risk of developing malignant neoplasms was observed among adolescents aged 15–17 years (OR 16,310 [95% CI 5,927–44,888]), followed by children under 14 years (OR 5,528 [95% CI 3,423–

8,925]). In comparison, the adult population demonstrated considerably lower risk levels (OR 2,461 [95% CI 2,309–2,622]). The EF of malignant neoplasms risk attributable to environmental pollution was estimated at 59,6–94,3%.

3. Considering the concentrations of chemical substances in mine water and the analysis of malignant neoplasms health risks, new approaches are required for the development of preventive measures and strategies, taking into account all determinants,

including the environmental factors.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Conceptual bases of psychocorrectional assistance to women in codependent marriage relationships

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## ABSTRACT

**Aim:** To develop a programme of psychocorrectional assistance for women in codependent marital relationships and to evaluate its effectiveness.

**Materials and Methods:** The study involved 48 women aged 25–45 years who were in codependent marital relationships. The basic course of psychocorrectional therapy consisted of ten sessions. The effectiveness of the psychocorrectional programme was assessed using the following psychodiagnostic methods: the Codependency Self-Evaluation Scale (CSIS), the Codependency Level Measurement Scale (D. Spann and D. Fisher), Self-Evaluation of Emotional States (A. Wessman, D. Ricks), Self-Evaluation of Mental States (H. Eysenck), and Definition of Self-Evaluation (S. Kovalev).

**Results:** As a result of the psychocorrectional sessions, the general mental state of the women improved. They reported feeling more energetic, confident, calm, and relaxed. Stabilisation of the new self-position in everyday and partner-related situations, as well as a strengthening of decision-making quality in relationships, was observed in 42 (87.5%) participants during long-term follow-up.

**Conclusions:** The developed comprehensive psychocorrectional programme demonstrated high effectiveness both in short- and medium-term outcomes and in the long term. This indicates the achievement of a positive personal transformation among the participants, serving as a psychoprophylactic factor against the risk of developing further codependent relationships. Future research is planned to focus on developing a psychocorrectional programme for men in codependent relationships and evaluating its effectiveness.

**KEY WORDS:** codependency, women, mental states, psychological assistance

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## INTRODUCTION

The relevance of this study is determined by the significant prevalence of codependent relationships and the absence of a clear algorithm for providing psychocorrectional assistance to individuals involved in such relationships. Codependent relationships develop on the basis of dysfunctional interpersonal interactions, fear of loneliness, and fear of rejection [1, 2]. They manifest at the cognitive, behavioral, emotional, and activity levels of the personality [3, 4]. Family codependency is commonly associated with substance abuse [5–7]. Furthermore, predictors of family codependency formation include immaturity of psychological defense mechanisms, impaired communication and role distribution within the family, attachment-related anxiety, and characteristics of early mother–child relationships [8, 9].

Women with a high level of codependency are characterized by low self-esteem, self-blame, preoccupa-

tion with their own shortcomings, loss of self-control accompanied by identity disturbance, heightened vulnerability, and persistent feelings of guilt [10, 11]. Psychocorrection enables individuals to reassess existing problems and identify effective ways of resolving them [9, 12].

Thus, although the problem of codependency has recently received considerable attention in the scientific literature, there remains a lack of comprehensive empirical research and practically oriented psychocorrectional models [11, 12]. The need to address these gaps served as the main motivation for conducting this study.

The scientific novelty of the research lies in the theoretical substantiation of the conceptual foundations of a psychocorrectional assistance program for women in codependent marital relationships. The practical significance of the study results is defined by the possibility of applying them to the provision of psychocorrectional

and psychoprophylactic support for women in codependent marital relationships.

The hypothesis of the study was based on the assumption that the developed psychocorrectional program will contribute to the improvement of the mental state and positive transformation of the personal characteristics of women in codependent marital relationships.

## AIM

The study aimed to develop a psychocorrectional assistance program for women in codependent marriage relationships and evaluate its effectiveness.

## MATERIALS AND METHODS

The research methodology was based on the integration of theoretical knowledge regarding the formation of codependent marital relationships and the practical experience of their psychocorrection, as presented in modern scientific literature [1, 9, 10].

The research design provided for a sequential, phased implementation. The research algorithm consisted of the following stages: theoretical and analytical, preparatory, diagnostic, psychocorrectional, processing and analysis of results, and finalization with the formulation of conclusions and recommendations.

The study was conducted in a family psychotherapy setting. The sample comprised 48 women aged 25–45 years who sought psychological counseling due to existing codependent patterns in marital relationships. Psychocorrectional work was carried out in a group format, forming six groups of eight women each.

The inclusion criteria for participation in the psychocorrectional group were as follows: psychodiagnostic identification of codependent patterns; readiness for group participation; stability of mental state; informed consent to take part; and adherence to group rules. The exclusion criteria were: acute psychotic conditions; active suicidal risk; active phase of chemical dependency; severe manifestations of post-traumatic stress disorder; and unwillingness to maintain confidentiality. No exclusion criteria were observed among the women selected for the study.

The psychocorrection program was based on an integrative approach that combined cognitive-behavioral techniques (attitude reappraisal, work with automatic thoughts), body-oriented practices (awareness and regulation of body signals), group dynamics and mutual reflection, art therapy and narrative tools, as well as metaphorical work and resource integration.

The basic psychocorrection cycle consisted of ten sessions, each lasting 90 minutes and held once per week.

Structured homework assignments were completed between sessions. The post-program support plan included: recommended micropractices for four to six weeks; mini-support groups meeting once every two weeks; individual sessions upon request; identification of personal triggers; and the development of personalized self-help strategies. Typically, post-program support and follow-up lasted from six to twelve months.

To assess the effectiveness of the psychocorrectional program, psychodiagnostic testing was conducted at the beginning and after the completion of its basic cycle using the following methods:

- Codependency Self-Assessment Scale (CSIS) [13] – a 20-item questionnaire adapted for use in Ukraine. The level of codependency is assessed as follows: 20–29 points – low; 30–39 points – medium; 40–59 points – high; 60–80 points – very high;

- Codependency Measurement Scale (D. Spann and D. Fisher) [14] – a 16-item questionnaire aimed at identifying dysfunctional and codependent relationships. The number of points from 16 to 32 indicates normal relationships; the range of 33–60 points corresponds to moderately expressed codependency; 61–96 points – strongly expressed codependency.

- "Definition of self-esteem" (S. Kovalev) [15] – a test questionnaire consisting of 32 statements; the level of self-esteem of the subject is determined as follows: 0–25 points – high; 26–45 points – average; 46–128 points – low;

- "Self-assessment of emotional states" (A. Wessman & D. Rix) [16] – a questionnaire containing 40 questions that allow you to determine the emotional state of the subject on four scales: "Calmness-Anxiety", "Energy, cheerfulness-Fatigue", "Elevation-Depression", "Self-Confidence-Feeling of Helplessness". The maximum possible number of points on each scale is 40. A total score of the emotional state is also calculated for all scales (from 26 to 40 points - high level, from 15 to 25 points - medium level, from 4 to 14 points - low level);

- "Self-assessment of mental states" (G. Eysenck) [17] – a questionnaire with 40 questions that allows you to determine the level of anxiety, frustration, aggressiveness and rigidity (0–7 points – low level; 8–14 points – medium level; 15–20 points – high level).

The study was conducted with the permission of the ethics committee of the Interregional Academy of Personnel Management. All study participants signed an informed consent document. Throughout the entire period of the study, strict adherence to the provisions of the Declaration of Helsinki and other international bioethics documents was ensured, in particular: confidentiality of personal information; anonymity of test results; the right to refuse any training exercise; stabilization algorithm during emotionally necessary; safe and tolerant rules of interaction in the group.

**Table 1.** Dynamics of psycho-emotional states of women with codependency in the process of psychocorrection

| Scale                    |                                         | Average score (n=48) |           | Student's t-test | p        |
|--------------------------|-----------------------------------------|----------------------|-----------|------------------|----------|
|                          |                                         | A                    | A1        |                  |          |
| A. Wessman & D. Rix [16] | Calmness-Anxiety                        | 3.7±0.46             | 6.4±0.52  | 11.96            | 0.0003   |
|                          | Energy, cheerfulness-Fatigue            | 3.8±0.45             | 6.1±0.47  | 363.66           | <0.00001 |
|                          | Elevation-Depression                    | 3.4±0.38             | 6.3±0.45  | 106.61           | <0.00001 |
|                          | Self-confidence-Feeling of helplessness | 2.9±0.47             | 6.4±0.51  | 36.29            | <0.00001 |
|                          | Overall assessment of the condition     | 13.8±1.76            | 25.2±1.95 | 25.28            | <0.00001 |
| G. Eysenck [17]          | Anxiety [17]                            | 15.6±1.42            | 10.1±1.52 | - 34.11          | <0.00001 |
|                          | Frustration [17]                        | 16.6±1.57            | 7.2±1.46  | - 71.65          | <0.0001  |
|                          | Aggressiveness [17]                     | 15.4±1.63            | 8.1±1.53  | - 73.00          | <0.00001 |
|                          | Rigidity [17]                           | 15.8±1.62            | 6.9±1.68  | - 13.49          | 0.0002   |

Notes: A – initial testing, A1 – retesting  
Source: compiled by the authors of this study

**Table 2.** Dynamics of the level of codependency and self-esteem of the women studied in the process of psychocorrection

| Scale                                              |  | Average score (n=48) |           | Student's t-test | p        |
|----------------------------------------------------|--|----------------------|-----------|------------------|----------|
|                                                    |  | A                    | A1        |                  |          |
| Codependency Level (CSIS) [13]                     |  | 71.3±2.87            | 35.4±2.84 | - 18.89          | <0.00001 |
| Codependency Level (Spann & D. Fisher Method) [14] |  | 75.4±2.89            | 46.5±2.72 | - 116.31         | <0.00001 |
| Self-esteem Level [15]                             |  | 84.2±3.24            | 45.1±2.88 | - 330.93         | <0.00001 |

Notes: A – initial testing, A1 – retesting  
Source: compiled by the authors of this study

We used the IBM SPSS Statistics v. 27.0 (Armonk, NY: IBM Corp., USA) for the empirical data analysis. Quantitative indicators were presented as mean ± standard deviation, and compared by the paired Student's t-test at baseline and after completion of the psychocorrectional program. A p-value <0,05 was considered as statistically significant.

FRAMEWORK

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RESULTS

The dynamics of the psycho-emotional states of women exhibiting manifestations of codependency during the psychocorrection process, as determined by the meth-

ods of A. Wessman and D. Rix [16] and G. Eysenck [17], are presented in Table 1. As shown in the table, the initial testing of women in codependent marital relationships according to the A. Wessman and D. Rix method [16] revealed a depressive state, feelings of helplessness, and increased anxiety and fatigue. Assessment using G. Eysenck's method [17] demonstrated high levels of anxiety, frustration, aggression, and rigidity among the participants.

As a result of the psychocorrection sessions, according to the methods of A. Wessman and D. Rix [16] and G. Eysenck [17], the mental state of the participants normalized. They began to feel more energetic, confident, calm, and relaxed. The overall assessment of their mental state (based on the A. Wessman and D. Rix questionnaire [16]) improved significantly. Testing according to G. Eysenck's method [17] showed that, following psychocorrection, the participants demonstrated a statistically significant decrease in the levels of anxiety, aggressiveness, and rigidity to average values. At the same time, their frustration index decreased to within the normal range, indicating increased self-esteem, greater resilience to failure, and readiness to overcome life's difficulties (Table 1).

The effect of the psychocorrection program on enhancing self-esteem was also confirmed using the S. Kovalev method [15] (Table 2). This table additionally



presents the dynamics of codependency level indicators, determined using the CSIS [13] and the D. Spann and D. Fisher [14] methods.

During the initial testing of the women studied, high scores according to this method indicated a very low level of their self-esteem. Repeated testing recorded a statistically significant and significant decrease in the index according to the S. Kovalev method [15], which indicated the normalization of self-esteem and corresponded to the upper limit of the range of its average values. The normalization of self-esteem occurred synchronously with a decrease in the level of codependency, which was confirmed by the dynamics of indicators according to the CSIS [13] and D. Spann & D. Fisher methods [14].

During follow-up observation, long-term results of psychocorrection in the form of stabilization of the new self-position in everyday and partner situations and improvement of the quality of choice in relationships were noted in 42 (87,5%) cases.

## DISCUSSION

Our study was motivated by the need for an in-depth study of the gender specifics of the problem of codependency and the development of effective approaches to providing psychocorrectional assistance to women stuck in codependent marital relationships. The formation of a person's personal qualities that determine her tendency to codependent relationships is determined primarily by socio-psychological factors [18]. At the same time, women who are in codependent marital relationships are characterized by increased anxiety, aggressiveness, depression, fatigue, lethargy, self-doubt and disruption of both interpersonal contacts and self-attitude [10, 11, 19], which is confirmed by the very low level of self-esteem we found in such women. Codependent relationships transform the substantive characteristics and value-semantic sphere of a woman, her worldview and image of "I" [20].

Accordingly, psychocorrectional assistance to women in codependent relationships, according to researchers, should be comprehensive, consistent, and coordinated [11]. There is evidence of the effectiveness of using interactive and role-playing psychodramatic techniques with reflection and fairy tale acting for the correction of codependent relationships [21]. When developing the author's psychocorrectional program, we took these recommendations into account.

Psychodiagnostic testing in dynamics and subsequent follow-up observation demonstrated the high effectiveness of the psychocorrectional program we developed and allowed us to determine three levels of achieved psychocorrectional results: short-term results

(increased awareness of one's own bodily and emotional signals, reduced automatism of self-contempt, gaining experience in safely setting boundaries and verbalizing one's own needs); medium-term results (normalization of self-esteem, reduced frequency of codependent reactions, expansion of the repertoire of assertive strategies and increased tolerance to emotions; long-term results (stabilization of the new self-position in everyday and partner situations and increased quality of choice in relationships).

The limitations of this study during the experimental period were associated with several factors, including a temporary increase in emotional reactivity at the outset, heterogeneity of the group in terms of readiness, and the need for individualized refinement of certain topics between sessions. It should also be emphasized that the psychocorrection program does not in any way replace pharmacological treatment or crisis intervention. Therefore, among the many women exhibiting manifestations of codependency who were under our observation, only 48 participants were selected for inclusion in the study. These participants fully completed all stages of the psychocorrection program and took part in both the initial testing and retesting, providing accurate responses to all questionnaire items.

This study focused on assessing the effectiveness of psychocorrection for codependent relationships among women; however, men also experience codependent relationship patterns. In our previous publications, we examined the gender-specific characteristics of codependency formation and highlighted the importance of considering these differences in the process of psychocorrection. Accordingly, the psychocorrection program presented in this study was designed specifically for women, while a separate study will be devoted to the development and evaluation of a psychocorrection program for men in codependent relationships.

## CONCLUSIONS

Direct and strong correlations were established between the level of codependency and the negative psycho-emotional states of the women studied, including feelings of helplessness, increased anxiety, depression, and fatigue, as well as high levels of frustration, aggression, and rigidity.

Stabilization of the new self-position in everyday and partner situations, along with the strengthening of decision-making quality in relationships, was confirmed in 42 (87.5%) cases during long-term observation. The high effectiveness of the developed comprehensive psychocorrection program was demonstrated in short-, medium-, and long-term perspectives, indicating posi-

tive personal transformation among the participants as a psychoprophylactic factor in preventing the formation of further codependent relationships.

Thus, the psychocorrection program we developed can be recommended for providing psychocorrec-

tional and psychoprophylactic assistance to women in codependent marital relationships. Future research is planned to focus on the development and evaluation of a psychocorrection program for men in codependent relationships.

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### CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Reproductive physiological amelioration of BPA - induced toxicity by resveratrol: Sperm quality preservation, DNA damage mitigation, and testicular protection

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## ABSTRACT

**Aim:** The object of the present study aimed to investigate the impact of Bisphenol A on the fragmentation, and histopathological changes, and evaluating the protective effects of resveratrol against Bisphenol A.

**Materials and Methods:** The study used forty adults male Wistar rats were that randomly divided into four groups: a control group, a Bisphenol A group administered at 50 mg/kg/day, a resveratrol group at 50 mg/kg/day, and a Bisphenol + resveratrol group at the same dosages for 60 consecutive days. The blood and tissue samples were collected for evaluating the sperm parameters, DNA integrity, Luteinizing hormone, Testosterone hormone, total antioxidant capacity, and histopathological section.

**Results:** The results showed that Bisphenol A exposure causes significantly disrupted in sperm motility, concentration, morphology, and DNA integrity, with severe histopathological change in testicular tissue and causes significantly increasing in luteinizing hormone and decreased testosterone levels. In contrast, the results illustrated the resveratrol play as protective effects by conserved the sperm quality, testicular histopathology and reducing DNA fragmentation.

**Conclusions:** The Bisphenol A-induced significantly increased in oxidative stress while resveratrol play significant protection against Bisphenol A which that induced testicular damage. The present finds proved that resveratrol is a potential therapeutic agent that can be used as an intervention against chemical substances in the environment and preserve the fertility

**KEY WORDS:** antioxidant, Bisphenol A, DNA fragmentation, infertility, reproduction, resveratrol, sperm quality, testicular tissue

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## INTRODUCTION

Interestingly, infertility is a world health challenge, which affects about 15% of couples in the world, since, that is several factors participate to infertility, including chemical substances of the environment that exposure to the reproductive system, and it is one of the most important factors affecting the sperm parameters [1-2]. Bisphenol A (BPA), a widely used industrial chemical found in plastics, food containers, and everyday items, has appeared as a significant environmental threat to male reproductive health. With its ability to mimic estrogen and disrupt androgen function, BPA interferes with the hypothalamic-pituitary-gonadal axis, altering hormonal balance and impairing critical processes such as spermatogenesis [3]. Studies in both humans and animal models have linked BPA exposure to decreased testosterone levels, reduced sperm quality, DNA damage, and oxidative stress, raising concerns about its

long-term impact on fertility [4-5]. Resveratrol (RES) is a natural polyphenolic compound that found in grapes, berries, and peanuts [6]. Moreover, the RES have a potent antioxidant property, which scavenges oxidative stress and decreases the adverse effects that are due to environmental toxins such as BPA [7]. Generally, the study illustrated that RES protects the tissue by scavenging reactive oxygen species, which improves the activity of endogenous antioxidant included enzymes, and subsequently reduces the lipid peroxidation, which finally that prevents cellular damage in the tissue [3, 8]. Additionally, the last studies illustrated that RES improved the mitochondrial function by increasing the production of ATP and by stabilizing the membrane of mitochondrial, then subsequently lead to enhance the sperm motility and viability. Furthermore, that RES play a critical role in inhibiting of the pro-apoptotic pathways which that promoting the activation of survival

signaling pathways, thus the RES improved the germ cell integrity and enhancing the spermatogenesis [9]. Several studies showed that RES acts as an anti-inflammatory which protects the testicular tissue from exposure to environmental contaminants and protects reproductive health [10]. Sperm parameters, including concentration, motility, viability, and morphology, are critical indicators of male fertility and are highly susceptible to environmental toxins which BPA disrupts endocrine function, leading to hormonal imbalances that impair spermatogenesis. Studies have consistently shown that BPA exposure reduces sperm concentration [5] compromises motility by impairing mitochondrial function and ATP production [9,11] decreases viability through oxidative stress-induced apoptosis [8,10] and increases abnormal morphology [3]. Thus, the present study aimed to evaluation the protective effect of the RES against the adverse effect of BPA that exposure the reproductive system in rats, which assessments the levels of luteinizing and testosterone hormonal, evaluation of sperm parameters (motility, morphology, and concentration), sperm DNA fragmentation and total antioxidant capacity levels. Additionally, evaluate the Histopathological Examination of Testicular Tissue.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

This present study included 40 adults' male Wistar rats that weighted (225–275 g) that housed under typical conditions with a 12/12-hour light-dark cycle and free access to food and water. After adaptation in two-week period, the rats were randomly divided into four equal groups (10 per group): a control group (C) receiving no treatment; a BPA group administered BPA at 50 mg/kg/day [3]; a resveratrol group (RES) administered RES at 50 mg/kg/day [12]; and combined group a BPA + resveratrol group (BPA + RES) co-administered BPA (50 mg/kg/day) and resveratrol (50 mg/kg/day). All treatment solutions were given orally via gavage for 60 consecutive days.

### ETHICS

All the procedures in this study, including animal husbandry, handling, and scarifying were performed according to the guidelines instructed by the Animal Ethics Committee of The Islamic University, Najaf, Iraq (2024-12-1/M12a). Ethical considerations were paramount throughout all stages of the study, and efforts were made to minimize potential harm to the experimental animals.

### TREATMENT PROTOCOL

To prepare the RES solution 3 g of carboxymethylcellulose (CMC) powder in 97 g of distilled water to create the stock solution of 3% CMC then stirred continuously at room temperature for 4 hours to ensure dissolved. separately, the amount of RES was dissolved in a 1% ethanol to enhance its solubility then RES-ethanol solution was then added to the 3% CMC solution and stirring continuously for 30–60 minutes in water bath at 37 °C. The final volume of the mixture was adjusted with distilled water to achieve the desired concentration, followed by an additional 15 minutes of stirring to ensure complete homogeneity and stored at room temperature and [13]. To prepare the BPA solution, the amount of BPA was weighed and dissolved in normal to dosing at 50 mg/kg body weight. The prepared BPA solution was stored in an amber container at room temperature until administration via oral gavage [3, 10].

### ANIMALS AND SAMPLE COLLECTION

At the endpoint of experimental at the 60-day experimental period, the animals were euthanized through an intramuscular overdose of a combination of Xylazine (Micopite, USA, 40 mg/kg) and Ketamine (Alpha Than, Netherlands, 90 mg/kg). The Blood samples about 5 mL were collected via cardiac-puncture and then the scrotal area was carefully cleansed with sterile normal saline before harvesting bilateral testicular and epididymal tissues [14–16]. The epididymal tail was incubated in 2 mL of normal saline at 37°C then by using anatomical micro-scissors, the tissue was divided into segments, and spermatozoa were extracted for subsequent evaluation of sperm motility, concentration, morphology, and DNA Integrity following the study's standardized methodology [17].

### SPERM EVALUATION

The sperm motility, morphology, and concentration were assessed as followed: for evaluate sperm progressive motility, a 10 µL aliquot of sperm suspension was placed on a dry, warmed slide and examined under a light microscope at 400× magnification [14], while the sperm morphology, 10–20 µL of sperm was mixed with an 20 µL eosin-nigrosine stain, smeared onto a warm slide, air-dried, and observed under a microscope, where sperm heads stained pink [16–18]. Sperm concentration was determined by weighing testicular tissue, mincing it, and suspending it in 1 mL of PBS. A diluted aliquot (1:1000) was prepared using a solution of normal saline 95%, formaldehyde 4%, and eosin stain 1% following the methodology of Smith and Mayer (1955)

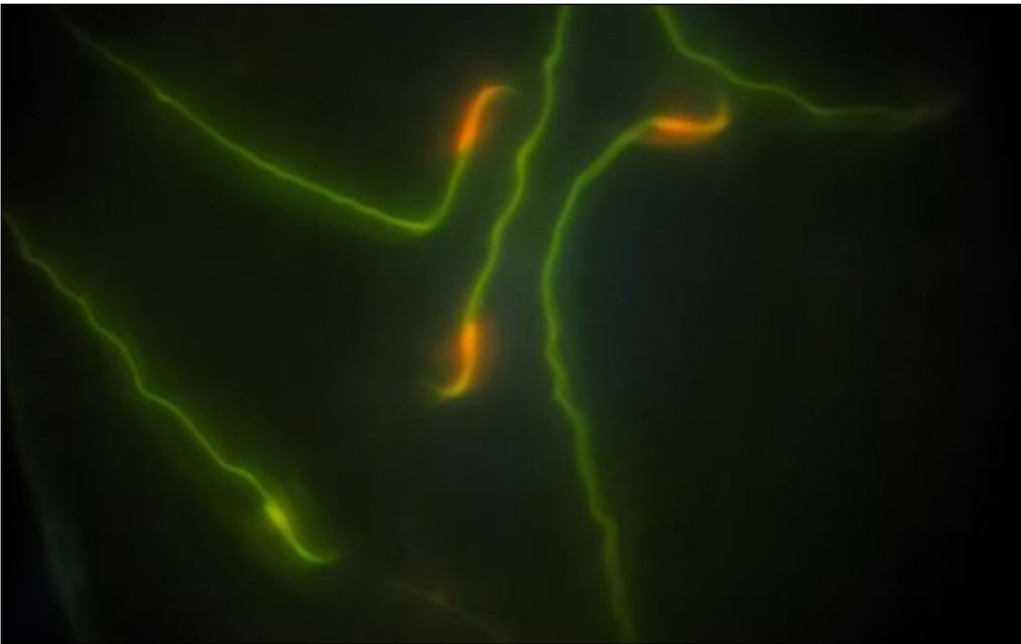


**Fig. 1.** Assessment of The Sperm Chromatin Integrity under Fluorescence that staining by Acridine Orange Staining. A Comparative Analysis of Condensed Chromatin (Green Fluorescence) and Denatured Chromatin (Red Fluorescence) 400X  
*Source: Own materials*

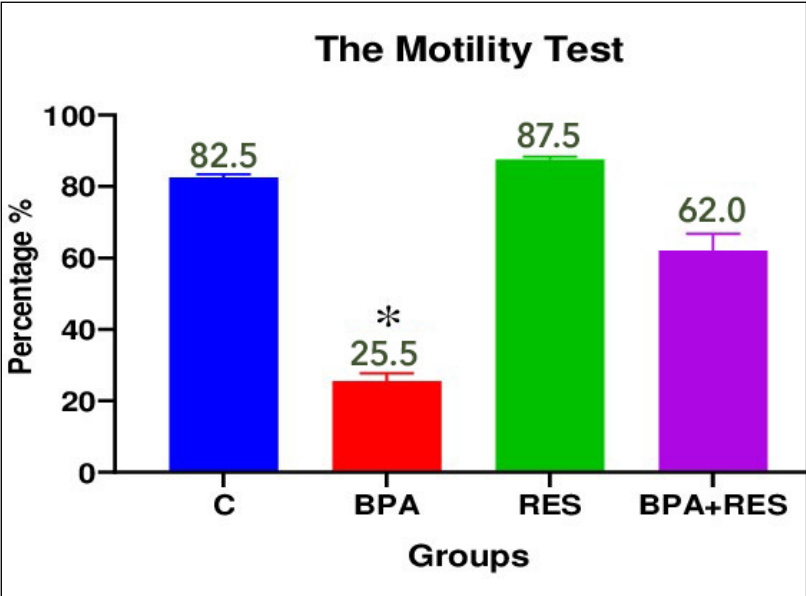


**Fig. 2.** Effects of Bisphenol A (BPA) and Resveratrol on Sperm Motility (%)  
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at p<0.05  
*Source: Own materials*

[19]. Sperm counting was performed using a Neubauer hemocytometer, adhering to the procedures outlined in previous studies [20].

**SPERM DNA FRAGMENTATION TEST**

For assessment the DNA fragmentation the sperm were fixation by drying a 10 µL of sample on a slide, and then immersing in a 3:1 methanol-acetic acid solution for 5 minutes, and fully dry [21]. After fixation the slide was stained by 0.01 of acridine orange for 2 minutes and washed with distilled water and left to dry. Finally, the slides examined under a fluorescent microscope showed sperm nuclei the green color mean with intact, and red that indicating fragmented, less condensed DNA, figure (1) [22].

**EVALUATION OF CIRCULATING LUTEINIZING AND TESTOSTERONE HORMONES**

For evaluation the LH and testosterone the blood was analyzed by using assay kits provided by (Sun Long Biotech Co., Ltd., China; SL1061Ra kit used for testosterone and the SL1093Ra kit for LH analysis.

**REDOX SYSTEM EVALUATION**

The redox system evaluation included the assessment of total antioxidant capacity (TAC) using specific assay kits (Cat No: BC1315) by using a spectrophotometer. The procedure involved sample preparation, serum dilution of the FeSO<sub>4</sub> standard solution, and preheating the spectrophotometer and reagents were added according to the protocol, and absorbance readings were used to construct a standard curve and calculate TAC values.



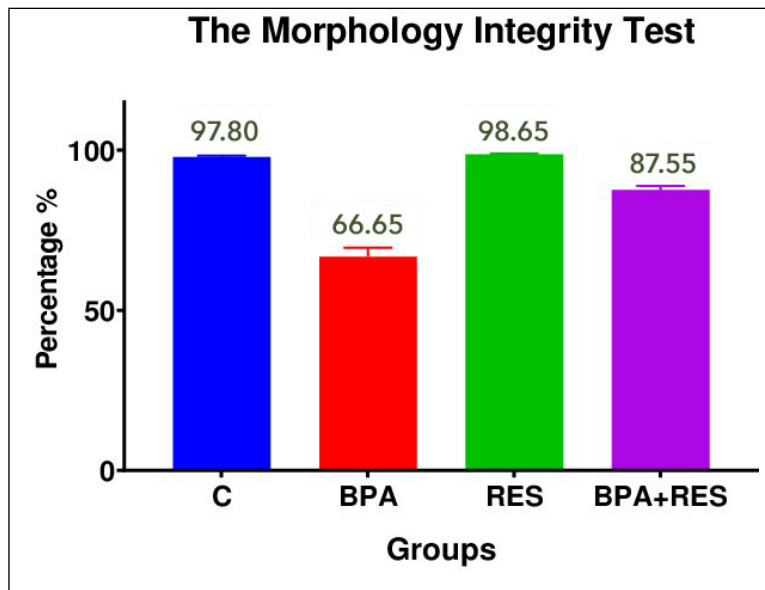


**Fig. 3.** Effects of Bisphenol A (BPA) and Resveratrol on Sperm Morphology (%)  
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p < 0.05$

Source: Own materials

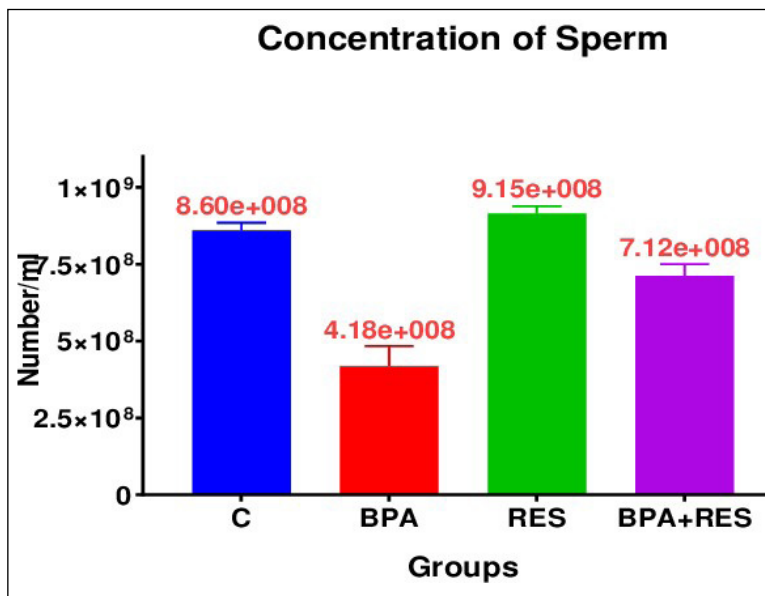


**Fig. 4.** Effects of Bisphenol A (BPA) and Resveratrol on Sperm concentration (%)  
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p < 0.05$

Source: Own materials

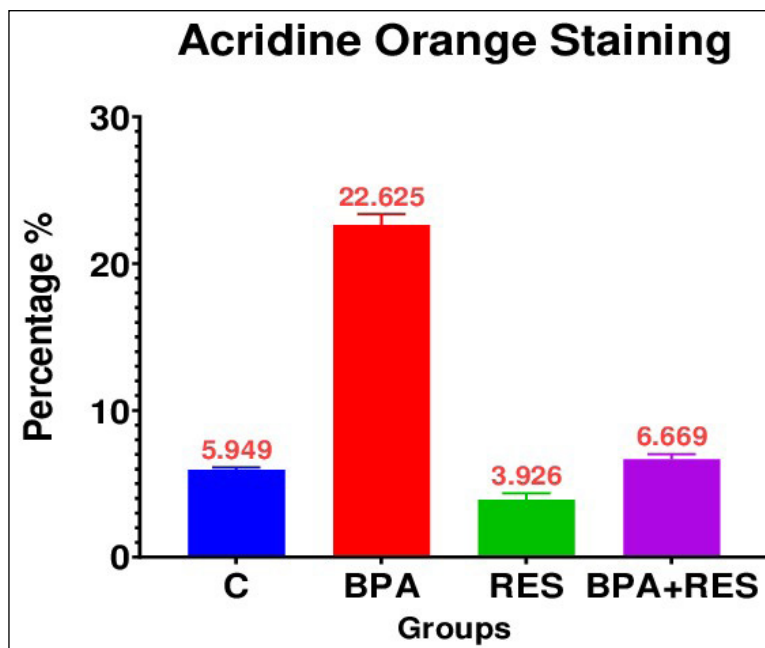


**Fig. 5.** Effects of Bisphenol A (BPA) and Resveratrol on Sperm DNA Fragmentation (%)  
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p < 0.05$

Source: Own materials

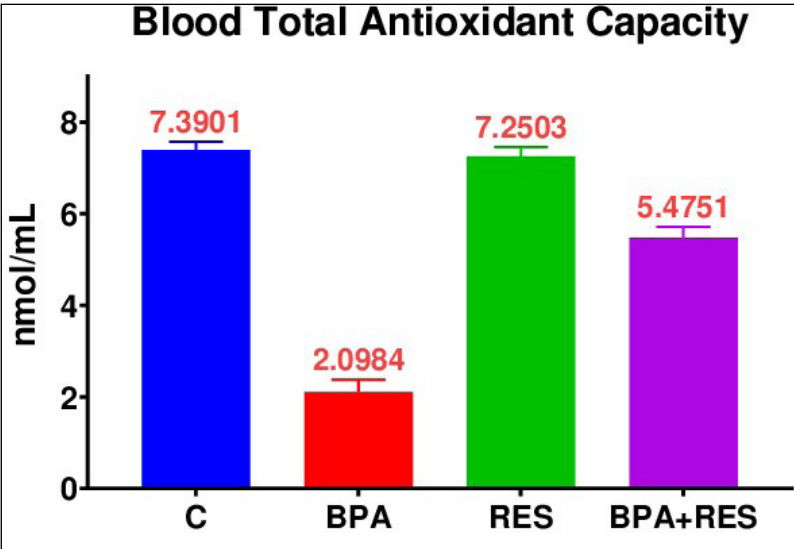


**Fig. 6.** Effects of Bisphenol A (BPA) and Resveratrol on Blood total Antioxidant capacity Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p<0.05$   
*Source: Own materials*

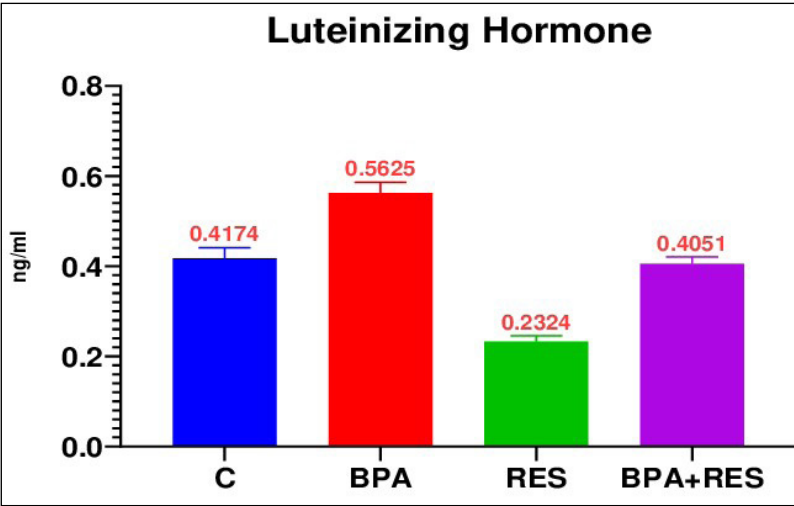


**Fig. 7.** Effects of Bisphenol A (BPA) and Resveratrol on LH Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p<0.05$   
*Source: Own materials*

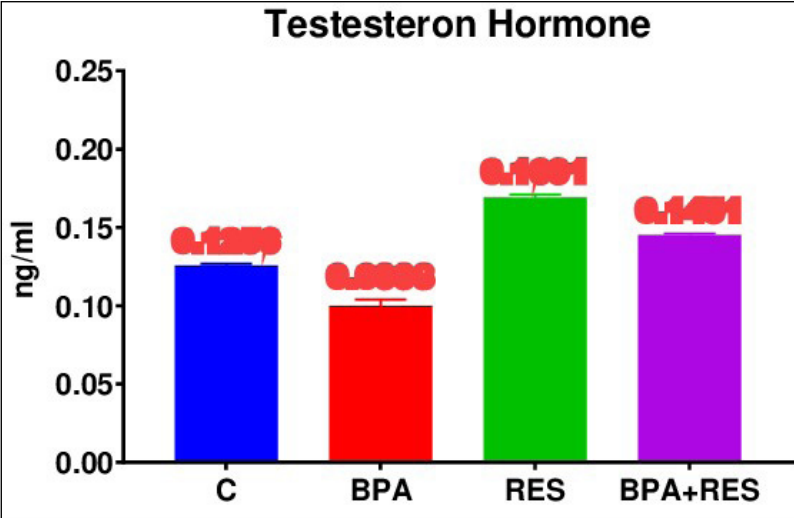


**Fig. 8.** Effects of Bisphenol A (BPA) and Resveratrol on Testosterone Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), \*Indicates significant differences between groups at  $p<0.05$   
*Source: Own materials*

**HISTOPATHOLOGICAL EXAMINATION OF TESTICULAR TISSUE**  
The testicular tissue was excised, longitudinally opened, and preserved in 10% buffered formalin for fixation. Following standardized histological procedures [23]

the samples were fixed at room temperature for 48 hours. The fixed tissues underwent graded alcohol dehydration, clearing in two stages of xylene, and embedding in liquid paraffin maintained at 56°C for two hours. Thin sections, 5 micrometers thick, were

prepared using a microtome. These sections were then dewaxed and stained with Harris Hematoxylin and Eosin (H&E) for detailed histological analysis. The stained slides were examined under a light microscope using magnifications of X4, X10, and X40, allowing for a comprehensive evaluation of the testicular tissue's histological structure.

## RESULTS

### SPERM EVALUATION

The Figure 2 illustrated the progressive sperm motility which showed significant variations among the experimental groups since C group exhibited the highest motility, with a mean value of  $(82.5 \pm 0.83)$  indicating normal physiological function, conversely, the BPA group showed a significant reduction in motility  $(25.5 \pm 2.167)$ , reflecting the detrimental effects of BPA on sperm motility. Interestingly, the RES group demonstrated the highest motility among all groups  $(87.5 \pm 0.8333)$ , and nonsignificant different compared with the control group. The combined treatment group (BPA + RES) showed a partial restoration of motility  $(62 \pm 4.726)$ , indicating that resveratrol mitigated, but did not fully counteract, the negative effects of BPA.

The evaluation of sperm morphology in Figure 3 revealed significant differences among the treatment groups. The control group exhibited morphology percentage  $(97.8 \pm 0.4163)$ , closely matched and non-significantly different from the used resveratrol-only RES group with  $98.65 \pm 0.2242$ , indicating no adverse impact on sperm structure. In contrast, the BPA group showed a significant decrease in sperm morphology  $(66.65 \pm 2.861)$ , highlighting the detrimental effects of BPA exposure on sperm structure. Notably, the co-administration of BPA and resveratrol BPA+RES demonstrated a marked improvement in morphology  $(87.55 \pm 1.248)$  compared to the BPA group, suggesting the protective role of resveratrol.

The evaluation of sperm concentration in figure (4) showed significant differences among the experimental groups since the C group showed a sperm concentration  $(8.60 \times 10^8 \text{ sperm/mL})$ , indicating normal spermatogenesis, in contrast, the BPA group showed significantly reduced in sperm concentration  $(4.18 \times 10^8 \text{ sperm/mL})$ , that mean BPA's toxic effects on spermatogenesis while on the other hand, the RES group exhibited the normal concentration  $(9.15 \times 10^8 \text{ sperm/mL})$ , a nonsignificant difference among the control group, suggesting that resveratrol not only counteracts oxidative damage but may enhance spermatogenic efficiency. Notably, the co-administration group (BPA+RES) demonstrated

normal sperm concentration  $(7.12 \times 10^8 \text{ sperm/mL})$  compared to the BPA group, highlighting the protective role of resveratrol.

### SPERM DNA FRAGMENTATION

The assessment of sperm DNA fragmentation in figure (5) revealed the significant differences across the experimental groups which the C group exhibited low levels of DNA fragmentation  $(5.949 \pm 0.1742)$ , indicative of intact chromatin integrity, in contrast, the BPA group showed a dramatic increase in DNA fragmentation  $(22.63 \pm 0.7494)$ , reflecting BPA's adverse effects on sperm DNA stability. The RES demonstrated the lowest levels of DNA fragmentation  $(3.926 \pm 0.4412)$ , suggesting a strong protective effect of resveratrol on chromatin integrity. The co-administration BPA+RES group exhibited a marked reduction in DNA fragmentation  $(6.669 \pm 0.3551)$  compared to the BPA group, indicating the partial restorative capacity of resveratrol.

### TOTAL ANTIOXIDANT CAPACITY LEVELS

The result of blood total antioxidant capacity (TAC) test showed in figure (6) that illustrated the C group exhibited the highest TAC levels  $(7.3901 \text{ nmol/mL})$ , indicating antioxidant capacity under normal physiological conditions while in contrast, the BPA group showed a significant decreased in TAC  $(2.0964 \text{ nmol/mL})$ , that proved the oxidative stress induced by BPA, in other hands RES group showed TAC levels  $(7.2503 \text{ nmol/mL})$  while he co-administered BPA + RES group exhibited TAC levels in  $(5.4751 \text{ nmol/mL})$  compared to the BPA group, indicating the partial ameliorative effect of resveratrol against BPA-induced oxidative stress.

### HORMONAL ANALYSIS

#### SERUM LEVELS OF LUTEINIZING

The result of analysis of serum LH levels in the figure (7) explained that is significant change between groups which C group  $(0.4174 \pm 0.02326)$ , indicative of normal level of LH which BPA group showed significant increase in LH levels  $(0.5625 \pm 0.02311)$ , which is indicated the disruptive effect of BPA on endocrine signaling pathway, in contrast, the RES group exhibited the normal LH levels  $(0.2324 \pm 0.01295)$  that proved that resveratrol enhance androgen feedback. In other hands, BPA + RES group showed a reduction in LH levels  $(0.4051 \pm 0.01547)$  significant different with the BPA group.



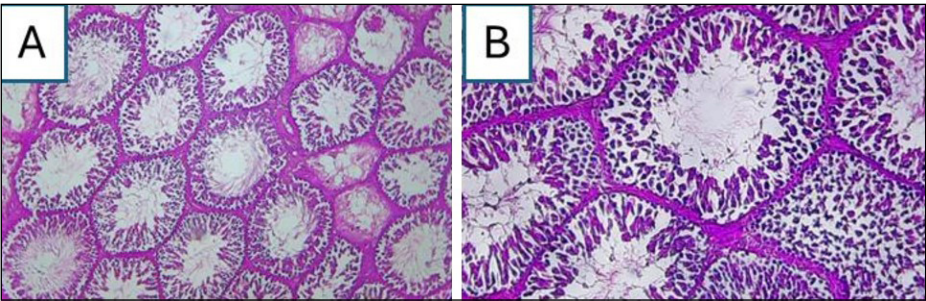


**Fig. 9.** Histological Section of Control Group Testicular Tissue (Normal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X  
*Source: Own materials*

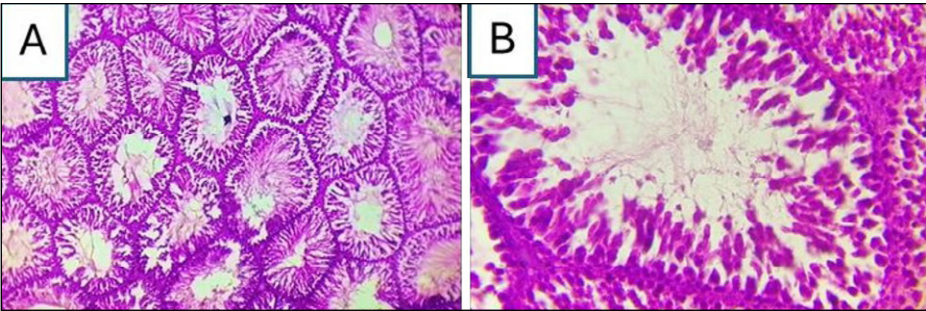


**Fig. 10.** Histological Section of RES Group Testicular Tissue (Normal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X  
*Source: Own materials*

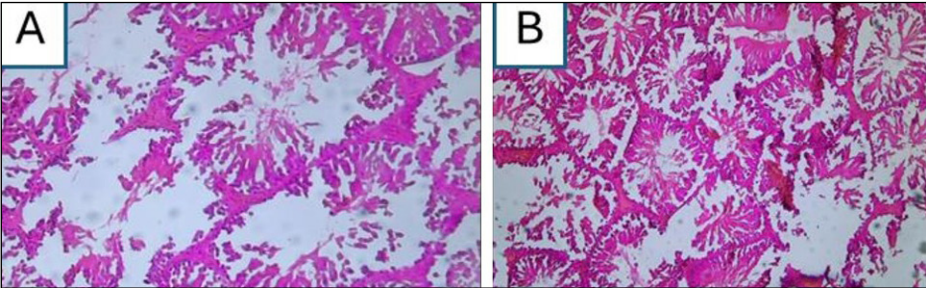


**Fig. 11.** Histological Section of BPA Group Testicular Tissue (Abnormal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X  
*Source: Own materials*

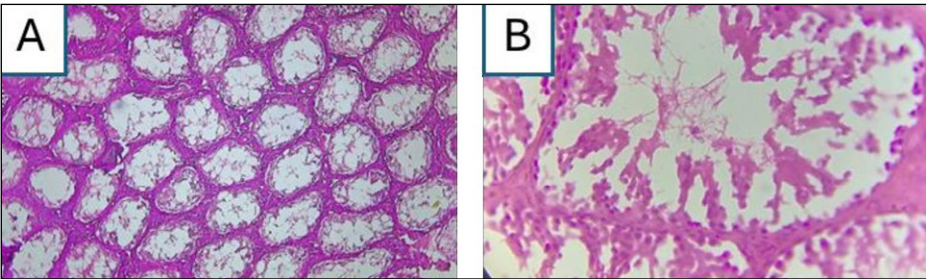


**Fig. 12.** Histological Section of BPA+RES group Testicular Tissue (Abnormal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X  
*Source: Own materials*

SERUM LEVELS OF TESTOSTERONE

The analysis of serum testosterone levels across the experimental groups revealed significant alterations due to Bisphenol A (BPA) exposure and the potential restorative effects of resveratrol. The control group (C) exhibited baseline testosterone levels ( $0.1256 \pm 0.1256$ ), reflecting normal androgen function and hypothalamic-pituitary-gonadal (HPG) axis activity. BPA exposure resulted in a reduction in serum testosterone levels ( $0.09979 \pm 0.09979$ ), indicating the detrimental effects of BPA on Leydig cell function and androgen synthesis. Conversely, the resveratrol-only group (RES) showed an increase in testosterone levels ( $0.1691 \pm 0.1691$ ), suggesting that resveratrol enhances testosterone production, likely through its antioxidative and protective

mechanisms. The co-administration group (BPA+RES) displayed an intermediate level of testosterone ( $0.1451 \pm 0.1451$ ), indicating that resveratrol partially mitigates BPA-induced disruptions (Fig. 8).

HISTOPATHOLOGICAL EVALUATION OF TESTICULAR TISSUE

The histopathological examination of testicular tissue of experimental groups sowed significant structural variations, reflecting the effects of BPA exposure and RES potential protective role. The C group, as shown in figure (9A-B), in contrast, the RES group figure (10A-B) displayed improved structural integrity, demonstrating the beneficial effects of resveratrol. Conversely, the BPA

group figure (11A-B) showed severe histopathological alterations, including disrupted seminiferous tubules and reduced germ cell populations. The BPA + RES group figure (12A-B) exhibited partial restoration of normal architecture, with noticeable improvement compared to the BPA group, though some abnormalities persisted.

## DISCUSSION

The present discussion highlights the findings related to BPA's detrimental effects on male reproductive parameters and the protective role of resveratrol, supported by comparisons with previous research which observed reduction in sperm motility in the BPA group aligns with previous studies demonstrating that BPA exposure significantly impairs sperm quality which disrupts mitochondrial function and ATP production by inducing oxidative stress and damaging mitochondrial DNA [11]. Additionally, BPA alters calcium ion homeostasis, which is crucial for flagellar movement and motility regulation [10]. The RES group showed an increase in motility, that surpassed the C group, which is in agreement with previous findings from studies that illustrated the antioxidant properties of RES which enhance the mitochondrial function and lead to increase the protection of ATP; moreover, RES scavenging of ROS, and protecting sperm from oxidative damage [10]. In the BPA + RES group, the protective effect of RES enmeshment the motility percentage which that suggests decreased the adverse effect of BPA. Which RES play critical role in upregulated the endogenous antioxidant enzymes and reduced oxidative stress in the sperm cell [3], however, the incomplete restoration indicates that the chronic exposure to BPA cause irreversible damage to sperm cells or mitochondrial dysfunction beyond the protective role capabilities of resveratrol [7, 10, 24].

The BPA-induced oxidative stress further damages the plasma membrane and axonemal structures essential for motility while the resveratrol mitigates these effects by targeting oxidative stress and inflammation that due to its antioxidant action prevents lipid peroxidation of sperm membranes and restores mitochondrial function. The present result agreement with previous studies where BPA is shown to disrupt sperm morphology by inducing oxidative stress, leading to lipid peroxidation, DNA fragmentation, and impaired chromatin condensation [7, 25-26]. The protective role of RES in the BPA+RES group is attributed to RES potent antioxidant properties, which decreased oxidative damage by enhancing the activity of antioxidant enzymes like SOD and catalase, reducing MDA levels, and preserving cellular integrity [27-28]. Similar studies have documented

resveratrol's ability to counteract BPA-induced damage, supporting its role in maintaining sperm structural integrity [7]. These findings align with previous studies showing that BPA induced oxidative stress, mediated by ROS and lipid peroxidation, damages testicular tissue and germ cells [7, 25], while Resveratrol's effectiveness is attributed to its potent antioxidant and anti-inflammatory properties, which mitigate ROS, enhance the activity of antioxidant enzymes the SOD and catalase, and preserve mitochondrial function. Previous research corroborates these results, emphasizing resveratrol's ability to restore sperm production by protecting Sertoli cells and Leydig cells from oxidative stress [2, 29].

The results of DNA Fragmentation in figure 5 are consistent with previous findings that highlight BPA's ability to induce DNA damage through the generation of ROS and oxidative stress, which disrupt chromatin structure and increases DNA fragmentation [3,8,30]. The mechanism of BPA adverse effect lead to increased lipid peroxidation, mitochondrial damage, and subsequently causes activation of apoptotic pathways [11, 30]. On other hands, RES have antioxidant properties that scavenging the ROS and improved the endogenous antioxidant enzymes finally prevent oxidative damage [31-32]. Previous studies proved that RES plays a critical role in improving sperm DNA integrity by stabilizing chromatin and reducing oxidative stress [22].

The findings of antioxidants in figure 6 approbate with previous research, where BPA exposure is associated with a decrease in TAC due to excessive production of ROS, which overwhelm the body's antioxidant defenses and lead to oxidative damage [10-11]. The RES plays a critical role in the enmeshment at the TAC level, which that due to the activity of endogenous antioxidant enzymes sand its ROS-scavenging properties [3, 33, 34]. The partial recovery observed in the BPA+RES group underscores the potential of resveratrol to mitigate oxidative stress, though complete restoration requires further exploration.

The LH findings of present study agreement with previous studies, which proved that BPA causes disrupting of the hypothalamic-pituitary-gonadal axis that by binding to estrogen and androgen receptor and lead to impairing normal feedback pathway and subsequently increased in the LH levels [10, 30]. As description in the present result showed significant increased TCA and inflammation that induced by BPA may further dysregulate hypothalamic function, contributing to defect LH levels [14-15, 35]. RES play a critical role in restoring hormonal balance by its antioxidant and anti-inflammatory properties, which protect the hypothalamus and pituitary from oxidative damage [3, 9]. The testosterone level in the result findings are consistent with previ-

ous studies showing that BPA disrupts testosterone synthesis by impairing Leydig cell function through oxidative stress, mitochondrial dysfunction, and direct interference with steroidogenic enzymes. BPA's estrogenic activity also disrupts the feedback mechanisms of the hypothalamic-pituitary-gonadal axis, leading to reduced luteinizing hormone (LH) stimulation and subsequent declines in testosterone. Resveratrol's ability to restore testosterone levels is attributed to its potent antioxidant and anti-inflammatory properties, which protect Leydig cells from oxidative damage and enhance steroidogenesis by normalizing enzymatic activities involved in androgen production. The results highlight the potential of resveratrol as a therapeutic agent to counteract BPA-induced reproductive toxicity. The histological section of the tests showed significant deferent which C group, as shown in figure (9), which exhibited normal histological structure. Furthermore, the histological section of seminiferous tubules that are enveloped by a thin basement membrane and intact interstitial blood vessels, in additionally the showed intact and mature spermatozoa within the lumens of tubules. While the epithelial layer maintained its structural integrity with Sertoli and Leydig cells and germ cells at various stages of spermatogenesis. That proved RES in 50 mg/kg for 60 days preservation of testicular tissue integrity, and protective the testicular histology. Furthermore, the antioxidative and anti-inflammatory properties of RES play a critical role in mitigating oxidative stress and preventing cellular damage [36–38]. These findings agreement to previous studies, which showed RES scavenge free radicals, reduce oxidative stress, and protect against testicular damage caused by environmental pollution [7, 39–40]. In contrast, the BPA group Figure (11) showed severe histopathological damage, which that is irregularly shaped and atrophied seminiferous tubules, and a reduction in germ cell count. While the tubular lumens of seminiferous showed immature germ cells and sperm debris, due to impaired spermatogenesis. The oxidative stress causes disrupt of Leydig and Sertoli cell that leads to DNA fragmentation and apoptosis in sperm cells [10–11]. The BPA + RES group in the figure

(12) showed amelioration in the histopathological section which observed in the BPA group. While some of seminiferous tubule shrinkage, interstitial expansion, and basement membrane thickening were noted but in lower effect. That illustrated structural improvements by RES. Resveratrol's antioxidant mechanisms, including its ability to enhance endogenous antioxidant enzyme activity and neutralize reactive oxygen species, likely contributed to the observed recovery [12, 41–42].

## CONCLUSIONS

This study demonstrates the profound adverse effects of Bisphenol A (BPA) on male reproductive health, evidenced by disrupted hormonal balance, reduced sperm quality, increased DNA fragmentation, and compromised testicular histopathology. BPA exposure significantly impaired testosterone levels, sperm motility, concentration, morphology, and DNA integrity, emphasizing its role as an endocrine-disrupting chemical that induces oxidative stress and cellular damage. The findings align with existing research indicating BPA's detrimental effects on the hypothalamic-pituitary-gonadal axis and testicular structure. Importantly, resveratrol (RES) exhibited remarkable protective effects against BPA-induced reproductive toxicity. Resveratrol treatment not only restored sperm quality and DNA integrity but also mitigated histopathological abnormalities in testicular tissue. Its antioxidant, anti-inflammatory, and anti-apoptotic properties were pivotal in reducing oxidative stress, enhancing mitochondrial function, and preserving germ cell integrity. The partial recovery observed in the BPA+RES group underscores resveratrol's potential as a therapeutic agent to counteract environmental toxicants like BPA. In conclusion, this study highlights the dual impact of BPA on male reproductive parameters and the promising protective role of resveratrol. Future research should further explore the mechanisms of resveratrol's action and its potential in preventing or reversing reproductive damage induced by other environmental toxins, aiming to preserve fertility and male reproductive health.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Histological and oncological determinants of postpancreatectomy acute pancreatitis following pancreatoduodenectomy

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## ABSTRACT

**Aim:** This study aimed to evaluate the incidence of postpancreatoduodenectomy acute pancreatitis (PPAP) and analyze its association with histological and oncological features of the resected tumors.

**Materials and Methods:** Data from 296 patients who underwent PD between 2014 and 2023 were analyzed. The intergroup analysis compared patients in the PPAP (n=126) and no-PPAP (n=170) groups regarding tumor histology, stage, differentiation, resection margin status, and type of PD.

**Results:** PPAP occurred in 42,6% of cases, and clinically relevant PPAP in 30,1%. PPAP was more common in patients with cystic pancreas neoplasms (22,4% in the PPAP group vs. 9,0% in the no-PPAP group,  $p=0,001$ ) and duodenal adenocarcinoma (4,0% vs. 0,6%,  $p=0,04$ ) and tended to occur more often in distal bile duct adenocarcinoma (10,4% vs. 4,8%,  $p=0,07$ ). In contrast, patients with pancreatic ductal adenocarcinoma had a lower PPAP rate (22,4% vs. 49,1%,  $p<0,0001$ ). The tumor size (T1-T4) and differentiation (G1-G4) did not affect PPAP incidence. A higher incidence of PPAP was observed in the N0 group (46,7% vs. 30,8%,  $p=0,03$ ), as well as in patients with R0 resections (91,0% vs. 70,3%,  $p=0,0006$ ). Standard PD was also associated with a higher frequency of this complication (the difference at borderline significance:  $p=0,05$ ).

**Conclusions:** The incidence of PPAP is associated with certain histological tumor types and the extent of surgery. Tumor size and grade of differentiation had no significant impact, while N0 lymph node status and R0 resection margins were associated with higher PPAP incidence.

**KEY WORDS:** pancreatoduodenectomy, postpancreatectomy acute pancreatitis, pancreatic tumors

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## INTRODUCTION

Pancreatoduodenectomy (PD) remains the main treatment method for tumors of the pancreas and periampullary structures. This surgical intervention is technically complex and is associated with a high incidence of significant postoperative complications (Clavien-Dindo  $\geq$  IIIa) [1]. One of the important aspects of the postoperative period is the development of postpancreatectomy acute pancreatitis (PPAP), which can influence the incidence and nature of other postoperative complications following PD. According to various authors, the incidence of clinically relevant forms of PPAP after PD ranges from 14,7% to 28% [2, 3].

Postoperative complications, particularly PPAP, significantly affect the duration of hospitalization. The overall complication rate after PD is 70,3%, increasing the average postoperative hospital stay from 22 to 30 days [4]. It has been proven that the incidence of

other serious postoperative complications of PD is significantly higher in patients with PPAP. However, the impact of PPAP on long-term outcomes after PD remains uncertain. Intergroup analysis did not reveal a significant difference in five-year overall survival or disease-free survival after PD [5].

According to the literature, benign diseases are identified in only 5–13% of patients who have undergone PD [6]. In the vast majority of patients, the final diagnosis is established postoperatively. Histologic analysis plays a key role in verifying the diagnosis, allowing not only the determination of the tumor's morphological type but also the assessment of prognosis and treatment effectiveness. Despite considerable attention to the impact of PPAP on disease progression, there is currently no convincing evidence of its association with the histologic type of tumor, its oncologic characteristics, or the extent of surgery.

## AIM

The study aimed to evaluate the incidence of PPAP in patients after PD, considering the histological and oncological characteristics of the pancreatic tumor.

## MATERIALS AND METHODS

The study was based on a retrospective analysis of data from patients who underwent PD over a 10-year period (2014–2023) at the Department of Surgery, University Hospital Schleswig-Holstein, Lübeck, Germany. A total of 296 patients were included in the analysis. The surgeries were performed for both malignant and benign diseases of the pancreas and peripancreatic structures.

The study was conducted in accordance with the ethical standards of the Helsinki Declaration (2004 revision, Tokyo). The study design was approved by the local Ethics Committee. All patients provided informed consent for the use of their personal data, examination results, and treatment outcomes. Data were collected and stored in an anonymized form.

The diagnosis of PPAP was made based on the ISGPS classification (2022), which defines it as inflammation of the pancreatic stump occurring within the first three days after surgery. Modified ISGPS criteria were used for diagnosis, in particular serum lipase levels, which have higher specificity in detecting acute pancreatitis [7]. An increase in serum lipase concentration ( $>60$  U/L) on postoperative days 2 and 3 was considered diagnostically significant. PPAP was classified into three degrees: biochemical (only changes in laboratory analyses), grade B (moderate complications), and grade C (severe, life-threatening conditions) [8]. The main characteristics of the study group are shown in Table 1.

The median age of patients who underwent PD was 69 years. Males predominated over females (61,15% vs. 38,85%). The majority of patients underwent pylorus-preserving PD (77%), compared to those who underwent the classic Whipple procedure (23%). The most common histological diagnosis was pancreatic ductal adenocarcinoma (37,5%). In total, 42,6% ( $n=126$ ) of patients developed PPAP, of whom 30,1% had clinically significant forms (grades B and C). In-hospital mortality rate was 7,8% (23/296 cases).

Before surgery, all patients underwent a comprehensive clinical and laboratory examination, which included blood tests, biochemical markers, tumor markers (CA 19-9, CEA), and the determination of alkaline phosphatase and gamma-glutamyltransferase levels. Serum lipase levels were assessed before surgery and in the early postoperative period (1-3 days), and in the case of hyperlipasemia, the levels were monitored until normalization. Drainage fluid was routinely analyzed

for bilirubin and amylase levels during the first three days of the postoperative period, with further analyses performed as needed. Instrumental diagnostic methods included gastroduodenoscopy, abdominal ultrasound, contrast-enhanced computed tomography, and magnetic resonance imaging. Macroscopic specimens obtained during surgery were subjected to pathomorphological examination.

All patients were divided into two groups: PPAP (126 patients) and no-PPAP (170 patients). An intergroup analysis was conducted in both groups depending on the histological type of the tumor, the stage of cancer according to the TNM classification, tumor grade (G1-G4), resection margin status (R0/R1), and the type of PD (standard or extended) [9, 10].

The data were processed using SPSS software (version 20, IBM). Qualitative variables (absolute and relative frequency [%]) were evaluated using the  $\chi^2$  test. For specific parameters, intergroup analyses were conducted in subgroups of patients for whom the corresponding data were available. The actual sample size used in each analysis is indicated below the respective figures. Differences were considered statistically significant at  $p < 0,05$ .

## ETHICS

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

## RESULTS

In most cases, the final diagnosis was not established before PD. Histological verification of pancreatic pathology was usually performed intraoperatively as a frozen section, as well as postoperatively. Histological variants of pancreatic pathology identified in the operated patients, depending on the presence or absence of PPAP, are shown in Fig. 1.

Among the histological findings obtained from postoperative macroscopic specimens, malignant variants of pancreatic pathology predominated. However, benign pathology was confirmed in some patients: CP was identified in 15,6% of patients in the no-PPAP group and in 11,2% of patients in the PPAP group ( $p=0.28$ ). Regarding histological variants of pancreatic tumors, the most common tumor types were PDAC (22,4% - 49,1%), CNP (9,0% - 22,4%), and AMPAC (9,0% - 12,0%). Other histological variants of pancreatic tumors were much less common.

The results of the intergroup analysis showed that in the PPAP group, compared to the no-PPAP group, CNP were more common (22,4% vs. 9,0%,  $p=0,001$ ), as well

**Table 1.** Baseline characteristics of the study group of patients undergoing pancreatoduodenectomy (n=296)

| Characteristics                         | Number (%)        |
|-----------------------------------------|-------------------|
| Age, years (median; percentiles)        | 69.0 (59.0; 77.0) |
| Gender:                                 |                   |
| • male                                  | 181 (61.15 %)     |
| • female                                | 115 (38.85 %)     |
| Type of PD:                             |                   |
| • pylorus-preserving PD                 | 228 (77.0 %)      |
| • Whipple procedure                     | 68 (23.0 %)       |
| Histological conclusion:                |                   |
| • chronic pancreatitis                  | 40 (13.5%)        |
| • cystic neoplasms of the pancreas      | 43 (14.5 %)       |
| • neuroendocrine tumors of the pancreas | 13 (4.4 %)        |
| • pancreatic ductal adenocarcinoma      | 111 (37.5 %)      |
| • ampullary adenocarcinoma              | 30 (10.1 %)       |
| • distal bile duct adenocarcinoma       | 21 (7.1 %)        |
| • duodenal adenocarcinoma               | 6 (2.0 %)         |
| • other                                 | 32 (10.8 %)       |

Source: compiled by the authors of this study

as DUOAC (4,0% vs. 0,6%,  $p=0,04$ ), and DBDAC (10,4% vs. 4,8%,  $p=0,07$ ). The latter showed only a trend toward significance in the analyzed groups. In addition, a significant decrease in cases with histologically confirmed PDAC was recorded in the PPAP group compared to no-PPAP group (22,4% vs. 49,1%,  $p<0,0001$ ). As previously mentioned, PDAC was the most common histological variant of pancreatic tumors in the operated category of patients.

Regarding the characteristics of the primary tumor according to the TNM classification system, the analysis of tumor size and location (T1–T4) in the groups (Fig. 2) showed that T3 (45,3% and 42,5% of cases in the no-PPAP and PPAP group, respectively) and T2 stages (35,9% and 35,6%, respectively) were the most common, while T1 and T4 were significantly less common (9,4% and 11,0% of cases, respectively). There was no statistically significant intergroup difference among the T stages in the analyzed patient groups.

The analysis of tumor spread to regional lymph nodes (N0–N2) according to the TNM staging system in the groups showed (Fig. 3) that N0 and N1 stages prevailed in both studied groups. Moreover, in the PPAP group compared to the no-PPAP group, the N0 stage was significantly more often recorded (46,7% vs. 30,8%,  $p=0,03$ ). In other groups, no significant intergroup differences were observed.

Regarding the assessment of tumor grade, the results of tumor cell differentiation (G1–G4) in the PPAP and no-PPAP groups of patients are presented in Fig. 4. The most common differentiation grade was G2, identified in 54,3% and 59,5% of cases, respectively. The G3 grade was less frequent, occurring in 28,6% and 28,8% of cases, respectively. The G1 grade was found in 17,1% and 9,9% of cases, while G4 was identified in

only 2 patients (1,8%) in the no-PPAP group. Analysis of tumor cell differentiation did not reveal significant statistical differences between the studied groups in the incidence of PPAP.

The assessment of the radicality of PD was based on the resection margin status (R). The results of the analysis of the resection margin status of the pancreas in the PPAP and no-PPAP groups are presented in Fig. 5. It was found that in 70,3% of cases in the no-PPAP group and in 91,0% of cases in the PPAP group, cancer cells were absent at the resection margins. Conversely, their presence was detected in 29,7% and 9,0% of cases, respectively ( $p = 0,0006$ ), indicating that cancer cells were significantly less frequently found at the resection margins in the PPAP group compared to the no-PPAP group.

The analysis of the type of PD in the PPAP and no-PPAP groups (Fig. 6) showed that in the no-PPAP patients, extended PD was performed more often (48,2% vs. 36,8%) and standard PD was performed less frequently (51,8% vs. 63,2%) (the difference at borderline significance:  $p=0,05$ ).

## DISCUSSION

The obtained results indicate a different incidence of PPAP after PD depending on the histological variants of pancreatic pathology. In this study, PPAP developed after both benign and malignant pancreatic pathologies. However, the incidence of PPAP varied significantly depending on the histological type of neoplasm, which aligns with findings from other studies.

In patients with chronic pancreatitis and PDAC, the frequency of PPAP was lower compared to other histologically verified pathologies. Postoperative PPAP was

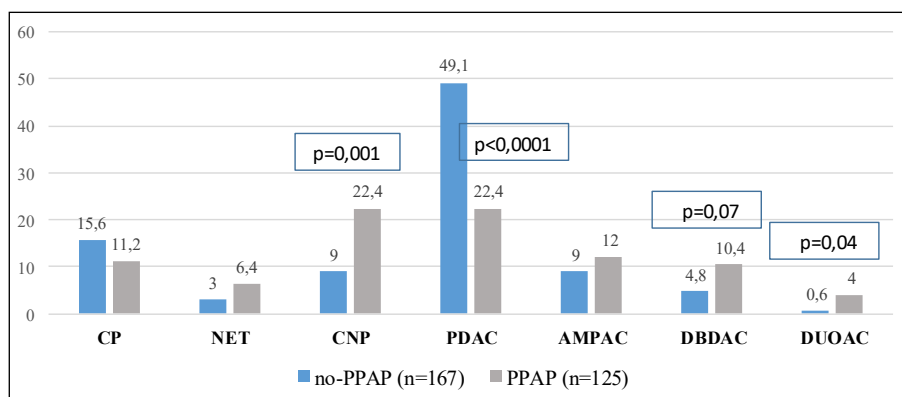


**Fig. 1.** Histologic variants of pancreatic pathology (malignant and benign) verified in the PPAP and no-PPAP groups (in %)

Notes: CP – chronic pancreatitis, NET – neuroendocrine tumors of the pancreas, CNP – cystic neoplasms of the pancreas, PDAC – pancreatic ductal adenocarcinoma, AMPAC – ampullary adenocarcinoma, DBDAC – distal bile duct adenocarcinoma, DUOAC – duodenal adenocarcinoma, PPAP – postpancreatectomy acute pancreatitis

Picture taken by the authors

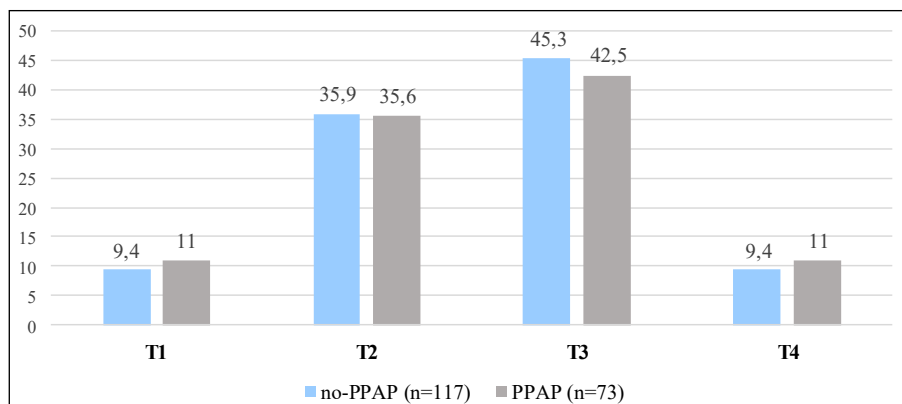


**Fig. 2.** The size of the primary tumor (T1-T4) according to the TNM staging system in the PPAP and no-PPAP groups (%)

Picture taken by the authors

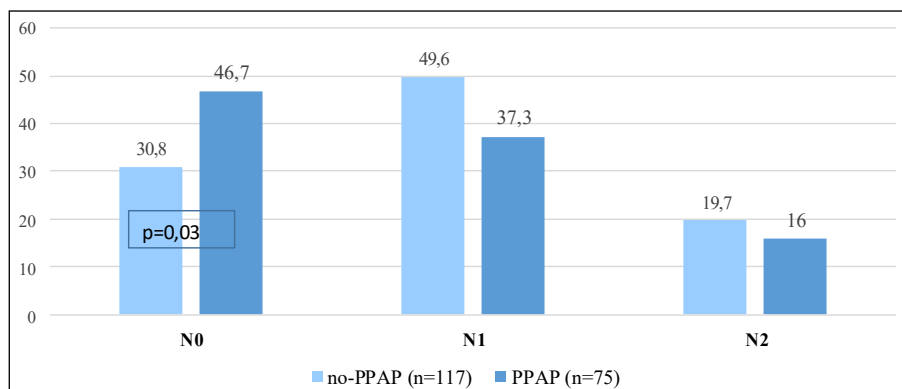


**Fig. 3.** Spread of the tumor process to regional lymph nodes (N0-N2) according to the TNM staging system in the PPAP and no-PPAP groups (in %)

Picture taken by the authors

significantly less frequent among patients with PDAC. Among patients with chronic pancreatitis, the frequency of PPAP was also lower, although this difference did not reach statistical significance. In all other histological variants of pancreatic pathology, the proportion of PPAP patients was higher than that of no-PPAP patients, with a significant difference observed in the CNP and DUOAC groups, while in the DBDAC group there was only a tendency toward a higher proportion of PPAP patients.

These findings highlight the varying impact of histological forms of pancreatic pathology on the prognosis of PPAP after PD.

A connection has been established between the inflammatory process in the pancreas and the development of malignant formations, which is associated with changes in the microenvironment in PDAC [11].

Patients with chronic pancreatitis have a significantly higher risk of developing PDAC [12]. The period between the diagnosis of CP and PDAC usually lasts from one to two decades [13]. Currently, research is ongoing to investigate the relationship between stromal and cancerous cells. It is known that PDAC is characterized by a pronounced desmoplastic stromal reaction surrounding cancer cells [14]. Stellate cells create a microenvironment that is favorable for tumor growth, immunosuppression, and metastasis [15]. It appears that the increased density of pancreatic tissue that develops in CP and PDAC contributes to a reduced risk of PPAP, which explains the lower incidence of PPAP in this study among patients with PDAC.

Currently, there are no studies on the incidence of PPAP at different stages of the oncological process. Although the size of pancreatic tumors is known to affect



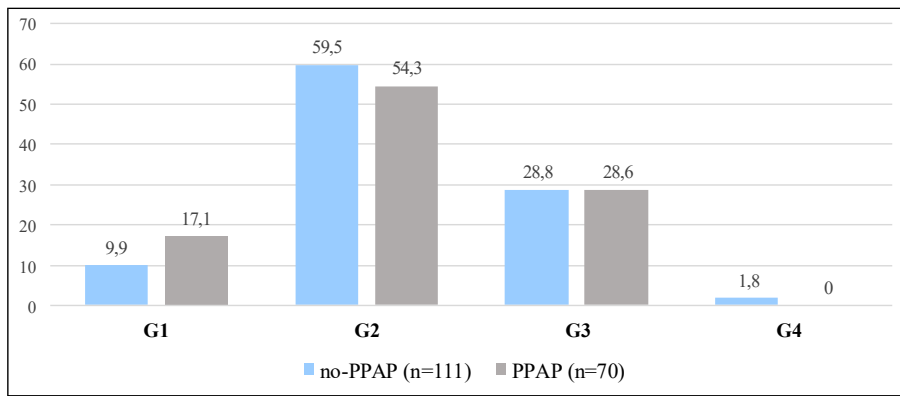


**Fig. 4.** Tumor differentiation (G1-G4) in the PPAP and no-PPAP groups (in %)  
Picture taken by the authors

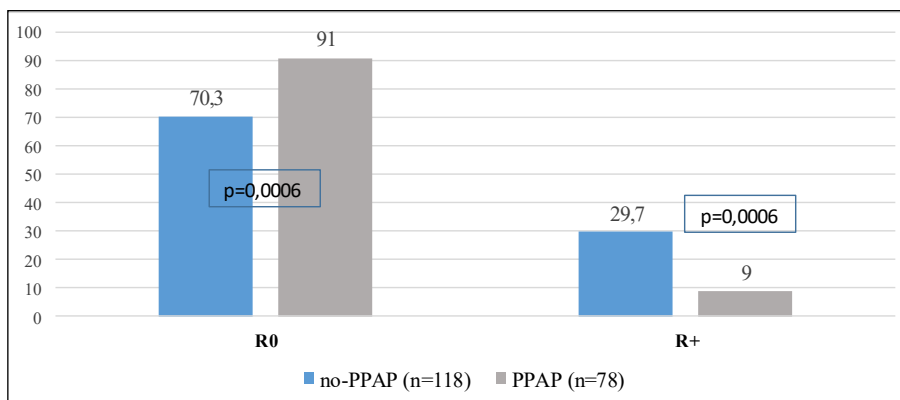


**Fig. 5.** Resection margin status (R) of the pancreas in the PPAP and no-PPAP groups (in %)  
Picture taken by the authors

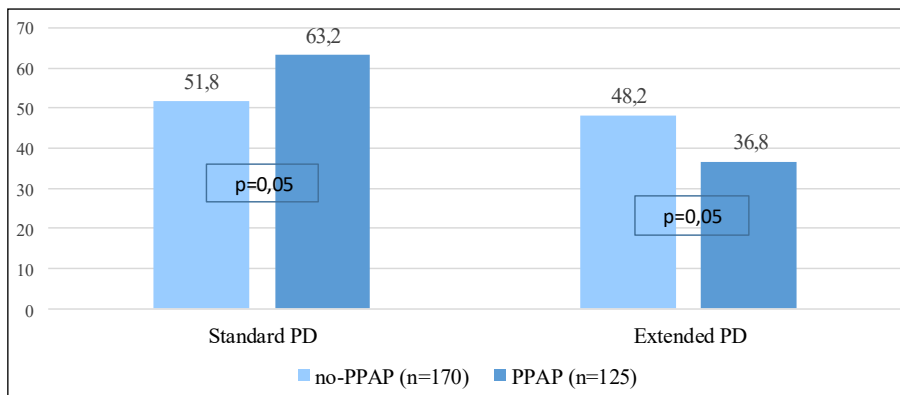


**Fig. 6.** Variant of pancreatoduodenectomy (standard or extended) in the PPAP and no-PPAP groups (in %)  
Picture taken by the authors

postoperative outcomes, its prognostic role remains controversial [16]. T-stage determination depends on the method [17], and tumors larger than 2 cm worsen the survival of patients with PDAC [18, 19].

The incidence of postoperative complications after PD at different TNM stages requires further investigation. Low tumor differentiation and lymph node metastases are recognized as independent predictors of poor survival in PDAC patients after PD [20]. Our analysis did not reveal a statistically significant difference in PPAP incidence among tumors sizes (T1–T4). However, patients with N0 were significantly more likely to develop PPAP, whereas different tumor grades (G) did not significantly affect the incidence of PPAP.

Resection margin status (R) is of great importance. Achieving R0 status should be the primary goal in the

surgical treatment of PDAC [21]. However, for a long time, the significance of R0 remained unclear, and its impact on prognosis varied depending on the analyzed datasets and different definitions of R0 [22, 23]. Some authors reported that both recurrence-free and overall survival were significantly higher in pancreatic cancer patients with R0-resected tumors [24]. At the same time, numerous studies have shown that positive resection margins (R1) reduce survival rates for all types of pancreatic cancer, including adenocarcinoma [25]. Recent studies have shown that in patients receiving neoadjuvant therapy, the prognostic significance of a positive margin is less important [26, 27]. In contrast, a recent population-based study by German authors showed that R0 status remained an independent predictor of overall and disease-free survival after PDAC resection [28].

In the available literature, data on the incidence of postoperative complications depending on different resection margin statuses (R) are limited. In our study, PPAP was significantly more common in patients with R0 resection. We suggest that this is related to a more radical surgical approach and, consequently, greater postoperative tissue injury.

Regarding the type of surgery, according to our data, standard PD was associated with a higher incidence of PPAP, while extended PD was associated with a lower incidence, and these differences were of borderline statistical significance. Similar results were obtained by De Reuver and colleagues (2015) [20]. In contrast, Hartwig W. et al. (2016) demonstrated that extended resections in patients with pancreatic cancer are associated with a higher complication rate [29]. On the other hand, according to Mitra A. et al. (2018), the incidence of severe postoperative complications (Clavien grades III, IV, and V) in patients after extended and standard pancreatectomies did not differ significantly [30].

Thus, the results of this study indicate the importance of an individualized approach to PD, taking into account the histological type of the tumor, TNM stage, R status, and extent of resection. Further research is necessary to gain a deeper understanding of the mechanisms underlying PPAP development and to improve surgical strategies.

## CONCLUSIONS

The incidence of PPAP varies significantly depending on the histologic type of pancreatic tumor. PPAP was more common in CNP and DUOAC, less common in patients with PDAC. Tumor size and grade of differentiation did not influence the incidence of PPAP. N0 status of regional lymph nodes and R0 resection margin status were associated with a higher incidence of PPAP. Standard pancreatoduodenectomy was associated with a higher incidence of PPAP compared with extended procedures.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Advances in clinical and metabolic profiling in children with type 1 diabetes and diabetic nephropathy

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## ABSTRACT

**Aim:** To stratify children with T1D according to their risk of developing and progressing DN using cluster analysis of clinical and laboratory indicators.

**Materials and Methods:** Cluster analysis was performed in a cohort of 60 children (mean age  $13 \pm 3$  years; 17 [28,3%] boys and 43 [71,7%] girls) with T1D, with ( $n = 30$ ) or without ( $n = 30$ ) early-stage DN, based on parameters such as T1D duration, estimated glomerular filtration rate, microalbuminuria (MAU), urinary kidney injury molecule-1 (KIM-1), and episodes of diabetic ketoacidosis (DKA).

**Results:** Three clusters with distinct renal risk profiles were identified in children with T1D with or without DN. The risk group for DN development in T1D children showed moderate T1D duration, random MAU and DKA episodes, and slightly increased KIM-1 levels. The risk group for further DN progression in children with early DN was characterized by prolonged T1D duration, pronounced MAU, frequent DKA, hyperfiltration, and markedly elevated urinary KIM-1.

**Conclusions:** Cluster analysis allows early identification of the risk group for DN in children with T1D and the risk group for DN progression in children with early DN. Urinary KIM-1 may be implemented as a biomarker of tubular injury, providing opportunities for earlier intervention before clinical nephropathy develops.

**KEY WORDS:** diabetic nephropathy, kidney injury molecule-1, children

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## INTRODUCTION

According to a study published in 2021, the global prevalence of diabetic nephropathy (DN) associated with type 1 diabetes mellitus (T1D) increased to 6,3 million cases in 2021. It is predicted that by 2041 this figure will reach 7 million cases, with incidence rates of 95,140 cases in 2021 and 115,000 expected by 2041 [1]. In Ukraine, there has been a significant increase in the age-standardized mortality rate from DN associated with T1D, from 1,9 per 100,000 population in 1990 to 12,4 in 2021, representing an increase of 560,2%. DN develops in 15-20% of children with T1D, and early manifestations may be asymptomatic [2-4].

In DN, functional disorders are associated with hyperglycemic damage to the glomerular endothelium, leading to increased basement membrane permeability and the onset of microalbuminuria (MAU). Activation of the renin-angiotensin-aldosterone system results in vasoconstriction, initial hyperfiltration, and a gradual decline in the estimated glomerular filtration rate (eGFR). Inflammatory processes are marked by elevated levels of the pro-inflammatory cytokines tumor necrosis factor (TNF- $\alpha$ ) and interleukin-1 (IL-1), which induce fibrosis and interstitial degradation [3]. Oxidative stress

causes injury to both the filtration and reabsorption epithelium of the nephron [4].

Various factors implicated in the development and progression of DN include metabolic and hemodynamic disorders, genetic predisposition, and inflammatory and fibrotic mechanisms [5]. However, glomerular lesions are considered the most dominant of the key pathomorphological changes in DN, determining the degree of impairment of the filtration capacity of the kidneys and closely correlating with the clinical picture. Modern studies also show that tubular-interstitial lesions are no less important in the pathogenesis of DN and may precede glomerular changes, especially in children. Several studies have highlighted the importance of kidney injury molecule-1 (KIM-1) as an early biomarker of tubular injury. Elevated urinary KIM-1 concentrations have been associated with the progression of chronic kidney disease and higher cardiovascular risk in patients with diabetes. However, most of the findings are based on studies in adults, and evidence in children with T1D and DN remains limited, emphasizing the importance of further research. In view of this, the search for new biomarkers and approaches to stratifying patients with T1D according to their risk of developing nephropathy is highly relevant [6,7].

## AIM

To evaluate clinical and metabolic profiling parameters in children with T1D to identify markers associated with the early onset and progression of DN.

## MATERIALS AND METHODS

To clarify the pathophysiological features and identify more informative diagnostic indicators, a cluster analysis was conducted in a cohort of 60 children (mean age [mean  $\pm$  standard deviation]  $13 \pm 3$  years; 17 [28,3%] boys and 43 [71,7%] girls), of whom 30 had T1D without signs of DN and 30 had early DN (pre-clinical, asymptomatic stage of kidney damage in T1D, with no major clinical symptoms yet apparent [8]). All patient data were documented, including medical history and systematic assessment. Urine albumin level (MAU) was assessed in 24-hour urine samples using standard methods (values less than 30 mg/24 h were considered normoalbuminuria, and 30–300 mg/24 h as MAU). The eGFR was calculated using the Schwartz formula [9]. The level of kidney injury molecule type 1 (KIM-1) in urine samples was determined using a commercial Human KIM-1 ELISA Kit (Cat. No. EH0210, 96 determinations; Wuhan Fine Biotech Co., Ltd, Wuhan, China).

The data were processed using Statistica v. 10.0 (originally developed by StatSoft Inc., USA), GraphPad Prism 10.5.0 for Windows (San Diego, CA, USA), and EZR v. 1.68. Quantitative data were expressed as median and interquartile range, and qualitative parameters as absolute and relative (%) frequency. Quantitative data were compared using the Mann-Whitney U test (for two unrelated samples) or the Kruskal-Wallis test with Dunn's post hoc test (for three unrelated samples). The frequency of binary parameters was compared using Fisher's exact test (for two unrelated samples) or Fisher's exact test with Bonferroni correction (for three unrelated samples).

Two-stage clustering was performed using Statistica v. 10.0 software. An intelligent clustering method was applied, in which the optimal number of clusters was determined automatically in two processes: initial clustering followed by hierarchical clustering. Hierarchical algorithms were used to estimate the optimal number of clusters based on silhouette width, log-likelihood distance calculation, and Bayesian Schwarz clustering. A p-value  $<0,05$  was considered statistically significant (after applying Bonferroni correction).

## ETHICS

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki, as well as

the recommendations established by ethical committee of the Bogomolets National Medical University (No. 4/2024). Prior to the start of the study, all participants received comprehensive information regarding the purpose, methods, potential risks, and benefits of participation. Participation in the study was based on the principle of informed consent, and all respondents signed an appropriate consent form before data collection began. Participants had the right to withdraw from the study at any time without providing a reason.

In the case of research involving minors or individuals with limited legal capacity, informed consent was obtained from their legal representatives.

In accordance with confidentiality standards, all data were collected anonymously and processed in compliance with the applicable data protection legislation, including the General Data Protection Regulation (GDPR), to ensure the protection of personal information. All information was used exclusively within the scope of this study and was aggregated for subsequent analysis of the results.

## FRAMEWORK

The work is carried out within the framework of the initiative research work of the Bogomolets National Medical University (Kyiv, Ukraine) «Optimization of diagnostics and treatment and rehabilitation algorithms for the impact on the development of somatic diseases in children under martial law in Ukraine» (state registration number: 0125U000004; date: 2025-2027).

## RESULTS

The analysis of clinical characteristics revealed that patients with DN were older and had a longer duration of diabetes compared to those with T1D without nephropathy (Table 1). The sex distribution was similar between groups. The frequency of diabetic ketoacidosis (DKA) and glycated hemoglobin (HbA1c) levels did not differ significantly. The T1D group was entirely represented by patients with normoalbuminuria, whereas in the DN group, 12 (40%) cases had normoalbuminuria. Moreover, the remaining 18 (60%) children with DN presented with MAU. The average urine albumin concentration (among detectable cases) was higher in the DN group. The eGFR was significantly increased in DN patients. Importantly, urine KIM-1 levels were almost two-fold higher in the DN group compared to the T1D group, indicating ongoing tubular injury in children with the pre-clinical stage of DN (Table 1).

In each group, three clusters were identified by re-modeling cluster analysis based on five variables. The

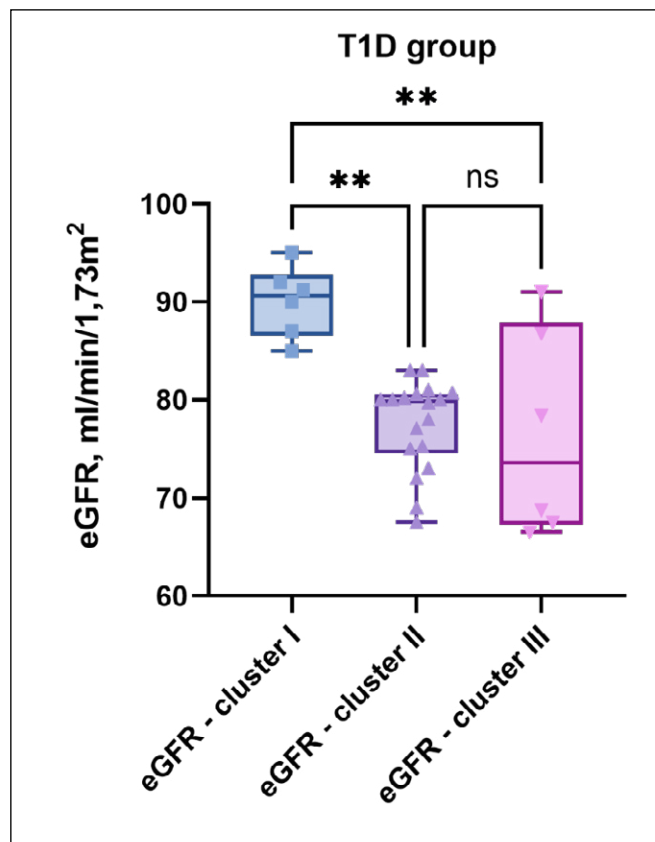


**Table 1.** Clinical characteristics of patients in the studied groups

| Characteristics                  | T1D (n=30)                  | DN (n=30)                     | p       |
|----------------------------------|-----------------------------|-------------------------------|---------|
| Age, years                       | 13 (9-15)                   | 14 (13-16)                    | <0.05   |
| Boys, n (%)                      | 9 (30)                      | 8 (27)                        | NS      |
| Girls, n (%)                     | 21 (70)                     | 22 (73)                       |         |
| DM duration, years               | 7 (4-9)                     | 11 (8-12)                     | <0.001  |
| DKA, episodes/year               | 2 (2-3)                     | 3 (2-4)                       | NS      |
| HbA1c, %                         | 8.6 (6.5-10.6)              | 8.6 (7.8-9.5)                 | NS      |
| Normoalbuminuria, n (%)          | 30 (100)                    | 12 (40)                       | <0.0001 |
| MAU, n (%)                       | 0                           | 18 (60)                       |         |
| Urine albumin level, mg/24 h*    | 15.2 (14.5-17.1)<br>N = 7** | 83.0 (75.0-92.0)<br>N = 18*** | <0.0001 |
| eGFR, ml/min/1.73 m <sup>2</sup> | 80.0 (75.0-85.0)            | 117.5 (114.0-120.0)           | <0.001  |
| Urine KIM-1, ng/ml               | 5.2 (2.8-6.2)               | 11.0 (8.4-13.2)               | <0.001  |

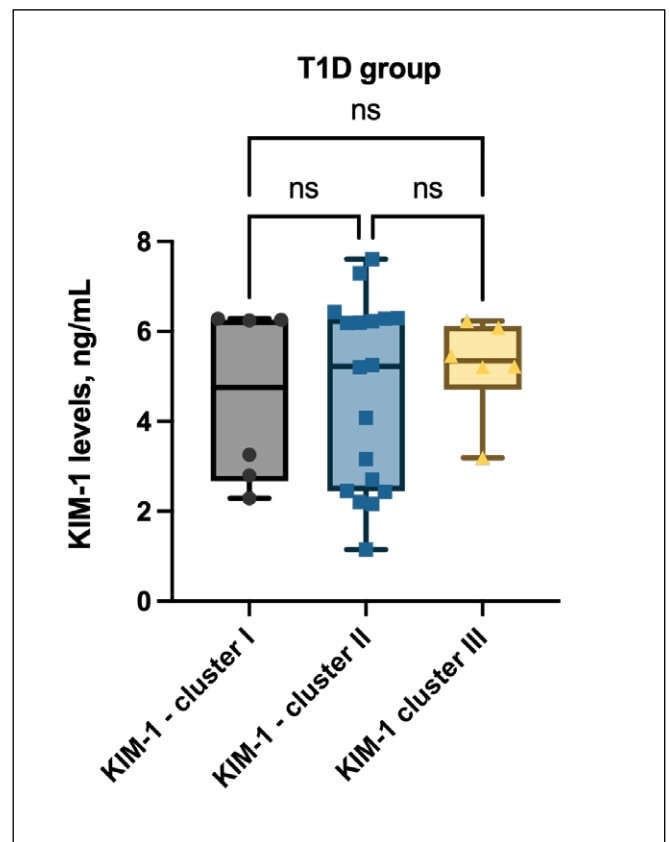
Note: NS – non-significant; \* – among the detectable cases; \*\* – urine albumin level (min-max) 6,1-20,1 mg/24 h; \*\*\* – urine albumin level (min-max) 65,0-95,0 mg/24 h

Source: compiled by the authors of this study

**Fig. 1.** eGFR levels in Clusters of the T1D group (box-and-whisker and dot plots [all the data]).

NS – non-significant; \*\* –  $p < 0,01$

Picture taken by the authors

**Fig. 2.** Urine KIM-1 levels in the clusters of T1D group (box-and-whisker and dot plots [all the data]).

NS – non-significant

Picture taken by the authors

clustered results were based on duration of diabetes, eGFR, MAU, DKA episodes per year, and KIM-1, a marker of tubular kidney injury. These five variables were selected because they reflect key clinical and pathophysiological dimensions of diabetic nephropathy progression.

Duration of T1D is a major determinant of complication risk and disease stage. eGFR represents renal function status, and MAU is a well-established early marker of glomerular damage. The frequency of DKA episodes per year reflects the degree of metabolic instability

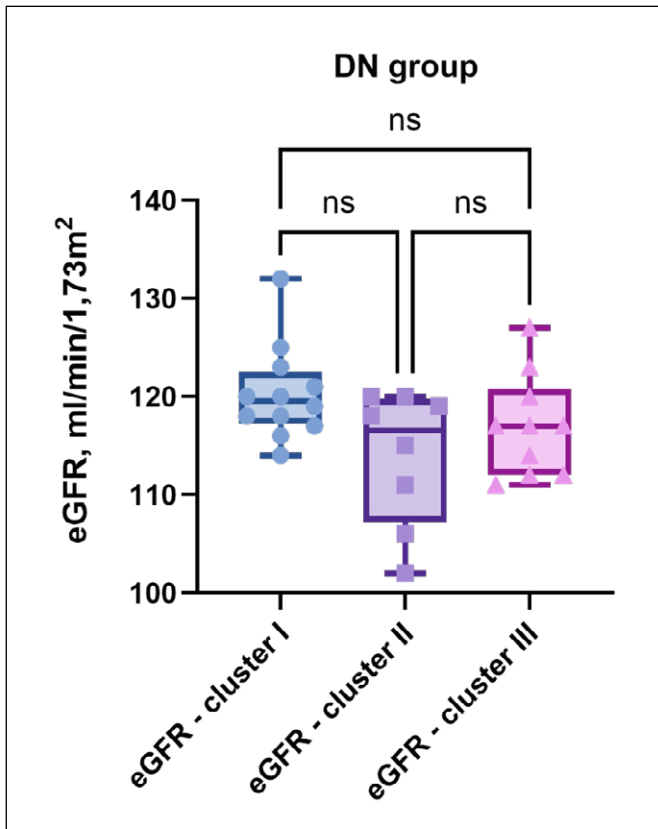


**Fig. 3.** eGFR levels in the Clusters of DN group (box-and-whisker and dot plots [all the data])  
 NS – non-significant  
 Picture taken by the authors

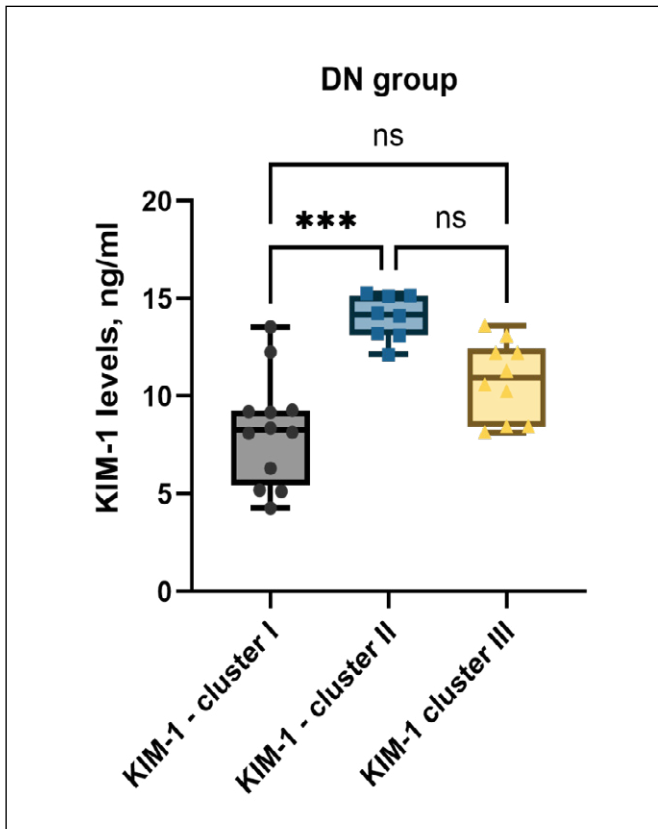


**Fig. 4.** Urine KIM-1 levels in clusters of DN group (box-and-whisker and dot plots [all the data])  
 NS – non-significant, \*\*\* –  $p < 0,001$   
 Picture taken by the authors

and quality of T1D compensation, which contributes to vascular and renal injury. Finally, KIM-1 was included as a sensitive tubular injury biomarker, providing additional information on early kidney damage beyond conventional markers [10-12].

The analysis of T1D duration across clusters in the T1D group showed no statistically significant differences: the median disease duration was 6 (4–10) years in Cluster I (n = 6), 6 (2–9) years in Cluster II (n = 18), and 8 (7–8) years in Cluster III (n = 6) ( $p = 0,657$ ).

The analysis of eGFR values across the three clusters in the T1D group revealed significant differences. Median eGFR was 90,6 (87,0–92,0) ml/min/1,73 m<sup>2</sup> in Cluster I, which was higher than the corresponding values in Cluster II (79,9 [75,0–80,6] ml/min/1,73 m<sup>2</sup>;  $p < 0,01$ ) and Cluster III (73,6 [67,5–86,8] ml/min/1,73 m<sup>2</sup>;  $p < 0,01$ ) (Fig. 1).

It should be noted that the majority of detectable cases of normoalbuminuria were observed in Cluster III (6 out of 7), despite only one case in Cluster I ( $p = 0,045$ ) and the absence of such cases in Cluster II ( $p < 0,0001$ ).

The frequency of DKA episodes per year did not differ significantly between clusters: Cluster I – 3 (1–3) episodes/year; Cluster II – 2 (2–3) episodes/year; and Cluster III – 3 (2–3) episodes/year ( $p = 0,567$ ).

Finally, urine KIM-1 levels were comparable across the three clusters. Median values were 4,8 (2,8–6,3) ng/ml in Cluster I, 5,2 (2,5–6,3) ng/ml in Cluster II, and 5,3 (5,2–6,1) ng/ml in Cluster III ( $p = 0,973$ ) (Fig. 2).

In the group of patients with DN, significant differences in the duration of type 1 diabetes were observed between the clusters: 8 (7–9) years in Cluster I (n=12), 13 (12–14) years in Cluster II (n=8), and 11 (9–11) years in Cluster III (n=10). The significant differences between Cluster I and Cluster II ( $p < 0,001$ ), and between Cluster II and Cluster III ( $p < 0,05$ ) were found.

In the DN group, the eGFR did not differ statistically significantly between the clusters: 119,5 (117,5–122,0) ml/min/1,73 m<sup>2</sup> in Cluster I, 116,5 (108,5–119,5) in Cluster II, and 117,0 (112,0–120,0) in Cluster III ( $p = 0,112$ ) (Fig. 3).

The analysis of MAU cases between clusters in the DN group revealed certain differences. No cases of MAU were observed in Cluster I. At the same time, all children in Clusters II and III had MAU ( $p < 0,0001$  vs. Cluster I for both comparisons). At the next step, higher urine albumin concentrations were observed in Cluster II compared to Cluster III: 92,0 (91,0–94,0) mg/24 h vs. 76,5 (71,0–80,0) mg/24 h, respectively ( $p < 0,0001$ ).

The level of urine KIM-1 differed significantly between the clusters. Median values were 8,3 (5,8–9,2) ng/ml in Cluster I, 14,2 (13,2–15,1) ng/ml in Cluster II, and 11,0 (8,5–12,2) ng/ml in Cluster III. A significant difference was found between Clusters I and II ( $p < 0,001$ ), while the difference between Clusters II and III showed a trend toward significance ( $p = 0,052$ ) (Fig. 4).

The frequency of DKA episodes significantly differed between clusters of the DN group. In particular, median values were 1 (1–2) episodes/year in Cluster I, 4 (3–4) episodes/year in Cluster II, and 4 (3–4) episodes/year in Cluster III. The significant differences were revealed between Cluster I and Cluster II ( $p < 0,01$ ), and between Cluster I and Cluster III ( $p < 0,001$ ) (Fig. 5).

The distribution of clusters within the T1D and DN groups is presented in Fig. 6. In the T1D group, Cluster II predominated, comprising 60% of patients, while in the DN group, the largest proportion fell into Cluster I (40%). Clusters I and III in the T1D group were equal, each representing 20%. In the DN group, the proportions were 40% in Cluster I and 33% in Cluster III (Fig. 6).

## DISCUSSION

This study revealed certain intercluster features regarding indicators of renal functional status and the course of T1D. The moderate average duration of T1D across all clusters of this group is consistent with other data [13]. In our study, Cluster III included patients with early signs of DN (decreased eGFR, albuminuria, and slightly elevated KIM-1).

Albuminuria increases with the progression of kidney disease and is therefore considered a marker of disease severity. MAU levels were significantly elevated in Cluster III, indicating initial impairment of glomerular barrier function in the patients of this cluster. Given the absence of clinical nephropathy in these patients, these changes can be interpreted as early predictors of kidney damage. Previous studies have shown that the first and highest peak of incidence occurs after 16 years of diabetes [14].

A modest increase in KIM-1 levels was observed in patients from all clusters. These findings are consistent with studies showing an association between urinary KIM-1 levels and the development of latent DN in children and adolescents with normoalbuminuria and T1D [15,16]. This also aligns with results from two pediatric cohorts with T1D of different durations, which found no significant differences in eGFR or MAU. These data support the idea that, in children without overt nephropathy, KIM-1 may serve as an independent marker of tubular damage without concurrent changes in glomerular function [17].

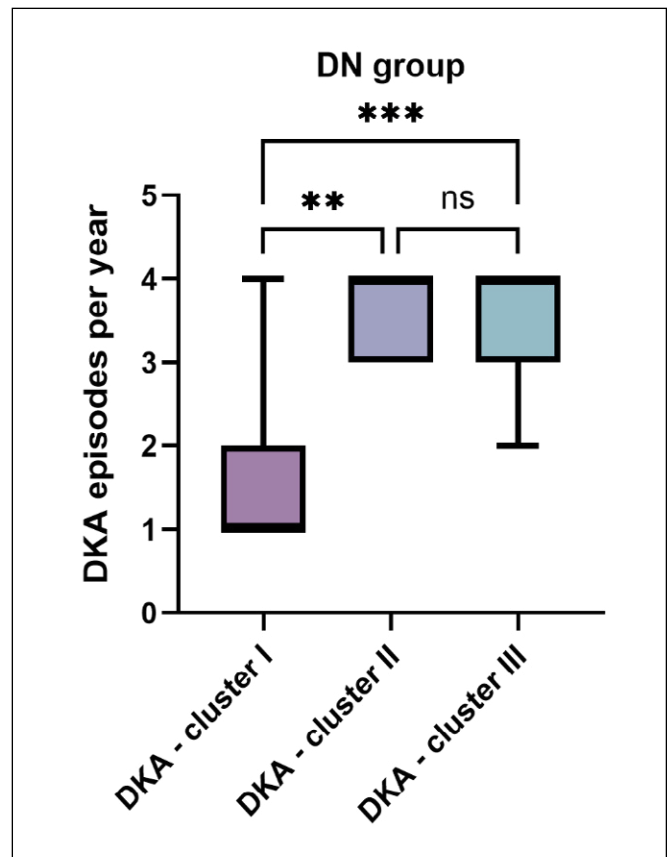


**Fig. 5.** DKA episodes/year in clusters of DN group (box-and-whisker plot) NS – non-significant, \*\* –  $p < 0,01$ , \*\*\* –  $p < 0,001$

*Picture taken by the authors*

Cluster analysis allowed us to identify a group of children with T1D (Cluster III), in whom, despite a moderate duration of the disease, risk factors for the development of DN were already observed: episodes of microalbuminuria and DKA, decreased eGFR, and increased KIM-1. Similar to previous data [18], a decrease in eGFR may precede the appearance of MAU. This is consistent with findings showing the existence of preclinical forms of DN with an isolated increase in KIM-1 [18,19].

In our study, the highest T1D duration in the DN group was observed in Cluster II. The lowest average duration in Cluster I may indicate an early onset of nephropathy, even with a relatively shorter disease history, or a more intensive progression of kidney damage. The latter may be due to genetic, immunological, or metabolic factors, as also evidenced by other research groups [20].

The highest levels of KIM-1 were found in Cluster II, which directly indicates the predominance of tubulointerstitial kidney damage. The difference between Clusters II and III, along with the data from An et al., confirms the association of elevated KIM-1 with albuminuria, glycemic instability, and early glomerular changes. At the same time, regression of MAU is accompanied by a decrease in KIM-1 [21].

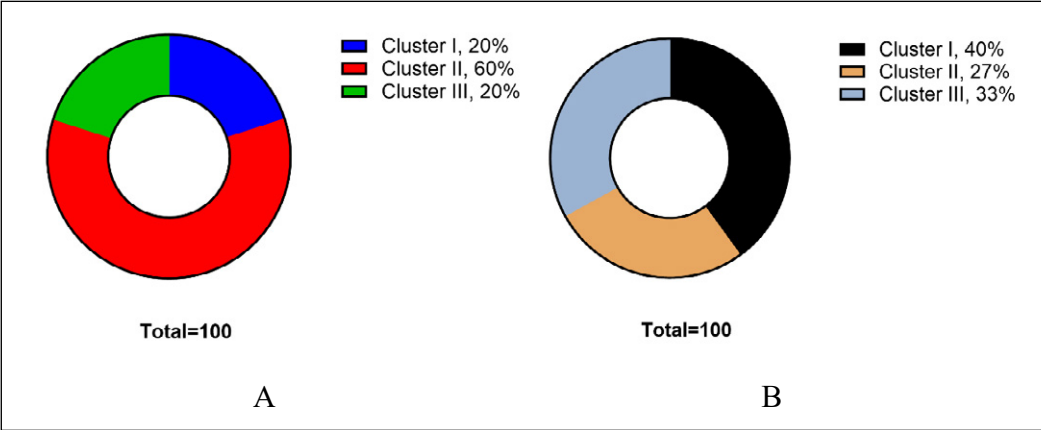


**Fig. 6.** The distribution (%) of clusters of patients in T1D (A) and DN (B) groups  
*Picture taken by the authors*

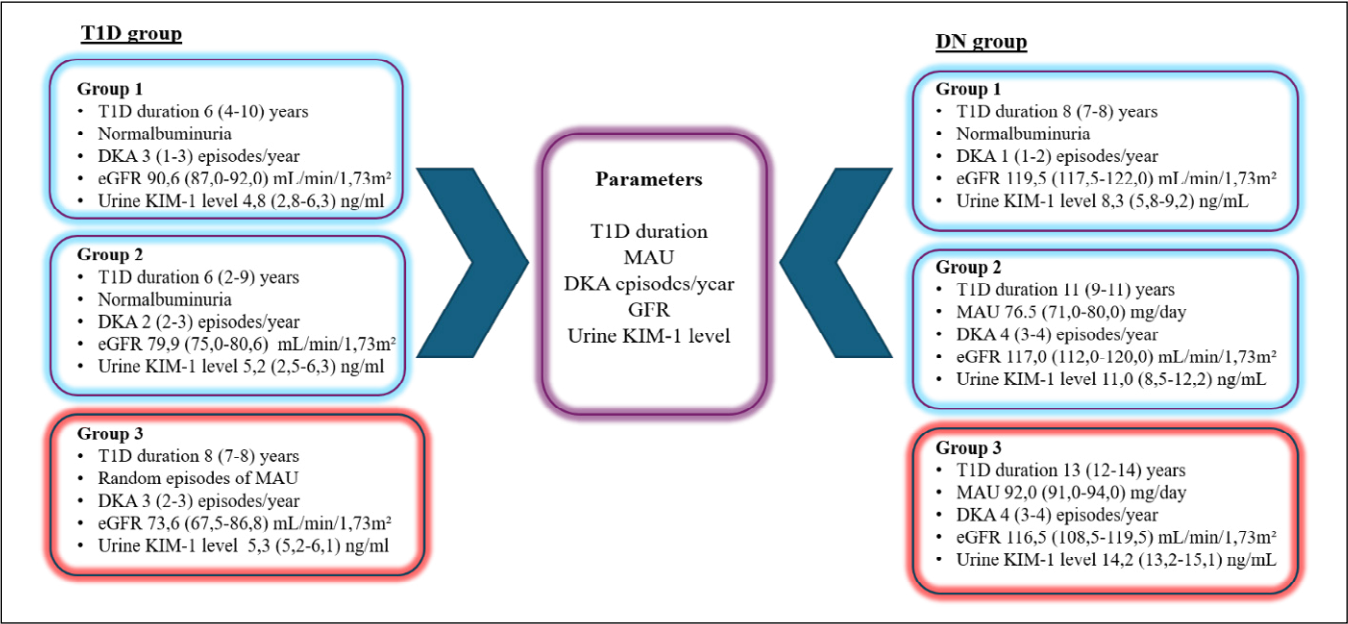


**Fig. 7.** Profiling of children with T1D according to the risk of developing DN based on clinical and biochemical parameters  
*Picture taken by the authors*

MAU was not recorded in patients in Cluster I, suggesting that the glomeruli were still intact or the lesions had not yet manifested. In contrast, MAU was significantly increased in patients in Clusters II and III, indicating progressive damage to glomerular structures. The difference in MAU between Clusters II and III showed only a tendency toward significance, which may be related to the stage of disease or characteristics of treatment. Although MAU is a classic confirmatory test for the diagnosis of DN, not all patients develop macroalbuminuria.

The frequency of DKA episodes was statistically higher in Clusters II and III, potentially reflecting a more latent course of diabetes, insufficient glycemic control, and/or poor adherence to treatment, as also indicated by other studies [20–22].

The results of our study allowed us to identify two risk groups. One group, identified among T1D patients, was characterized by a moderate duration of T1D, sporadic

MAU and DKA episodes, and KIM-1 levels exceeding the normal range. We speculate that this group can be considered a DKA risk group for further renal injury and DN development. In children from the DN group, we identified a risk group for subsequent DN progression, characterized by a longer duration of T1D, pronounced MAU, frequent DKA episodes, hyperfiltration, and markedly elevated urinary KIM-1 levels (Fig. 7).

Further studies should focus on a comprehensive analysis of the role of xanthine oxidase and KIM-1 as potential markers of early tubulointerstitial kidney damage in children with T1D and DN.

### CONCLUSIONS

1. The cluster analysis verified distinct subgroups of children with T1D. Even with moderate disease duration, early renal changes were detected, including decreased eGFR, MAU, and elevated urinary KIM-1.

2. In children from the DN group, a risk group for subsequent DN progression, characterized by long T1D duration, pronounced MAU, frequent DKA episodes, hyperfiltration, and a marked rise in urinary KIM-1 levels.
3. Urinary KIM-1 measurement can be considered as a complementary biomarker to MAU for early detection, monitoring, and risk stratification of DN in children with T1D.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Antibacterial activity of new azole derivatives incorporating Etodolac

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## ABSTRACT

**Aim:** This research paper investigates the synthesis and antibacterial activity of novel azole derivatives incorporating Etodolac.

**Materials and Methods:** The study focused on the reaction of Etodolac with three different aromatic amines: o-phenylene diamine, 2-aminophenol, and 2-aminothiophenol, resulting in three new compounds (1-3). The antibacterial activity of these compounds was assessed against *Escherichia coli*, *Klebsiella*, *Staphylococcus aureus*, and *Streptococcus mutans*.

**Results:** Compound 1 demonstrated the most potent antimicrobial activity against all four bacteria, exhibiting a remarkably low MIC against *Escherichia coli*.

**Conclusions:** The results of this research suggest that incorporating Etodolac into the synthesis of azole derivatives can lead to potent antimicrobial agents. Further exploration of these compounds is warranted to fully understand their potential therapeutic applications.

**KEY WORDS:** anti-bacterial agents, azoles, drug synergism, Etodolac, structure-activity relationship

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## INTRODUCTION

Azole derivatives have emerged as a significant class of compounds in medicinal chemistry due to their diverse biological activities. These compounds have demonstrated efficacy against a broad range of infections, including bacterial, fungal, malarial, and viral infections [1]. Furthermore, azole derivatives exhibit potential in the treatment of cancer and inflammation [2]. Some of azoles derivatives show antibacterial activity Research has highlighted the antibacterial activity of certain azole derivatives [3-6]. Others exhibit both antibacterial and antifungal properties [7-8], while some possess antifungal activity alone [9]. Additionally, azole derivatives have demonstrated cytotoxic [10], and anticancer properties, as well as antimicrobial activity [11]. Etodolac, a non-steroidal anti-inflammatory drug (NSAID), is commonly used to alleviate pain and inflammation [12]. Derivatives of Etodolac have been shown to exhibit a range of biological activities, including antimicrobial [13], anti-inflammatory [14-16], cytotoxic [17-18] and anticancer activity [19-22]. Etodolac derivatives have also been explored as enzyme inhibitors and

have demonstrated a reduced ulcerogenic potential, an important factor in NSAID development [23].

## AIM

This research paper investigates the synthesis and antibacterial activity of novel azole derivatives incorporating Etodolac

## MATERIALS AND METHODS

All reactions were conducted in oven-dried glassware. Commercial grade solvents and reagents were purified by distillation prior to use. Thin-layer chromatography (TLC) using silica gel GF 254 on microscopic glass slides coated with silica gel was employed to monitor reaction progress. Melting points were determined using an electrothermal melting point apparatus with open capillary tubes. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded at ambient temperature using Bruker 400 MHz spectrometers. Samples were prepared by dissolving the compounds in deuterated chloroform (CDCl<sub>3</sub>) with

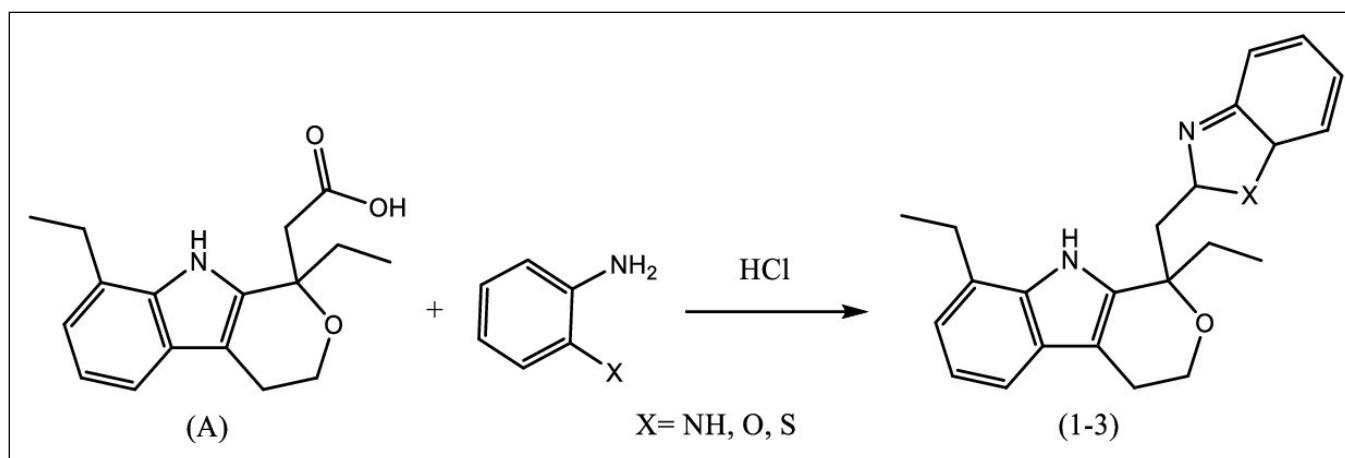


**Fig. 1.** Synthesis of azole derivatives

Source: Own materials

tetramethylsilane (TMS) as an internal standard. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm).

## GENERAL PROCEDURE FOR SYNTHESIS OF TITLE COMPOUND [24]

To synthesize the desired product, Etodolac (A) was reacted with three different aromatic amines: *o*-phenylene diamine, 2-aminophenol, and 2-aminothiophenol. The reaction was carried out in an acidic environment using 4N hydrochloric acid and heated under reflux at 100°C for 5 hours with constant stirring. The progress of the reaction was monitored using thin-layer chromatography (TLC). Once the reaction was complete, the mixture was carefully neutralized with a 10% sodium hydroxide solution. The resulting solution was then cooled in an ice bath for 5 minutes to promote precipitation of the product. The solid product was then isolated by filtration and dried.

## RESULTS

This study included the synthesis of some azole derivatives through the reaction of Etodolac (A) with different aniline derivatives (*o*-phenylene diamine, 2-aminophenol and 2-aminothiophenol) in the presence of HCl as shown in Figure 1.

### SYNTHESIS OF ORGANIC COMPOUNDS (1-3)

#### SYNTHESIS OF 1-((2,7A-DIHYDRO-1H-BENZO[D]IMIDAZOL-2-YL) METHYL)-1,8-DIETHYL-1,3,4,9-TETRAHYDROPYRANO[3,4-B] INDOLE (1)

This compound Fig 2 was synthesized by reacting Etodolac (A) with *o*-phenylene diamine in the presence of

HCl as both solvent and catalyst. The resulting product is a brown powder with a melting point of 91°C. Structure (1). The compound (1) was identified by:

FTIR (KBr) ( $\text{cm}^{-1}$ ):  $\nu$ : N-H (3518-3489), C-H aliphatic (2995-2892), C-H aromatic (3080), C = C (1584, 1482), C = N (1681), C-N (1340-1000), C-O (1301-992).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 12.88 (1H, s, N-H), 11.67 (1H, s, N-H of <sup>indole</sup>), 8.32-7.07 (6H, m, C-H <sup>aromatic</sup> and N-H of imidazole), 3.69 (2H, m,  $\text{CH}_2$ -O), 3.02 (2H, s,  $\text{CH}_2$ ), 2.71 (2H, q,  $\text{CH}_2$ ), 2.62 (2H, t,  $\text{CH}_2$ - $\text{CH}_2$ -O), 1.67 (2H, q,  $\text{CH}_2$ - $\text{CH}_3$ ), 1.18 (3H, t,  $\text{CH}_3$ - $\text{CH}_2$ ), 0.89 (3H, t,  $\text{CH}_3$ - $\text{CH}_2$ ).

#### SYNTHESIS OF 1-((2,7A-DIHYDROBENZO[D] OXAZOL-2-YL) METHYL)-1,8-DIETHYL-1,3,4,9-TETRAHYDROPYRANO[3,4-B] INDOLE (2)

This compound Fig 3 was synthesized by reacting Etodolac (A) with 2-aminophenol in the presence of HCl as both solvent and catalyst. The resulting product is a brown powder with a melting point of 90°C. Structure (2). The compound (2) was identified by:

FTIR (KBr) ( $\text{cm}^{-1}$ ):  $\nu$ : N-H (3525-3492), C-H aliphatic (3000-2899), C-H aromatic (3092), C = C (1590, 1482), C = N (1677), C-N (1344-989), C-O (1310-993).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm): 12.80 (1H, s, N-H), 11.65 (1H, s, N-H of <sup>indole</sup>), 8.30-7.06 (6H, m, C-H <sup>aromatic</sup>), 3.70 (2H, m,  $\text{CH}_2$ -O), 3.03 (2H, s,  $\text{CH}_2$ ), 2.73 (2H, q,  $\text{CH}_2$ ), 2.60 (2H, t,  $\text{CH}_2$ - $\text{CH}_2$ -O), 1.66 (2H, q,  $\text{CH}_2$ - $\text{CH}_3$ ), 1.19 (3H, t,  $\text{CH}_3$ - $\text{CH}_2$ ), 0.89 (3H, t,  $\text{CH}_3$ - $\text{CH}_2$ ).

#### SYNTHESIS OF 1-((2,7A-DIHYDROBENZO[D] THIAZOL-2-YL) METHYL)-1,8-DIETHYL-1,3,4,9-TETRAHYDROPYRANO[3,4-B] INDOLE (3)

This compound Fig 4 was synthesized by reacting Etodolac (A) with 2-aminothiophenol in the presence of

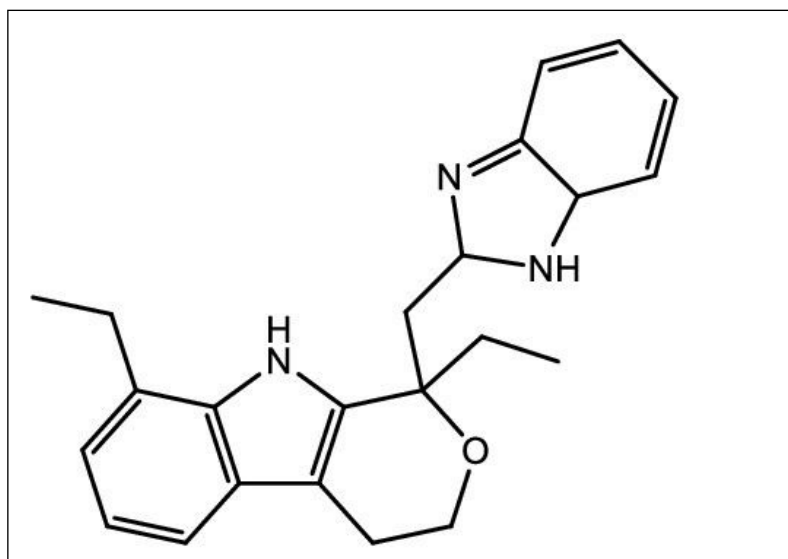


**Fig. 2.** Structure of compound (1)  
*Source: Own materials*

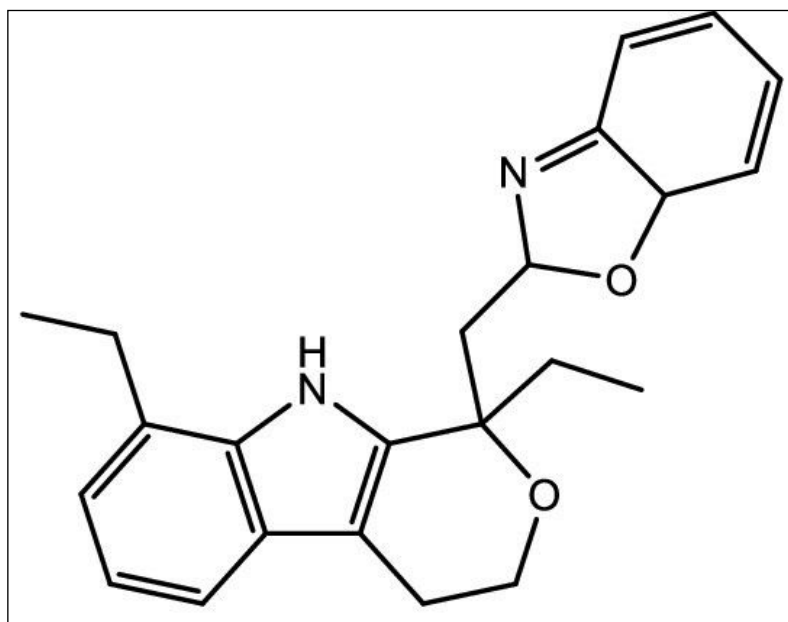


**Fig 3.** Structure of compound (2)  
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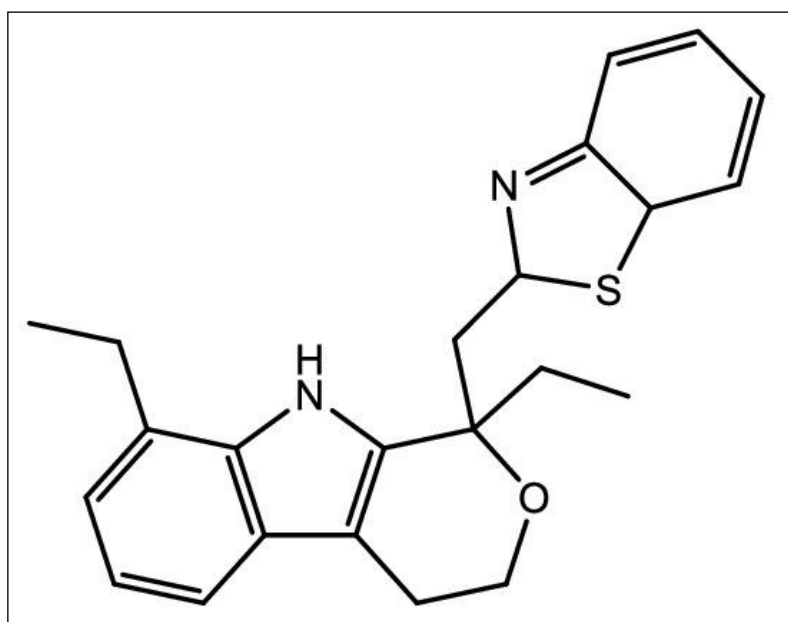


**Fig. 4.** Structure of compound (3)  
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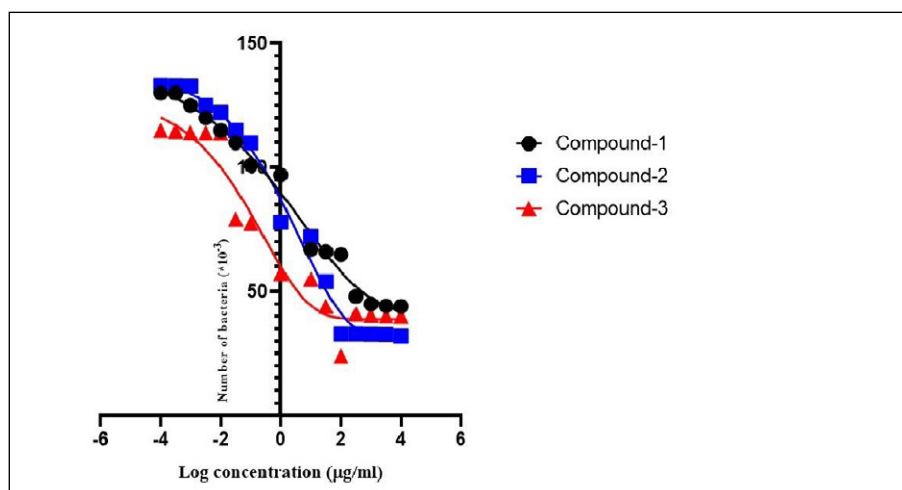


**Fig. 5.** Antimicrobial biological activity of Compounds 1, 2, and 3 against *E. coli*  
Source: Own materials

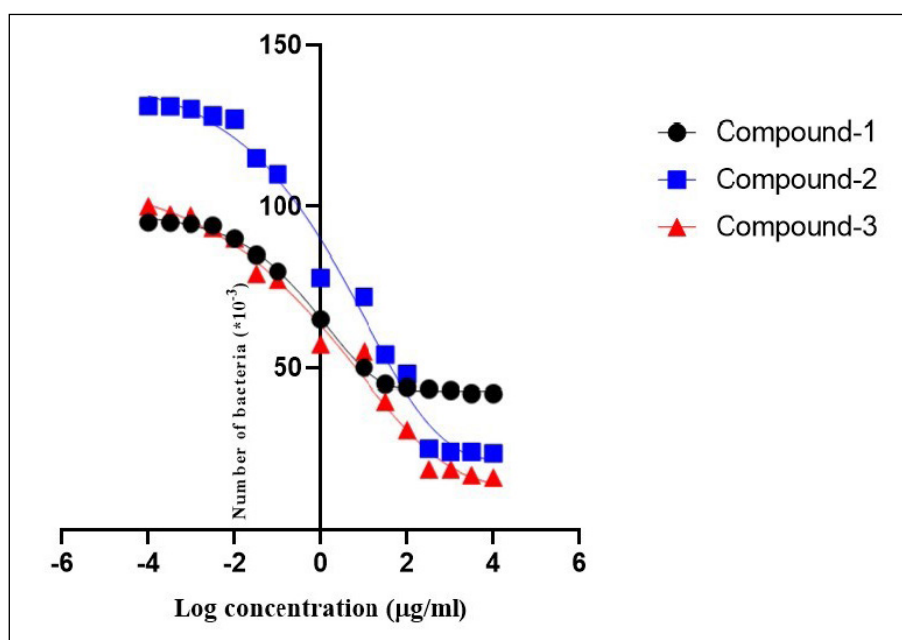


**Fig. 6.** Antimicrobial Biological Activity of Compounds 1, 2, and 3 against *Klebsiella*  
Source: Own materials

HCl as both solvent and catalyst. The resulting product is a brown powder with a melting point of 89°C. Structure (3). The compound (3) was identified by:

FTIR (KBr) (cm<sup>-1</sup>): ν: N-H (3525-3492), C-H aliphatic (3003-2890), C-H aromatic (3050), C=C (1580, 1482), C=N (1677), C-N (1344-989), C-O (1310-993), C-S (605-701).

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 12.80 (1H, s, N-H), 11.65 (1H, s, N-H of indole), 8.30-7.06 (6H, m, C-H aromatic), 3.73 (2H, m, CH<sub>2</sub>-O), 3.05 (2H, s, CH<sub>2</sub>), 2.73 (2H, q, CH<sub>2</sub>), 2.63 (2H, t, CH<sub>2</sub>-CH<sub>2</sub>-O), 1.66 (2H, q, CH<sub>2</sub>-CH<sub>3</sub>), 1.18 (3H, t, CH<sub>3</sub>-CH<sub>2</sub>), 0.89 (3H, t, CH<sub>3</sub>-CH<sub>2</sub>).

## ANTIBACTERIAL ACTIVITY

The antibacterial properties of compounds 1–3 were evaluated against both Gram-negative and Gram-positive bacteria. The results are summarized below:

*Escherichia coli* (Gram-negative): Compound 1 showed the strongest activity, with a very low MIC value (9.089

μg/mL). Compound 3 was moderately active (173.9 μg/mL), while compound 2 was weak (908.1 μg/mL) (Fig. 5).

*Klebsiella* (Gram-negative): Again, Compound 1 was the most effective (21.3 μg/mL). Compound 2 and 3 showed much weaker activity (583.6 μg/mL and 993.2 μg/mL, respectively) (Fig. 6).

*Staphylococcus aureus* (Gram-positive): Compound 3 showed the best activity in this case (568.4 μg/mL), followed by compound 1 (844.2 μg/mL) and compound 2 (1271 μg/mL) (Fig. 7).

*Streptococcus mutans* (Gram-positive): Compound 2 performed best (583.6 μg/mL), compared to compound 3 (844.2 μg/mL) and compound 1 (1220 μg/mL) (Fig. 8).

## DISCUSSION

Our results demonstrate that modifying Etodolac into new azole derivatives can lead to compounds with promising antibacterial activity, but their effectiveness varies depending on the bacterial species.

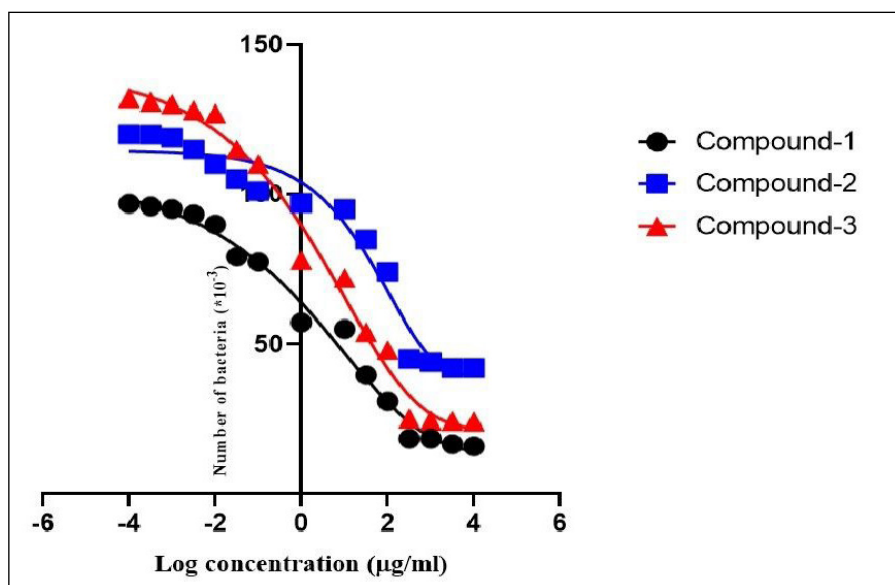


**Fig. 7.** Antimicrobial Biological Activity of Compounds 1, 2, and 3 against *Staphylococcus aureus*

Source: Own materials

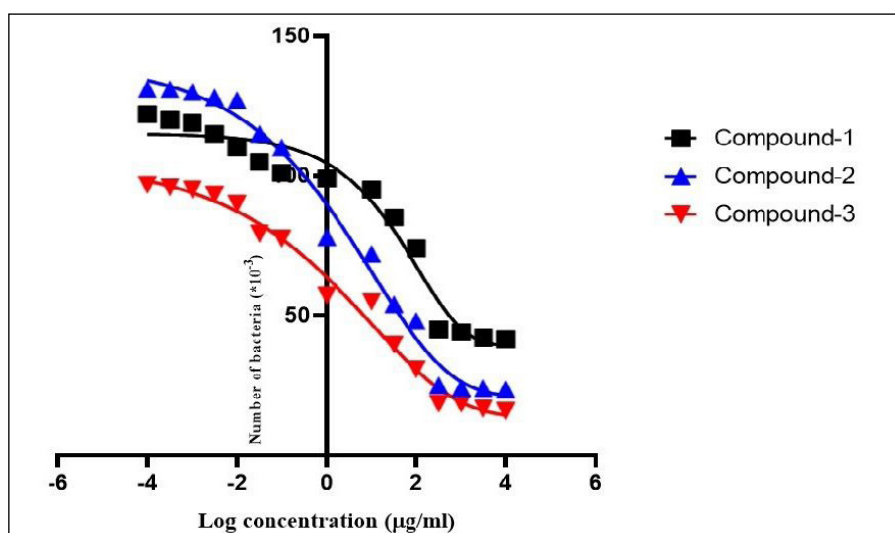


**Fig. 8.** Antimicrobial Biological Activity of Compounds 1, 2, and 3 against *Streptococcus mutans*

Source: Own materials

Compound 1, containing the benzimidazole ring, was the most effective overall, particularly against Gram-negative Bacteria *E. coli* and *Klebsiella*. This suggests that the benzimidazole moiety may enhance interactions with bacterial targets in Gram-negative strains. On the other hand, Compound 3, with a thiazole ring, showed stronger effects against the Gram-positive Strain *S. aureus*, possibly due to the sulfur atom increasing lipophilicity and membrane penetration. Interestingly, Compound 2, the oxazole derivative, displayed generally weaker activity but was the most active against *S. mutans*.

Compared with previously reported azole derivatives [3–6], the very low MIC value of Compound 1 against *E. coli* is particularly notable, highlighting its potential as a strong antibacterial candidate. However, the relatively high MICs observed for the Gram-positive bacteria indicate that further structural optimization is needed to achieve broader-spectrum activity.

These findings align with earlier studies showing that Etodolac derivatives can gain new biological properties beyond their original use as anti-inflammatory drugs [12–14]. Taken together, our study suggests that Etodolac-based azole derivatives are worth pursuing as potential antibacterial agents, although more detailed mechanistic studies and in vivo testing will be essential to confirm their therapeutic relevance.

## CONCLUSIONS

This work demonstrates that modifying Etodolac into new azole derivatives can yield compounds with meaningful antibacterial properties. The benzimidazole derivative showed particular promise against Gram-negative strains, while the thiazole and oxazole analogues exhibited selective effects against Gram-positive bacteria. These findings suggest that Etodolac can serve as a useful scaffold for the development of novel antibacterial agents. Future efforts should aim to optimize these derivatives and evaluate their potential in preclinical studies.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Crisis communication potential of medical institutions of Ukraine in war conditions: An empirical analysis

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## ABSTRACT

**Aim:** To analyze the Ukrainian and international experience of HF communications in ES to identify effective solutions that can be implemented in Ukraine in order to increase the readiness of HF to function in crisis conditions and improve interaction with key partners.

**Materials and Methods:** A survey of 870 managers and employees from 45 healthcare facilities in different regions of Ukraine was conducted to assess the level of management during emergencies. The author's questionnaire was developed taking into account the World Health Organization (WHO) recommendations on crisis communication. The survey was conducted in April–May 2025 remotely, in compliance with the principles of volunteerism, anonymity and bioethics (informed consent, confidentiality, prevention of harm). The instrument has been initially tested and has not been used in other studies, which confirms its novelty.

**Results:** The results of the self-assessment of internal communication effectiveness show that 51% of respondents assessed communication as "rather effective," indicating a basic level of functioning with potential for improvement. Additionally, 62,2% of respondents indicated that their facilities have internal communication protocols in place that are regularly updated, suggesting institutional maturity and a systematic approach to risk management. However, only 20% of healthcare facilities conduct crisis communication training regularly, which is a critically low figure under martial law. Most hospitals coordinate their actions with local authorities (77,8%), other healthcare facilities (60%), and emergency services and police (57,8%). Only 17,8% of healthcare facilities reported active coordination with the Ministry of Health of Ukraine, and even fewer with international humanitarian organizations (6,7%) and non-state partners (8,9%).

**Conclusions:** The communication function in Ukrainian healthcare is not sufficiently institutionalized. WHO principles on communication in emergencies are only partially integrated and implemented in a fragmented manner. Personnel training in strategic communication is irregular, and coordination across management levels is weak. The military context further complicates the situation.

**KEY WORDS:** communications, healthcare facilities, war, empirical analysis

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## INTRODUCTION

Emergency situations (ES) and martial law create difficult conditions for the effective functioning of the healthcare system, making it hard for the population to have uninterrupted access to medical services [1]. Effective communication is particularly important for coordinating actions, providing timely information, maintaining trust, and responding to events quickly [2].

During armed conflicts, healthcare facilities (HF) face challenges that go beyond usual practice, requiring the administration to make pre-developed decisions and ensure proper internal and external communication [3].

However, in crises, particularly during the war in Ukraine, there are often shortcomings in response due to inconsistency of actions, lack of resources, problems with digital technologies, and insufficient levels of ad-

aptation. This leads to delays in care, loss of trust, and increased health and life risks. In such circumstances, communication becomes a key tool for coordination and saving lives. The relevance of this study lies in the need to identify practical shortcomings in interaction with authorities, emergency services, and international partners.

The research topic aligns with national policy priorities and international health management strategies in emergencies, addressing global health safety challenges.

## AIM

The aim of the study was to analyze the Ukrainian and international experience of HF communications in ES

to identify effective solutions that can be implemented in Ukraine in order to increase the readiness of HF to function in crisis conditions and improve interaction with key partners.

## MATERIALS AND METHODS

During the study, a survey was conducted among the staff of 45 HF (a total of 870 questionnaires) across different regions of Ukraine to assess the quality of management in ES and to identify key challenges in the communication processes of medical institutions based on their practical experience

Among the 45 HF: outpatient clinics – 32 (216 questionnaires), district hospitals – 7 (237 questionnaires), regional hospitals – 3 (256 questionnaires), others – 3 (161 questionnaires).

Among the staff, the number of questionnaires: administration – 74, heads of departments – 241, specialists – 382, other employees – 173.

The author's questionnaire was developed taking into account the World Health Organization (WHO) recommendations on crisis communication [4, 5]. The survey was conducted in April–May 2025 remotely, in compliance with the principles of volunteerism, anonymity and bioethics (informed consent, confidentiality, prevention of harm). The instrument has been initially tested and has not been used in other studies, which confirms its novelty (Annex).

The questionnaire covered five thematic blocks:

1. Internal communication and coordination – assessment of the quality of communication between departments, protocols and staff awareness;
2. Communication channels – availability of basic and backup means of communication, instructions in case of failures;
3. External coordination – interaction with authorities, other HF and international structures;
4. Cooperation with international organizations – experience and barriers in interaction with humanitarian partners;
5. Feedback – the need for instructions, difficulties in communicating with staff, patients and partners.

Representatives of HF from different regions of Ukraine were involved in the survey. Criteria for inclusion in the sample:

- the respondent's membership in the administrative, managerial or coordinating personnel of the HF;
- working experience in an emergency or martial law;
- readiness to provide complete answers to all questions of the questionnaire.

In this study, the authors adhered to the ethical Principles of Medical Research Involving Humans

as set forth in the Declaration of Helsinki of the World Medical Association (VMA, 1964) and current Ukrainian regulatory documents. The study protocol was approved by the Commission on Ethics of Medical and Biological Research of the L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety of the Ministry of Health of Ukraine (State Enterprise).

## FRAMEWORK

The study was conducted within the framework of the research work "Scientific substantiation of medical criteria of chemical and food safety; toxicological and hygienic studies of chemicals, pesticides and agrochemicals, polymers, materials and products; medical and sanitary regulation of dangerous factors in the objects of human life environment" (№ 0123U102087; 2023-2027).

## RESULTS

The survey of healthcare facility (HF) employees conducted in the study allowed us to assess the actual state of internal and external communication, identify barriers, evaluate the level of staff awareness, the degree of process formalization, and the technical readiness of institutions to act in crisis conditions. Most of the responses were received from the Kyiv, Lviv, Odessa, and Chernihiv regions, which allows us to take regional features into account in further analysis.

Municipal institutions accounted for the overwhelming majority of respondents – 80%, indicating the active participation of primary and secondary links subordinate to local self-government bodies. Private institutions accounted for 11,1% of the sample, demonstrating a growing awareness of the role of crisis communication in private medicine. State institutions subordinate to central executive authorities accounted for 8,9%.

The results of self-assessment of the effectiveness of internal communication between HF units during ES are shown in Fig. 1.

51,0% of respondents rated communication in their institutions as rather effective, which indicates a basic level of functioning with the potential for improvement. The answer "partially effective" was chosen by 26,7%, indicating fragmentation or instability of communication processes. Only 15,6% considered communication to be very effective – structured and sustainable, and 6,7% of respondents considered it to be absolutely ineffective (Fig. 1).

An important component of emergency response is preparation: creating message templates, instructions

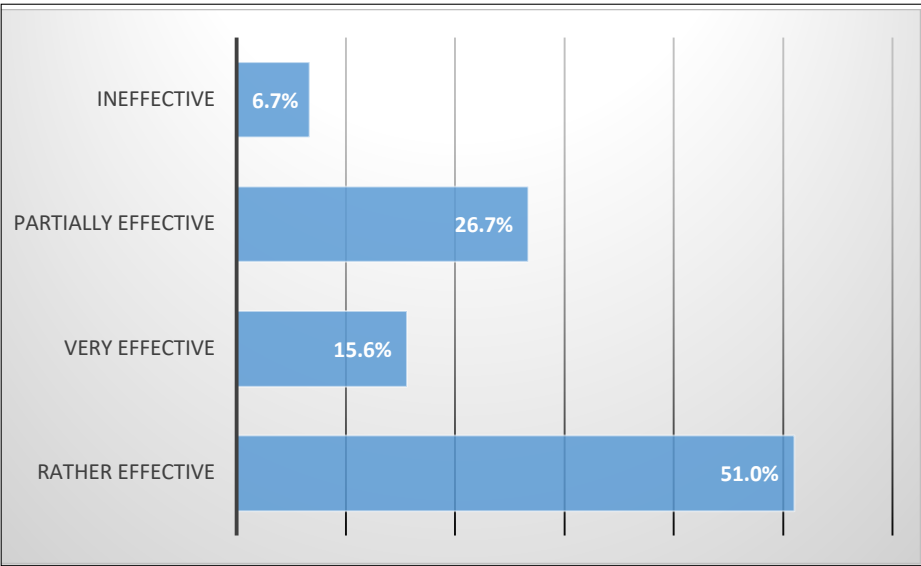


**Fig. 1.** Self-assessment of the internal communication effectiveness between HF divisions (n=870)  
*Picture taken by the authors*

for various scenarios, and training personnel. This is especially relevant for healthcare facilities that operate under constant threats of shelling, occupation, and evacuation [4]. Fig. 2 presents the results of a study on the availability of internal communication protocols in the HF in case of ES (among the 45 HF).

The 62,2% of respondents noted that their institutions have internal emergency communication protocols and are regularly updated, which indicates institutional maturity and a systematic approach to risk management; 17,8% indicated that documents need to be updated, probably due to their obsolescence or failure to take into account new threats related to the war; 11,1% reported partial or fragmentary availability of instructions, which may indicate informal approaches; 8,9% of institutions do not have any protocols, which creates serious risks for management in times of crisis (Fig. 2).

The 35,6% of respondents reported that internal communication protocols in HF were reviewed in the last 6 months; 35,5% updated their documents over the past year, which is acceptable, although in conditions of high threat dynamics, updates should be more frequent; 17,8% said that the protocols were last reviewed more than a year ago, which creates risks of outdated algorithms; 11,1% of respondents admitted that the protocols were never reviewed, which threatens the effectiveness of communication in ES.

The frequency of protocol revisions is an indicator of readiness for the challenges of military operations, emergencies, and destabilization. According to WHO recommendations, protocols should be regularly reviewed, tested, and adapted [6–10]. The results of the study show that in Ukraine, only a third of HF adhere to such cyclicity.

To improve the effectiveness of the communication

policy, it is important to institutionalize its function: develop a unified communication policy of the Ministry of Health, create permanent strategic communication units in HF, and implement performance monitoring [4,11]. To the question “Is your institution responsible for coordinating actions in crisis situations?” 66,7% of respondents answered “yes,” indicating the existence of institutional responsibility; 22,2% indicated partial availability, and 11,1% reported the absence of those responsible, creating a critical risk in crises.

Among the 45 HF, only 20% regularly conduct crisis communication training, which is a critically low figure under martial law. 46,7% perform such training sporadically, reducing its effectiveness; 22,2% did not conduct any training at all, creating serious risks in emergencies; and 11,1% found it difficult to answer, which may indicate a lack of information or formal conduct without real involvement of staff.

The survey results show that most HF coordinate their actions with local authorities (77,8%), other medical facilities (60%), as well as ES and the police (57,8%) in an emergency situation.

At the same time, only 17,8% of institutions reported active coordination with the Ministry of Health of Ukraine, and even fewer with international humanitarian organizations (6,7%) and non-state partners (8,9%).

The results of the survey on the provision of medical institutions with instructions and recommendations on crisis communication from key institutions, in particular the Ministry of Health, the National Health Service of Ukraine, the Military Administrations, etc., show a moderate shortage of regulatory and methodological support from the central executive authorities (Fig. 3).

The largest share of HF (37,8%) rated the level of provision of instructions on crisis communication as “3”

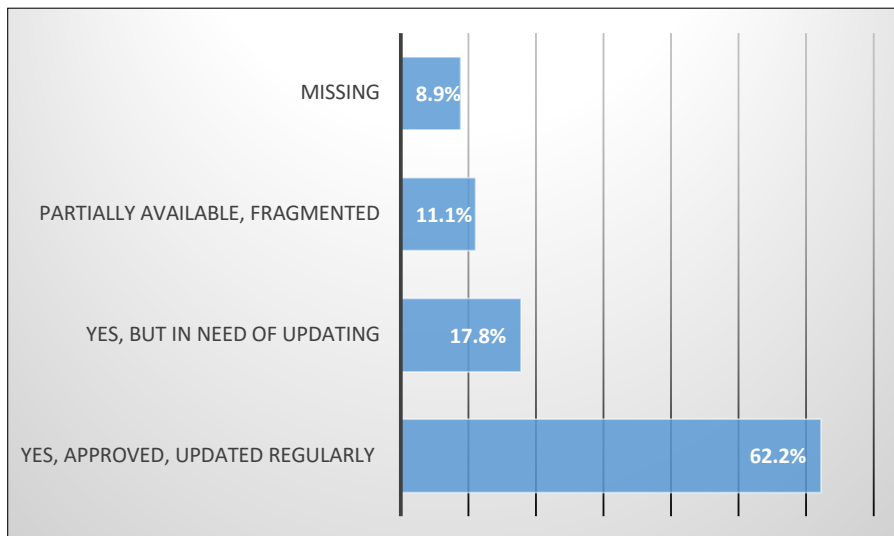


**Fig. 2.** Responses regarding the availability of approved internal emergency instructions or communication protocols (n=870)

*Picture taken by the authors*

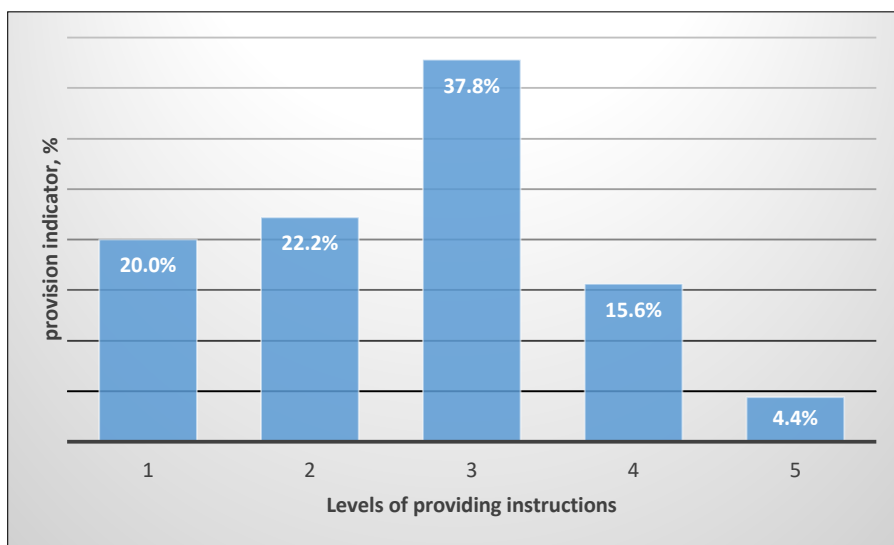


**Fig. 3.** Assessment of the availability of crisis communication instructions from the Ministry of Health, the National Health Service of Ukraine, and Military Administrations (n=45)

*Picture taken by the authors*

on a five-point scale (Annex, item 16). The 22,2% gave a rating of "2", and 15,6% – "4", that is, above average, but with comments. Only 20% consider the recommendations quite sufficient (rating "1"), and 4,4% said that there are no such instructions at all (rating "5") (Fig. 3). Approximately 80% of respondents do not consider the available materials to be fully sufficient, which indicates the lack of unified, accessible and adapted documents at different levels of the healthcare system.

In terms of coordination with external partners, only 24,4% of respondents confirmed the official appointment of responsible persons for each key partner; 28,9% reported a partial organization of coordination, 8,9% – a complete absence of such persons, and 37,8% could not answer, which indicates a lack of internal communication

and formalization of procedures. This indicates the lack of a unified approach to external interaction, which contradicts WHO recommendations [4]. The lack of a systematic practice of consolidating external coordination functions complicates communication in crises. A significant proportion of uncertainty about those responsible indicates a lack of policy and distribution of responsibilities. In order to harmonize with international standards, it is necessary to formalize external interaction, appoint those responsible for communication with key partners and conduct explanatory work among staff. This will strengthen the readiness of institutions for a crisis response and strengthen confidence in the healthcare system [4, 11].

The results of the survey on the mechanisms of regular information exchange between HF and local authorities in

times of peace and crisis revealed a contradictory situation. Only 37,8% of respondents reported the existence of an effective, formalized mechanism with a documented basis and clear channels, in line with WHO recommendations [4,11]. However, 33,3% indicated that the exchange takes place only informally, depending on personal contacts, which makes the system vulnerable when personnel change or the crisis worsens. Another 28,9% described the relationship as episodic, without a stable order of interaction, creating risks of delayed information transmission or duplication of decisions during peak periods.

In crisis conditions, flexible operational channels dominate: phone calls and meetings are used by 51,1% of respondents, indicating the predominance of informal exchange, which is effective in urgent cases but lacks documentation and continuity of information. Electronic communication (correspondence, instant messengers) is used by 28,9%, reflecting a desire for systematization. Only 11,1% of respondents use working groups or headquarters, and personal meetings account for 8,9%.

Risk communication should be integrated into the crisis management and planning system. For Ukraine, given the armed conflict and challenges, the implementation of the principles of this framework is strategically necessary.

## DISCUSSION

The problem of effective HF communications in wartime is multifaceted. Internal communications among personnel require clear organization in the face of infrastructure destruction and the loss of traditional communication channels. At the same time, there is a need to promptly inform the population about the availability of medical care, evacuation procedures, and risks during military operations [4].

The burden on the healthcare system is increasing due to the large number of casualties, both military and civilian. Currently, HF operate in emergency mode: surgical departments are overloaded, medical materials, personnel, and equipment are insufficient, and evacuation is often difficult. Therefore, it is critically important to have established internal communication and alternative communication channels, including connections with local authorities and military administrations [6-9].

In 2011, WHO created a tool for hospital administrators and emergency managers that lists priority actions for rapid response, defining internal and external communication as a key component of the response [10]. In 2017, WHO published the framework documents *Communication for Health: WHO Strategic Communication Framework* [11], *Strategic Framework for Emergency Preparation* [12], and *Public Health Emergency Risk Communication: WHO Guidelines on Emergency Risk Communication Policy and Practice (ERC)* [4], which outline basic principles, approaches, and tools for

ensuring effective preparation and response of the health system to crisis events.

The provisions of the above-mentioned WHO documents have formed the basis for numerous scientific developments and practical solutions in the field of crisis communication, confirming their effectiveness in responding to emergencies, particularly outbreaks of infectious diseases such as monkeypox, yellow fever, dengue fever, Zika virus, and Ebola disease [13-16].

In Ukraine, strategic communications received regulatory formalization after 2015 through a joint roadmap with NATO, inclusion in the *Military Doctrine* (2015) [17], the *Information Security Doctrine* (2017) [18], and the current *Information Security Strategy* (2021) [19]. These documents define strategic communications as the coordinated use of state communication tools to promote national interests, particularly in the context of hybrid threats, information attacks, and disinformation.

In 2021, the Center for Strategic Communications and Information Security was established in Ukraine under the Ministry of Culture and Information Policy, coordinating anti-crisis communications, countering information threats, and developing communication capacity. At the same time, the executive branch has specialized divisions that provide interdepartmental coordination of strategic communications policy. HF communication, based on the principles of strategic communications, should combine truthfulness, speed, adaptation to audience needs, and consistency with government and security structures. Its goal is not only to inform but also to maintain trust in the healthcare system, strengthen social cohesion, and influence population behavior (e.g., evacuation, vaccination, donation). In these circumstances, strategic communications in healthcare are becoming a key tool for countering crises, manipulation, and maintaining the legitimacy of state policy [20].

A number of communication provisions in the healthcare system under martial law contain an Emergency Response Plan approved by the order of the Ministry of Health of Ukraine dated 21.12.2023 No. 2172 "On approval of the Emergency Response Plan of the Ministry of Health of Ukraine in the field of medical protection of the population and sanitary and epidemiological welfare of the population" [21]. The key advantages of the plan include a hierarchical communication structure that ensures coherence of actions, prompt decision – making and avoidance of duplication of information, integration of HF into the national civil protection system, which contributes to coordination with other structures: authorities, law enforcement agencies, military administrations.

## PERSPECTIVES FOR FURTHER RESEARCH

Improving crisis communication will increase the readiness of medical institutions to act in ES and strengthen



public confidence in the healthcare system. Communication readiness should be a key element of national security, which requires a strategic approach, resource support and continuous improvement.

## CONCLUSIONS

The conducted study comprehensively assessed the communication readiness of Ukrainian HF to respond to emergencies under martial law. Based on the analysis

of WHO documents, Ukrainian standards, and a survey of healthcare workers, the following conclusions were drawn: the communication function in healthcare is not sufficiently institutionalized; WHO principles on emergency communication are only partially integrated into Ukrainian standards and are implemented in a fragmented manner; staff training in strategic communication is irregular; coordination across management levels is weak; and the military context further complicates the situation.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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## ANNEX

**QUESTIONNAIRE: COMMUNICATIONS OF HEALTHCARE FACILITIES IN WARTIME**

(List of questions used in this study)

**1. Region / town:** \_\_\_\_\_

**2. Form of ownership of the institution**

- ☐ State
- ☐ Municipal
- ☐ Private
- ☐ Other:

**3. What level of medical care does the institution you work in belong to?** *(You can choose several options)*

- ☐ Emergency medical care
- ☐ Primary medical care
- ☐ Secondary (specialized) medical care
- ☐ Tertiary (highly specialized, high-tech) medical care
- ☐ Palliative medical care
- ☐ Medical rehabilitation
- ☐ Other (please specify): \_\_\_\_\_

**4. What category of employees do you belong to?** *(Choose one option)*

- ☐ Administration representative
- ☐ Head of department
- ☐ Employee
- ☐ Specialist
- ☐ Responsible for communications

**5. How would you rate the effectiveness of communication between departments in your institution during emergencies?**

- ☐ Very effective
- ☐ Rather effective

☐ Partially effective

☐ Ineffective

☐ Not implemented

**6. Does your institution have approved internal instructions or protocols for communication in case of an emergency?**

☐ Yes, approved and updated regularly

☐ Yes, but need updating

☐ Partially available, fragmented

☐ Not available

**7. When were the internal communication protocols last reviewed or updated**

☐ Within the last 6 months

☐ Within the last year

☐ More than a year ago

☐ Never reviewed

**8. Does your institution have designated persons responsible for coordinating actions in crisis situations?**

☐ Yes, responsible persons are officially designated

☐ Partially, but not all roles are defined

☐ No

**9. What departments or categories of personnel are involved in crisis management and communication? \_\_\_\_\_**

**10. Has training been conducted or training of personnel on actions in case of emergency situations (military actions, evacuation, communication damage)?**

☐ Yes, regularly

☐ Yes, occasionally

☐ No, not conducted

☐ I don't know

**11. How do you assess the awareness of personnel with internal communication procedures in crisis situations?**

☐ High - personnel are well-oriented

☐ Medium - partially oriented

☐ Low - many do not know how to act

☐ Hard to say

**12. Is there an internal staff notification system (messenger groups, mailing lists, internal network, etc.)?**

☐ Yes, reliable and operational

☐ Yes, but needs improvement

☐ Partially available

☐ Not available

**13. What are the main difficulties you have observed in organizing internal communication during crisis situations? \_\_\_\_\_**

**14. With which external structures does your institution coordinate during crisis situations? (multiple options are possible)**

☐ Local authorities

☐ Other healthcare facilities

☐ Emergency services (emergency services, police )

☐ Ministry of Health

☐ International humanitarian organizations

☐ Non-governmental partners (foundations, volunteers)

☐ Other (please specify): \_\_\_\_\_

**15. How quickly do external partners or authorities respond to your facility's requests in emergency situations?**

☐ Very quickly (up to 1 hour)

☐ Moderately quickly (within 24 hours)

☐ With delays (1–3 days)

☐ Slow or not at all

**16. How much do you think there are enough instructions/recommendations on crisis communication from the Ministry of Health, the National Health Service, military administrations, etc.? (where 1 is quite enough, 5 is completely absent)**

| 1                        | 2                        | 3                        | 4                        | 5                        |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

# Circulating T-cell immunoglobulin and mucin domain-3 (TIM-3) levels and (TIM-3) gene polymorphism in systemic lupus erythematosus patients

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## ABSTRACT

**Aim:** To determine the soluble TIM-3 levels and how a variation in the -574 locus gene in the TIM-3 gene's promotor region contributes to SLE.

**Materials and Methods:** 180 participants participated in the current study: 90 SLE patients and 90 clearly healthy controls (HCs) from October 2024 to February 2025. These subjects selected from Medical City, Baghdad, Iraq. Participant's genomic DNA was collected from blood sample. Every sample was diagnosed with SLE. The enzyme-linked immunosorbent assay (ELISA) was utilized to determine the quantity of soluble TIM-3 (sTIM-3) in the serum of SLE and HC patients. The TIM-3 gene's rs10515746 gene fragment, which corresponds to the -574 locus, was amplified and genotyped using the allele-specific polymerase chain reaction (AS-PCR).

**Results:** Patients had significantly higher TIM-3 levels (median = 396.5 ng/ml, range = 198-698 ng/ml), than controls (median of 1618 ng/ml and a range of 1306-1999 ng/ml). The GT-TT genotypes were more prevalent in patients than controls (98.89% vs. 93.33%), with a significant difference (OR = 6.36, 95%CI = 0.75-53.92). At the allelic level, compared to controls, patients' frequencies of the mutant allele (T) were noticeably higher (90.56% vs. 79.44%) (OR = 2.15, 95%CI = 1.15-4.03, p = 0.016).

**Conclusions:** Individuals with SLE have significantly higher levels of soluble TIM-3, which suggests that the protein has a role in the pathophysiology of the condition and that the T allele of rs10515746 is a risk factor for SLE.

**KEY WORDS:** systemic lupus erythematosus, T-cell immunoglobulin and mucin domain-3, Enzyme-Linked-Immunosorbent Assay, polymorphism

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## INTRODUCTION

Immune checkpoint receptors for co-stimulatory or co-inhibitory chemicals are crucial components of the immune system. It appears that the ligand galectin-9 (Gal-9) and T-cell immunoglobulin and mucin-domain-containing molecule 3 (TIM-3) are implicated in the pathophysiology of autoimmune disorders as negative checkpoint receptors [1]. A single transmembrane domain, an intracellular domain, and an N-terminal variable immunoglobulin (IgV)-like and mucin-like domain in the extracellular region are all present in TIM-family proteins. Transmembrane glycoprotein TIM-3 is primarily generated by Th1 and Th17 cells but not Th2. TIM-3 is vital for immunological tolerance since the TIM-3/Gal-9 pathway promotes apoptosis, which

governs Th1 immunity, and preventing this connection makes autoimmunity worse [2, 3]. Additionally, immune cells including dendritic cells, macrophages, and natural killer cells express TIM-3 on their surface as well, when these cells overexpress TIM-3, it impairs the immunological function of the aforementioned immunocytes [4]. There have been reports linking TIM-3 polymorphism and malfunction to a number of diseases, including cancer, autoimmune illness, infections, and allergies [5]. The autoimmune condition known as systemic lupus erythematosus (SLE) is typified by multisystem inflammation and antibodies to nuclear and cytoplasmic antigens. The prevalence ranges from 20 to 50 per 100,000 women in the US. Numerous organs impacted by the illness, and the musculoskeletal,



cutaneous, renal, central nervous, hematologic, cardiac, and gastrointestinal systems are among the clinical manifestations [6]. Systemic lupus erythematosus (SLE), it is thought that autoreactive T-cells are triggered by impaired self-tolerance, and that autoreactive B-cells are then encouraged to create auto-antibodies. The TIM-3 genes' polymorphism may affect the immunological response [7]. Recent achievement in cancer treatment using immune checkpoint antagonists has shown that the importance of immune co-inhibitory or co-inhibitory systems in anti-tumor immune responses; however, Similar to autoimmune illnesses, immune-intercede adverse events (irAEs) are commonly caused by inhibiting inhibitory immunological checkpoint receptors [8]. Autoimmune diseases, nevertheless, restricting immune responses by either blocking co-stimulatory signals or enhancing co-inhibitory signals is a hopeful implementation. paradigm. [9]. Creation of immunological complexes due to the generation of antinuclear antibodies (ANA) [10] encourage plasmacytoid dendritic cells to express interferon alpha (IFN- $\alpha$ ) [11]. Type I interferon (IFN) system activation, this emerges as IFN- $\alpha$  or IFN-inducible genes (IFN signatures) in the bloodstream, and SLE-associated autoantibodies are linked to the etiology of SLE and disease activity in SLE patients [11]. The TIM-3 ligand Gal-9 undergoes elevation in SLE patients, which coincides with the proliferation of interferon-signature genes. These data suggest that Th1 and Th17 immune responses significantly influenced by TIM-3/Gal-9 pathway, and that autoimmune disorders worsen when TIM-3/Gal-9 connection is blocked [12-14]. A previous study looked at the connection between the expression of TIM-3 on SLE peripheral blood mononuclear cells (PBMCs) patients and the activity of SLE illness [15]. A-disintegrin-like and metalloproteinase with thrombospondin type 1 motifs (ADAM) 10 or ADAM17 separates the TIM-3 stalk region, TIM-3 can be released from the cell surface, resulting in a soluble form of TIM-3 (sTIM-3), which is higher in autoimmune disease patients' serum [16-18]. Human TIM members are encoded by three genes (HAVCR1, HAVCR2, and TIMD4, which respectively encode TIM1, TIM3, and TIM4). The TIM-3 polymorphism simulates the process that gives rise to specific immunological diseases through modifications to the interconnection between TIM-3 and its ligand [19]. Human HAVCR2 variants in both the coding and non-coding parts have been interrelation with autoimmune and allergy disorders. The genome harbors several variations, notably +4259T/G (rs1036199) in the coding area and -1516G/T (rs10053538) and -574G/T (rs10515746) in the promoter site correlated with gastrointestinal cancer and rhinitis [20-21], pancreatic cancer and renal cell

carcinoma. TIM-3 protein expression was detected by immunohistochemistry in women with breast cancer, according to another study. It eventually emerged that rs10053538 had a considerably greater likelihood of breast cancer than the wild-type genotype [22]. For another immune checkpoint such as Programmed cell death-1 (PCD-1), a specific type of SNP in the PD-1 promoter region that corresponds to rs38084323 has been linked to the *Hepatitis B virus* (HBV), according to one investigation [23]. In the present study, we assessed the levels of circulating sTIM-3 in both healthy control volunteers and SLE patients. Also looked into how a single nucleotide variation in the -574-locus gene in the promoter region of the TIM-3 gene affects SLE and controls.

## AIM

In the present study, we assessed the levels of circulating sTIM-3 in both healthy control volunteers and SLE patients. We also looked into how a single nucleotide variation in the -574 locus gene in the promoter region of the TIM-3 gene affects SLE and controls.

## MATERIALS AND METHODS

### THE STUDY POPULATION

Hundred and eighty subjects enrolled in this case-control study, including 90 SLE patients and 90 healthy controls (HCs). The study carried out at Autoimmune Disease Center in Medical City-Baghdad-Iraq during period from October 2024 to February 2025. All groups gave their documented consent prior to participating in the study. Systemic lupus erythematosus was diagnosed clinically and confirm by Indirect immunofluorescence on Hep-2 cells remain the method of choice for detection of anti-nuclear antibodies. The ANA test has a very high (almost 100%) sensitivity for SLE but a low specificity because ANA can be seen in a variety of clinical disorders and even in healthy individuals. Anti-dsDNA antibodies seen in 40–80% of SLE patients and are very specific for the disease. The Farr assay is the preferred technique for anti-ds DNA; however, its usefulness is limited due to the requirement of employing radioactive material. The research's ethical permission was obtained by the University of Diyala's College of Medicine's scientific and ethical committees.

### DATA AND SAMPLE COLLECTION

All participants' demographic information, such as age, sex, and body mass index (BMI), residency was gath-

ered through in-person interviews using a pre-made questionnaire. Extracted two halves of five milliliters of venous blood. Serum was separated in a gel tube, with the first part (3 ml) used for the Enzyme-Linked Immunosorbent Assay to detect sTIM-3. Two milliliters of the second ingredient were put in an EDTA tube and stored at -80°C until the DNA extraction process for -574 locus of promoter area of TIM-3 gene amplification was required.

**Serum preparation and storage:** To ensure full coagulation, for ten minutes, the tubes centrifuged at 1900-x g at 4°C after kept for 20 minutes at ambient temperature (15–25°C), finally, the supernatant was carefully transferred to a fresh tube in order to identify sTIM-3.

## T-CELL IMMUNOGLOBULIN AND MUCIN DOMAIN-3 (TIM-3) DETERMINATION USING ELISA

Sandwich enzyme-linked immune-sorbent assay technology served as the foundation for this kit. The 96-well plate was pre-coated with an anti-HAVCR2 antibody. The detecting antibody was the biotin-conjugated anti-HAVCR2 antibody. The wells were then filled with the pilot samples and standards. Wash buffer was applied. To remove the unbound conjugates subordinate incubation. After that, a biotinylated detection antibody was added to engage with the coated antibody's HAVCR2 conjugate. HRP-Streptavidin was gathered after unbound conjugates cleaned off. TMB substrates introduced to observe the HRP enzymatic response subordinate a third washing. HRP stimulated the production of a blue product from TMB, which became yellow when a stop solution was added. Measure the O.D. absorbance at 450 nm with a microplate reader. A standard curve was created to determine the sample's HAVCR2 content. The OD450 value is proportionate to the target substance's concentration.

## ISOLATION DNA AND ALLELE-SPECIFIC - POLYMERASE CHAIN REACTION (AR-PCR)

A commercially available Gsync DNA extraction kit (Cat. No. GS100, Geneaid, Taiwan) was used to extract DNA from the genome. It adheres to the procedure that was recorded at the time of its development. At 260 nm/280 nm (A260/A280), the amount of DNA extracted from the samples was determined using a Biospec Nano spectrophotometer. Using allele-specific PCR (AS-PCR), the -574 locus (rs10515746) was investigated. Three distinct primers were employed to accomplish this.

Forward 1 (F1)

5'-GGCTTATGCTGGGAGTTGCT-3'

Forward 2 (F2)

5'-GGCTTATGCTGGGAGTTGCG-3'

Reverse for the F1 and F2 (R) series.

5'-GGT GTCTGATTGCCAGTGATTC-3' [24].

The T alleles amplified using the F1 and R primers, and the G alleles amplified using the F2 and R primers. All of the expatiate fragments have a total length of 539 bp, and the samples were generally not up to par for AS-PCR using F1/R and F2/R. Overall, the segments that were magnified were 539 bp in length, each sample had to be optimized for AS-PCR by following several steps. Consequently, the 25 µl final volume produced the following nucleotide mixture on reactivity expansion: 12.5 µl of master mix (Promega, USA), 2 µl of primers (1.0 µl from the forward side and 1.0 from the reverse side), and 1.0 µl of DNA template which were all accomplished using water that has been double distilled. The target sequence amplified using the touchdown PCR method, which involved applying heat. A thermal cycler was utilized for this purpose under the following circumstances: 5 minutes at 94°C, 30 seconds at 94°C, 30 seconds at 60°C for annealing, 30 seconds at 72°C for extension, 30 seconds at 72°C for cycling, and 7 minutes at 72°C for final extension. In order to identify the amplified product for gel electrophoresis, it subjected to 1.5 g agarose in 100 ml 10x Tris-borate-EDTA (TBE) (Promega, USA) and developed with ethidium bromide.

## STATISTICAL ANALYSIS

The analyses were complete utilizing the statistical program SPSS 25.0 (SPSS, Chicago). The Student t-test was used for analysis, and the continuous data's mean and standard deviation were shown. The Chi-square test applied to examine categorical variables, which reported as numbers and percentages. Using binary logistic regression, the association between SLE and rs10515746 in the promoter region of the TIM-3 gene was evaluated. Based on this test, the odds ratio (OR) and related 95% CI were calculated. A p-value of less than 0.05 indicated that a change was considered statistically significant.

## RESULTS

### DEMOGRAPHIC FEATURES OF THE PATIENTS

The relationship between demographic characteristics and SLE is shown in Table 1. The mean age of 51.12±10.59 years for the patients was considerably lower than the mean age of 55.26±15.85 years for the controls. While controls had a more balanced male-to-female ratio (58.89% male, 41.11% female), SLE patients

**Table 1.** Features of the research population's demographics

| Variables                                  | Patients (n=50)           | Controls (n=50)           | p-value |
|--------------------------------------------|---------------------------|---------------------------|---------|
| Age, years<br>Mean±SD<br>Range             | 51.12±10.59<br>16.0-82    | 55.26±15.85<br>9.0-85     | 0.041   |
| Gender<br>Male<br>Female                   | 16(17.78%)<br>74(82.22%)  | 37(41.11%)<br>53(58.89%)  | 0.001   |
| BMI, kg/m <sup>2</sup><br>Mean±SD<br>Range | 25.03±3.81<br>18.78-34.37 | 25.13±3.76<br>20.34-31.46 | 0.819   |
| Residence<br>Rural<br>Urban                | 27(30%)<br>61(67.78%)     | 29(32.22%)<br>63(70%)     | 0.747   |

SD: standard deviation, BMI: body mass index

Source: Own materials

**Table 2.** Median level of sPD-1 in patients with SLE and controls

| Variables                                    | Patients (n=90)                      | Controls (n=90)                 | p-value |
|----------------------------------------------|--------------------------------------|---------------------------------|---------|
| sPD-1, ng/ml<br>Mean ± SD<br>Median<br>Range | 1630.5±175.07<br>1618.0<br>1306-1999 | 396.5±96.05<br>396.5<br>198-698 | <0.001  |

Source: Own materials

**Table 3.** The prevalence of multiple genotypes and allele of rs10515746 polymorphism in SLE patients and control

| Rs10515746 Polymorphism | Patients (n=90) | Controls (n=90) | P-value | OR(95%CI)        |
|-------------------------|-----------------|-----------------|---------|------------------|
| Genotypes               |                 |                 |         |                  |
| GG                      | 1(1.11%)        | 6(6.67%)        | 0.256   | 1.0              |
| GT                      | 15(16.67%)      | 25(27.78%)      | 0.065   | 7.19(0.88-64.25) |
| TT                      | 74(82.22%)      | 59(65.56%)      | 0.032   | 3.6(0.39-32.87)  |
| HWE                     | 0.808           | 0.654           |         |                  |
| Dominant model          |                 |                 |         |                  |
| GG+GT                   | 31(34.44%)      | 16(17.78%)      | 0.012   | 2.43(1.22-4.86)  |
| TT                      | 59(65.56%)      | 74(82.22%)      |         |                  |
| Recessive model         |                 |                 |         |                  |
| GT+TT                   | 89(98.89%)      | 84(93.33)       | 0.09    | 6.36(0.75-53.92) |
| GG                      | 1(1.11%)        | 6(6.67%)        |         | 1.0              |
| Alleles                 |                 |                 |         |                  |
| T                       | 163(90.56%)     | 143(79.44%)     | 0.016   | 1.0              |
| G                       | 37(20.56%)      | 17(9.44%)       |         | 2.15(1.15-4.04)  |

Source: Own materials

are overwhelmingly male 82.22%. At  $p = 0.001$ , this difference is extremely significant. There is no discernible difference between the mean BMI of SLE patients ( $25.03 \pm 3.81$  kg/m<sup>2</sup>) and controls ( $25.13 \pm 3.76$  kg/m<sup>2</sup>) ( $p = 0.819$ ).

### STIM-3 SERUM LEVEL IN SLE PATIENTS

It was discovered that the data pertaining to the blood levels of sTIM-3 was not regularly distributed. As a result, the median and range of these data were used, and the non-parametric Mann Whitney U test was used

for analysis. In patients with SLE, Patients' TIM-3 was significantly greater than controls' (median = 396.5 ng/ml, range = 198-698 ng/ml) *versus* patients (median = 1618 ng/ml, range = 1306-1999 ng/ml) (Table 2).

### MOLECULAR ASSAY

This study examined the relationship between SLE infection and the SNP rs10515746 in the TIM-3 gene's promoter region. PCR is used for allele-specific genotyping. Figure 1 displays the results of the PCR. GG, GT, and TT were the three genotypes in which the SNP was



**Fig. 1.** Genotype patterns of rs10515746 in the promoter region of TIM-3 gene allele-PCR conceived under UV transilluminator. M: DNA marker, lanes 1, 3: TT genotype, lanes 2 and 6: GT genotype, lanes 5 and 7; GG genotype

Source: Own materials

found. These genotypes' distribution in patients and controls was in good agreement with Hardy-Weinberg equilibrium.

## ASSOCIATION OF RS10515746 WITH SLE INFECTION

The homozygous genotype (TT) was more frequent in patients with SLE (82.22%) than controls (65.56%) with a significant difference ( $p=0.032$ ). Although the heterozygous genotype (GT) was less common in patients than controls (16.76% vs. 27.78%), the difference was not significant. The GT-TT genotypes were more prevalent in patients than controls (98.89% vs. 93.33%), with a significant difference ( $OR=6.36$ ,  $95\%CI=0.75-53.92$ ), suggesting that this polymorphism has recessive inheritance. At the allelic level, patients had a significantly higher frequency of the mutant allele (T) than controls (90.56% vs. 79.4%) ( $OR=2.15$ ,  $95\%CI=1.15-4.03$ ,  $p=0.016$ ) (Table 3).

## DISCUSSION

This study revealed SLE patients were significantly younger than the controls ( $51.12\pm10.59$  years), with an average age of  $55.26\pm15.85$  years. Females make up 82.22% of SLE patients, and this difference is statistically significant  $p=0.001$ . Comparable with past report was found Men's and women's anticipated rate of incidence are 0.9 (95% CI 0.7 to 1.1) and 1.9 (95% CI 1.7 to 2.2) per 100,000 person-years, respectively. In the male population, the age-specific incidence rate of SLE peaks at 2.2 (95% CI 1.0 to 3.4) per 100,000 person-years between ages of 65 and 70. The incidence peaks at 3.6 (95% CI 2.9 to 4.3) cases per 100,000 person-years

in women aged 20 to 25 [25]. Critically differentiated T-lymphocytes' surface expression of TIM-3 has related to the pathophysiology of autoimmune disorders driven by Th1 by negatively controlling T-cell response. Autoimmune complex deposition and autoantibody synthesis are hallmarks of SLE, a Th1-dependent human autoimmune illness [26-27]. In this work, we showed that SLE patients had considerably higher serum sTIM-3 levels than HCs. It has proposed that the TIM-3/Gal-9 co-inhibitory pathway is substantial autoimmune modulator [28]. SLE disease activity correlates with T-cell TIM-3 expression; furthermore, it has shown that SLE patients have a larger CD3+CD4+TIM3+ T-cell subset than HCs. Patients with SLE also showed high serum levels of Gal-9 [29]. Patients with SLE also showed elevated serum levels of Gal-9 [30-31]. It can conclude that the stimulation of the TIM-3/Gal-9 pathway functions as an anti-immune mediator in patients with SLE. By lowering the levels of anti-double stranded DNA antibodies, intraperitoneal Gal-9 treatment improved the proteinuria and arthritis in lupus-prone mice [32]. On the other hand, this route can modulated by the sTIM-3 that released from TIM-3 conveyed on the immune cell surface [33]. T-cell exhaustion may caused by the TIM-3/Gal-9 interconnection; however, sTIM-3 appears to have different effects against this feedback process. It should note that a soluble form of a receptor might not necessarily cause receptor preventing; additional research is required to identify the origin of serum sTIM-3 and its function in the pathophysiology of SLE. The current study accordance to previous study examined the soluble TIM-3 levels were considerably greater in 65 Japanese subjects with SLE. The study's clinical data and medical histories were gathered by looking through the patients' electronic medical records compared to those

in controls (1363 pg/mL; IQR, 1097–1673;  $p=0.0015$ ) and SLE patients (2123 pg/mL (IQR, 229–7235) [34]. In contrast to recent study, Jin et al. revealed that there was not any interesting association between SLEDAI scores and sTIM-3 levels that elevated serum IL-17 levels linked to elevated serum sTIM-3 scales in SLE subjects, and that serum sTIM-3 scales were discernable lower in SLE patients with lupus nephritis [35]. This study found the predominance of homozygous genotype (TT) was significantly greater in SLE patients versus to controls. Patients were less likely than controls to have the heterozygous (GT) genotype, but the variation was not statistically considerable. These results indicated a relationship between Iraqi SLE-patients and the -574 locus polymorphism. Numerous investigations have shown that the aberrant behavior and loss of function of CD8+ T-cells in relation to SLE caused by the immunoglobulin-and mucin domain-containing molecule-3 (TIM-3). Similar findings from our work Cai and associates were showed the elevated expression of TIM-3 ligand (galactin-9) mRNA in SLE patients suggested that TIM proteins may have a role in the pathophysiology of SLE [36]. While, Li et al reported the systemic lupus erythematosus in a Chinese population is not linked to polymorphisms of the TIM-1 and TIM-3 genes [37]. According to Guo et al., Chinese people are susceptible to renal cell carcinoma (RCC) due to TIM-3 polymorphisms [38]. TIM-3's binding to its ligand, galectin-9, can make the Tim-3 protein more expressed when regulatory

T-cells (Treg) are stimulated. This causes helper T-cells to die, and a decrease in TIM-3 linked to a rise in Th2 cells and a decrease in Th1 cells [39]. Investigations into the association of TIM3 SNPs with several autoimmune diseases, including type I diabetes and Ankylosing Spondylitis (AS), have been conducted [40]. Unfortunately, no prior research has examined the relationship between the genes of the -574 locus and SLE infection. Nonetheless, it may be inferred that the presence of thymine rather than guanine in the -574 locus of the TIM-3 gene's promoter area may enhance the transcription of this gene, possibly as a result of an increase in RNA polymerase to the promoter region. Overexpression of TIM-3 transcriptions can hinder T-cell function and increase the likelihood that SLE will cause the illness.

## CONCLUSIONS

Our findings demonstrate that sTIM-3 levels correlated with SLE disease activity and that circulating levels of sTIM-3 are higher in SLE patients than in healthy persons. According to these results, the T allele of rs10515746 is thought to be a risk factor for SLE infection. The association between the GT+TT genotype and patients with this genotype may benefit from closer monitoring and more aggressive therapies to achieve better disease control. Screening for the rs10515746 polymorphism could be useful in identifying disease activity, enabling personalized treatment approaches.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Biochemical changes in oral fluid in individuals with generalized periodontitis living in different environmental conditions

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## ABSTRACT

**Aim:** To study the biochemical changes in the oral fluid of individuals with generalized periodontitis living in different environmental conditions.

**Materials and Methods:** In order to investigate biochemical changes in the oral liquid of people diagnosed with generalized periodontitis associated with the impact of Cd, we examined 113 people aged from 20 to 59 years. Generalized periodontitis was detected in 83 (73,45%) patients: initial – 19 (22,9%) cases, stage I – 32 (38,55%), and stage II – 32 (38,55%). All the examined individuals were further divided into two groups. The first group (n=52) consisted of people from certain areas in the Ivano-Frankivsk region of Ukraine with elevated Cd content. The second group (n=61) included individuals who lived in areas of the Ivano-Frankivsk region where the Cd content in the environment (soil) did not exceed the maximum permissible concentration. We estimated the content of calcium (Ca), inorganic phosphorus (Pi), and the hydroxyproline level in the oral liquid of the enrolled subjects.

**Results:** A decrease in Ca content in the oral liquid of patients with generalized periodontitis was found, which was accompanied by an increase in Pi and hydroxyproline, observed in both study groups as compared to controls. These changes were associated with the progression of generalized periodontitis severity.

**Conclusions:** The obtained results indicate a certain similarity in the biochemical changes of oral fluid among individuals residing in areas with different environmental conditions, thus requiring further research.

**KEY WORDS:** oral liquid, biochemical studies, cadmium

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## INTRODUCTION

Many studies have proven that environmental conditions have a significant impact on human health. Numerous studies demonstrate that the prevalence of somatic and dental diseases in the population is on the rise in ecologically polluted areas [1–11]. Particularly, they indicate that residents of environmentally polluted regions belong to risk groups and have a high incidence of diseases of the digestive, endocrine, cardiovascular, and hematopoietic systems [10–13].

An environmental increase in toxic compounds, heavy metals in particular, leads to deterioration in the health of adults and children [1, 3, 4, 9, 12–16]. We focused on studying cadmium (Cd) regarding its impact on the periodontal tissues. Cd has the ability to accumulate in the human body, creating toxic concentrations and causing long-term negative health consequences. Moreover, Cd intoxication causes various health effects, leading to the development of malignant tumours, cardiovascular diseases, and diabetes mellitus [12–14]. Furthermore,

not only employees of enterprises that have direct contact with xenobiotics are in the risk group, but also other people living in areas contaminated with heavy metals. It was found that the content of Cd exceeded the maximum permissible concentration by 1.2 times in some areas of the Ivano-Frankivsk region of Ukraine (in particular, the village of Horokholyno, Bohorodchany district) [4].

Interest in Cd is due to its toxic properties and ability to interact with vital elements such as calcium (Ca) and zinc, and also to influence phosphate and Ca metabolism. Recent epidemiological studies show that dental [1–3, 5, 7, 9, 14, 17–19] and somatic diseases [10–13] significantly increased in areas contaminated with salts of heavy metals.

Although the role of toxic compounds is of great importance for many countries nowadays, it is especially vital for Ukraine because the ecological situation in the country during the ongoing war has become extremely difficult due to air, water, and soil pollution and the

damage done to industrial facilities. The population and ecosystems of Ukraine feel the terrible consequences of missile attacks on factories and thermal power plants.

## AIM

The aim of our research was to study the biochemical changes in oral fluid of individuals with generalized periodontitis living in different environmental conditions.

## MATERIALS AND METHODS

In order to study the effect of Cd on biochemical changes that occur in the oral liquid in generalized periodontitis, we examined 113 people aged from 20 to 59 years. Among them were 54 (47,79%) men and 59 (52,21%) women. Generalized periodontitis was detected in 83 (73,45%) patients: initial – 19 (22,9%) cases, stage I – 32 (38,55%), and stage II – 32 (38,55%). Consequently, 30 (26,55%) people were free from generalized periodontitis (relatively healthy controls).

All the examined individuals were divided into two groups. The first group (n=52) consisted of people from areas with elevated Cd content (the village of Horokholy-no, Bohorodchany district, and the city of Ivano-Frankivsk, Ukraine), where a local increase in Cd levels was detected (conditionally contaminated areas): healthy controls – 15 cases; initial periodontitis – 7; stage I – 15; and stage II – 15.

The second group (n=61) consisted of individuals who lived in areas where the Cd content in the environment (soil) did not exceed the maximum permissible concentration (Bohorodchany and Ivano-Frankivsk districts of the Ivano-Frankivsk region, Ukraine) (conditionally clean areas): healthy controls – 15 cases; initial periodontitis – 12; stage I – 17; and stage II – 17.

The clinical examination was conducted at the Department of Dentistry of the Educational and Scientific Institute of Postgraduate Education, Ivano-Frankivsk National Medical University (head of the department: Prof. Ivan V. Paliichuk). The investigation also included a complete clinical examination and biochemical analysis of oral fluid.

Biochemical analyses of oral fluid were conducted spectrophotometrically at the G. Babenko Bioelementology Center of Ivano-Frankivsk National Medical University (head: Prof. Hanna M. Ersteniuk). The key indicators that characterize the depth of destructive processes in periodontal tissues and phosphate-Ca metabolism were studied, in particular: the content of Ca, inorganic phosphorus (Pi), as well as the determination of hydroxyproline, which reflects the state of collagen fibres.

The determination of Ca concentration in oral fluid is based on the ability of Ca to form a blue complex with Arsenazo III dye in a neutral medium, which has an absorption maximum at 590–650 nm.

The concentration of Pi was determined using a standard reagent kit («Simko Ltd», Ukraine). The principle of the method is based on the interaction of Pi with ammonium molybdate to form a phosphorus–molybdate complex, which has an absorption maximum at a wavelength of 340 nm, the value of which is proportional to the concentration of Pi.

The determination of hydroxyproline was conducted in order to assess the state of collagen fibres and bone tissue. The principle of the method is based on the oxidation of hydroxyproline by hydrogen peroxide to pyrrole in an alkaline solution in the presence of copper ions, removal of excess hydrogen peroxide, and the formation of a pink colour with paradimethylaminobenzaldehyde in an acidic medium. The intensity of the colour is proportional to the concentration of hydroxyproline.

The data were analysed using Statistica v. 14.0 (TIBCO Software Inc., USA) and EZR v. 1.68 software. Quantitative variables were presented as mean  $\pm$  standard error of the mean. We used the ANOVA test (with Tukey's HSD post hoc test) for independent samples (to compare four independent groups of subjects). An adjusted p-value <0,05 was considered statistically significant.

## ETHICS

All studies were conducted in accordance with the requirements of the Declaration of Helsinki, the Convention on Human Rights and Biomedicine, and Ukrainian regulatory documents. The research protocols were approved by the Bioethics Commission of Ivano-Frankivsk National Medical University.

## FRAMEWORK

The study was carried out within the framework of two researches: the research «Clinical effectiveness of complex treatment of diseases of hard dental tissues and periodontium in the population from ecologically bad regions», which was financed by the state budget of Ukraine (state registration number 0118U004144, implementation period 2018-2020); and the research «Complex morpho-functional study and substantiation of modern technologies for the treatment and prevention of dental diseases», which is financed from the state budget of Ukraine (state registration number: 0121U109242, implementation period: 2021 – 2023).

## RESULTS

The obtained data demonstrated a decrease in Ca content in the oral fluid of individuals in both research groups who were diagnosed with different stages of generalized periodontitis, which was also accompanied by a statistically significant increase in Pi and hydroxyproline levels (Table 1).

**Table 1.** The biochemical indicators of oral liquid in the healthy controls and patients with generalized periodontitis from the studied ecological regions,  $M \pm m$ 

| Investigated indicator | Ecological region | Healthy (controls)  | Patients              |                          |                           | P                                                                                                                          |
|------------------------|-------------------|---------------------|-----------------------|--------------------------|---------------------------|----------------------------------------------------------------------------------------------------------------------------|
|                        |                   |                     | Initial periodontitis | I stage of periodontitis | II stage of periodontitis |                                                                                                                            |
| Ca, mmol/l             | Group I (N=52)    | 1.68 ± 0.06<br>n=15 | 1.39 ± 0.05<br>n=7    | 1.18 ± 0.02<br>n=15      | 1.05 ± 0.02<br>n=15       | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} = 0.025$<br>$p_{2-4} < 0.001$                      |
|                        | Group II (N=61)   | 1.63 ± 0.06<br>n=15 | 1.34 ± 0.03<br>n=12   | 1.19 ± 0.02<br>n=17      | 1.0 ± 0.02<br>n=17        | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} = 0.039$<br>$p_{2-4} < 0.001$<br>$p_{3-4} = 0.003$ |
| $P_i$ , mmol/l         | Group I (N=52)    | 5.97 ± 0.03<br>n=15 | 6.59 ± 0.09<br>n=7    | 6.86 ± 0.04<br>n=15      | 7.06 ± 0.03<br>n=15       | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} = 0.003$<br>$p_{2-4} < 0.001$<br>$p_{3-4} = 0.004$ |
|                        | Group II (N=61)   | 5.97 ± 0.04<br>n=15 | 6.48 ± 0.05<br>n=12   | 6.76 ± 0.04<br>n=17      | 7.02 ± 0.03<br>n=17       | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} < 0.001$<br>$p_{2-4} < 0.001$<br>$p_{3-4} < 0.001$ |
| Hydroxyproline, mmol/l | Group I (N=52)    | 4.55 ± 0.23<br>n=15 | 11.68 ± 0.15<br>n=7   | 14.97 ± 0.17<br>n=15     | 24.94 ± 0.32<br>n=15      | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} < 0.001$<br>$p_{2-4} < 0.001$<br>$p_{3-4} < 0.001$ |
|                        | Group II (N=61)   | 4.25 ± 0.27<br>n=15 | 11.46 ± 0.11<br>n=12  | 14.73 ± 0.19<br>n=17     | 24.20 ± 0.34<br>n=17      | $p_{1-2} < 0.001$<br>$p_{1-3} < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{2-3} < 0.001$<br>$p_{2-4} < 0.001$<br>$p_{3-4} < 0.001$ |

Notes:  $p_{1-2}$  – significance of difference between initial periodontitis and controls;  $p_{1-3}$  – significance of difference between stage I periodontitis and controls;  $p_{1-4}$  – significance of difference between stage II periodontitis and controls;  $p_{2-3}$  – significance of difference between initial and stage I periodontitis;  $p_{2-4}$  – significance of difference between initial and stage II periodontitis;  $p_{3-4}$  – significance of difference between stages I and II periodontitis

Source: compiled by the authors of this study

According to the study results, there was a 17,4% decrease in Ca content in the oral fluid of patients with the initial stage of generalized periodontitis compared to the control group in both investigated regions. In the first group, with generalized periodontitis of stage I, there was a 30% decrease in Ca level, and in the second group – a 27% decrease, as compared to the control group. In individuals with generalized periodontitis of stage II, these indicators were 37,5% and 38,6%, respectively (Table 1). Since Ca metabolism is closely related to phosphate metabolism, we also examined the level of  $P_i$  in the oral fluid of the examined individuals. The content of  $P_i$  increased by 10,4% in

those with initial generalized periodontitis within the first group and by 8,6% within the second group compared to the control group (Table 1).

Considering generalized periodontitis of the first stage, the  $P_i$  level increased by 14,7% in the first group and by 13,2% in the second group, in contrast to the control group. In cases of generalized periodontitis of the second stage, the indicators increased by 18,25% and 17,6%, respectively (Table 1).

We also found a significant rise in hydroxyproline levels in individuals with initial generalized periodontitis: 2,6 times in the first group and 2,7 times in the second

group, as compared to the indicators of individuals from the control group. These changes in hydroxyproline are associated with the severity of generalized periodontitis. In particular, in generalized periodontitis of stage I, the hydroxyproline content exceeded the indicators in the control group by 3,3 and 3,5 times, respectively (Table 1).

There was also an increase in the concentration of hydroxyproline in individuals diagnosed with generalized periodontitis of stage II, which confirmed the tendency to rise with the worsening of the pathological process. Indeed, in the first group, the amount of hydroxyproline increased 5,5 times, and in the second group – 5,7 times, as compared to the corresponding indicators of the control group (Table 1).

## DISCUSSION

Our research revealed a certain similarity in the biochemical changes observed among individuals residing in areas with different environmental conditions. We assume that this may indicate that, in addition to Cd, a combination of various factors influences these biochemical alterations – for instance, stress-related factors that may be associated with cadmium exposure. In our study, the Cd concentration in the examined area was 1,2 times higher than in the environmentally clean region, which may explain the observed similarity of changes. Some inconsistency in the results has also been reported by other researchers; however, they emphasize that a high concentration of Cd plays a decisive role in the development of pathology. In particular, scientists [1] have found that periodontitis, a microbiome-driven chronic inflammatory disease that destroys the supporting apparatus of the teeth, is influenced by various environmental factors, including exposure to heavy metals such as lead and Cd. The authors indicate that the results highlight the importance of reduced environmental exposure to these heavy metals as part of preventive strategies for periodontal diseases. Further research is needed to explore the underlying biological

mechanisms and to evaluate potential interventions in order to reduce exposure-associated periodontitis [1].

Scientists [2] conducted a study aimed at examining the relationship between internal exposure to Cd and lead and periodontitis in a representative sample of adults who participated in the Fourth Korea National Health and Nutrition Examination Survey (KNHANES). A total of 1966 individuals aged over 19 years who took part in the survey were examined. A multivariate logistic regression analysis revealed an association between Cd and periodontitis [2].

It was confirmed that there is a connection between the effects of heavy metals, in particular Cd, and somatic health [10, 12]. It has been shown that an omnipresent environmental metal, cadmium, can cause oxidative stress in diabetic cardiomyocytes, leading to iron accumulation, glutathione depletion, lipid peroxidation, and, ultimately, exacerbation of ferroptosis and disruption of cardiac function. Moreover, Cd-induced hyperglycaemia can enhance the circulation of advanced glycation end products [12].

## CONCLUSIONS

Over the last decades, the number of eco-dependent diseases has been on the rise, as evidenced by research around the world. Our biochemical study of oral liquid showed a decrease in Ca content in individuals with generalized periodontitis compared to individuals with healthy periodontium, which correlates with the data of other researchers. The results demonstrated that the content of Pi in individuals with generalized periodontitis also increased, as did the level of hydroxyproline, compared with the indicators of individuals from the control group. However, the results obtained in our study indicate a certain similarity in biochemical changes, which may be attributed to stress-related factors; therefore, this issue requires further research. Since generalized periodontitis is one of the main problems of modern dentistry, the obtained data can be used in treatment planning and prophylaxis of periodontal disorders.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Interaction between gut microbiota and antibiotic resistance in patients with PTSD: Data from a cohort study

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## ABSTRACT

**Aim:** To analyze the composition of gut microbiota, antibiotic resistance profiles of isolated strains, and biochemical blood parameters in individuals with and without PTSD.

**Materials and Methods:** A cohort study was conducted involving 82 participants: 40 patients diagnosed with PTSD and 42 without PTSD (control group). All subjects completed the PCL-5 questionnaire and underwent clinical examination, biochemical blood testing, and microbiological analysis of fecal samples. Bacterial identification and antibiotic susceptibility testing were performed using standardized culturomic and EUCAST methods.

**Results:** PTSD patients showed a significantly higher prevalence of opportunistic microorganisms, including *Klebsiella pneumoniae* and *Enterobacter cloacae*, and a reduction in beneficial strains such as *Lactobacillus casei*. Increased resistance to carbapenems and macrolides was noted in the PTSD group. Biochemical analysis revealed hyperglycemia, elevated liver enzymes, decreased total protein, and increased markers of inflammation. These findings demonstrated significant correlations between gut dysbiosis, antibiotic resistance, and systemic metabolic alterations.

**Conclusions:** The study confirms a link between gut microbiota composition, antimicrobial resistance, and metabolic dysregulation in PTSD patients. These results may inform the development of integrated diagnostic approaches and targeted interventions in PTSD management.

**KEY WORDS:** PTSD, gut microbiota, antibiotic resistance, dysbiosis, biochemical markers

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## INTRODUCTION

Post-traumatic stress disorder (PTSD) is a complex neuroimmune condition that does not always develop even after severe trauma exposure. It emerges as a result of traumatic experiences and excessive stress, affecting patients' cognitive functions, emotional state, and physical health. Studies, particularly in the context of the war in Ukraine, confirm that PTSD significantly reduces quality of life, disrupts social adaptation, and increases the risk of comorbid disorders, including depression and generalized anxiety disorder [1, 2].

Recent studies (2019–2025) demonstrate increased interest in the impact of microbiota on mental health, particularly regarding disorders such as post-traumatic stress disorder (PTSD) [3, 4]. It has been proven that the microbiota plays a key role in the regulation of the "gut-brain" and "gut-immunity" axes, interacting with the central nervous and immune systems through metabolites (e.g., short-chain fatty acids – butyrate, propionate, acetate), neurotransmitters (serotonin,  $\gamma$ -aminobutyric acid), and inflammatory cascade mechanisms [5].

Changes in the composition of microbiota cause systemic inflammation and can influence the levels of both systemic and neuroinflammation. These inflammatory processes, in turn, affect the development of depressive and anxiety disorders and can alter the body's response to stress [6].

Moreover, experimental models demonstrate that microbiota transplantation from individuals with PTSD to germ-free rats can induce behavioral changes characteristic of increased anxiety and hyperarousal [7].

Alongside microbiota, biochemical blood markers play an important role in the diagnosis and understanding of PTSD pathophysiology, reflecting metabolic state, inflammatory activity, and the functioning of internal organs. Changes in levels of glucose, liver enzymes (ALT, AST), creatinine, protein, leukocytes, and platelets may result from chronic stress as well as indicate systemic effects of an altered gut microbial composition. This is supported by the findings by Zhou et al. (2020), who demonstrated that systemic inflammation associated with PTSD is accompanied by biochemical changes in

the blood, including hyperglycemia, liver and kidney dysfunction [8].

Similar changes have also been described in the paper of Foster et al. (2022), who emphasize the connection between metabolic processes, microbial profile, and the psycho-emotional state of an individual [9].

Thus, combining microbiological analysis with screening of biochemical markers enables a more comprehensive understanding of the physiological state of patients with PTSD and identifies potential targets for therapeutic intervention.

As was mentioned, the gut microbiota is not only a defined component of immune defense but also a key regulator of the “gut-brain” axis. Disruptions in the composition and functions of the gut microbiota, particularly dysbiosis, can lead to enhanced inflammatory processes, impaired intestinal barrier function, and altered production of neurotransmitters [10–12].

Consequently, it may contribute to the worsening of psycho-emotional disorders, including PTSD.

At the same time, it has been proven that dysbiosis can increase the risk of intestinal colonization by opportunistic microorganisms, among which multi-drug-resistant bacterial strains are often found [13]. In patients with PTSD, who often undergo treatment in hospital settings, the risk of colonization by resistant opportunistic microorganisms increases significantly. This is associated both with the widespread use of antibiotics and with microbiota changes induced by chronic stress [14]. Moreover, broad-spectrum antibiotics reduce microbiota diversity, leading to an imbalance between beneficial and opportunistic microorganisms.

Thus, disruption of the gut microbiota not only intensifies inflammatory processes and affects mental health but also creates preconditions for the development of antibiotic resistance, complicating the course of the underlying disease and requiring a comprehensive treatment approach.

The use of probiotics may help restore microbiota balance and reduce the risk of antibiotic resistance. Studies have shown that certain probiotic strains are capable of inhibiting survivance of multidrug-resistant bacteria and disrupting their biofilms, thereby reducing antibiotic resistance [15–18].

The data obtained indicates the potential of gut microbiota as a therapeutic target in the correction of mental disorders, which justifies usage of probiotics, prebiotics, and dietary recommendations in a comprehensive approach to psychiatric therapy.

## AIM

To analyze the composition of gut microbiota, antibiotic resistance profiles of isolated strains, and biochemical blood parameters in individuals with and without PTSD.

## MATERIALS AND METHODS

The study included 82 individuals, divided into two main groups: a group of patients diagnosed with PTSD (n=40) and a group without signs of PTSD (n=42), which served as the control. Patients were hospitalized at Uzhhorod City Multidisciplinary Hospital and the regional psychiatric facility in Berehove (September–December 2024). All individuals provided written informed consent prior to enrollment. Each participant underwent a comprehensive assessment that included the administration of the PTSD Checklist for DSM-5 (PCL-5), a clinical medical examination, and laboratory testing. Laboratory evaluations consisted of general and biochemical blood tests to support the clinical findings and explore potential physiological correlations of PTSD.

Gut microbiota was examined by culturomic technique by using of plating of fecal samples, serial dilution on selective and chromogenic nutrient media [19]. After cultivation at 37 °C for 24–48 hours, colony-forming units (CFUs) of bacteria were counted. Fecal samples were collected in sterile containers and transported to the laboratory. Bacterial identification was carried out based on morphological, biochemical, and serological characteristics.

The study of antibiotic resistance of microorganisms was conducted using the disk diffusion method in accordance with EUCAST standards (2023) [20, 21]. A standard set of disks with 12 key antibiotics was used, covering representatives of carbapenems, beta-lactams, macrolides, fluoroquinolones, and aminoglycosides.

Statistical analysis was performed using SPSS v.26. The  $\chi^2$  test was used for frequency comparisons, the Mann–Whitney U test for quantitative variables, and Pearson’s correlation analysis (r) was applied.

## ETHICS

The research was conducted in accordance with ethical standards. All patients gave informed consent.

## RESULTS

The results of the microbiological analysis of fecal samples indicate alterations in the gut microbiota of patients with PTSD. Compared to the control group, differences were noted both in the spectrum of isolated microorganisms and in their antibiotic resistance.

Microbiological examination of fecal samples revealed significant changes in the gut microbiota composition of patients with PTSD (n=40) compared to the control group (n=42). These differences included the spectrum of isolated microorganisms, their concentration profiles, and antibiotic resistance.

In the control group, the dominance of representatives of the normal microbiota with minimal presence

**Table 1.** Species composition and concentration of generous microorganisms in feces of control group participants

| Microorganism species               | Detection frequency (%) | Number of isolates (n) | Concentration (CFU/mL; M $\pm$ SD) |
|-------------------------------------|-------------------------|------------------------|------------------------------------|
| <i>Escherichia coli</i>             | 45%                     | 19                     | $(2.1 \pm 0.3) \times 10^6$        |
| <i>Bacteroides thetaiotaomicron</i> | 27%                     | 11                     | $(7.8 \pm 1.2) \times 10^7$        |
| <i>Lactobacillus casei</i>          | 18%                     | 8                      | $(1.3 \pm 0.2) \times 10^6$        |
| <i>Enterobacter cloacae</i>         | 5%                      | 2                      | $(9.5 \pm 1.0) \times 10^3$        |
| <i>Staphylococcus aureus</i>        | 7%                      | 3                      | $(1.2 \pm 0.3) \times 10^4$        |

Source: compiled by the authors of this study

**Table 2.** Microorganisms identified by advanced analysis (n=40)

| Isolates                                      | Number of patients (n=40) |
|-----------------------------------------------|---------------------------|
| <i>Klebsiella pneumoniae</i>                  | 7                         |
| <i>Enterobacter cloacae</i>                   | 5                         |
| <i>Proteus mirabilis</i>                      | 4                         |
| <i>Staphylococcus aureus</i>                  | 8                         |
| <i>Corynebacterium spp.</i>                   | 3                         |
| <i>Lactobacillus casei</i> (decreased number) | 13                        |

Source: compiled by the authors of this study

**Table 3.** Susceptibility of *Klebsiella pneumoniae* (n=7)

| Nº | Antibiotic          | EUCAST Breakpoints (mm) |      | Inhibition Zone (mm $\pm$ SD) |
|----|---------------------|-------------------------|------|-------------------------------|
|    |                     | S $\geq$                | R <  |                               |
| 1  | Meropenem           | $\geq 22$               | < 22 | $11.0 \pm 1.6$                |
| 2  | Imipenem/Cilastatin | $\geq 22$               | < 19 | $13.5 \pm 2.1$                |
| 3  | Ceftriaxone         | $\geq 25$               | < 22 | $12.3 \pm 2.0$                |
| 4  | Azithromycin        | $\geq 17$               | < 13 | $11.8 \pm 2.4$                |
| 5  | Levofloxacin        | $\geq 23$               | < 19 | $10.9 \pm 1.5$                |

Source: compiled by the authors of this study

of opportunistic forms was observed (Table 1). Specifically, *Escherichia coli* was detected in 45% of samples (19 isolates) at an average concentration of  $(2.1 \pm 0.3) \times 10^6$  CFU/mL. A significant portion of the microbial composition also consisted of *Bacteroides thetaiotaomicron* (27%; 11 isolates) at amount of  $(7.8 \pm 1.2) \times 10^7$  CFU/mL. *Lactobacillus casei*, as key beneficial microbes, were isolated in 18% of samples (8 isolates) at a level of  $(1.3 \pm 0.2) \times 10^6$  CFU/mL.

Among the opportunistic microorganisms in the control group, *Enterobacter cloacae* (5%; 2 isolates) and *Staphylococcus aureus* (7%; 3 isolates) were found sporadically, with concentrations of  $(9.5 \pm 1.0) \times 10^3$  CFU/mL and  $(1.2 \pm 0.3) \times 10^4$  CFU/mL, respectively. These values only slightly exceeded reference levels and were not clinically significant, indicating the preservation of eubiotic balance in participants without signs of PTSD.

Further analysis of the microbiological profile of patients with PTSD (n=40) included the quantitative determination of key representatives of commensal and opportunistic microbiota. The study covered

four main bacterial groups: physiologically important commensals (*E. coli* lac+, *Enterococcus faecalis*, *Lactobacillus casei*) and opportunistic pathogens (*Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus* MRSA). Additionally, for a subset of patients (n=13), an extended evaluation was conducted, including detailed antibiogram and microbiological characterization of the isolates.

*E. coli* lac+ was detected in all samples, with concentrations ranging from  $10^7$  to  $10^8$  CFU/mL, which corresponds to physiological norms for a major facultative anaerobe. This indicator suggests the preservation of a baseline level of obligate intestinal microbiota even in the presence of dysbiosis. At the same time, the quantity of *Lactobacillus casei* in most patients was below the reference range ( $10^6$ – $10^8$  CFU/mL). This indicates a reduction in the mucosal barrier function of the intestine and suggests weakened colonization resistance.

*Enterococcus faecalis* concentrations varied widely – from  $10^3$  to  $10^8$  CFU/mL. This discrepancy may reflect individual characteristics of microbial status as well as responses to

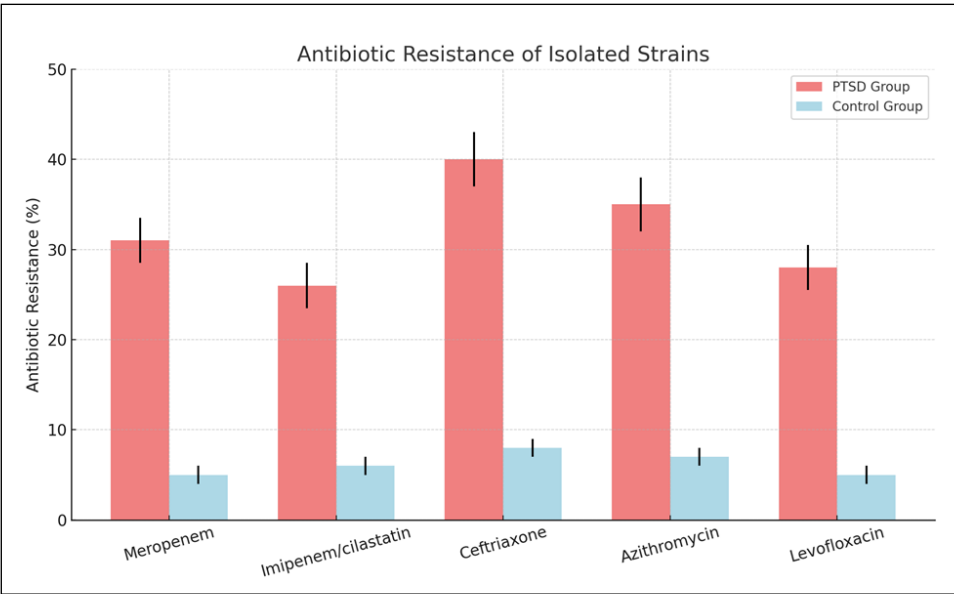


**Fig. 1.** Antibiotic resistance of isolated strains  
Picture taken by the authors

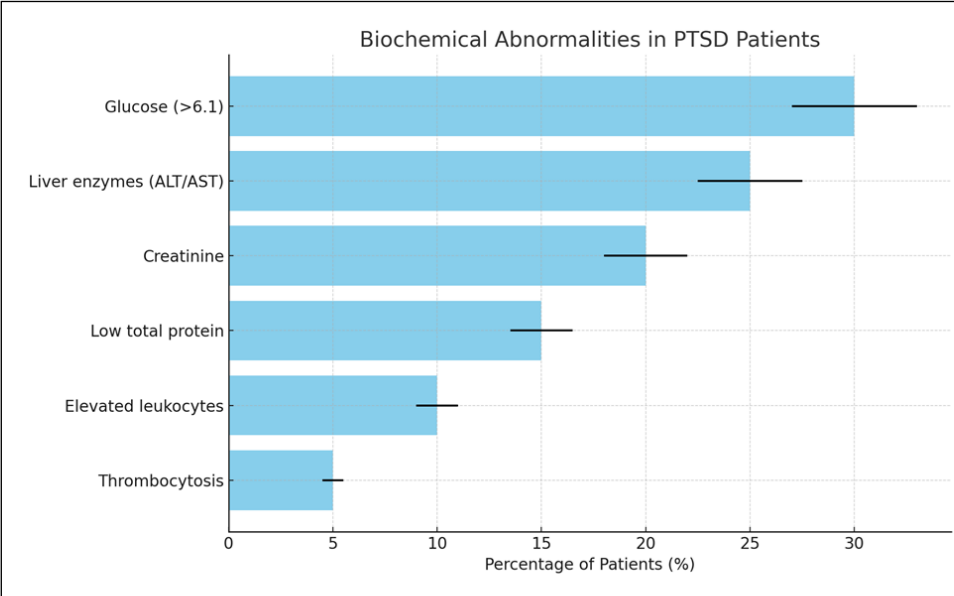


**Fig. 2.** Biochemical abnormalities in PTSD patients  
Picture taken by the authors

external factors, including antibiotic therapy, stress, and dietary changes. The most significant microbiological burden was associated with opportunistic members of the *Enterobacteriaceae* family, particularly *Klebsiella pneumoniae* and *Enterobacter cloacae*. These accounted for more than 65% of all isolated strains, with concentrations mostly exceeding  $10^6$  CFU/mL. Such values significantly surpass the clinically acceptable level ( $<10^4$  CFU/mL) and may indicate active colonization of the intestinal mucosa and a translocation potential of these strains.

In 13 patients, *Staphylococcus aureus* was identified, including methicillin-resistant strains (MRSA). Concentrations of this pathogen exceeded  $10^6$  CFU/mL, which may point to endogenous colonization of the skin or mucous membranes. Considering many patients' medical histories, including prolonged hospitalizations and repeated antibiotic use, the probability of nosocomial origin for these strains is high.

In an in-depth study of participants (n=40), which included antibiotic resistance profiling and refined bacteriological identification, these same trends were confirmed. Specifically, among all isolated strains, the proportion of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis* together accounted for more than 65% of the total. These species are associated with opportunistic infections, often multidrug-resistant, and are capable of forming stable biofilms, which complicates their elimination.

Further analysis of the microbiological profile of patients with PTSD (n=40) included quantitative determination of the main representatives of commensal and opportunistic microbiota. Specifically, among all isolated strains, the proportion of *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Proteus mirabilis* together accounted for over 65% of the total. These species are associated with opportunistic infections, are often

**Table 4.** Susceptibility profile of *Enterobacter cloacae* (n=5)

| Nº | Antibiotic          | EUCAST Breakpoints (mm) |      | Inhibition Zone (mm ± SD) |
|----|---------------------|-------------------------|------|---------------------------|
|    |                     | S ≥                     | R <  |                           |
| 1  | Meropenem           | ≥ 22                    | < 22 | 12.3 ± 1.8                |
| 2  | Imipenem/Cilastatin | ≥ 22                    | < 19 | 14.2 ± 2.1                |
| 3  | Cefepime            | ≥ 27                    | < 24 | 13.5 ± 1.9                |
| 4  | Cefotaxime          | ≥ 20                    | < 17 | 12.9 ± 2.4                |
| 5  | Ciprofloxacin       | ≥ 25                    | < 22 | 13.8 ± 2.0                |
| 6  | Amikacin            | ≥ 18                    | < 18 | 16.0 ± 2.3                |

Source: compiled by the authors of this study

**Table 5.** Susceptibility profile of *Staphylococcus aureus* (n=8)

| Nº | Antibiotic    | EUCAST Breakpoints (mm) |      | Inhibition Zone (mm ± SD) |
|----|---------------|-------------------------|------|---------------------------|
|    |               | S ≥                     | R <  |                           |
| 1  | Azithromycin  | ≥ 13                    | < 13 | 11.9 ± 2.1                |
| 2  | Ciprofloxacin | ≥ 50                    | < 17 | 13.2 ± 2.4                |
| 3  | Levofloxacin  | ≥ 50                    | < 22 | 14.4 ± 1.7                |
| 4  | Linezolid     | ≥ 21                    | < 21 | 22.3 ± 1.1                |
| 5  | Fusidic acid  | ≥ 24                    | < 24 | 25.1 ± 1.5                |

Source: compiled by the authors of this study

multidrug-resistant, and form stable biofilms, which complicate their elimination (Table 2).

*Bifidobacterium* spp. were not detected in the fecal samples. Antibiotic susceptibility testing showed a high level of resistance in the PTSD group (n=40) to the following drugs:

- Meropenem: 31% resistant strains;
- Imipenem/cilastatin: 26%;
- Ceftriaxone: 40%;
- Azithromycin: 35%;
- Levofloxacin: 28%.

In contrast, in the control group, only a few isolates showed resistance to the mentioned drugs (not exceeding 5–8%) (Fig.1).

According to the results of this analysis, the proportion of isolates resistant to meropenem was 69.2% (28 out of 40 patients), more than double the overall rate. Similar values were observed for levofloxacin – also 69.2% (28 out of 40), ceftriaxone – 38.5% (15 out of 40), azithromycin – 38.5%, and imipenem/cilastatin – 38.5%.

Such a level of resistance observed in PTSD patients not only aligns with overall trends in the study but also highlights the severe microbiological profiles within the subgroup exhibiting pronounced colonization. In particular, all *Klebsiella pneumoniae* strains (n=7) were resistant to at least four classes of antibiotics–carbapenems, fluoroquinolones, macrolides, and beta-lactams (Table 3).

A similar resistance profile was observed in *Enterobacter cloacae* (n=5)–none of the isolates were susceptible to ceftriaxone, levofloxacin, or imipenem (Table 4).

In 80% of *Staphylococcus* spp. isolations (6 out of 8), an MRSA-like phenotype was identified with multiple resistance to fluoroquinolones, macrolides, and ampicillin, while partial susceptibility to linezolid and fusidic acid was retained (Table 5).

These results suggest that, under PTSD-modified immune status and hospital environmental conditions, a stable bacterial colonization burden is formed, dominated by multidrug-resistant Enterobacteriaceae and Gram-positive cocci. Of particular concern is the detection of *Corynebacterium* spp., which was found to be completely resistant to all tested drugs, including linezolid, vancomycin, and rifampicin. Although this was a single case, it illustrates the risk of emerging super-resistant isolates in patients with a history of repeated hospitalizations.

In the control group (n=42), such pathogens were detected much less frequently, and resistance to key antibiotics did not exceed 5–8% in any case. Compared to the PTSD group, these differences were statistically significant ( $p < 0.05$ ;  $\chi^2$ ), allowing PTSD-associated microbial colonization to be considered an independent risk factor in the formation of multidrug-resistant status.

Thus, antibiotic resistance rates in the PTSD group were found to be significantly higher than in the control group, with particularly high resistance to carbapenems and macrolides. This pattern suggests colonization by microorganisms that potentially pose an epidemiological threat in hospital environments.

Additionally, a biochemical blood analysis was performed, the results of which confirmed systemic chang-

es characteristic of PTSD patients (Fig. 2). In 40% of individuals, blood glucose levels exceeded the normal range ( $6.23 \pm 0.88$  mmol/L), indicating hyperglycemia likely associated with activation of the stress response. Liver enzymes (ALT, AST) were elevated in 33% of participants, suggesting liver dysfunction. Creatinine levels were above normal in 25% of individuals, and total protein was decreased in a significant number of patients, which may indicate catabolic processes or insufficient protein intake. An increase in leukocytes was observed in 20% of individuals, and thrombocytosis in 15%, pointing to activation of inflammatory processes.

Thus, biochemical blood analysis reflects systemic changes that suggest a physiological response to stress and a possible influence of microbial imbalance. In particular, elevated glucose may result from activation of gluconeogenesis via the HPA (hypothalamic–pituitary–adrenal) axis, while elevated ALT and AST levels indicate hepatocyte cytolysis, likely linked to the effects of bacterial endotoxins. Decreased protein levels may point of to disrupted protein metabolism or malabsorption syndrome, which often accompanies inflammatory bowel disorders.

In patients diagnosed with PTSD, changes in renal function (elevated creatinine) and reduced total protein were also recorded, indicating possible catabolic processes. Additionally, thrombocytosis and elevated leukocyte counts were observed in some patients, consistent with a probable chronic inflammatory state. Therefore, the results of microbiological analysis are directly related to biochemical markers that indicate a systemic impact of PTSD on the body.

## PRINCIPAL COMPONENT ANALYSIS AND CORRELATION ANALYSIS

For integrative assessment of the relationships between gut microbiota, antibiotic resistance, and blood biochemical parameters in patients with PTSD, a principal component analysis (PCA) was conducted. This approach allowed the identification of the main factors driving the variability of the parameters studied and the establishment of key axes linking microbiota composition and systemic metabolic markers.

The analysis revealed that the first principal component (PC1) accounted for 42% of the data variance and was most strongly associated with elevated levels of opportunistic Enterobacteriaceae (particularly *Klebsiella pneumoniae*, *Enterobacter cloacae*), as well as high antibiotic resistance (notably to carbapenems and macrolides). At the same time, it showed a negative correlation with the abundance of probiotic forms (*Lactobacillus casei*, *Bifidobacterium spp.*). The second

component (PC2), which explained 25% of the variance, was predominantly driven by blood biochemical parameters, including elevated glucose, ALT, AST, and creatinine levels.

Pearson correlation analysis demonstrated statistically significant associations between increased abundance of opportunistic bacteria (*Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*) and the following biochemical indicators:

- Glucose level ( $r = 0.58$ ;  $p < 0.01$ )
- ALT level ( $r = 0.49$ ;  $p < 0.05$ )
- Creatinine ( $r = 0.52$ ;  $p < 0.05$ )
- Elevated leukocytes ( $r = 0.45$ ;  $p < 0.05$ )

Conversely, the abundance of *Lactobacillus casei* had an inverse correlation with glucose level ( $r = -0.47$ ;  $p < 0.05$ ) and liver enzymes ( $r = -0.42$ ;  $p < 0.05$ ), suggesting a potential protective role of probiotic bacteria against the development of metabolic disturbances.

Additionally, a high level of antibiotic resistance in strains (*Klebsiella pneumoniae*, *Enterobacter cloacae*) to meropenem and ceftriaxone was directly correlated with increased intestinal colonization by these microorganisms ( $r = 0.61$ ;  $p < 0.01$ ) and the frequency of hospitalizations ( $r = 0.54$ ;  $p < 0.05$ ).

Thus, the results of the analysis support the hypothesis of a unified pathophysiological complex that links microbiome alterations, the development of antibiotic resistance, and systemic metabolic disorders in patients with PTSD. The findings underscore the need for a comprehensive treatment approach for such patients, which includes simultaneous assessment of microbiota status, antibiotic resistance levels, and key blood biochemical parameters.

## DISCUSSION

The results of our study are consistent with the findings of Cryan & Dinan (2019), who demonstrated that microbiota directly affects central nervous system functioning through the regulation of key neurotransmitters [22]. Specifically, the authors describe that metabolic products of certain gut bacteria, including *Lactobacillus* and *Bifidobacterium*, can stimulate the synthesis of gamma-aminobutyric acid (GABA) – the main inhibitory neurotransmitter involved in reducing anxiety. They also report effects on tryptophan metabolism and serotonin synthesis neurotransmitter that plays a crucial role in mood regulation. Through the “gut-brain axis,” cortisol secretion – the primary stress hormone is also modulated, and its concentration can be influenced by the microbiota. Therefore, changes in gut microbial composition may directly affect emotional state, anxiety, and the ability to adapt to stress.



Foster et al. (2022) likewise emphasize that the state of microbiota is closely linked to immune system modulation, particularly through effects on regulatory T-cells and the production of anti-inflammatory cytokines [23]. The authors highlight that shifts in microbial composition may promote either activation or suppression of the immune response, which, in turn, affects the hypothalamic–pituitary–adrenal (HPA) axis and cortisol secretion regulation – the key stress hormone. Under normal conditions, symbiotic microbiota maintains a balance between pro-inflammatory and anti-inflammatory signals, whereas dysbiosis disrupts this balance, potentially contributing to the development of anxiety and depressive disorders, including PTSD. Moreover, their research shows that some bacterial metabolites, especially short-chain fatty acids (SCFAs), play an important role in reducing systemic inflammation and improving stress adaptation through effects on the nervous and endocrine systems.

In the study by Kelly et al. (2016), a reduction in *Faecalibacterium prausnitzii* – one of the key anti-inflammatory members of the microbiota – was observed in PTSD patients, correlating with increased levels of inflammatory cytokines [24]. Our results confirm these findings, as we observed an increase in gram-negative pathogens capable of triggering inflammatory pathways via LPS (lipopolysaccharides).

Furthermore, according to WHO and CDC guidelines [25, 26], the detected resistance to carbapenems is an indicator of critically important health threats. Our findings underscore the need to adapt treatment protocols based on epidemiological context and local resistance profiles.

Another important observation concerns the role of hospitalization and living conditions, as noted by Zhou et al. (2020), who emphasized that psychiatric patients are at increased risk of colonization with multidrug-resistant microbiota due to frequent hospitalizations, prolonged inpatient stays, and repeated antibiotic treatments [27].

Thus, the results of this study add new evidence supporting the hypothesis of a link between mental health, microbiota, and antibiotic resistance. They underscore the importance of an integrative approach to PTSD

treatment that includes microbiological and nutritional monitoring.

## CONCLUSIONS

1. PTSD patients showed significant gut dysbiosis, with increased levels of opportunistic bacteria (*Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus mirabilis*, *Staphylococcus aureus*) and reduced commensals (*Lactobacillus casei*, *Bifidobacterium spp.*), potentially contributing to psycho-emotional disturbances via gut–brain axis disruption.
2. In a PTSD subgroup, 77% had clinically significant colonization by multidrug-resistant Gram-negative pathogens. Some patients exhibited co-colonization with multiple strains, including *Corynebacterium spp.* resistant to all tested antibiotics.
3. High antibiotic resistance was observed in the PTSD subgroup: meropenem and levofloxacin resistance reached 69.2%; azithromycin, ceftriaxone, and imipenem/cilastatin resistance was 38.5%. *K. pneumoniae* isolates showed resistance to  $\geq 4$  antibiotic classes.
4. Biochemical analyses indicated stress-related metabolic shifts – hyperglycemia, elevated ALT/AST, dysproteinemia, creatinemia, and inflammation – correlating with Enterobacteriaceae levels and antibiotic resistance.
5. PCA revealed that microbial resistance and opportunistic colonization were primary factors driving patient variability; higher probiotic abundance inversely correlated with pathological biochemical markers.
6. Strong correlations were identified between Enterobacteriaceae levels and glucose ( $r = 0.58$ ), ALT ( $r = 0.49$ ), and creatinine ( $r = 0.52$ ), implicating dysbiosis and endotoxemia in PTSD pathogenesis.
7. The findings support a multidisciplinary PTSD treatment approach involving microbiota screening, resistance profiling, biochemical monitoring, and personalized probiotic/nutritional interventions.
8. Multidrug-resistant colonization may serve as a predictive marker for PTSD severity and prognosis, highlighting the role of gut microbiota in systemic and neuropsychiatric dysfunction.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Tangeretin ameliorates renal ischemia reperfusion injury via regulating oxidative stress and Notch1/Jagged1 signaling in male rats

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## ABSTRACT

**Aim:** This study was performed to investigate the potential nephroprotective effect of Tangeretin on bilateral renal I/R injury in male rats.

**Materials and Methods:** Forty male rats were split into four groups of ten (sham, control, DMSO, and Tangeretin). The sham group underwent a median laparotomy under anaesthesia without inducing ischemia/reperfusion; the control group underwent clamping for thirty minutes on the bilateral renal artery, followed by two hours of reperfusion; the vehicle group received DMSO one hour before induction of ischemia; and the Tangeretin group received 5 mg/kg of Tangeretin one hour before ischemia. Biochemical parameters (KIM1, IL-1 $\beta$ , and TNF- $\alpha$ , F2-isoprostane, GSH, and caspase-3) were measured using an ELISA approach. Furthermore, histological alterations were examined, and the Notch/Jagged1 signalling pathway was assessed using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

**Results:** Tangeretin pre-treatment reduced kidney damage molecules (KIM1, IL-1 $\beta$ , and TNF- $\alpha$ , F2-isoprostane, GSH, and caspase-3) while increasing anti-oxidant indicators and decreasing inflammatory and apoptotic markers. Improving histological outcomes and significantly decreasing Notch1 and Jagged-1 gene expression in kidney tissues during renal ischemia/reperfusion injury.

**Conclusions:** Tangeretin has significant nephroprotective advantages in renal IRI by decreasing the Notch pathway and exhibiting anti-apoptotic, antioxidant, and anti-inflammatory properties.

**KEY WORDS:** renal IRI, Tangeretin, inflammation, oxidative stress, Notch1, Jagged-1, apoptosis and nephroprotection

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## ABBREVIATIONS

IRI: Renal Ischemia-reperfusion Injury

AKI: Acute Kidney Injury

mPTP: mitochondrial Permeability Transition Pore

NICD: Notch Intracellular Domain

KIM: Kidney Function Measures

## INTRODUCTION

Renal I/R injury results from a sudden, brief disruption of the kidney's blood supply, which is followed by the blood returning to normal and reoxygenation the tissue. This leads to several pathological changes, including glomerular damage and significant tubular damage, which is shown by nuclear sequestration, tubular dilatation, brush border defects, tubular formation, interstitial oedema, medullary congestion, and necrosis. Overproduction of (ROS) from broken tissue during I/R results in oxidative stress, and blood flow during reperfusion produces oxygen free radicals that cause apoptosis through lipid peroxidation and oxidative

damage to proteins and DNA, which ultimately causes damage to kidney tissue [1-2]. Following an ischaemic insult, a considerable drop in mitochondrial ATP production, membrane potential, and the subsequent opening of the mitochondrial permeability transition pore (mPTP) [3]. The levels of ATP in proximal tubule cells fall to a trough within minutes of ischemia [4]. Furthermore, the drop in ATP levels may restrict the action of Na<sup>+</sup>/K<sup>+</sup>-ATPase, resulting in intracellular Na<sup>+</sup> build-up and subsequent Ca<sup>2+</sup> influx, leading to accumulation in mitochondrial. Following reperfusion, large increases in ROS and Ca<sup>2+</sup> levels inside the mitochondria which cause mitochondrial dysfunction [5]. Reactive oxygen species, such as the hydroxyl radical, interact with DNA and damage the ribose-phosphate backbone. Furthermore, reactive oxygen species' interactions with lipid bilayers remove hydrogen atoms from unsaturated fatty acids attached to phospholipid; this process is called lipid peroxidation, which leads to the reduction of the integrity and functionality of cellular membranes [6]. As a result, the mPTP releases cytochrome C, which initiates the caspase

cascade and the mitochondrial death pathway [7]. Notch signalling pathway; mammals possess Notch ligands such as Jagged-1, Jagged-2, Delta-like 1 (DLL1), DLL3, and DLL4, as well as four Notch receptors (Notch1-4). The receptor and its ligands are transmembrane proteins that have many extracellular domains [8]. The interaction with ligand cause modulation of the receptor which lead to stimulating two proteolytic processes. The extracellular region sheds as a result of primary cleavage, which is carried out by metalloproteases from the ADAM family. The  $\gamma$ -secretase complex catalyses the second cleavage, which takes place within the membrane domain. The Notch intracellular domain (NICD) is then cleaved and transported to the nucleus, where it forms a transactivation complex [9]. This approach eliminates co-repressing complexes via co-activators, resulting in the transcription of Notch target genes, which comprise two families of transcriptional factors: hairy/enhancer-of-split linked with YRPW motif (Hey) (HEY1 and HEY2) and hairy-enhancer of split (Hes) (HES1 and HES5) [10-13]. Furthermore, during ischemia reperfusion, the activation of the Notch pathway by ROS leads to the activation of metalloproteases (ADAM17), which indirectly triggers the discharge of NICD. NICD translocate to the nucleus and promotes transcription of its target genes, causing the development of fibrosis [14]. Tangeretin, a natural bioactive compound known as (5,6,7, 8,4-pentamethoxyflavone), which is a member of polyethoxylated flavones [15-16]. It is located evidently in citrus fruit peels and dried tangerine peels [17]. It exhibits different effects, which include antioxidant, anti-inflammatory, antidiabetic, anticancer, and neuroprotective results [18-20], moreover, Tangeretin exhibits neuroprotective consequences against ischemic stroke by increase the activity of superoxide dismutase and the survival of endothelial cells of human brain micro vascular. This action affects in reduced level of reactive (ROS) and malondialdehyde (MDA), which decrease apoptosis and subsequently reduce brain injury [21-22]. In kidney tissue, Tangeretin significantly decreased inflammatory cytokines and lipid peroxides with the improving the levels of both enzymatic and nonenzymatic antioxidants [23]. This normalization included antioxidant enzymes which include Catalase (CAT), Superoxide dismutase (SOD), Glutathione reductase (GR), and Glutathione peroxidase (GPx) in diabetic rats [24].

## MATERIALS AND METHODS

### PREPARATION OF ANIMALS

We obtained 40 male Wistar albino rats aged 8 to 12 weeks from the University of Kufa's Faculty of Science, each weighing 200 to 300 g. The rats lived at Kufa University's Faculty of Science Animal Facility. The animals were housed in cages

with a 12-hour light/dark cycle, a temperature of  $22 \pm 2^\circ\text{C}$ , and humidity levels ranging from 60–65%. The rats were given a typical meal that included commercial ordinary chow pellets and tap water. All experimental methods at the University of Kufa followed Institutional Animal Care and Use Committee (IACUC) guidelines.

### STUDY DESIGN

Forty male Wistar albino rats were randomly assigned to four groups of ten each: sham, control, DMSO, and Tangeretin. The sham group underwent a median laparotomy under anaesthesia with no ischemia or reperfusion in the kidneys. While the IRI group (control group) underwent clamping in bilateral renal arteries for thirty minutes, followed by two hours of reperfusion, the vehicle group received DMSO intraperitoneally one hour before the induction of ischemia [25-26]. In the Tangeretin group, all ten rats were pre-treated with Tangeretin 5 mg/kg [27] and for anaesthesia during the procedure, the rats received an intraperitoneal injection of 10 mg/kg xylazine and 100 mg/kg ketamine [28].

### SAMPLING TECHNIQUES

#### TISSUE SAMPLING FOR BIOCHEMICAL ANALYSIS

The kidney tissues were stored at  $-80^\circ\text{C}$  until homogenised with a high-intensity ultrasonic liquid processor in 1:10 W/V phosphate buffered saline with 1% Triton X-100 and a protease inhibitor cocktail. The homogenate samples were centrifuged at  $4^\circ\text{C}$  for 15 minutes at 14000 rpm [29-30], then we analysed the supernatants for kidney injury markers (KIM, TNF- $\alpha$ , IL-1 $\beta$ , F2-isoprostane, GSH, and caspase-3).

#### TISSUE SAMPLING FOR IMMUNOHISTOCHEMISTRY

This approach makes use of formalin-fixed, paraffin-embedded slides. Primary antibodies target particular antigens, while labelled secondary antibodies assess target protein expression. The antigen-antibody combination is detected using a labelling approach that is either fluorescent or enzymatic. IHC analysis is based on the labelled streptavidin-biotin staining technique (LSAB).

### ANALYSIS OF NOTCH SIGNALING PATHWAY BY QRT-PCR.

Notch-1 and Jagged-1 expression levels in the kidney were determined using qRT-PCR. We extracted around 50-100 mg of kidney tissue using the Easy-spin™ (DNA-

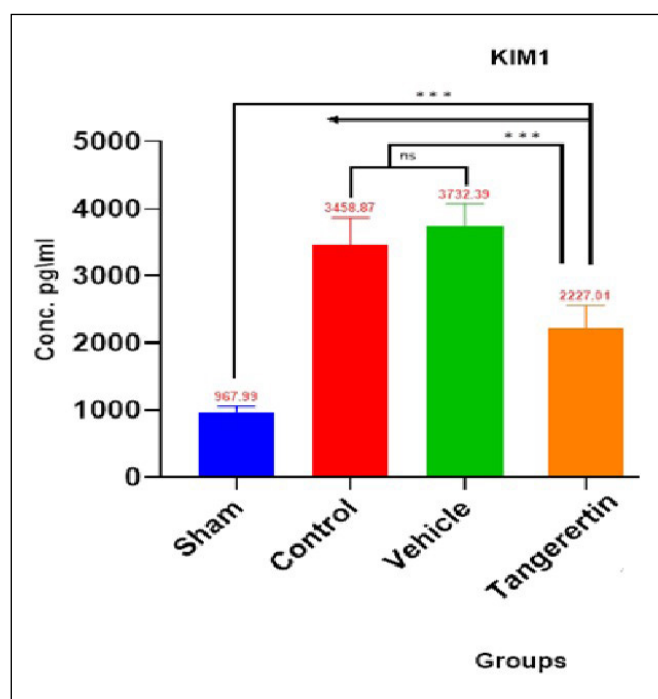


**Fig. 1.** Impact of Tangeretin on levels following renal IR, tissue KIMI (pg/ml) among groups, Mean  $\pm$  SD, n=10

\*\*\* $p \leq 0.001$  vs. sham; \*\*\* $p \leq 0.001$  vs. control/vehicle

Source: Own materials

free) total RNA extraction Kit (Intron/Korea) according to the manufacturer's procedure. cDNA was synthesised using reverse transcription using the Add Script cDNA Synthesis Kit according to the manufacturer's instructions. PCR was carried out in accordance with the GoTaq® RT-qPCR System instructions. The sequences of the primer for Jagged1 (F) AACTGGTACCGGTGCGAA (R) TGATGCAAGATCTCCCTGAAAC; Notch1 (F) CACCCATGACCACTACCCAGTT (R) CCTCGGACCAATCAGAGATGTT [31] and for GAPDH (F) ATGACTCTACCCACGGCAAG [32] for specific gene amplification. The  $2^{-\Delta\Delta Ct}$  method was used to measure fold changes in gene expression. Gene expression is measured as a relative fold change from GAPDH, an internal control reference gene.

## INVESTIGATIONS

### BIOCHEMICAL MARKERS ANALYSIS

By using Elisa kits from SunLong Biotech Co.LTD, China, to evaluate the levels of KIM1, IL-1 $\beta$ , and TNF- $\alpha$ , F2-isoprostane, GSH, and caspase-3.

### STATISTICAL ANALYSIS

The statistical analysis for this study was carried out using Graph Pad Prism version 8 software. After de-

termining the normal distribution of data. To make numerous comparisons across groups, the one-way ANOVA test was utilised using parametric variables, and the Bonferroni procedure was chosen for the Post Hoc test. P-values less than 0.001 were deemed statistically significant in all tests, all data are presented as mean  $\pm$  SD.

## RESULTS

The ischemia lasted 30 minutes, with two hours of reperfusion. Prior to one hour of ischemia, the rats received DMSO, Tangeretin (5 mg/kg), or were left untreated (sham and control groups). Several biochemical indicators were used to assess the degree of kidney injury.

### IMPACT ON KIDNEY INJURY MOLECULE (KIM1)

Following renal I/R, the IRI (control) group had significantly greater tissue KIM1 levels than the Sham group. Tangeretin treatment resulted in a significant decline in KIM1 levels in tissues (Fig. 1)

### IMPACT ON KIDNEY TISSUE INFLAMMATORY MARKERS

The IRI group showed significantly higher levels of TNF- $\alpha$  and IL-1 $\beta$  in their tissues than the sham group. The Tangeretin group dramatically reduced tissue inflammatory parameters (IL-1 $\beta$  and TNF- $\alpha$ ) (Fig. 2 A-B).

### IMPACT ON OXIDATIVE AND APOPTOTIC MARKERS

F2-isoprostane, GSH, and caspase-3 levels in rat kidney tissue were significantly increased in the IRI group compared to the Sham group. Tangeretin treatment increased antioxidant marker (GSH) levels in renal tissue while reduced apoptotic marker (Caspase-3) levels when compared to the control group (IRI) (Fig. 3 A-C).

### HISTOPATHOLOGICAL FINDINGS

The Sham group reported much lower damage estimates than the other groups (\*\*\*,  $p < 0.001$  vs. Sham). Tangeretin showed much less damage than the control and DMSO groups (\*\*\* $p < 0.001$  vs. induced groups), indicating a nephroprotective effect (Fig. 4).

The control group had a significant abnormality in renal structure, with severe renal tissue architecture changes such as tubular increased cytoplasmic eosin-



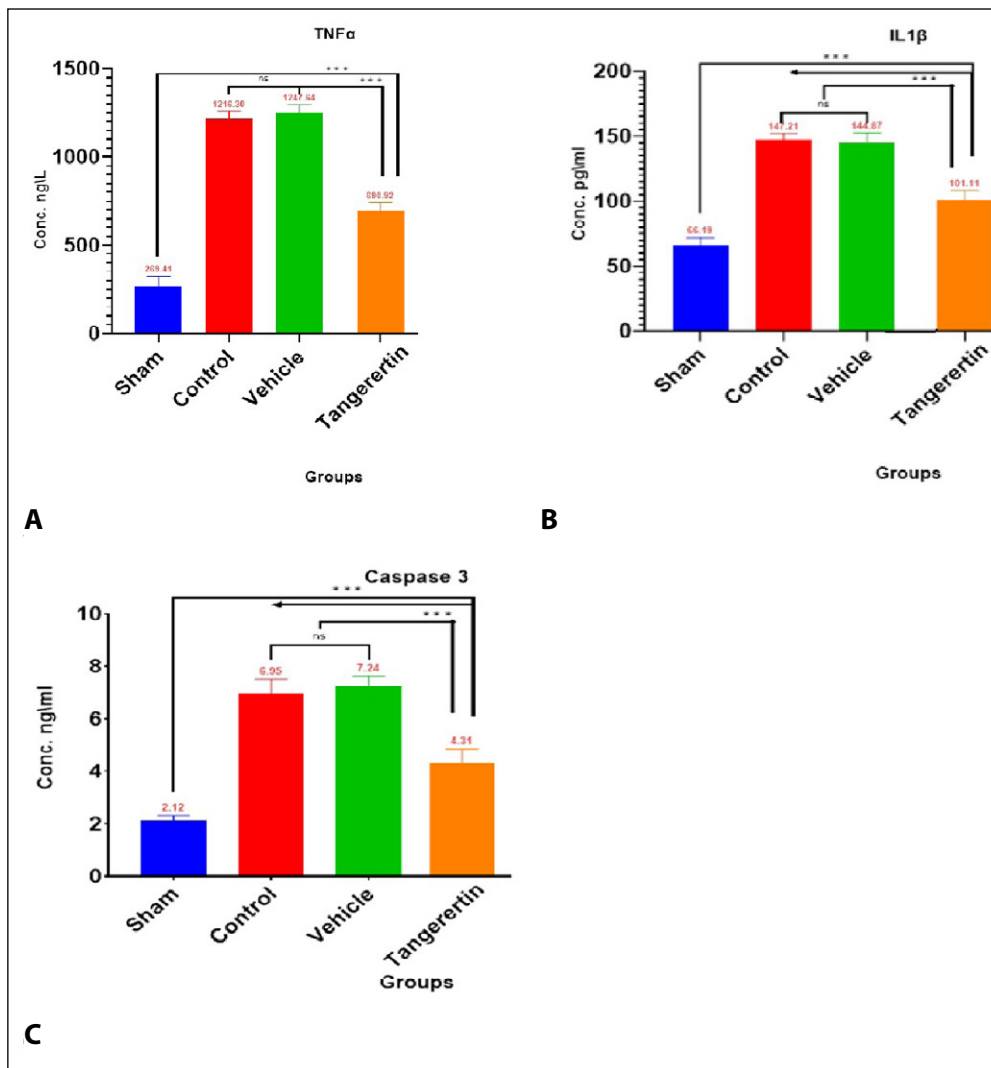


**Fig. 2.** Effect of Tangeretin on TNF- $\alpha$  (A) and IL-1 $\beta$  (B) levels after renal IR, including tissue TNF- $\alpha$  (ng/L) and IL-1 $\beta$  (pg/ml) between groups,  $n = 10$ , mean  $\pm$  SD \*\*\* $p \leq 0.001$  vs. sham; \*\*\* $p \leq 0.001$  compared control/vehicle

Source: Own materials

ophilia, cellular swelling, tubular epithelium degeneration, vascular congestion, eosinophilic cast, and cytoplasmic vacuoles (score = 4 and representing > 75% of damage). The group that got DMSO exhibited comparable pathology to the IRI group. The Tangeretin group showed moderate alterations in renal architecture (scoring = 2) (Fig. 5A-F).

The Sham group had a normal morphological appearance tissue without histological change while control and vehicle groups had severe morphological change in renal tubules include increased cytoplasmic eosinophilia, eosinophilic cast, cytoplasmic vacuoles and cytoplasmic swelling. Tangeretin pre-treated group the renal tubules damage with score 2 characterized by involving 30% of the examined damaged tubules.

## IMPACT ON NOTCH1 AND JAGGED1 M RNA EXPRESSION

Notch1 and Jagged-1 expression levels in renal tissue were significantly greater in the control and vehicle

groups compared to the sham group ( $p < 0.001$ ). However, there was no significant difference between the vehicle and control groups. Tangeretin showed significantly lower levels ( $p < 0.001$ ) compared to the sham, control, and vehicle groups (Fig. 6).

## DISCUSSION

Ischemia is a quick transitory drop in blood flow to the organ and subsequent reoxygenation, which is followed by a severe oxidative stress and inflammatory response to hypoxia and reperfusion, disrupting organ function [33] cause exacerbation in tubular and glomerular damage due to strong inflammatory response, called "reperfusion injury" [34]. This research aims to explain the nephroprotective impact of Tangeretin in the kidneys through decrease inflammation, oxidative stress, and apoptotic pathway. The current investigation found that renal IRI raised tissue levels of KIM1, IL-1 $\beta$ , and TNF- $\alpha$ , F2-isoprostane, and caspase-3 with decreased GSH in kidneys of rats. Interestingly, pre-treated with Tangeretin

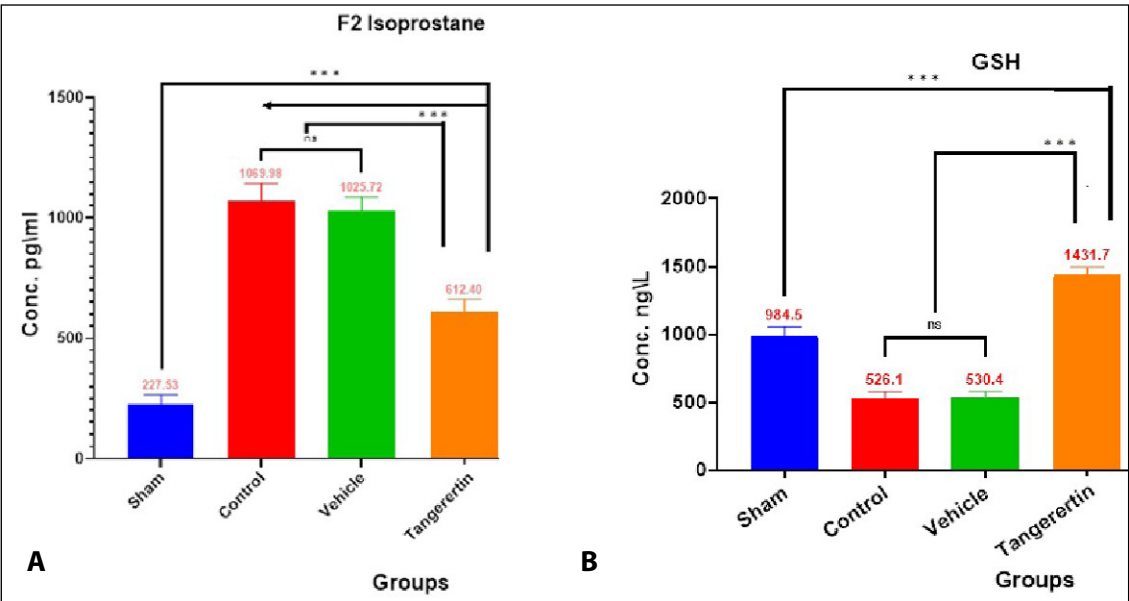


**Fig. 3.** Effect of Tangeretin on levels F2-isoprostane, GSH, and caspase-3 following renal IR, tissue F2-isoprostane (pg/ml), GSH (ng/L) and caspase-3 (ng/ml) between groups, Mean  $\pm$  SD, n=10, \*\*\*p $\leq$ 0.001 vs. sham; \*\*\*p $\leq$ 0.001 vs. control and vehicle  
Source: Own materials

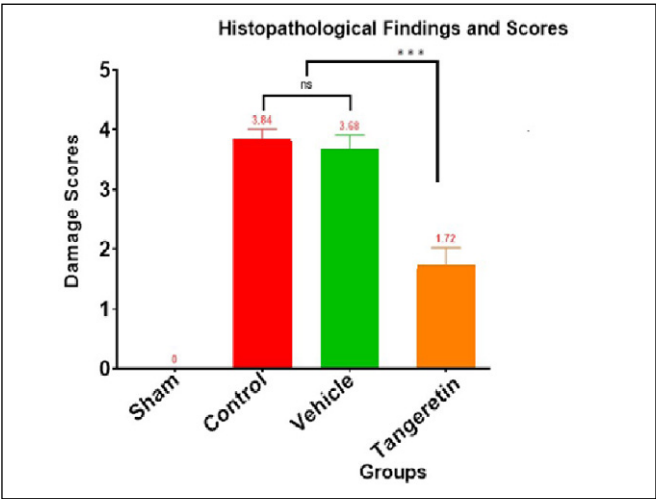


**Fig. 4.** Mean of Histopathological kidney injury score \*\*\*p < 0.001 vs. control & vehicle groups, the Kruskal-Wallis test was used for analysis, data are expressed as mean  $\pm$  SD  
Source: Own materials

increased the level of antioxidant (GSH) with reduced levels of KIM1, oxidative stress marker (F2-isoprostane), apoptotic marker (Caspase-3), and inflammatory markers (IL-1 $\beta$ , TNF- $\alpha$ ). According to [35], increasing the level of KIM-1 in the proximal tubule in early kidney damage has the advantage of being a sensitive, specific, and ideal biomarker marker for early diagnosis of kidney injury, including acute kidney injury (AKI), as well as a prognostic predictor. The results indicate that Tangeretin has a protective effect on kidney function measures (KIM) after renal IRI. The [36] found that Tangeretin reduce sig-

nificantly renal tissue levels of KIM-1 in cisplatin induced nephrotoxicity of rats kidney tissue which reflects amelioration in the kidney functions. The important parameters for detection of inflammation in RIRI are (TNF- $\alpha$ , IL-1 $\beta$ ). This result agreement with [37] which shown that the level of TNF- $\alpha$  was increased after 30 minutes of ischemia followed by 2 hours of reperfusion in a rat model. The reference [38] demonstrated that elevation of the inflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) as a result of hypoxia due to decreased in renal supply which cause infiltrate of different inflammatory cells into the injured tissue. Pre-treatment with Tangeretin significantly suppresses production of inflammatory markers TNF $\alpha$  and IL-1 $\beta$ . This consistent with a study by [36] revealed that Tangeretin decreases NF- $\kappa$ B activity lead to significantly decline in TNF- $\alpha$  and IL-1 $\beta$  protein levels in kidney tissue which alleviates oxidative stress and renal inflammation in cisplatin-induced nephrotoxic rats and enhancement of renal protective mechanisms of renal tubular cell injuries. The research showed significant elevation in F2 Isoprostane in renal tissue of the IRI group, that mean there is increase in the level of ROS formation due to oxidative stress which consist with study by [39] have established that during IRI, inflammation and tubular cell injury can be caused by a burst of (ROS); hence, lowering oxidative stress can prevent damage in IRI by assessing F2-isoprostane. The pre-treatment group with Tangeretin exhibited a substantial decrease in the level of F2 Isoprostane, and no prior study has demonstrated the effect of Tangeretin on (F2) in RIRI damage in a rat animal model. The reference [40] demonstrated that

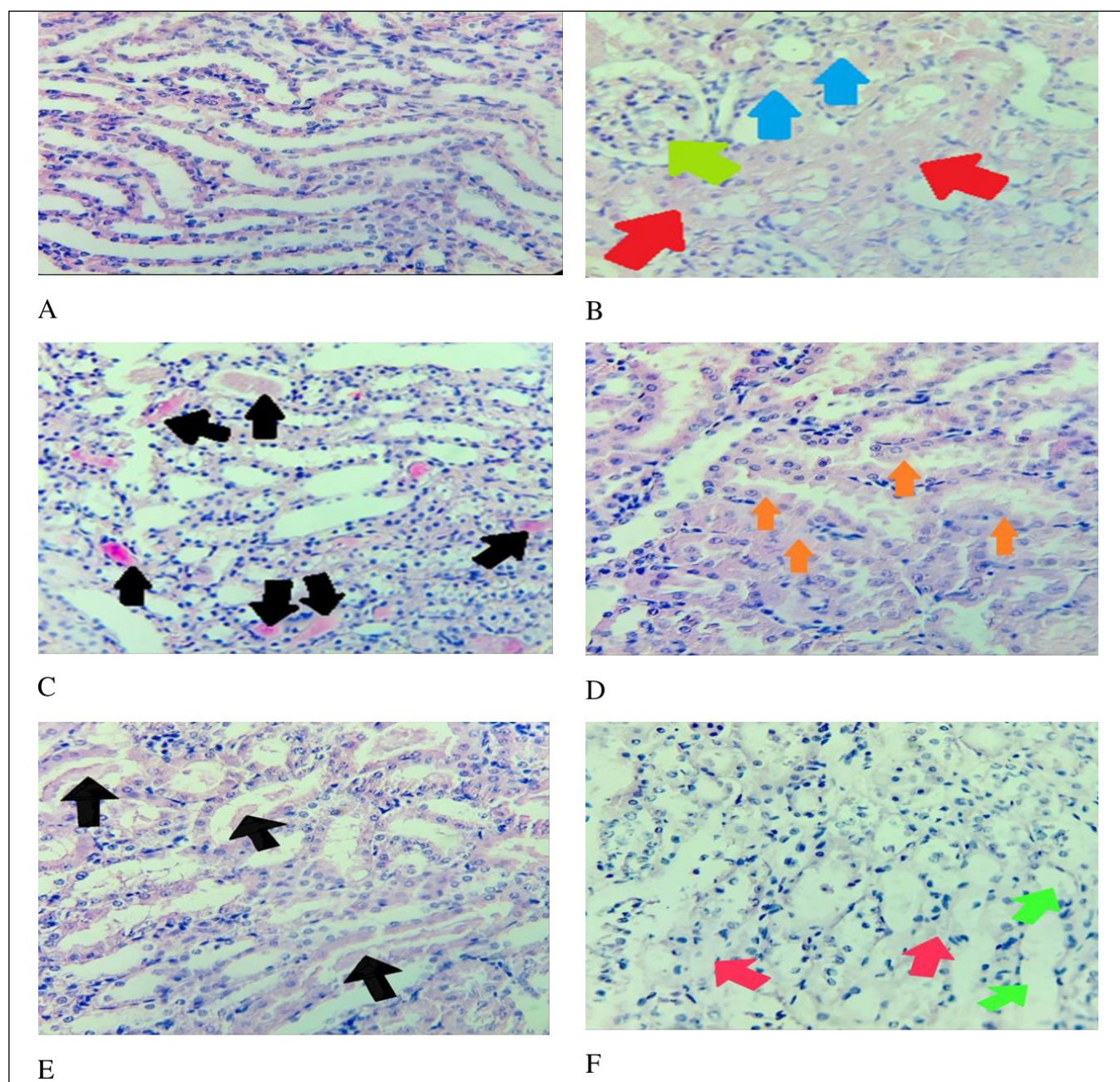


**Fig. 5.** Histopathological analysis of renal tissues, (A) Sham group has normal renal tubules, (B, C) Control group, renal tubules with score 4 damage, cytoplasmic swelling and increased cytoplasmic eosinophilia (red arrows), cytoplasmic vacuoles (blue arrows), eosinophilic cast (black arrows), normal glomerulus (green arrow), (D, E) Vehicle group, renal tubules with score 4 damage, Cytoplasmic swelling and increased cytoplasmic eosinophilia (red arrows), eosinophilic cast (black arrows), vascular congestion (orange arrow), (F) Tangeretin group renal tubules with score 2 damage, damaged tubules (red arrows), normal tubules (green arrows), H&E. X400

Source: Own materials

Tangeretin downstream the protein expression of nitric oxide (NO) in renal tissues, which cause attenuating renal oxidative stress and inflammation therefore have protective actions against cisplatin-induced renal injury in rats. The research showed significant reduction in Glutathione (GSH) in renal tissue of IRI group, this result agreement with [41] which indicated that GSH levels were considerably lower in the renal IRI and vehicle groups as compared to the sham group in response

to renal ischemia reperfusion, indicating that this is due to a decline in the kidney's capacity to serve as an antioxidant. The significantly higher the level of (GSH) in renal ischemic tissues of group that pre-treated with Tangeretin, [36] shown that Tangeretin pre-treatment group significant increased the levels of CAT, GSH and concentrations of SOD in renal tissues which alleviates renal oxidative stress and inflammation, these effects reduces renal nitric oxide (NO) and malondialdehyde



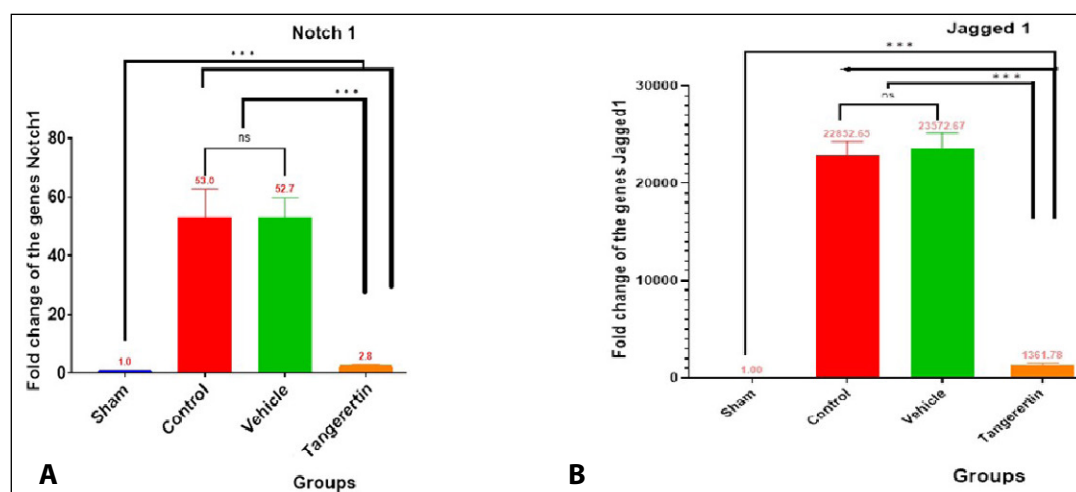


**Fig. 6.** (A) The bar graph Notch gene expression, (B) The bar graph Jagged-1 gene expression, All data were expressed as mean  $\pm$  SD (n = 10), Sham group vs. vehicle & control groups \*\*\*p < 0.001, Tangeretin treated group vs. vehicle & control groups, \*\*\*p < 0.001.

Source: Own materials

1(MDA) levels with concomitantly increases GSH concentrations which ameliorative nephrotoxic effect by cisplatin in the renal tissue and improve renal function. The significant increase of Caspase-3 in current study agreement with [42] shown that after thirty minutes of renal ischemia after that two hours of reperfusion in rats model the significant increase in Caspase-3 level with a significant decrease in Bcl-2, which explain the results that apoptosis is a conclusive process in renal IRI and the stimulation of caspase-3 have an important role for programmed cell death because the equilibrium between antiapoptotic (Bcl-2) proteins and pro-apoptotic (Bax) is essential for the survival of cells, the disruption. The significant decreased in Caspase-3 level in Tangeretin pre-treated group shown the nephroprotective effect of Tangeretin after renal IRI, the research by [43] revealed that the pre-treated group with Tangeretin can attenuate cisplatin induced apoptosis in hepatic injury tissues in rats model which inhibited the apoptotic markers Bax and caspase with increased the anti-apoptotic Bcl-2 with suppression of TNF- $\alpha$  and oxidative stress cause attenuation of hepatic tissue apoptosis. Furthermore, histological examinations confirmed Tangeretin have protective properties in kidney injury. These examinations demonstrated a significant reduction in renal tissue damage, including decrease in infiltration of inflammatory cells, and damage of renal tubules in rats treated with Tangeretin compared to control and vehicle groups, there has been no research to determine the effect of Tangeretin on reducing tissue damage in unilateral or bilateral renal ischemia/reperfusion injury models in rats or mice. The findings in the current study were parallel to the histopathological results through the ability of Tangeretin to decrease oxidative and the inflammatory markers and the results agreement with study by [36] which showed that Tangeretin pre-treatments groups markedly improved histological changes in the kidneys

against oxidative stress and inflammations induced by cisplatin through reduced serum levels of blood urea nitrogen (BUN) and creatinine with declined renal levels of the inflammatory cells into the perivascular and sub vascular areas. Moreover, in the present study the level of Notch gene expression in renal tissue of IRI group significantly greater than sham group, this finding is consistent with [44] that show there was a significant upregulation of Notch expression in the endothelial cells and increased cleaved Notch1 was found in all parts of the post ischemic kidney with strongest signal intensity in proximal tubules. The reference [45] revealed that Notch signaling plays a key role in the developing kidney by regulation of ontogenetic nephron segmentation which strongly reduced in the adult kidney but reactivation of Notch has been appeared in chronic and acute kidney injury. The level of Notch1 pathway gene expression was significantly lower in Tangeretin treated group than IRI control group, there is no previous study demonstrate the effect of Tangeretin on Notch1 Jagged1 gene expression in renal ischemia reperfusion injury in an animal model. The reference [46] demonstrated that Tangeretin could specifically inhibit the expression of Notch1 and Jagged1 in CD4 + T cells which can mitigate allergic rhinitis by inhibiting the expression of Notch1 and Jagged1.

The data in the present study adds novel insights about the effects of Tangeretin to protect the tissue damage due to ischemia/reperfusion in kidney as show by [40] that Tangeretin markedly attenuates cisplatin-induced histopathological alterations in renal tissues and preserved the renal architecture with mitigation of renal leukocyte recruitment, the changes in renal tissues due to free radical generation along with lowering of cellular anti-oxidants enhanced renal cellular damage, tangeretin which have antioxidant action reversed the generation of lipid peroxides and NO with augmented the cellular GSH and GPx.

## CONCLUSIONS

Tangeretin shown a nephroprotective effect due to ischemia/reperfusion (I/R). Tangeretin exhibited anti-apoptotic, antioxidant, and anti-inflammatory characteristics by

significant decreased in inflammatory, oxidative apoptotic markers and Notch1 and Jagged-1 expression in kidney tissues after renal ischemia/reperfusion damage. Therefore it may be a promising treatment people with RIRI.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# The impact of wartime factors on harmful habits among the adult population depending on their psycho-emotional state

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## ABSTRACT

**Aim:** To analyse patterns of alcohol and tobacco consumption among adult Ukrainians, including variations based on their psycho-emotional state, using an anonymous survey conducted during martial law.

**Materials and Methods:** The study employed an online survey based on the "Study of Eating Behaviour and Health Status of the Ukrainian Population" questionnaire. The final sample comprised 1,101 adult Ukrainian respondents: 212 (19,3%) men and 889 (80,7%) women, with a mean age of  $36 \pm 13$  years. Participants were divided into Group I ( $n = 400$ ; 36,3%) – adults with probable PTSD, and Group II ( $n = 701$ ; 63,7%) – without signs of probable PTSD.

**Results:** The study revealed a high prevalence of harmful habits among adults (nearly 80,0% consumed alcohol and 30,0% smoked). Respondents with probable PTSD symptoms were 2.5 times more likely to report increased alcohol consumption (34,0% vs. 13,5%;  $p < 0,001$ ) and 1.5 times more likely to report increased smoking (65,0% vs. 45,0%;  $p = 0,009$ ) compared with those without such symptoms. Only 7,0% of alcohol consumers and 32,4% of regular smokers who had visited a doctor in the past year reported receiving advice to quit these habits.

**Conclusions:** The onset of the full-scale war in Ukraine has negatively affected adult lifestyles, notably increasing alcohol and tobacco use. Most respondents with harmful habits reported not receiving medical advice on reducing or quitting them.

**KEY WORDS:** alcohol, smoking, post-traumatic stress disorder

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## INTRODUCTION

The full-scale invasion of Ukraine by the terrorist state has become a global stressor for Ukrainians, introducing tangible risks of a technological, environmental, psychological, political, and military nature. These factors have triggered adverse changes in people's health, including its psychological components.

According to Ukraine's electronic healthcare system, the number of post-traumatic stress disorder (PTSD) cases in 2023 increased nearly fourfold compared with 2021. In the first two months of 2024 alone, 3292 cases were recorded—almost equalling the total for 2021 (3167) [1]. Harmful habits such as alcohol consumption and smoking remain highly prevalent and have severe socio-medical consequences [2–5]. Alcohol use contributes to over 200 diseases and injuries and accounts for approximately 5% of the global disease burden; among individuals under 40 years, 13,5% of deaths are alcohol-related. In Ukraine, per capita alcohol consumption

among those aged  $\geq 15$  years is 8,6 liters annually [6–8]. A WHO-supported survey conducted in December 2023 showed that 77,4% of adults had consumed alcohol in the past year, 50,8% drank at least monthly, and the highest prevalence (86,3%) was observed among respondents aged  $\leq 29$  years [9].

Tobacco use is among the most harmful lifestyle factors, linked to chronic diseases and premature mortality [10–20]. According to WHO, tobacco kills about 50% of its users, causing over 8 million deaths annually, including 1,3 million from second-hand smoke; in 2020, 22,3% of the global population used tobacco [19]. A national survey conducted in April 2023, "The Use of Tobacco and Nicotine Products in Ukraine," found that 27,4% of adults were current users, including 23,0% daily and 6,8% occasional users [20, 21].

Given the above data, it is evident that a pressing issue in Ukraine today is the analysis of the impact of wartime factors on lifestyle and health.

## AIM

To analyze the characteristics of alcohol and tobacco consumption among adult Ukrainians, including the influence of their psycho-emotional state, through an anonymous survey conducted during martial law.

## MATERIALS AND METHODS

The study used a secure online survey based on the questionnaire "Study of Dietary Behaviour and Health Status of the Ukrainian Population" [22]. The questionnaire, information sheet, and consent form were approved by the Institute's Bioethics Committee (Protocol No. 13, 27 Oct 2021). Following the full-scale invasion on 24 Feb 2022, additional questions on wartime impact, alcohol, and tobacco use were incorporated and approved by the Academic Council (Protocol No. 7, 3 Aug 2022).

Respondents received an information sheet explaining the study and provided online informed consent. Participation was voluntary, with withdrawal permitted at any stage. The survey was conducted from December 2022 to January 2024 among Ukrainian adults ( $\geq 18$  years) using snowball sampling. Data collection and database formation followed international Good Epidemiological Practice standards.

The final sample included 1,101 adults (212 [19,3%] men and 889 [80,7%] women; mean age  $36 \pm 13$  years). The age distribution was as follows: 18–30 years – 37,4%; 31–40 – 30,0%; 41–50 – 18,7%; 51–60 – 8,3%; 61–70 – 4,5%; and  $>70$  – 1,1%. Responses to questions about alcohol and tobacco/nicotine consumption, including changes since the onset of the full-scale invasion, were used to assess harmful habits.

To explore possible associations between alcohol/tobacco consumption and changes in psycho-emotional states during the full-scale invasion, respondents completed a block of questions from the Unified Clinical Protocol for Primary, Secondary, and Tertiary Medical Care "Reaction to Severe Stress and Adaptation Disorders. Post-Traumatic Stress Disorder" [23]. The block included seven binary ("yes"/"no") questions;  $\geq 4$  positive responses indicated probable PTSD. The internal consistency index (Cronbach's alpha) for this block of questions in the studied sample was 0,625.

Participants were divided into two groups: Group I – 400 (36,3%) adults with probable PTSD, and Group II – 701 (63,7%) without. Harmful habits were assessed via self-reported alcohol and tobacco use; only valid responses were analyzed.

Statistical data analysis was performed using IBM SPSS Statistics v. 27.0 (Armonk, NY, IBM Corp., USA). Quantitative data were presented as mean  $\pm$  standard

deviation, and qualitative parameters as absolute and relative (%) frequencies. The Student's *t*-test was used to compare quantitative data between two independent samples. Qualitative parameters between two independent groups were compared using the  $\chi^2$  test (with subsequent z-test for subcategory frequency comparison) or Fisher's exact test (for binary parameters). A *p*-value  $< 0,05$  was considered statistically significant.

## ETHICS

The study was conducted in accordance with the standards outlined in the Helsinki Declaration of the World Medical Association «Ethical Principles for Medical Research Involving Human Subjects». Informed consent to participate in the survey was obtained from all participants before the study began. No violations of ethical and deontological principles regarding the respondents, staff involved at any stage of the study, institutional regulations, or current Ukrainian legislation were identified.

## FRAMEWORK

The study was conducted within the framework of the research project "Studying the Impact of Armed Aggression by the Russian Federation on the Health of Children and Women in Ukraine" (state registration No. 0124U000209; research implementation period: 2024–2025) at the Department of Medical and Psychosocial Problems of Family and School-Age Children's Health, State Institution "Ukrainian Center of Maternity and Childhood of the National Academy of Medical Sciences of Ukraine".

## RESULTS

The study groups were comparable by gender (women: 80,0% vs. 81,2%; *p* = 0,636), marital status (married: 62,2% vs. 60,4%; *p* = 0,692), and education level (higher education: 75,1% vs. 75,6%; *p* = 0,858). Additionally, respondents from Group I were slightly but not meaningfully older than those from Group II ( $38 \pm 13$  vs.  $36 \pm 13$  years, respectively; *p* = 0,022).

The study revealed that the majority of respondents (79,8%) consume alcohol. Among the 1101 surveyed adults, 242 (22,0%) reported drinking alcohol a few times per year, 287 (26,1%) 1–2 times per month, 195 (17,7%) 3–4 times per month, 135 (12,3%) 2–3 times per week, and 20 (1,8%) daily. A total of 100 respondents (9,1%) reported never consuming alcohol, while 122 (11,1%) had quit drinking. Among those who quit (*n* = 122), 74 (60,7%) made the decision independently,

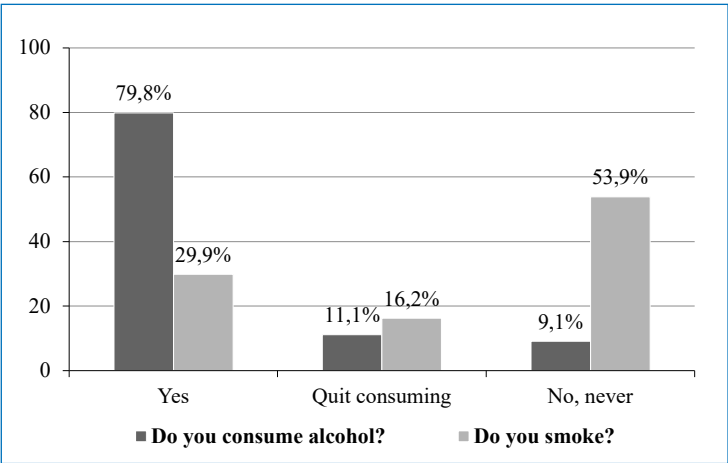


**Fig. 1.** The distribution (%) of respondents' answers regarding alcohol consumption and tobacco/nicotine product use after combining response options (n=1101)  
*Picture taken by the authors*

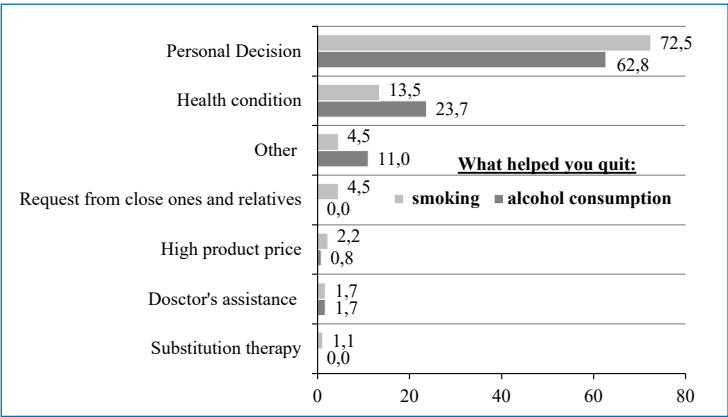


**Fig. 2.** The distribution of respondents' answers regarding reasons that helped quit harmful habits (alcohol consumption and smoking)  
*Picture taken by the authors*

28 (23,0%) quit due to health deterioration, a few (7 [5,7%]) cited high alcohol prices or support from a healthcare provider as their reason, and 13 (10,6%) were undecided about the reasons for quitting.

Analysis of another harmful habit—smoking—showed that nearly one-third of the adult Ukrainian cohort (1,101 individuals) smoked tobacco-containing cigarettes: 329 respondents (29,9%). Among the smokers, 58 (5,3%) smoked but not daily, 53 (4,8%) consumed 1–7 cigarettes per day, 57 (5,2%) 8–20 cigarettes per day, and 22 (2,0%) smoked more than one pack per day. Additionally, 139 respondents (12,6%) reported using electronic cigarettes or similar devices. Half of the respondents (593 individuals, 53,9%) stated they had never smoked, while 179 (16,2%) reported quitting smoking (Fig. 1).

It was established that among the 879 individuals who consumed alcohol, only 116 (13,2%) had attempted to quit within the past 12 months. Among respondents who visited a doctor during the last year, only 49 (5,6%) reported receiving advice to reduce or stop drinking alcohol, while 155 (17,8%) indicated that they had not visited a doctor during this period.

Of the 179 respondents who quit smoking within the previous 12 months, the most frequently cited reason was a self-motivated decision, reported by

129 individuals (72,5%). For 24 respondents (13,5%), health concerns served as the primary motivation. In rare cases, respondents quit smoking at the request of relatives, due to the high cost of cigarettes, with the help of a healthcare provider, or through replacement therapy. Eight individuals (4,5%) were undecided about their reason for quitting.

For further analysis of smoking cessation attempts, individuals who used electronic cigarettes or smoked infrequently (n=197) were excluded. Among the remaining 132 regular cigarette smokers, only 26 (19,7%) had attempted to quit this harmful habit within the past year. The number of respondents who regularly smoked cigarettes, visited a doctor within the past 12 months, and received advice to quit smoking was 24 (18,2%).

A descriptive analysis of the identified reasons that helped adult Ukrainians quit harmful habits is presented in Fig. 2.

A specific block of questions focused on possible relationships between wartime factors, lifestyle features (harmful habits), and the psycho-emotional state of Ukrainians.

It was found that since the beginning of the war, every fifth respondent reported increased alcohol consumption, i.e. 187 individuals (21,4%), while 685 (78,6%) indicated no changes. Among cigarette smokers, every

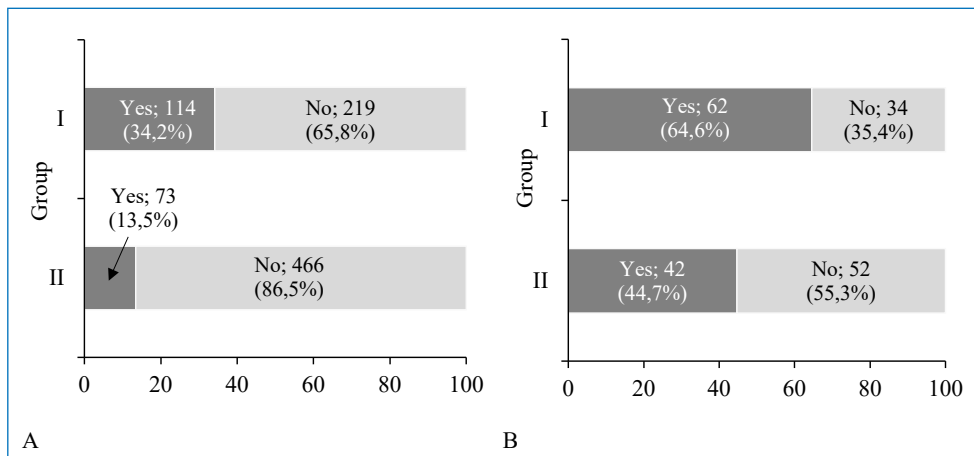


**Fig. 3.** The distribution of respondents (available data) by answers to the question «Have you started consuming more alcohol due to the onset of military actions?» ( $p < 0,001$ ) (A). The distribution of respondents (available data) by answers to the question «Have you started smoking more due to the onset of military actions?» ( $p = 0,009$ ) (B). Group I – the signs of probable PTSD; Group II – no probable signs of PTSD  
Picture taken by the authors

**Table 1.** The distribution of responses to questions on alcohol consumption depending on the psycho-emotional state of respondents

| Questions and responses                                                                                  |                                                                       | PTSD           |                | p      |
|----------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|----------------|----------------|--------|
|                                                                                                          |                                                                       | Group I (Yes)  | Group II (No)  |        |
| Do you consume alcohol?                                                                                  | Yes, n/N (%) <sup>z</sup>                                             | 336/400 (84.0) | 543/701 (77.4) | 0.033  |
|                                                                                                          | Stopped drinking alcohol, n/N (%)                                     | 36/400 (9.0)   | 86/701 (12.3)  |        |
|                                                                                                          | No, never, n/N (%)                                                    | 28/400 (7.0)   | 72/701 (10.3)  |        |
| Have you tried to quit alcohol consumption within the past 12 months                                     | Yes, n/N (%)                                                          | 64/336 (19.0)  | 52/543 (9.6)   | <0.001 |
|                                                                                                          | No, n/N (%)                                                           | 272/336 (81.0) | 491/543 (90.4) |        |
| Have you been advised to reduce or stop alcohol consumption during a medical visit in the past 12 months | Yes, n/N (%) <sup>z</sup>                                             | 26/336 (7.7)   | 23/543 (4.2)   | <0.001 |
|                                                                                                          | No, n/N (%) <sup>z</sup>                                              | 223/336 (66.4) | 428/543 (78.8) |        |
|                                                                                                          | Have not visited a doctor in the past 12 months, n/N (%) <sup>z</sup> | 87/336 (25.9)  | 92/543 (17.0)  |        |
|                                                                                                          | Yes, n/N* (%)                                                         | 26/249 (10.4)  | 23/451 (5.1)   |        |
|                                                                                                          |                                                                       |                |                | 0.013  |

Note: \* – among healthcare providers' visitors; z – statistically significant difference by z-test ( $p < 0,05$ )

Source: compiled by the authors of this study

**Table 2.** The distribution of responses to questions on tobacco and nicotine product use depending on the psycho-emotional state of respondents, n (%)

| Questions and responses                                                               |                                 | PTSD           |                | p      |
|---------------------------------------------------------------------------------------|---------------------------------|----------------|----------------|--------|
|                                                                                       |                                 | Group I (Yes)  | Group II (No)  |        |
| Do you smoke?                                                                         | Yes, n/N (%) <sup>z</sup>       | 147/400 (36.7) | 182/701 (26.0) | <0.001 |
|                                                                                       | Stopped smoking, n/N (%)        | 67/400 (16.8)  | 112/701 (16.0) |        |
|                                                                                       | No, never, n/N (%) <sup>z</sup> | 186/400 (46.5) | 407/701 (58.0) |        |
| Have you tried to quit smoking within the past 12 months?                             | Yes, n/N* (%)                   | 14 (21.5)      | 12 (17.9)      | 0.665  |
|                                                                                       | No, n/N* (%)                    | 51 (78.5)      | 55 (82.1)      |        |
| Have you been advised to quit smoking during any medical visit in the past 12 months? | Yes, n/N* (%)                   | 14 (40.0)      | 10 (25.6)      | 0.220  |
|                                                                                       | No, n/N* (%)                    | 21 (60.0)      | 29 (74.4)      |        |

Note: \* – available data among regular smokers; z – statistically significant difference by z-test ( $p < 0,05$ )

Source: compiled by the authors of this study

third person smoked more, i.e. 104 individuals (54,7%), while 86 (45,3%) made no changes to their smoking habits.

The next stage of the scientific study involved examining potential links between harmful habits and changes in the psycho-emotional state of adults during the full-scale invasion of Ukraine by the Russian Federation. Respondents were divided into two groups based on psycho-emotional state criteria: Group I – respondents with signs of PTSD ( $n = 400$ ); Group II – respondents without signs of PTSD ( $n = 701$ ).

The findings revealed that most adults, regardless of their psycho-emotional state, consumed alcohol, with a higher frequency in Group I compared to Group II (84,0% and 77,4%, respectively;  $p = 0,010$ ) (Table 1). Among consumers, attempts to quit alcohol during wartime were reported by 19,0% of those with PTSD symptoms compared to 9,6% of those without PTSD symptoms ( $p < 0,001$ ). Overall, fewer than 10,0% of respondents received recommendations from healthcare providers to reduce alcohol consumption (49 of 700 [7,0%] visitors), particularly 5,1% in Group II versus 10,4% in Group I ( $p = 0,013$ ) (Table 1).

The characteristics of respondents' use of tobacco and nicotine products depending on their psycho-emotional state are presented in Table 2. It was also found that among the surveyed Ukrainians, 36,7% of adults with PTSD symptoms and 26,0% of those without such symptoms smoked ( $p < 0,001$ ). Among regular smokers with available data, 21,5% and 17,9% had attempted to quit smoking within the last 12 months, respectively. Moreover, during visits to a doctor, 40,0% of respondents in Group I and 25,6% in Group II reported receiving advice to quit smoking (in total, 24 of 74 [32,4%] respondents), which was higher than the proportion of similar advice provided regarding alcohol consumption.

It was determined that, due to the onset of military actions, respondents with PTSD symptoms were twice as likely (34,2%) to report an increase in alcohol consumption compared to those without PTSD symptoms (13,5%) ( $p < 0,001$ ) (Fig. 3).

The beginning of military actions acted as a trigger for increased consumption of tobacco and nicotine products among the majority of individuals in Group I (64,6%) compared to 44,7% in Group II ( $p = 0,009$ ) (Fig. 3).

## DISCUSSION

The present study analyzed the impact of wartime factors on the prevalence of harmful habits among the adult population of Ukraine and established a connection with changes in their psycho-emotional state. The

analysis revealed a high prevalence of these habits: nearly 80,0% of adult respondents consumed alcohol, and 30,0% smoked. These figures are alarming and generally consistent with the results of other national surveys conducted in Ukraine during the same period. Specifically, a WHO-supported sociological survey conducted in December 2023 found that 77,4% of Ukrainians had consumed alcohol in the past 12 months, which is almost identical to our findings (79,8%) [24]. A similar consistency was observed for smoking: our study identified 29,9% of respondents as smokers, while a sociological survey in April 2023 recorded 27,4% as current users of tobacco products [21]. This close alignment of results supports the representativeness of our sample and the validity of the data obtained.

The key and most revealing finding of our study is the direct correlation between probable symptoms of PTSD and the dynamics of substance use. It was established that respondents with probable PTSD symptoms not only had a higher prevalence of harmful habits (84,0% consumed alcohol and 36,7% smoked, compared to 77,4% and 26,0% in the group without PTSD symptoms, respectively) but were also significantly more likely to increase their consumption. Individuals with probable signs of PTSD were 2,5 times more likely to report an increase in alcohol consumption (34,2% vs. 13,5%) and nearly 1,5 times more likely to report an increase in smoking intensity (64,6% vs. 44,7%) compared to those without PTSD symptoms. These findings convincingly indicate that alcohol and tobacco are being used as maladaptive coping strategies for stress. The results correlate with numerous international studies demonstrating that individuals who have experienced traumatic events often turn to substance use as a form of self-medication to alleviate symptoms of anxiety, intrusive memories, and emotional numbness characteristic of PTSD [20, 21, 25].

Against the backdrop of a significant rise in diagnosed PTSD cases in Ukraine, which increased nearly fourfold in 2023 compared to 2021, our findings point to the formation of a dangerous trend. Attempts to "self-medicate" the manifestations of psychological trauma with alcohol and nicotine do not solve the underlying problem but rather deepen it, creating the risk of developing a dual diagnosis—the comorbidity of PTSD and chemical dependency. This, in turn, complicates the treatment of both conditions and significantly worsens the long-term health prognosis for the individual.

Particularly concerning is the fact that the healthcare system appears to be not optimally addressing this problem. Our study revealed that the vast majority of respondents with harmful habits had not received any medical recommendations to reduce or quit them. In



particular, only 7,0% of alcohol consumers who had visited a doctor received such advice, and only 32,4% of regular smokers were advised to quit. This indicates a significant gap in primary healthcare delivery and highlights the urgent need to implement screenings for substance use and brief interventions during routine medical visits, especially when working with patients living under conditions of chronic stress.

Certain limitations of our study should be acknowledged. First, the use of the “snowball sampling” method for respondent recruitment and the significant predominance of women in the sample (80,7%) may limit the applicability of the results to the entire adult population of Ukraine. Second, the diagnosis of probable PTSD symptoms was conducted using a screening questionnaire rather than a clinical interview, which is the gold standard. Nevertheless, the findings clearly illuminate a serious public health issue. They underscore the ur-

gent need for the development and implementation of comprehensive psychological support and stress prevention programs that offer healthy alternatives for coping with the consequences of trauma, as noted in our perspectives for future research. The integration of mental health and substance abuse services is critically important for overcoming the negative health consequences of the war on the nation.

## CONCLUSIONS

It can be stated that the onset of the full-scale military operation in Ukraine, as a traumatic stress factor, negatively affected the lifestyle of adults, particularly by increasing alcohol and tobacco consumption. At the same time, the majority of respondents with harmful habits did not receive medical recommendations to reduce or quit these behaviors.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Silibinin nephroprotective effects in renal ischemia reperfusion injury in rats *via* regulation of Notch-1

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## ABSTRACT

**Aim:** To evaluate nephroprotective effects of Silibinin in renal ischemia -reperfusion injury in rats by regulation of Notch-1 and Jagged-1.

**Materials and Methods:** 28 male Wistar albino rats were randomly divided into four groups, (seven rats / group): sham group (laparotomy only), control group (ischemia for 30 min / 2 hrs reperfusion), DMSO vehicle group (rats were injected intraperitoneally with 10% DMSO 1 hour before ischemia, then ischemia for 30 min / 2 hrs reperfusion, Silibinin treatment group (rats were injected with Silibinin (60 mg/kg) intraperitoneally 1 hour before ischemia, then "ischemia for 30 min / 2 hrs reperfusion. The kidneys were harvested after 2 hours of reperfusion for assessment of kidney injury molecule-1 (KIM-1), interleukin 1  $\beta$  (IL-1 $\beta$ ), TNF- $\alpha$ , F2-Isoprostane, and glutathione (GSH) by ELISA technique; Notch-1 and Jagged-1 mRNA levels were assessed by reverse transcription-polymerase chain reaction (RT-PCR), BAX and Bcl2 protein expression were assessed by immunohistochemistry (IHC).

**Results:** Silibinin demonstrated considerable amelioration of renal ischemia reperfusion injury as evidenced by the significant reduction of KIM-1, IL-1 $\beta$ , TNF  $\alpha$ , F2-Isoprostane, in rat kidneys pretreated with Silibinin, GSH level was significantly elevated in rat kidneys pretreated with silibinin. Furthermore silibinin pretreatment significantly reduced Notch-1 and Jagged-1 mRNA expression after renal ischemia reperfusion.

**Conclusions:** Silibinin offers protection against renal ischemia reperfusion injury due to its antioxidant, anti-inflammatory, and anti-apoptotic effects, those effects are associated with the downregulation of Notch-1 and its ligand, Jagged-1.

**KEY WORDS:** renal ischemia reperfusion injury, silibinin, Notch-1, Jagged-1

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## ABBREVIATIONS

RIRI: Renal Ischemia Reperfusion Injury

AKI: Acute Kidney Injury

DGF: Delayed Graft Function

ROS: Reactive Oxygen Species

MAM: Mastermind Transcription Factor

NICD: Notch Intracellular Domain

Hes: hairy and enhancer of split

Hey: hairy/enhancer-of-split linked with YRPW motif

NF- $\kappa$ B: nuclear factor kappa B

ELISA: Enzyme Linked Immunosorbent Assay

## INTRODUCTION

Acute kidney injury (AKI) is characterized by a sudden decrease in kidney function, which leads to internal milieu disruptions and uremic toxin retention, which primarily affects the immunological, cardiovascular, and neurological systems [1]. One of the major contributors to acute kidney injury (AKI) is renal ischemia-reperfusion injury (RIRI). It often occurs during kidney transplantation, where it may

cause delayed graft function (DGF) that can lead to loss of vital kidney tissue and activates immune responses that can provoke rejection, ultimately leading to the loss of the graft [2]. In addition to kidney transplants, RIRI can also develop in other conditions associated with reduced blood flow or oxygen supply, such as thrombotic disorders, cardiac surgeries, traumas, and sepsis [3-4]. Reperfusion of an ischemic kidney worsens oxidative stress and inflammation, resulting in damage to DNA and proteins, ultimately causing cell apoptosis and necrosis. The main mechanisms behind this process include the release of pro-inflammatory substances, activation of genes related to apoptosis, production of reactive oxygen species (ROS), and excess calcium levels [5-6]. The Notch signaling pathway plays a crucial role in regulating important developmental processes, such as lateral inhibition, lineage decisions, and the formation of boundaries, in addition to contributing to homeostasis and regeneration in adult tissues. It is a type of juxtacrine signaling pathway where both the receptors and ligands are transmembrane proteins that facilitate communication between neighboring

cells. For trans-signaling events to occur, direct contact between the cells is necessary, allowing the receptor to bind with its ligand [7]. Signal transduction occurs at the moment of ligand binding to the receptor which is followed by two successive proteolytic cleavages leading to the release of the Notch Intracellular Domain (NICD) which Translocates to the nucleus to enhance transcription of Notch target genes [8]. While Notch signaling is notably reduced in the adult kidney, research indicates that both acute and chronic kidney injuries can lead to a reactivation of Notch signaling [9] analysis of the kidneys of mice after induction of renal IRI showed an increased expression of processed Notch-2, Delta-1, and Hes-1 mRNA and protein. This indicates that Notch2 is involved in the proliferation of epithelial cells [10]. Silibinin is the primary biologically active component of silymarin which is a specific combination of polyphenolic flavonoids extracted from milk thistle extract. It is a mixture of two diastereomers (A and B). Silibinin's potent anti-hepatotoxic effects have led to its widespread use as a dietary supplement due to its great human acceptability. Milk thistle plant has been utilized for liver disease therapy for over 2,000 years [11]. The nephroprotective effects of silibinin in cisplatin induced AKI were examined by study which revealed that silibinin ameliorated cisplatin-induced AKI via decreasing ROS-mediated MAPK signaling pathway activation, which was confirmed using the inhibitor N-acetylcysteine. Moreover, the protective effect of silibinin against cisplatin-induced ROS generation through the antioxidant transcription factor nuclear factor-erythroid 2-related factor 1 (Nfe2l1), rather than Nfe2l2, mediates HO1 expression [12]. Limited number of studies were performed to examine the nephroprotective effects of silibinin in renal IRI. Deng et al have shown that silibinin ameliorated renal dysfunction and inflammation in mice model of renal IRI by inhibiting ferroptosis through binding to ferritin heavy chain 1 (FTH1) [13].

## AIM

The present study aims to investigate the potential nephroprotective effects of silibinin in renal ischemia reperfusion injury rat model and to investigate the role of Notch signaling pathway in mediating these effects.

## MATERIALS AD METHODS

### ANIMAL PREPARATION AND ETHICAL CONSIDERATIONS

The research received approval from the Institutional Animal Care and Use Committee (IACUC) at Kufa University. A total of twenty-eight male Wistar albino rats, each

weighing between 250-300 grams, were obtained from the Animal Resources Centre at the College of Science at Kufa University. The rats were housed in the university's animal facility, maintained at a controlled environment of  $25\pm 2^{\circ}\text{C}$  and 60-65% humidity, with a 12-hour light/dark cycle. They were provided with unrestricted access to water and standard laboratory chow.

### STUDY DESIGN

Following a two-week acclimation period, the rats were divided into four groups, each comprising seven rats as follows: Sham group: rats were subjected to anesthesia and surgery without the induction of renal ischemia, control group: rats were subjected to 30 minutes of bilateral renal pedicle clamping to induce renal ischemia, followed by a two-hour reperfusion period, DMSO vehicle group in which rats were injected intraperitoneally (i.p.) with 10% DMSO the vehicle for silibinin 1 hour before induction of ischemia followed by bilateral renal pedicles clamping for 30 minutes and then reperfusion for two hours, and silibinin treatment group: rats were injected with silibinin (60 mg/kg) [14] dissolved in 10% DMSO i.p. 1 hour before induction of ischemia followed by bilateral renal pedicles clamping for 30 minutes and then reperfusion for two hours.

### BILATERAL RENAL ISCHEMIA REPERFUSION INJURY MODEL

Rats were anesthetized using an intraperitoneal injection of a mixed solution containing ketamine at a dosage of 100 mg/kg and xylazine at 10 mg/kg. They were then placed in cages maintained at a stable temperature to prevent hypothermia. Once the loss of righting reflex was confirmed, the rats were laid on their backs, and their limbs and tail were secured with stickers to ensure stability during the surgical procedure. After making a midline laparotomy incision, the intestines were retracted to expose the abdominal cavity and renal pedicles [15]. The renal pedicles were carefully separated, and microvascular clamps were applied around them. Within one to two minutes, the kidney's color shifted from red to deep purple, indicating ischemia [15]. After a thirty-minute period, the clamps were taken off to allow blood flow to return, initiating the reperfusion phase. The abdominal incision was then closed using 3/0 silk sutures. Following two hours of reperfusion, the rats were anesthetized once more, and both kidneys were excised. The rats were then euthanized using an excessive dose of ketamine. The left kidney was kept in liquid nitrogen for the assessment of experimental parameters.

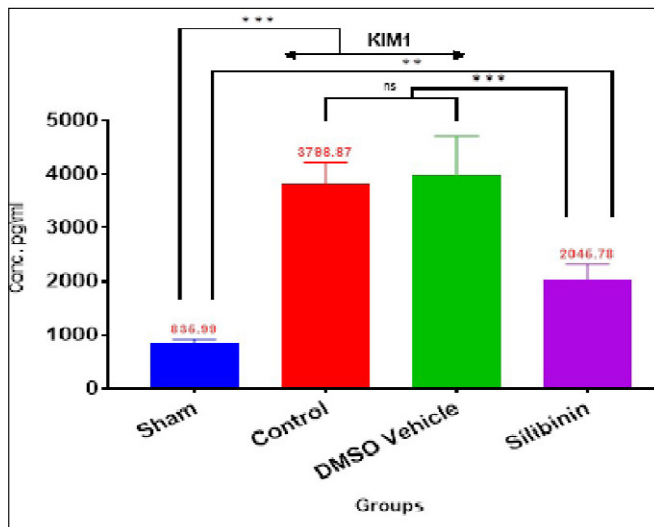


**Fig. 1.** Effect of silibinin on KIM-1 renal tissue concentration (pg/ml), no. of animals in each group=7

\*\* $p < 0.01$ , \*\*\* $p < 0.001$

Source: Own materials

## EVALUATION OF KIM-1, IL-1 $\beta$ , TNF $\alpha$ , F2 ISOPROSTANE, AND GSH RENAL TISSUE CONCENTRATION BY ELISA TECHNIQUE

After surgically removing the left kidney from each rat, the organ was rinsed with cold normal saline to ensure that all blood clots were eliminated. Next, the kidney was cut into two halves. One half was subjected to homogenization using a high-intensity ultrasonic liquid processor, mixed in a 1:10 (w/v) dilution of phosphate-buffered saline (PBS) that included 1% Triton X-100 and a cocktail of protease inhibitors. The homogenate

was subsequently centrifuged at 4°C for 15 minutes at 22,132  $\times g$ . The supernatant was collected and distributed into Eppendorf tubes for further analysis of KIM1, IL-1 $\beta$ , and TNF- $\alpha$ , GSH, and F2 Isoprostane using the ELISA technique. The kits were purchased from SunLong Biotech Co.LTD, China. (KIM-1 kit Catalogue No.: SL0433Ra, TNF- $\alpha$  kit Catalog No.: HS-EL0012Ra, IL-1 $\beta$  kit Catalog No.: EL0040Ra, F2 isoprostane kit catalogue NO.:SLD2059Ra, GSH kit Catalogue No.:SL1410Ra).

## IMMUNOHISTOCHEMISTRY (IHC) ANALYSIS OF BAX AND BCL2

The Immunohistochemistry analysis was performed in The Middle Euphrates Cancer Research Unit at Kufa University. Formalin fixed paraffin embedded section slides were used for this method. Primary antibodies were used to prob the sections against the required antigens. The primary antibodies were purchased from Elabscience<sup>®</sup>, USA (BAX catalog NO. E-AB-13814), (Bcl2 catalog NO. E-AB-22004).

Labeled secondary antibodies were then added to assess the target protein expression. An enzymatic label was used to detect the antigen-antibody combination. The IHC analysis depended on labelled streptavidin-biotin staining method (LSAB). H score calculations depends on the intensity of staining and the proportion of cells affected; the staining intensity is given a score range from (0-3). Zero for no stain, 1 denotes weak intensity staining, 2 denotes moderate staining, while 3 represent strong staining, the H score for each sample is obtained by multiplying the staining intensity score by the proportion of cells affected.

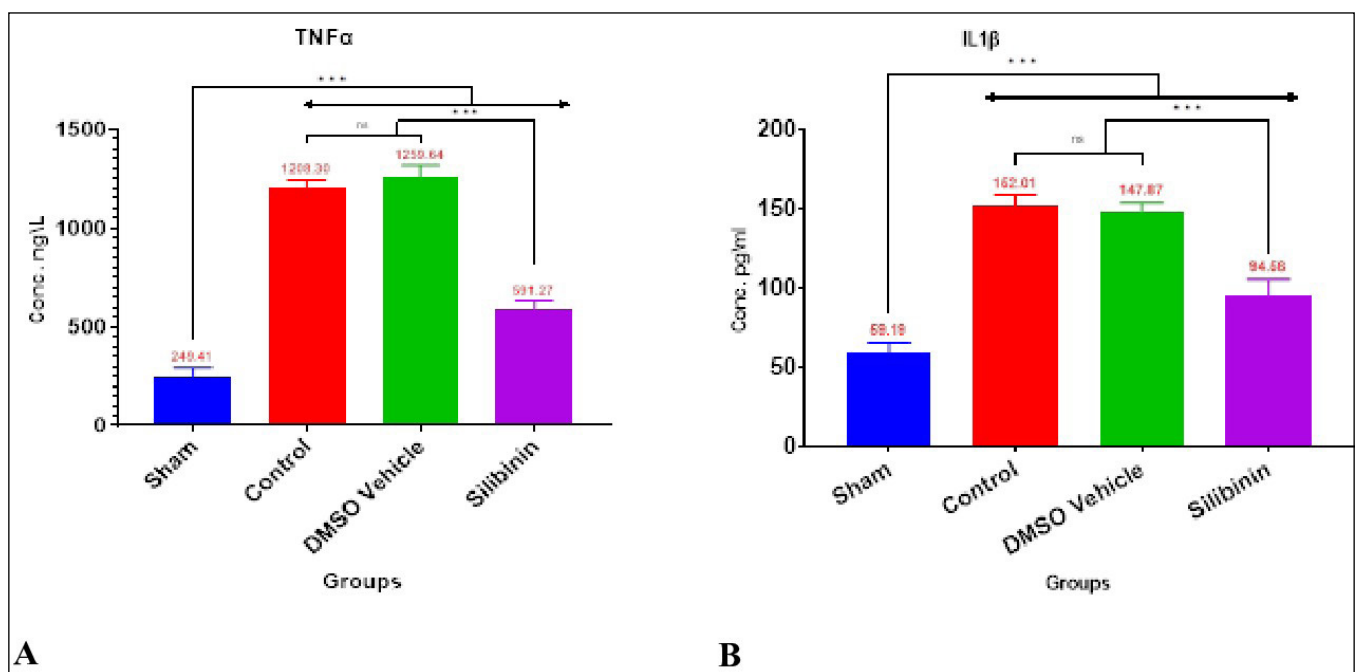


**Fig. 2.** Effect of silibinin on (A): TNF $\alpha$  renal tissue concentration (pg/ml), (B): IL1 $\beta$  renal tissue concentration, no. of animals in each group=7,

\*\* $p < 0.01$ , \*\*\* $p < 0.001$

Source: Own materials



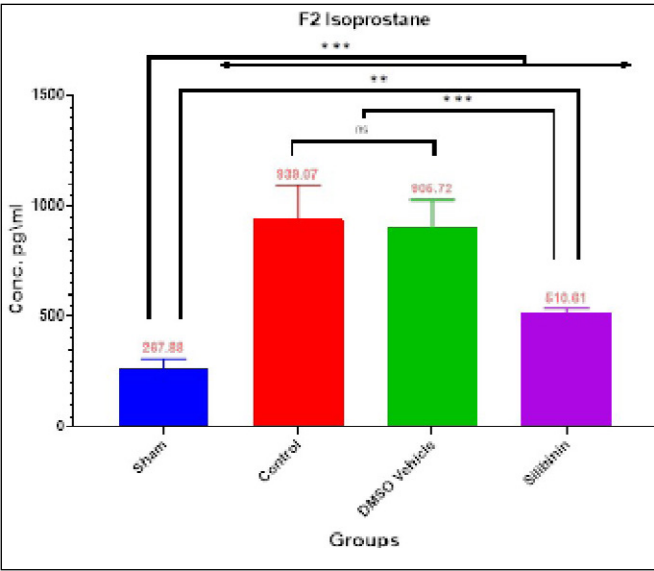


**Fig 3.** Effect of silibinin on F2 Isoprostane renal tissue concentration (pg/ml), no. of animals in each group=7  
\*\*p<0.01, \*\*\*p<0.001  
Source: Own materials

**ESTIMATION OF NOTCH1 AND JAGGED1 MRNA EXPRESSION BY RT-PCR**  
We used the quantitative real-time PCR (qRT-PCR) technique to accurately evaluate the expression levels of Notch1 and Jagged1 mRNA. Initially, we extracted total RNA with precision using the Easy-spin™ (DNA-free) total RNA extraction kit. The high-quality RNA was subsequently converted into complementary DNA (cDNA) with the AddScript cDNA Synthesis Kit. For the quantitative real-time PCR process, we employed the GoTaq® RT-qPCR System to guarantee dependable results and specific primers: Notch1 for: CACCCATGACCACTACCCAGTT, Notch1 rev: CCTCGGACCAATCAGAGATGTT, Jagged1 for: AACTGGTACCGGTGCGAA, Jagged 1 rev: TGATGCAAGATCTCCCTGAAAC [16] and GAPDH for: ATGACTCTACCCACGGCAAG, GAPDH rev: CTGGAAGATGGTGATGGGTT [17].

**STATISTICAL ANALYSIS**  
Statistical analysis of this study was performed by Graph Pad Prism version 8 software. Parametric variables were tested using the one-way ANOVA test with multiple comparisons between groups being made by Post Hoc. tukey Test.

**RESULTS**

**SILIBININ ATTENUATES KIM-1 RAT RENAL TISSUE CONCENTRATION AFTER RENAL I/R**  
KIM-1 renal tissue concentration was considerably (p<0.001) increased after renal ischemia /reperfusion (renal I/R) in comparison with sham group. Notably, this increase was

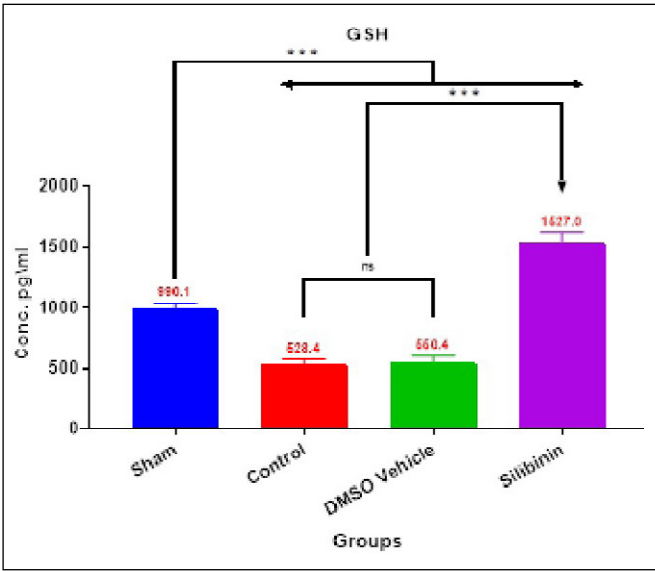


**Fig. 4.** Effect of silibinin on GSH renal tissue concentration (pg/ml), no. of animals in each group=7  
\*\*p<0.01, \*\*\*p< 0.001  
Source: Own materials

attenuated by silibinin pretreatment in a dose of (60 mg/kg) i.p., however, silibinin treatment group showed significant difference (p<0.001) when compared to sham group (Fig. 1).

**SILIBININ ATTENUATES IL-1B AND TNFA RENAL TISSUE CONCENTRATIONS AFTER RENAL I/R**  
Following renal ischemia/reperfusion, the concentration of both IL-1β and TNFα in the renal tissue of rats were significantly increased (p < 0.001). Pretreatment with silibinin at a dosage of 60 mg/kg administered i.p. notably reduced the levels of these markers. However, the silibinin treatment group still showed significantly elevated levels (p< 0.001) when compared to the sham group (Fig. 2A-B).

**SILIBININ REDUCES F2 ISOPROSTANE RENAL TISSUE CONCENTRATION AFTER RENAL I/R**  
As shown in figure 4, sham group showed significantly lower F2 Isoprostane renal tissue concentration as compared to control group. However, silibinin treatment group was considerably lower p<0.001 than control group, but it was still significantly higher p<0.001 than sham group (Fig. 3).

**SILIBININ IMPROVES GSH LEVELS IN RATS RENAL TISSUE AFTER RENAL I/R**  
Interestingly, I/R significantly (p< 0.001) decreased GSH rat renal tissue concentration relative to sham group. Pre-treatment with silibinin in a dose of 60 mg/kg i.p. caused considerable (p< 0.001) elevation in GSH renal tissue concentration in comparison with control group, (Fig. 4).



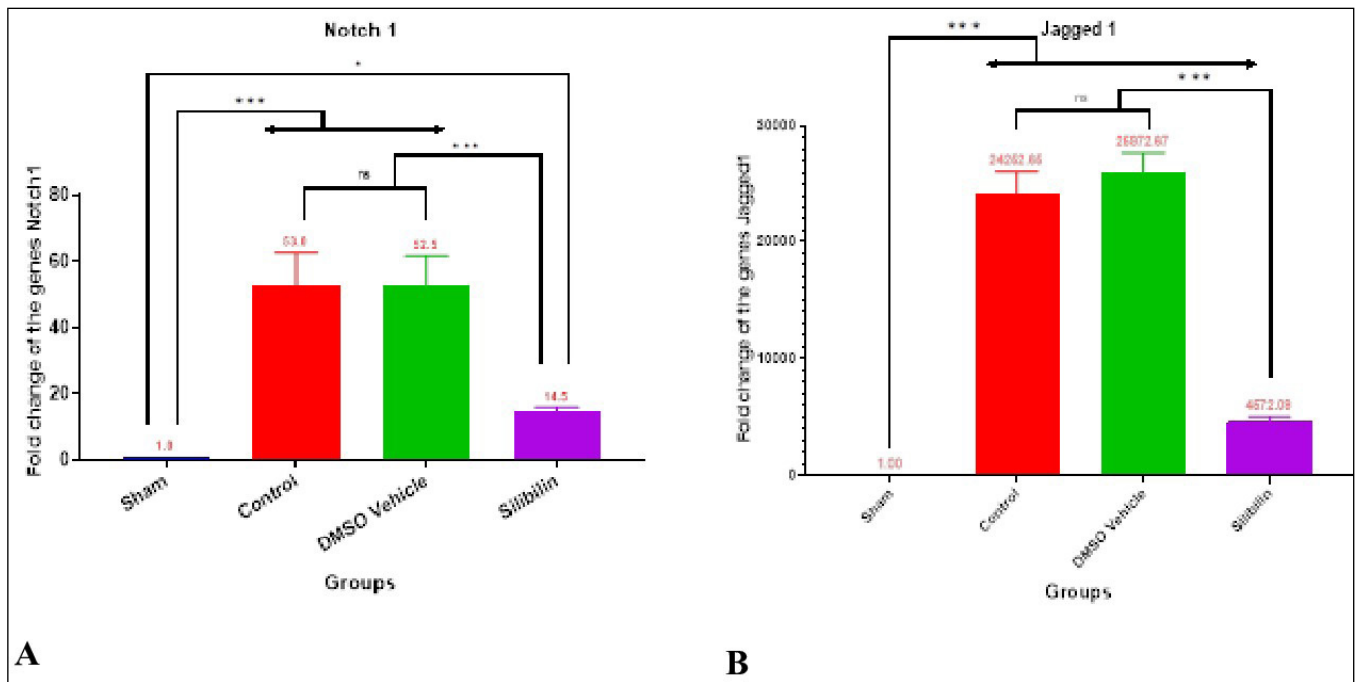


**Fig. 5.** Effect of silibinin on (A): Notch1 mRNA expression level in renal tissue, (B): Jagged-1 mRNA expression level in renal tissue, no. of animals in each group=7

\*\* $p < 0.01$ , \*\*\* $p < 0.001$

Source: Own materials

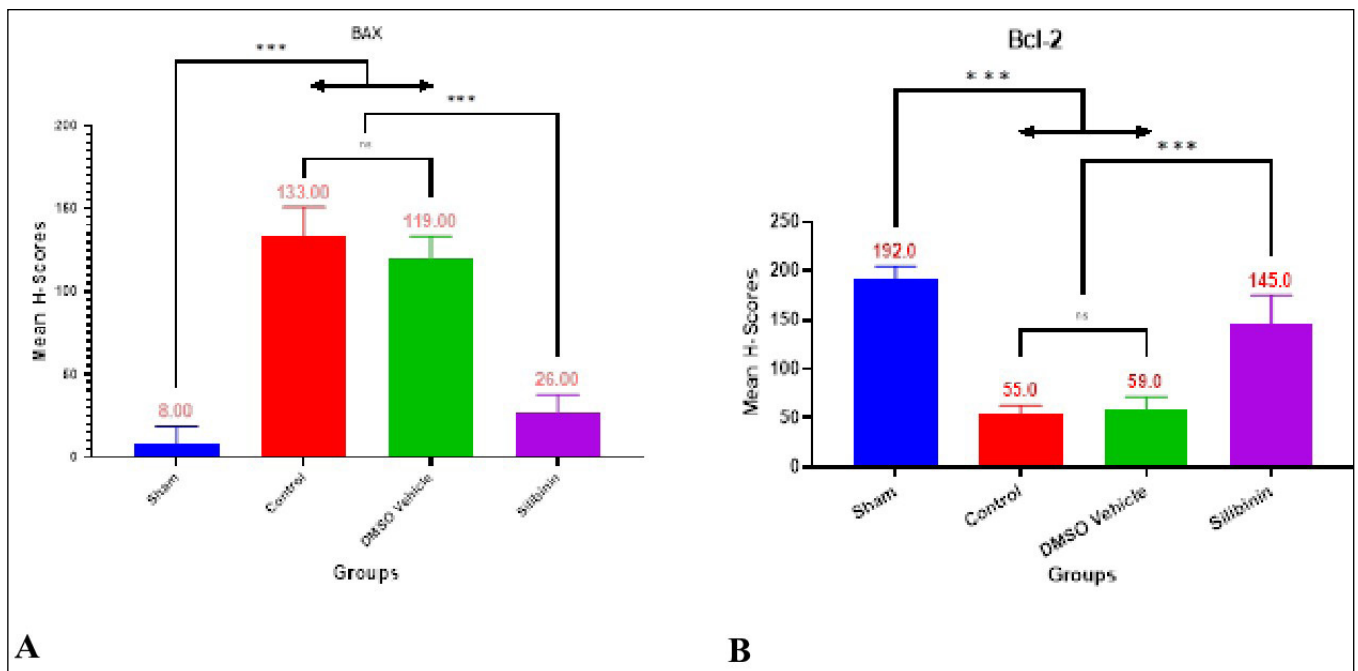


**Fig. 6.** Effect of silibinin on protein expression of (A): BAX, (B): Bcl2 in renal tissue, no. of animals in each group=7

\*\* $p < 0.01$ , \*\*\* $p < 0.001$

Source: Own materials

### SILIBININ REDUCES MRNA EXPRESSION OF NOTCH-1 AND JAGGED-1 IN RATS RENAL TISSUE AFTER RENAL I/R

Both Notch-1 and its ligand Jagged-1 showed a significant increase  $p < 0.001$  in the renal tissue of rats

following ischemia/reperfusion (I/R). This increase was notably reduced  $p < 0.001$  by pretreatment with silibinin in a dose of 60 mg/kg administered intraperitoneally (Fig. 5A-B).

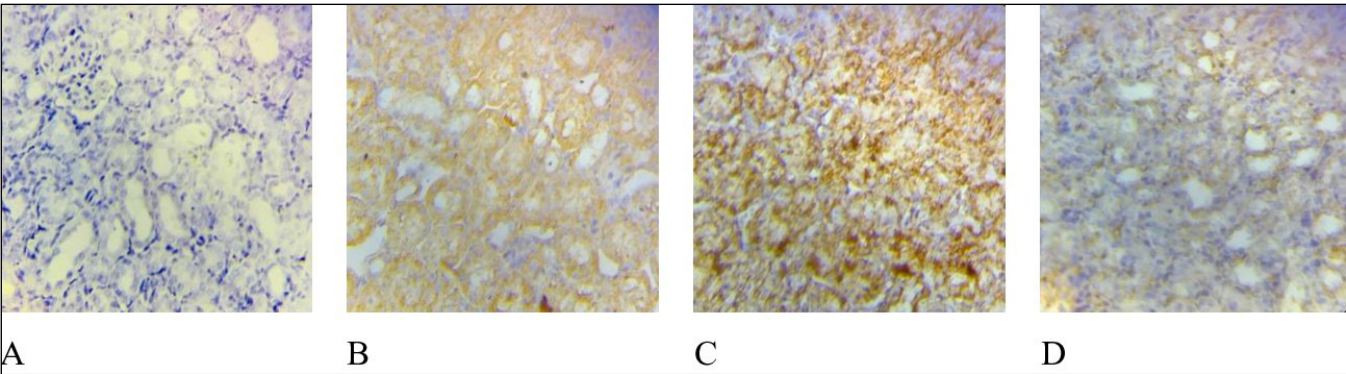


**Fig. 7.** BAX immunohistochemical staining, X400, (A): sham group, negative stain IHC, (B): Control group. Positive moderate intensity cytoplasmic staining in 80% of the examined cells, (C): DMSO Vehicle group. Positive moderate intensity cytoplasmic staining in 70% of the examined cells, (D): silibinin treatment group. Positive weak intensity cytoplasmic staining in 10% of the examined cells.  
*Source: Own materials*

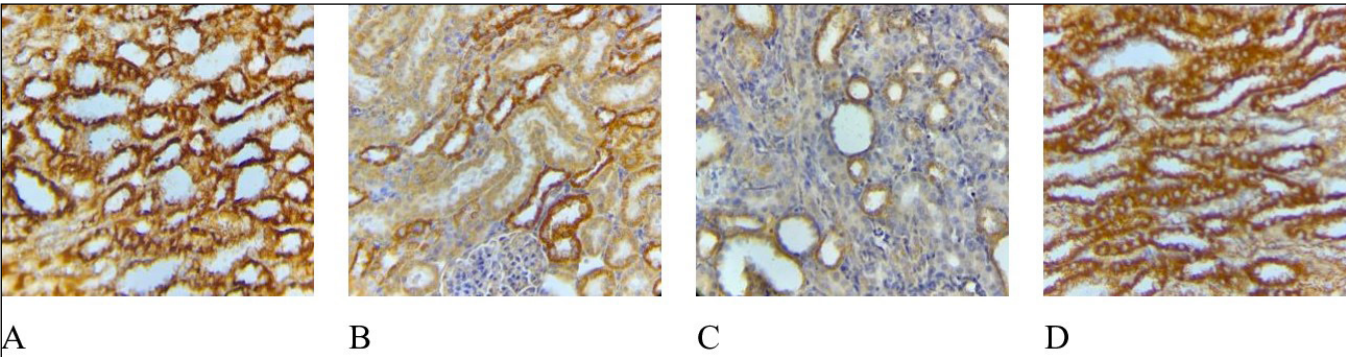


**Fig. 8.** Bcl2 immunohistochemical staining, X400, A: sham group Positive strong intensity cytoplasmic staining in 70% of the examined cells, B: control group. Positive strong intensity cytoplasmic staining in 35%% of the examined cells, C: DMSO vehicle group Positive moderate intensity cytoplasmic staining in 20% of the examined cells, D: silibinin treatment group, Positive strong intensity cytoplasmic staining in 60% of the examined cells.  
*Source: Own materials*

SILIBININ ATTENUATES APOPTOSIS BY DECREASING BAX AND INCREASING BCL2 PROTEIN EXPRESSION

The expression level of apoptotic marker BAX in renal tissue of sham group was significantly ( $p<0.001$ ) low in comparison with control group, while silibinin pretreatment caused considerable lowering ( $p < 0.001$ ) in BAX protein expression when compared to control group (Fig. 6A, Fig. 7A-D). On the other hand, the anti-apoptotic marker Bcl2 protein expression is significantly decreased in control group in comparison with sham group, meanwhile, silibinin pretreatment showed significant  $p<0.001$  elevation in Bcl2 protein expression in comparison with control group (Fig. 6B, Fig. 8A-D).

DISCUSSION

Earlier research has shown that renal ischemia-reperfusion injury (IRI) is strongly linked to oxidative stress and inflammatory reactions which are particularly related to the damage of renal tubular epithelial cells (TECs) that plays a significant role in the overall damage

to the kidneys [18]. Numerous molecular pathways have been proposed to be involved in the pathological processes that result in cellular damage related to ischemia-reperfusion injury (IRI). Furthermore, basic research has identified various targets that may help slow the progression of kidney disease. Despite these promising discoveries, only a few have been effectively validated in clinical trials, and they do not provide a substantial alternative to dialysis or transplantation. In the current study, we evaluated the nephroprotective effects of silibinin in renal IRI and the association of Notch signalling pathway in mediating these effects. The present research indicated a notable increase in the levels of KIM-1 within renal tissue following renal ischemia/reperfusion (I/R). Since KIM-1 is absent in healthy kidney tissue and is uniquely produced in injured proximal tubules, it acts as a highly sensitive, specific, and accurate early marker of renal tubular damage [19], validating the efficacy of the IRI model used in this study, silibinin treatment in a dose of (60mg /kg) i.p.1 hour before induction of ischemia resulted in significant reduction in KIM-1 renal tissue level when compared

to control group and each of the vehicle groups which may reflect the nephroprotective effects of silibinin. This study comes into agreement with another study of renal IRI in mice, in which silymarin that is a mixture of both silibinin isomers was found to decrease KIM-1 protein expression in addition to other markers of oxidative stress, inflammation through inhibition of apoptotic pathways and NF- $\kappa$ B [20]. In this experimental study, comparisons between the sham and control groups showed that both TNF- $\alpha$  and IL1 $\beta$  levels were significantly increased in the control groups. This supports the notion that renal inflammation was present due to renal IRI. It has become clear that a substantial amount of the harm observed following renal ischemia-reperfusion (IR) is caused by oxidative stress in the mitochondria and the generation of reactive oxygen species (ROS) during the reperfusion phase. These ROS, such as superoxide, peroxynitrite, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), activate inflammatory cells, leading to the release of interleukins (ILs), tumor necrosis factor (TNF- $\alpha$ ), and a variety of other inflammatory substances [21]. This elevation in inflammatory markers was significantly attenuated by silibinin pretreatment. This reduction in TNF- $\alpha$  level and other inflammatory cytokines could be due to the inhibition of NF- $\kappa$ B pathways which has been documented by many studies [20–22]. This study also assessed a marker of oxidative stress known as F<sub>2</sub>-isoprostane, which is produced through a non-enzymatic reaction resulting from the significant peroxidation of arachidonic acid due to free radicals. Research conducted by the National Institutes of Health (NIH) in the United States has recognized Isoprostane as reliable biomarkers for oxidative stress. Furthermore, the study illustrated that measuring bioactive F<sub>2</sub>-isoprostanes in bodily fluids provides a novel noninvasive method for investigating the biological effects of free radicals [23]. In the present study renal tissue concentration of F<sub>2</sub>-isoprostane was considerably elevated after renal I/R. Meanwhile silibinin pretreatment significantly reduced F<sub>2</sub> Isoprostane renal tissue concentration after renal IR which reflects the potential antioxidant capacity of silibinin. The antioxidant effects of silibinin were proposed by many studies. In cisplatin induced AKI model, silibinin decreased ROS-mediated MAPK signaling pathway activation, in addition silibinin countered the inhibition of nuclear factor-erythroid 2-related factor 1 (Nfe2l1) caused by cisplatin by influencing its transcription and modifications after translation [24]. Another marker of oxidative stress was evaluated in the current study which is glutathione (GSH). It inhibits lipid peroxidation and scavenges free radicals (LPO). Some detoxifying enzymes and antioxidants require GSH as a cofactor, it aids in the renewal

of ascorbate and tocopherols [25]. Our study has shown a considerable reduction in GSH renal tissue concentration after renal IR which could be attributed to the depletion of the tissue antioxidative capacity by the huge amounts of ROS produced during the reperfusion phase as a result of the defect in the mitochondrial electron transport chain [26]. Interestingly, silibinin pretreatment significantly ameliorated GSH renal tissue concentration after renal IR. Our study agrees with another study that evaluated the nephroprotective effects of silibinin in renal IRI in mice showing significant elevation in GSH level in rat kidney tissue after IR in silibinin treated mice and in mouse renal tubule tissue culture treated with silibinin in addition to improving other markers of oxidative stress like MDA and SOD, those effects were suggested to be mediated through inhibiting ferroptosis by binding Ferritin heavy chain 1 (FTH1) [27]. Previous research has indicated a close relationship between apoptosis and the pathological process of renal ischemia-reperfusion injury (IRI) [28]. The fate of cells following renal IRI is determined by the balance between the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2, both of which are part of the Bcl-2 family [29]. Excessive reactive oxygen species (ROS) trigger stress pathways in both the mitochondria and the endoplasmic reticulum. When mitochondrial damage occurs, the rise in Bax levels and the fall in Bcl-2 levels alter the Bax to Bcl-2 ratio in the outer mitochondrial membrane, leading to the release of cytochrome c. This release activates the apoptosis executioner enzyme caspase-3, ultimately resulting in mitochondrial-dependent apoptosis [30]. In this experimental study, immunohistochemical analysis of the apoptotic activator BAX and its antagonist Bcl2 revealed significant upregulation in BAX renal tissue protein expression level after renal IR while Bcl2 was significantly downregulated after renal IR. The results of this study clearly align with a previous study on the protective effects of Allicin in rat model of renal ischemia reperfusion injury which documented the increase in caspase-3, BAX and the decrease in Bcl2 renal tissue protein expression after renal IR [31]. On the other hand, silibinin pre-treatment resulted in significant increase in Bcl2 renal tissue protein expression while decreasing BAX protein expression in renal tissue after IR. Silibinin may improve apoptosis by decreasing oxidative stress and scavenging ROS, this can prevent the activation of mitochondrial apoptotic pathways by reducing BAX and enhancing Bcl2 levels [32]. The findings of this study agree with another study which assessed the nephroprotective effects of silymarin in renal ischemia reperfusion injury in mice which have shown that silymarin enhanced Bcl2 protein expression while lowered both

Bax and cleaved caspase-3 [33]. In order to assess the effect of silibinin on Notch-1 signaling pathway, we estimated Notch-1 and its ligand Jagged-1 mRNA expression in all study groups. Our results have shown significant elevation in Notch-1 and Jagged-1 mRNA expression after IR. This study comes in line with a previous study which evaluated the nephroprotective effects of Notch inhibitor DAPT in renal I/R injury induced in diabetic rats showing that both Notch-1 and its ligand Jagged-1 mRNA expression in renal tissues were elevated after renal I/R [34]. Notably, pretreatment with silibinin resulted in a significant reduction of Notch-1 and Jagged-1 mRNA levels in renal tissues following renal IR. In an *in vivo* and *in vitro* model of hepatocellular carcinoma, silibinin exerted potent antitumor activity which was accompanied by Notch-1 down regulation [35]. The inhibition of oxidative stress and NF- $\kappa$ B

activation by silibinin [36] could provide a reasonable explanation to the down regulation of Notch-1 by this supplement since they are documented regulators of Notch-1 and Jagged-1 [37].

## CONCLUSIONS

Results of this study may provide additional evidence regarding the role of inflammation, oxidative stress, and apoptosis in the development of renal ischemia/reperfusion (I/R) injury, indicating that the Notch-1 signaling pathway may be involved in these processes. Silibinin appears to offer protective benefits against renal I/R injury due to its antioxidant, anti-inflammatory, and anti-apoptotic properties. Furthermore, the protective effects of silibinin were linked to a decrease in the expression of Notch-1 and its ligand Jagged-1.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Adaptive volleyball as a means of physical education and sports rehabilitation of combatants

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## ABSTRACT

**Aim:** To determine the impact of adaptive volleyball training sessions on the psycho-emotional and functional state of combatants.

**Materials and Methods:** The research involved 64 service members participating in combat operations who underwent rehabilitation in 2024–2025 after injuries and damages to the musculoskeletal system. The duration of the research was 6 weeks. The research employed theoretical, empirical, and mathematical statistical methods.

**Results:** It has been found that adaptive volleyball training sessions have a positive effect on the indicators of psycho-emotional and functional state of combatants. A significant improvement in the indicators of emotional state ( $p \leq 0.001$ ) by 1.33 points and neuro-emotional stress ( $p \leq 0.05$ ) by 4.43 points has been found. The functional state of the respiratory system in combatants also improved by 5.6 seconds in the Stange test and by 2.9 seconds in the Genchi test. The indicators of the functional state of the cardiovascular system in combatants, according to the Rufier Index, have also improved by 0.83 c. u. after the use of adaptive volleyball training sessions. Still, the changes were not significant ( $p > 0.05$ ) due to the short period of the research.

**Conclusions:** It has been established that adaptive volleyball is an effective means in the framework of physical education and sports rehabilitation for individuals who have suffered musculoskeletal injuries. It has been proven that adaptive volleyball training sessions have a positive effect on the indicators of the psycho-emotional and functional state of combatants.

**KEY WORDS:** adaptive volleyball, combatant, psycho-emotional state, functional state, physical education and sports rehabilitation

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## INTRODUCTION

The consequences of the hostilities in Ukraine have led to an increase in the number of people with disabilities, including those with musculoskeletal disorders. The vast majority are service members and employees of other law enforcement agencies. After receiving medical care, including surgical intervention, these individuals require further comprehensive rehabilitation measures to restore their self-care functions, motor skills, and social activities. To this end, healthcare facilities, the Ministry of War Veterans Affairs, and related agencies are expanding and diversifying rehabilitation services.

Physical education and sports rehabilitation is essential and effective. It is considered a system of measures

developed through the use of physical exercises aimed at restoring and compensating for the body's functional capabilities, improving physical and mental health, and overall well-being [1, 2]. Exercise is beneficial for people who have been injured, wounded, and are undergoing rehabilitation; people with disabilities, as it prevents secondary complications (muscle atrophy, osteoporosis) and helps to improve psycho-emotional and functional state [3, 4]. An essential task in the rehabilitation of combatants who have suffered musculoskeletal injuries through physical culture and sports is to restore mobility in joints, strengthen muscles, improve coordination and endurance, and reduce pain through vigorous physical activity [5, 6].

One type of physical education and sports rehabilitation is adaptive sports [7]. By adapting traditional sports to people with disabilities and involving them in participation, it is possible to more effectively overcome the effects of injuries, wounds, post-traumatic stress disorder, increase self-esteem, reduce symptoms of depression and tension, improve the process of socialization, etc. [8]. The adaptive sports program includes various game-based activities, such as adaptive volleyball, wheelchair basketball, amputee football, swimming, track and field, cycling, archery, table tennis, etc.

The analysis of modern scientific works gives grounds to state that adaptive volleyball is an effective means of physical culture and sports rehabilitation of people with musculoskeletal disorders [9, 10]. This sport is a complex coordination sport, involves various muscle groups, requires concentration, quick thinking, and rapid reaction. The key feature of adaptive volleyball is moving around the court with support on the hands and buttocks. During training, players develop the ability to coordinate their actions, taking into account anatomical and physiological features; develop skills in performing several technical and tactical actions necessary for a successful game; develop special physical qualities, in particular speed, agility during the performance of complex coordination movements [11]. Adaptive volleyball is the only team sport for people with amputations and other musculoskeletal injuries that is cultivated in many countries and is part of the Paralympic Games. Experts say that people without notable physical limitations can also play and train in this sport, since the specifics of adaptive volleyball do not provide any advantage on the court [12].

Scientists [10, 13] note that the inclusion of adaptive volleyball in the rehabilitation program for service members with lower limb amputation is a logical response to the challenges of today, as this area helps to expand the functional capabilities of a person, promotes faster recovery of physical and mental health, and more effectively overcomes the effects of stress. Other researchers emphasize the effectiveness of the impact of adaptive game sports on the psycho-emotional state and socialization of participants, including people with disabilities [14].

The need to study the impact of adaptive volleyball as a means of physical culture and sports rehabilitation on the psycho-emotional and functional state of combatants has prompted our research.

## AIM

The aim is to determine the impact of adaptive volleyball training sessions on the psycho-emotional and functional state of combatants.

## MATERIALS AND METHODS

### PARTICIPANTS

The research, which was conducted in 2024-2025, involved 64 service members participating in combat operations who were undergoing the final stage of rehabilitation after injuries and damages to the musculoskeletal system, in particular the lower extremities and spine, and were recommended for physical training and sports rehabilitation based on medical reports. The rehabilitation activities were conducted at rehabilitation centers in Kyiv, Kyiv Oblast, and Zhytomyr. Adaptive volleyball training sessions were held at the sports bases of the National Academy of Internal Affairs (NAIA) and S. P. Koroliov Zhytomyr Military Institute (ZhMI) five times a week. The results were analyzed at the Department of Physical Education and Sports Rehabilitation of S. P. Koroliov ZhMI and the Department of Special Physical Training of the NAIA. The psycho-emotional and functional state of combatants was tested at the beginning of their arrival at the training bases of the educational institutions and after six weeks of systematic training.

### RESEARCH METHODS

The research employed theoretical, empirical (psycho-diagnostic, functional methods), and mathematical statistical methods. Two psycho-diagnostic methods were used to study the dynamics of psycho-emotional state indicators in the participants of the experiment: "Self-Assessment of Emotional State" (developed by A. Wessman and D. Ricks) and "Assessment of Neuro-Emotional Stress" (proposed by T. A. Nemchyn). The "Self-Assessment of Emotional State" method is designed for self-assessment of the emotional states of combatants on a ten-point scale. The participants were asked to choose from each of the following statements ("Resilience-anxiety", "Vigor-fatigue", "Elation-depression", "Self-confidence-helplessness") the one that most accurately reflects their emotional state during the research period and mark the corresponding number in the form. The integral indicator of the emotional state of combatants was determined using the appropriate formula. If the emotional state indicator is within 8-10 points, it can be interpreted as very good; within 6-7 points – as good; 4-5 points – as worsened; 1-3 points – as poor.

The "Assessment of Neuro-Emotional Stress" method comprises 30 characteristics of this condition, categorized into three degrees of severity: A (low), B (average), and C (high). By choosing one of the three answer options (A, B, C), the participants of the experiment

**Table 1.** Dynamics of the indicators of psycho-emotional and functional state of combatants in the process of adaptive volleyball training sessions (n = 64)

| Initial stage                           | Final stage  | Reliability of the difference |           |
|-----------------------------------------|--------------|-------------------------------|-----------|
| X ± m                                   | X ± m        | t                             | p         |
| Level of emotional state, points        |              |                               |           |
| 5.31 ± 0.21                             | 6.64 ± 0.19  | 4.70                          | p ≤ 0.001 |
| Level of neuro-emotional stress, points |              |                               |           |
| 61.24 ± 1.52                            | 56.81 ± 1.47 | 2.10                          | p ≤ 0.05  |
| Stange test, seconds                    |              |                               |           |
| 54.7 ± 2.15                             | 60.3 ± 1.98  | 1.92                          | p ≥ 0.05  |
| Genchi test, seconds                    |              |                               |           |
| 38.9 ± 1.22                             | 41.8 ± 1.08  | 1.78                          | p ≥ 0.05  |
| Rufier Index, c. u.                     |              |                               |           |
| 7.27 ± 0.48                             | 6.44 ± 0.45  | 1.26                          | p ≥ 0.05  |

Notes: X – arithmetic mean; m – standard error; t – Student's t-test value; p – p-value

Source: compiled by the authors of this study

assessed their state of neuro-emotional stress at the current time. The data were processed by summing the points: for answer A – 1 point, B – 2 points, and C – 3 points. The range from 30 to 50 points characterizes a low level of neuro-emotional stress, 51-70 points – average; from 71 to 90 points – high.

The functional capabilities of the participants were assessed using the Stange test (duration of breath holding during inhalation), the Genchi test (duration of breath holding during exhalation), and the Rufier Index. Breath-holding tests allow for assessing the functional capabilities of the respiratory system. The level of functional capabilities of individuals was assessed by the duration of breath holding during inhalation: more than 60 seconds (excellent), 40-60 seconds (good), 30-40 seconds (satisfactory), and less than 30 seconds (low). During exhalation, a breath-hold of more than 40 seconds indicates an excellent level of functional capacity; 30-40 seconds – good; 25-30 seconds – satisfactory; less than 25 seconds – low. The Rufier test determines the level of cardiovascular reserve during any physical activity. After 3-5 minutes of rest in a sitting position, the combatant's heart rate was counted for 15 seconds. The data obtained were recorded, and the participant was asked to perform 30 squats with arms extended forward for 45 seconds. At the end of the squats, the employee sat down, and the heart rate was counted for the first 15 seconds and the last 15 seconds of the first minute of recovery. The functional capabilities of the cardiovascular system were assessed by the Rufier Index (RI) using the formula:  $RI = ((P1 + P2 + P3) - 200) / 10$ , where P1 is the resting heart rate, and P2, P3 are the heart rate in the first 15 and last 15 seconds of the first minute after squats. The assessment of the RI was

carried out according to the following criteria: ≤ 0 c. u. (athletic heart); 0.1-5.0 c. u. (excellent); 5.1-10.0 (good); 10.1-15.0 (satisfactory); 15.1-20.0 (unsatisfactory).

## STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The reliability of the difference between the indicators was determined using the Student's t-test. The results were presented as X ± m, where X is the arithmetic mean, m is the standard error. The reliability of the difference was set at p<0.05. All statistical analyses were performed using STATISTICA 6.1 software package (number AGAR909E415822FA), adapted for medical and biological research.

## ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the NAIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

## RESULTS

The results of the study of the dynamics of the emotional state of combatants before and after two-week adaptive volleyball training sessions are presented in Table 1. It was found that the integral indicator of the emotional state of the participants of the experiment, which was determined by the indicators of each scale

("Resilience-anxiety", "Vigor-fatigue", "Elation-depression", "Self-confidence-helplessness"), before the beginning of adaptive volleyball training sessions was estimated as worsened ( $5.31 \pm 0.21$  points), and at the final stage – as good ( $6.64 \pm 0.19$  points). Positive changes of 1.33 points are estimated to be reliable ( $p \leq 0.001$ ).

The results of the study on neuro-emotional stress in combatants, according to the method proposed by T. A. Nemchyn, provide grounds for stating a positive trend. In particular, at the initial stage, this indicator was  $61.24 \pm 1.52$  points, at the final stage –  $56.81 \pm 1.47$ . The obtained data indicate a positive influence of adaptive volleyball training sessions on the level of neuro-emotional stress in participants of the experiment. The difference between the fixed indicators before and after the two-week training sessions is reliable, with a mean difference of 4.43 points ( $p \leq 0.05$ ). The level of neuro-emotional stress during the experiment was estimated as average. The obtained results testify to the improvement of the indicators of the psycho-emotional state of participants of combat actions during adaptive volleyball training sessions by both methods. This may be due to the influence of physical activity experienced by the participants of the experiment during the performance of specific motor actions of this sport. Exercise helps to reduce cortisol in the body and stimulates the production of endorphins. The cohesion, emotional support of each other that accompanies the game and the positive psychological atmosphere on the playground also have a positive effect on the participants' psycho-emotional state.

The analysis of the Stange test, which assessed the time of breath holding during inspiration, showed improvement at the final stage of the experiment; however, no significant difference was found ( $p > 0.05$ ). The level of functional capabilities of the respiratory system of combatants at the initial stage was assessed as good. The increase in the test was 5.6 seconds and reached  $60.3 \pm 1.98$  seconds, which corresponded to a high level. The analysis of the Genchi test gives grounds to state a similar dynamics – the level of functional capabilities of the respiratory system of combatants was assessed as good ( $38.9 \pm 1.22$  seconds). The increase in the test value was 2.9 seconds ( $41.8 \pm 1.08$  seconds), but the difference is not significant compared to the initial stage of the experiment ( $p > 0.05$ ). The average value of the Genchi test in the participants of the experiment at the final stage indicates an excellent assessment of the functional capabilities of the respiratory system.

The analysis of the Rufier Index testifies to a good level of functional capacities of the cardiovascular system in the participants of the experiment and positive dynamics after the use of adaptive volleyball training sessions;

however, changes in the results are unreliable ( $p > 0.05$ ). At the initial stage, the Rufier Index of the combatants was  $7.27 \pm 0.48$  c. u., and at the final stage, it improved by 0.83 c. u. to reach  $6.44 \pm 0.45$ . The obtained data allow us to assert that adaptive volleyball training sessions positively influence indicators of psycho-emotional and functional state of combatants. Functional reserves of the cardiovascular system gradually increased, since it is impossible to achieve significant changes in such a short period of time.

## DISCUSSION

The relevance of the research is confirmed by the publications of other scientists [15, 16], who argue that the outbreak of hostilities in Ukraine has exacerbated the rehabilitation direction in the field of physical education, as the problem of returning combatants to everyday life is significant. One of the key areas of physical education and sports rehabilitation is adaptive sports [7, 17], which is now becoming widespread in Ukraine thanks to government initiatives and the activities of public organizations.

The obtained results confirm the data of other scientists on the positive impact of adaptive sports in general and physical exercises in particular on improving the psycho-emotional and functional state of veterans [8, 18]. Involvement in adaptive sports is one of the most effective tools for the rehabilitation and social integration of veterans and combatants [7, 8]. Scientists are convinced that adaptive sports for combatants, veterans, and people with disabilities is not only physical activity, but also an essential tool for their rehabilitation, reintegration, and return to a full life. It is one of the key areas of involvement for such persons in physical education and sports, as well as the formation of a culture of conscious choice and adherence to an active and healthy lifestyle [19].

Experts are convinced that the development of adaptive sports is aimed at improving services in the field of physical culture and sports and building barrier-free accessibility, which has become a new value for many Ukrainians during the war. Adaptive sport makes it easier to compensate for physical, mental, and social changes in a person; the use of metered exercise activates the processes of readaptation and helps improve a person's communication and social skills. According to researchers, the use of adaptive sports in the rehabilitation system for service members with lower limb amputation leads to an increase in functional mobility, psychological stability, and daily activity [20]. The results of our research do not contradict the findings of other scientists, but rather expand and supplement them.

## CONCLUSIONS

Based on the study of scientific literature, it is stated that adaptive sport is an essential means of physical education and sports rehabilitation of combatants who have been injured, wounded, and are undergoing rehabilitation, as well as persons with disabilities. Exercise provided by this sport prevents secondary complications, helps to restore joint mobility, strengthen muscles, improve coordination and endurance, reduce pain, and is a tool for their physical, psychological, and social rehabilitation and return to full life. It has been established that it is effective to use adaptive volleyball for people who have suffered musculoskeletal injuries, people with amputations of the lower one or both limbs, in the framework of physical education and sports rehabilitation. It has been found that adaptive volleyball training sessions have a positive effect on the indicators of the psycho-emotional and functional state of combatants. A significant improvement in the indicators of emotional state ( $p \leq 0.001$ ), which was assessed at the final stage of the experiment as good, and neuro-emotional stress ( $p \leq 0.05$ ) was noted. The

level of neuro-emotional stress in the participants of the experiment during the training was estimated as average.

Positive changes in the functional state of the experiment participants were observed, but these changes were not statistically significant ( $p > 0.05$ ). The level of functional capabilities of the respiratory system of the combatants, as assessed by the Stange test at the final stage of the experiment, was found to be high ( $60.3 \pm 1.98$  seconds). According to the Genchi test, it was rated as excellent ( $41.8 \pm 1.08$  seconds). According to the Rufier Index at the final stage of the experiment, the functional reserves of the cardiovascular system were assessed as good –  $6.44 \pm 0.45$  c. u., but the changes in the results were also not reliable ( $p > 0.05$ ) due to the short period of the research.

## PROSPECTS FOR FURTHER RESEARCH

We see prospects for further research in studying the impact of training sessions in other types of adaptive sports on the psycho-emotional and functional state of combatants, including persons with disabilities.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Peculiarities of stress symptoms manifestation in future law enforcement officers under martial law

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## ABSTRACT

**Aim:** To conduct a comparative analysis of stress symptoms in police cadets depending on the year of their academic training and service activities during the war.

**Materials and Methods:** The research involved 276 male cadets from the 1<sup>st</sup> to 4<sup>th</sup> training years. Stress symptoms in the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets were assessed before and after their practical training sessions at training grounds, and the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets – before and after their internships in police units. Research methods: theoretical analysis of literature sources, psycho-diagnostic methods, statistical methods.

**Results:** It has been found that the most pronounced manifestation of all stress symptoms was observed in the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets, especially after completing the tasks of their academic training and service activities. This is due to insufficient psycho-emotional resilience to the stressful factors of academic training and service activities in junior cadets, inadequate skills in utilizing existing means for stress prevention during task performance under martial law, and a low level of stress resilience. Among the cadets studied, the most significant stress level was observed in the junior training years, both before and after their assignments.

**Conclusions:** The research shows that there is an urgent need to develop cadets' stress resilience to ensure the effectiveness of their academic training and further service activities in practical police units, as well as to develop skills in the use of available means to prevent stressful phenomena in the process of their academic training and service activities.

**KEY WORDS:** stress, stress symptoms, mental health, cadets, law enforcement officers, war

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## INTRODUCTION

Today in Ukraine, the problem of stress has become particularly relevant, and the ability to cope with it has become a universal need. In our society, there are many different professions, including those where the performance of professional duties takes place in extreme conditions, which can lead to a person's stressful state. This is especially true of law enforcement officers, whose service activities are carried out in rather difficult conditions: an aggressive communication environment, lack of working time, high responsibility for performance, and the occurrence of various kinds of extreme situations [1]. The problem has significantly aggravated since the beginning of the full-scale invasion of Ukraine by the Russian occupiers, which has led to a significant expansion of the range of duties and powers of law enforcement officers during martial law in Ukraine [2]. Every day, law enforcement officers face many specific stressors, most of which are continuous and long-term.

And, as a result, overwork leads to fatigue, general and occupational diseases, and premature aging [3, 4].

Scientists [5, 6] identify the most typical stressors in law enforcement activities as external, organizational, service-related, and personal factors. External stressors cause frustration related to the practice of judicial proceedings, media activities, and negative attitudes towards the police by the public. Organizational stressors are related to low salaries, excessive paperwork, and a lack of administrative support. Service-related stressors arise from the constant exposure to tragedy and people in distress, as well as the fear, danger, and boredom inherent in the job. Personal stressors include concerns about the individual's success and safety as a police officer, pressure from superiors on their service behavior, and stressors arising from police work: health, family problems, and alcohol dependence. Performing service duties under challenging conditions of martial law, where there is a threat to the health and life of not

only the individual but also others, leads to negative consequences, primarily in the mental sphere. Strict requirements for the actions of law enforcement officers and strict legal regulation of their service activities are one of the levers of negative impact on their mental health [7, 8]. This requires them to have a high level of psychological readiness for service activities, which is formed while studying at higher educational institutions with a specific learning environment (HEIs with SLE).

At the same time, for cadets – future law enforcement officers, the period of their academic training and service activities at the HEI with SLE is also accompanied by the negative impact of several negative stressors. Stress in cadets – future law enforcement officers occurs due to high psycho-emotional tension, life risks, strict requirements for service activities, and insufficient psychological preparedness, which can lead to emotional burnout, reduced motivation, and health problems [9, 10]. A high level of anxiety about the future of their relatives and friends, a constant threat to life and health, frequent sirens about missile danger and air raids, power outages, mobile communications, and the need to study remotely are not a complete list of causes of stress in future law enforcement officers [11]. In addition, a high level of responsibility for academic training outcomes, a significant increase in practical training sessions at training grounds, and internships in practical police units lead to a rise in stress levels among cadets – future law enforcement officers. According to scientists [12, 13], stress can manifest itself in various symptoms, both at the psychological and physiological levels. However, the insufficient level of study of the peculiarities of stress symptoms manifestation in cadets – future law enforcement officers during martial law in Ukraine led to the choice of the research topic.

## AIM

The aim is to conduct a comparative analysis of stress symptoms in police cadets depending on the year of their academic training and service activities during the war.

## MATERIALS AND METHODS

### PARTICIPANTS

The research involved 276 cadets of the 1<sup>st</sup> (n = 76), 2<sup>nd</sup> (n = 72), 3<sup>rd</sup> (n = 65) and 4<sup>th</sup> (n = 63) training years of Kharkiv National University of Internal Affairs (Ukraine, Kharkiv, KhNUIA), majoring in the “Law” specialty at the first (bachelor’s) level of higher education. For the 1<sup>st</sup>

and 2<sup>nd</sup> training year cadets, stress symptoms were assessed before their practical training sessions at training grounds (lasting 2-4 weeks) and after returning from them, for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets – before and after their internships in police units (lasting 4 weeks). The research was conducted in the academic year 2024-2025, and all respondents were male.

### RESEARCH METHODS

Theoretical analysis and generalization of literature sources, psycho-diagnostic methods (test of Yu. V. Shcherbatykh), statistical methods. The method of theoretical analysis and generalization of literary sources was used to conduct an analytical review of scientific sources on the outlined range of issues (23 sources (2010-2025) from MedLine, Scopus, Web of Science databases were analyzed).

To study the peculiarities of stress symptoms manifestation in future law enforcement officers during the performance of their academic training and service tasks under martial law, we used a test of Yu. V. Shcherbatykh, which allows the study of various signs of stress – intellectual, behavioral, emotional and physiological according to the answers to the questionnaire consisting of four groups of questions of 12 symptoms each [14]. For the presence of one of the intellectual and behavioral symptoms, the respondents rated it at 1 point; emotional – 1.5 points, and physiological – 2 points. The total maximum score for the entire list can theoretically reach 66. A score of 0 to 5 points is considered good, meaning there is no significant stress at this point in life. A score of 6 to 12 points means that a person is experiencing moderate stress, which can be compensated for by rational use of time, periodic rest, and finding the best way out of the situation. A score of 13 to 24 points indicates a fairly pronounced tension of the emotional and physiological systems of the body, which arose in response to severe stress that could not be compensated for. In this case, unique methods of coping with stress are required. A score of 25 to 40 points indicates a state of severe stress, which requires the help of a psychologist or psychotherapist to overcome it successfully. This stress level suggests that the body is already close to the limit of its ability to resist stress. A score of more than 40 points indicates that the body is moving to the third, most dangerous stage of stress – the depletion of adaptive energy reserves.

### STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The compliance of the sample data distribution with the Gauss’ law was assessed using

**Table 1.** The dynamics of intellectual symptoms manifestation of stress in cadets – future law enforcement officers of different training years ( $n = 276$ ) during their academic training and service activities under martial law (before and after practical training sessions at training grounds for the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets; before and after internships in police units for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets), %

| Intellectual symptoms                                        | 1 <sup>st</sup> training year<br>( $n = 76$ ) |       | 2 <sup>nd</sup> training year<br>( $n = 72$ ) |       | 3 <sup>rd</sup> training year<br>( $n = 65$ ) |       | 4 <sup>th</sup> training year<br>( $n = 63$ ) |       |
|--------------------------------------------------------------|-----------------------------------------------|-------|-----------------------------------------------|-------|-----------------------------------------------|-------|-----------------------------------------------|-------|
|                                                              | Before                                        | After | Before                                        | After | Before                                        | After | Before                                        | After |
| Prevalence of negative thoughts                              | 32.9                                          | 53.9  | 31.9                                          | 48.6  | 27.7                                          | 43.1  | 26.9                                          | 41.2  |
| Difficulty concentrating                                     | 19.7                                          | 26.3  | 19.4                                          | 23.6  | 16.9                                          | 21.5  | 15.8                                          | 17.5  |
| Deterioration of memory performance                          | 18.4                                          | 22.3  | 16.7                                          | 18.1  | 18.4                                          | 20.0  | 14.3                                          | 15.9  |
| Constant, fruitless revolving of thoughts around the problem | 35.5                                          | 56.6  | 33.3                                          | 51.4  | 30.8                                          | 47.7  | 30.1                                          | 44.4  |
| Increased distraction                                        | 23.7                                          | 42.1  | 23.6                                          | 43.1  | 21.5                                          | 38.4  | 19.0                                          | 34.9  |
| Difficulties in decision-making                              | 34.2                                          | 51.3  | 29.2                                          | 45.8  | 27.7                                          | 44.6  | 23.8                                          | 38.1  |
| Bad dreams, nightmares                                       | 26.3                                          | 31.6  | 23.6                                          | 26.4  | 21.5                                          | 24.6  | 20.6                                          | 23.8  |
| Frequent errors in calculations                              | 17.1                                          | 18.4  | 16.7                                          | 18.1  | 13.8                                          | 16.9  | 12.7                                          | 14.3  |
| Passivity, desire to shift responsibility                    | 30.3                                          | 59.2  | 31.9                                          | 56.9  | 29.3                                          | 55.4  | 30.2                                          | 53.9  |
| Violation of logic, confused thinking                        | 10.5                                          | 11.8  | 9.7                                           | 11.1  | 9.2                                           | 10.8  | 7.9                                           | 9.5   |
| Impulsiveness, unreasonable decisions                        | 25.0                                          | 31.6  | 26.3                                          | 29.2  | 24.6                                          | 29.2  | 25.3                                          | 26.9  |
| Narrowing the “field of view”, possible options for action   | 9.2                                           | 13.2  | 8.3                                           | 9.7   | 7.7                                           | 9.2   | 7.9                                           | 9.5   |

Source: compiled by the authors of this study

the Shapiro-Wilk  $W$  test. The reliability of the difference between the indicators was determined using the Student's  $t$ -test. The reliability of the difference was set at  $p < 0.05$ . All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

## ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the KhNUA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

## FRAMEWORK

This scientific article was carried out according to the plan of the research work of the National Academy of Internal Affairs for 2020-2026 “Psychological, pedagogical and sociological support of law enforcement officers” (state registration number 0113U008196).

## RESULTS

The results of the assessment of the dynamics of intellectual, behavioral, emotional, and physiological

symptoms of stress in cadets – future law enforcement officers of different training years during their academic training and service activities under martial law are presented in Table 1, Table 2, Table 3, Table 4, respectively. During the study of intellectual symptoms of stress, it was found that even before the beginning (practical training sessions at training grounds and internships in police units), many cadets had symptoms that emphasize the presence of stress even in the usual mode of organizing the academic training process in the HEI with SLE: predominance of negative thoughts (from 26.9 % in the 4<sup>th</sup> training year to 32.9 % in the 1<sup>st</sup> training year), constant, fruitless revolving of thoughts around the problem (from 30.1 % in the 4<sup>th</sup> training year to 35.5 % in the 1<sup>st</sup> training year), difficulty making decisions (from 23.8 % in the 4<sup>th</sup> training year to 34.2 % in the 1<sup>st</sup> training year), bad dreams (from 20.6 % in the 4<sup>th</sup> training year to 26.4 % in the 1<sup>st</sup> training year), desire to shift responsibility to another (from 30.2 % in the 4<sup>th</sup> training year to 30.3 % in the 1<sup>st</sup> training year), impulsivity, unreasonable decisions (from 25.3 % in the 4<sup>th</sup> training year to 26.3 % in the 2<sup>nd</sup> training year) (Table 1).

The most pronounced deterioration of this trend is observed among junior cadets: almost half of the surveyed 1<sup>st</sup> and 2<sup>nd</sup> training year cadets after returning from training grounds have the following symptoms: prevalence of negative thoughts (53.9 % and 48.6 % respectively), constant, fruitless revolving of thoughts

**Table 2.** The dynamics of behavioral symptoms manifestation of stress in cadets – future law enforcement officers of different training years (n = 276) during their academic training and service activities under martial law (before and after practical training sessions at training grounds for the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets; before and after internships in police units for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets), %

| Behavioral symptoms                               | 1 <sup>st</sup> training year<br>(n = 76) |       | 2 <sup>nd</sup> training year<br>(n = 72) |       | 3 <sup>rd</sup> training year<br>(n = 65) |       | 4 <sup>th</sup> training year<br>(n = 63) |       |
|---------------------------------------------------|-------------------------------------------|-------|-------------------------------------------|-------|-------------------------------------------|-------|-------------------------------------------|-------|
|                                                   | Before                                    | After | Before                                    | After | Before                                    | After | Before                                    | After |
| Loss of appetite or overeating                    | 9.2                                       | 10.5  | 8.3                                       | 9.7   | 7.7                                       | 9.2   | 6.3                                       | 7.9   |
| Increase in errors when performing common actions | 14.5                                      | 19.7  | 12.5                                      | 13.8  | 13.8                                      | 16.9  | 9.5                                       | 14.2  |
| Faster or slower speech                           | 6.6                                       | 9.2   | 6.9                                       | 8.3   | 3.1                                       | 3.1   | 3.2                                       | 3.2   |
| Voice trembling                                   | 2.6                                       | 3.9   | 1.4                                       | 1.4   | 0                                         | 0     | 0                                         | 0     |
| Increase in conflict situations                   | 10.5                                      | 14.5  | 8.3                                       | 13.8  | 6.1                                       | 7.7   | 4.8                                       | 4.8   |
| Chronic lack of time                              | 19.7                                      | 26.3  | 19.4                                      | 22.2  | 13.8                                      | 16.9  | 14.2                                      | 15.9  |
| Less time to spend with friends                   | 7.9                                       | 9.2   | 5.6                                       | 6.9   | 3.1                                       | 4.8   | 0                                         | 3.2   |
| Loss of attention to one's appearance, untidiness | 6.6                                       | 7.9   | 1.4                                       | 2.8   | 0                                         | 0     | 0                                         | 0     |
| Antisocial, conflict behavior                     | 9.2                                       | 10.5  | 8.3                                       | 13.8  | 6.1                                       | 7.7   | 3.2                                       | 4.8   |
| Low productivity of activities                    | 26.3                                      | 32.9  | 25.0                                      | 27.8  | 21.5                                      | 24.6  | 17.5                                      | 19.0  |
| Sleep disturbances, insomnia                      | 7.9                                       | 10.5  | 6.9                                       | 8.3   | 6.1                                       | 7.7   | 4.8                                       | 6.3   |
| More intensive smoking, alcohol consumption       | 6.6                                       | 9.2   | 6.9                                       | 13.8  | 7.7                                       | 10.7  | 7.9                                       | 9.5   |

Source: compiled by the authors of this study

around the problem (56.6 % and 51.4 % respectively), difficulties in making decisions (51.3 % and 45.8 % respectively), passivity and desire to shift responsibility (59.2 % and 56.9 % respectively).

The study also revealed a deterioration in cognitive functions in cadets under the influence of stressors of their academic training and service activities. The changes are not too pronounced, but still negative: difficulty concentrating (from 1.7 % in the 4<sup>th</sup> training year to 6.6 % in the 1<sup>st</sup> training year), memory impairment (from 1.6 % to 3.9 %), increased distraction (from 15.9 % to 19.0 %), logic disorders, confused thinking (from 1.3 % to 1.6 %). This shows that cadets' academic training and service activities under martial law, especially practical training sessions at training grounds and internships in practical police units, are extreme, leading to stress and increased anxiety, which negatively affects cadets' intellectual activity, their cognitive processes and the results of their training at the HEI with SLE.

The analysis of the dynamics of behavioral symptoms of stress in cadets shows that they also tend to deteriorate in the process of their academic training and service activities in cadets of all training years, but the negative changes are less pronounced compared to intellectual symptoms (Table 2).

The most pronounced behavioral symptoms of stress among cadets are: low productivity (from 17.5 % in the 4<sup>th</sup> training year to 26.3 % in the 1<sup>st</sup> training year),

chronic lack of time (from 14.2 % in the 4<sup>th</sup> training year to 19.7 % in the 1<sup>st</sup> training year), increase in errors in performing habitual actions (from 9.5 % in the 4<sup>th</sup> training year to 14.5 % in the 1<sup>st</sup> training year), increase in conflict situations (from 4.8 % in the 4<sup>th</sup> training year to 10.5 % in the 1<sup>st</sup> training year), loss of appetite or overeating (from 6.3 % in the 4<sup>th</sup> training year to 9.2 % in the 1<sup>st</sup> training year). Among the cadets of different training years, behavioral symptoms of stress are most pronounced among the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets. At the same time, after completion of tasks at training grounds and in practical police units, the greatest negative increase in the manifestation of behavioral symptoms of stress was also found in junior cadets: low productivity (32.9 % in the 1<sup>st</sup> training year and 27.8 % in the 2<sup>nd</sup> training year), chronic lack of time (26.3 % in the 1<sup>st</sup> training year and 22.2 % in the 2<sup>nd</sup> training year), increased errors in performing habitual actions (19.7 % in the 1<sup>st</sup> training year and 13.8 % in the 2<sup>nd</sup> training year), etc. Also noteworthy are the following behavioral symptoms of stress in cadets, which also have negative dynamics during martial law academic training and service activities, such as: antisocial, conflict behavior; sleep disturbances; loss of attention to their appearance, untidiness; more intensive smoking, alcohol consumption. Such symptoms require taking measures to prevent stress in the cadet environment to improve and quickly restore both the physical and

**Table 3.** The dynamics of emotional symptoms manifestation of stress in cadets – future law enforcement officers of different training years (n = 276) during their academic training and service activities under martial law (before and after practical training sessions at training grounds for the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets; before and after internships in police units for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets), %

| Emotional symptoms                                  | 1 <sup>st</sup> training year<br>(n = 76) |       | 2 <sup>nd</sup> training year<br>(n = 72) |       | 3 <sup>rd</sup> training year<br>(n = 65) |       | 4 <sup>th</sup> training year<br>(n = 63) |       |
|-----------------------------------------------------|-------------------------------------------|-------|-------------------------------------------|-------|-------------------------------------------|-------|-------------------------------------------|-------|
|                                                     | Before                                    | After | Before                                    | After | Before                                    | After | Before                                    | After |
| Worry, anxiety                                      | 21.1                                      | 27.6  | 19.4                                      | 25.0  | 20.0                                      | 24.6  | 19.1                                      | 22.2  |
| Suspicion                                           | 6.6                                       | 7.9   | 5.6                                       | 6.9   | 4.6                                       | 4.6   | 3.2                                       | 3.2   |
| Gloomy mood                                         | 19.7                                      | 30.3  | 18.1                                      | 29.2  | 16.9                                      | 27.7  | 15.9                                      | 25.4  |
| Feeling of constant longing, depression             | 10.5                                      | 13.2  | 9.7                                       | 12.5  | 7.7                                       | 9.2   | 7.9                                       | 9.5   |
| Irritability, bouts of anger                        | 15.8                                      | 26.3  | 13.9                                      | 26.3  | 13.8                                      | 24.6  | 12.7                                      | 22.2  |
| Emotional “stupidity”, indifference                 | 7.9                                       | 9.2   | 5.6                                       | 6.9   | 4.6                                       | 6.1   | 4.7                                       | 4.7   |
| Cynical, inappropriate humor                        | 13.2                                      | 14.5  | 13.9                                      | 13.9  | 7.7                                       | 7.7   | 6.3                                       | 6.3   |
| Decreased sense of self-confidence                  | 6.6                                       | 7.9   | 6.9                                       | 8.3   | 4.6                                       | 4.6   | 0                                         | 3.2   |
| Decreased life satisfaction                         | 3.9                                       | 6.6   | 2.8                                       | 5.6   | 3.1                                       | 4.6   | 1.6                                       | 1.6   |
| Feelings of alienation, loneliness                  | 14.5                                      | 19.7  | 13.9                                      | 18.1  | 12.3                                      | 16.9  | 9.5                                       | 14.3  |
| Loss of interest in life                            | 0                                         | 0     | 0                                         | 0     | 0                                         | 0     | 0                                         | 0     |
| Decreased self-esteem, dissatisfaction with oneself | 5.3                                       | 6.6   | 2.8                                       | 4.2   | 1.5                                       | 3.1   | 1.6                                       | 3.2   |

Source: compiled by the authors of this study

psycho-emotional state of cadets after performing the tasks of their academic training and service activities under martial law.

The assessment of emotional symptoms of stress in cadets – future law enforcement officers of different training years during their academic training and service activities under martial law showed the presence of negative dynamics of the manifestation of most emotional symptoms in cadets of all training years after their practical training sessions at training grounds and internships in police units (Table 3).

The most pronounced at the beginning of the research and, especially, after the cadets returned from the training grounds and internships were the following emotional symptoms of stress: worry, anxiety (from 19.1-21.1 % before practical training sessions at the training grounds and internships to 22.2-27.6 % after), gloomy mood (from 15.9-19.7 % to 25.4-30.3 %), irritability, bouts of anger (from 12.7-15.8 % to 22.2-26.3 %), feelings of alienation, loneliness (from 9.5-14.5 % to 14.3-19.7 %). Other emotional symptoms (suspicion, a sense of constant longing, depression, indifference, decreased self-esteem, guilt, self-dissatisfaction, decreased self-confidence, etc.) also tend to worsen during the cadets' academic training and service activities under martial law, but the level of their manifestation and the magnitude of the change are not sufficiently pronounced. The worst level of emotional symptoms of stress, both before and after practical training sessions at training grounds and internships, was

found in junior cadets. It is important to add that such an emotional symptom as loss of interest in life was not detected in any of the cadets surveyed.

The study of physiological symptoms of stress in cadets – future law enforcement officers shows that, as with the previous symptoms, there is a tendency to increase the number of cadets of all training years with physiological symptoms of stress after returning from their training exercises at training grounds and internships in police units (Table 4).

The most pronounced physiological symptoms of stress were: increased fatigue (before the tasks – from 11.1 % in the 4<sup>th</sup> training year to 19.7 % in the 1<sup>st</sup> training year; after – from 15.9 % in the 4<sup>th</sup> training year to 31.6 % in the 1<sup>st</sup> training year); decreased immunity, frequent malaise (before – from 9.5 % in the 4<sup>th</sup> training year to 14.5 % in the 1<sup>st</sup> training year; after – from 12.7 % in the 4<sup>th</sup> training year to 21.1 % in the 1<sup>st</sup> training year); feeling of muscle tension (before – from 7.9 % in the 4<sup>th</sup> training year to 13.2 % in the 1<sup>st</sup> training year; after – from 9.5 % in the 4<sup>th</sup> training year to 19.7 % in the 1<sup>st</sup> training year); digestive disorders (before – from 6.3 % in the 4<sup>th</sup> training year to 11.8 % in the 1<sup>st</sup> training year; after – from 7.9 % in the 4<sup>th</sup> training year to 18.4 % in the 1<sup>st</sup> training year); weight gain or loss (before – from 7.9 % in the 4<sup>th</sup> training year to 9.2 % in the 1<sup>st</sup> training year; after – from 11.1 % in the 4<sup>th</sup> training year to 13.2 % in the 1<sup>st</sup> training year). The most pronounced manifestation of physiological

**Table 4.** The dynamics of physiological symptoms manifestation of stress in cadets – future law enforcement officers of different training years ( $n = 276$ ) during their academic training and service activities under martial law (before and after practical training sessions at training grounds for the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets; before and after internships in police units for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets), %

| Physiological symptoms                     | 1 <sup>st</sup> training year<br>( $n = 76$ ) |       | 2 <sup>nd</sup> training year<br>( $n = 72$ ) |       | 3 <sup>rd</sup> training year<br>( $n = 65$ ) |       | 4 <sup>th</sup> training year<br>( $n = 63$ ) |       |
|--------------------------------------------|-----------------------------------------------|-------|-----------------------------------------------|-------|-----------------------------------------------|-------|-----------------------------------------------|-------|
|                                            | Before                                        | After | Before                                        | After | Before                                        | After | Before                                        | After |
| Pain of an uncertain nature, headache      | 6.6                                           | 9.2   | 5.6                                           | 6.9   | 4.6                                           | 6.1   | 3.2                                           | 4.7   |
| Increase or decrease in blood pressure     | 3.9                                           | 6.6   | 2.8                                           | 5.6   | 0                                             | 4.6   | 0                                             | 3.2   |
| Rapid or irregular pulse                   | 6.6                                           | 7.9   | 2.8                                           | 5.6   | 0                                             | 0     | 0                                             | 0     |
| Disorders of digestive processes           | 11.8                                          | 18.4  | 9.7                                           | 15.3  | 7.7                                           | 10.8  | 6.3                                           | 7.9   |
| Impaired breathing                         | 0                                             | 0     | 0                                             | 0     | 0                                             | 0     | 0                                             | 0     |
| Feeling of tension in the muscles          | 13.2                                          | 19.7  | 12.5                                          | 18.1  | 10.8                                          | 15.4  | 7.9                                           | 9.5   |
| Increased fatigue                          | 19.7                                          | 31.6  | 18.1                                          | 29.2  | 12.3                                          | 18.5  | 11.1                                          | 15.9  |
| Hand tremors, cramps                       | 2.6                                           | 5.2   | 2.8                                           | 4.2   | 0                                             | 0     | 0                                             | 0     |
| Appearance of allergies, other skin rashes | 3.9                                           | 5.2   | 0                                             | 2.8   | 0                                             | 3.1   | 0                                             | 0     |
| Increased sweating                         | 7.9                                           | 11.8  | 4.2                                           | 6.9   | 3.1                                           | 4.6   | 3.2                                           | 4.7   |
| Increase or loss of body weight            | 9.2                                           | 13.2  | 8.3                                           | 12.5  | 7.7                                           | 9.2   | 7.9                                           | 11.1  |
| Decreased immunity, frequent ailments      | 14.5                                          | 21.1  | 12.5                                          | 18.1  | 10.8                                          | 15.4  | 9.5                                           | 12.7  |

Source: compiled by the authors of this study

symptoms of stress was found in junior cadets. The least pronounced physiological symptoms in cadets in the process of their academic training and service activities under martial law were: violation of freedom of breathing; trembling of hands, cramps; allergies, other skin rashes; changes in blood pressure; rapid or irregular pulse.

The study of the level of stress in cadets according to the test of Y. V. Shcherbatykh shows that before performing the tasks of academic training and service activities under martial law, all the studied groups showed a moderate level of stress (in the range from 6 to 12 points) (Table 5).

After completing the tasks of academic training and service activities, a statistically significant deterioration in the level of stress was found in the cadets of all training years ( $p \leq 0.05$ ). At the same time, while the difference between the stress level of senior (the 4<sup>th</sup> and the 3<sup>rd</sup> training year) cadets before and after their internships in police units is 2.1 and 2.0 points, the difference between the stress level of junior (the 2<sup>nd</sup> and the 1<sup>st</sup> training year) cadets before and after their practical training sessions at training grounds is 2.7 and 3.3 points, respectively. The level of stress in cadets of all training years after completing the tasks of their academic training and service activities, although worsened, is still within a moderate level. It was also found that at the beginning of the research there was no significant difference in the level of stress between

the cadets of all training years ( $p > 0.05$ ), and at the end of the research, a significantly worse level of stress was found in the 1<sup>st</sup> training year cadets compared to the 4<sup>th</sup> training year by 2.4 points, which indicates an insufficient level of stress resilience in junior cadets and the need to find effective means and methods to increase it during their academic training and service activities in the HEI with SLE during the war.

## DISCUSSION

Scientists [4, 15, 16] argue that stress is an integral part of modern human life, thanks to which adaptation to environmental conditions occurs, which is carried out through a universal complex of neurohumoral reactions. However, during war, stress symptoms are more pronounced than in peacetime and have a more negative impact on both mental and somatic health. According to scientists [11, 17], one of the most stressful professions is law enforcement. The stressfulness of the profession of law enforcement officers is permanent. This is due to constant participation in exhausting law enforcement activities, systematic risk to their own life and health, conflict environment, lack of stability of tasks, publicity of activities, the need to make quick decisions, use of physical force, special equipment, weapons, etc. [18]. These signs and operational activities of police officers confirm our opinion that systematic work on stress prevention and counteracting negative



**Table 5.** Dynamics of stress level in cadets – future law enforcement officers (n = 276) before and after performing tasks of their academic training and service activities under martial law, points

| Cadets' training years | Stress level |            | $\Delta$ | t / p      |
|------------------------|--------------|------------|----------|------------|
|                        | Before       | After      |          |            |
| 1 <sup>st</sup> (n=76) | 8.1±0.72     | 11.4±0.99  | 3.3      | 2.70/≤0.05 |
| 2 <sup>nd</sup> (n=72) | 7.5±0.66     | 10.2±0.93  | 2.7      | 2.37/≤0.05 |
| 3 <sup>rd</sup> (n=65) | 7.2±0.61     | 9.4±0.90   | 2.0      | 2.02/≤0.05 |
| 4 <sup>th</sup> (n=63) | 6.9±0.58     | 9.0±0.88   | 2.1      | 2.00/≤0.05 |
| t / p 1-2              | 0.61/>0.05   | 0.98/>0.05 |          |            |
| t / p 2-3              | 0.33/>0.05   | 0.62/>0.05 |          |            |
| t / p 3-4              | 0.36/>0.05   | 0.32/>0.05 |          |            |
| t / p 1-4              | 1.31/>0.05   | 2.02/≤0.05 |          |            |

Notes:  $\Delta$  – difference between the studied indicators; t – value of Student's t-test; p – level of statistical significance of differences; t / p 1-2 ... 1-4 – value of Student's t-test and level of significance between the indicators of cadets of the respective training year

Source: compiled by the authors of this study

factors of law enforcement should begin in the cadet environment, and therefore – at the stage of forming a professional personality. This will contribute to the rapid recovery of both the physical and psycho-emotional state of law enforcement officers after performing the tasks of their academic training and service activities under martial law.

Today, cadets of HEIs with SLE are forced to study in conditions of dispersion in remote areas from their permanent deployment, in conditions of constant air raids, in air-raid shelters and hiding-places, without proper conditions for their training and rest, which significantly affects the level of manifestation of various stress symptoms. The academic training and service activities of cadets of HEIs with SLE during the war, accompanied by the negative impact of a number of stress factors, led to an increase in the level of stress in cadets – future law enforcement officers, which is reflected in the deterioration of their psychophysical health, the deterioration of the quality of their academic training activities and the effectiveness of their performance of service tasks [19]. This is confirmed by our research results. In particular, an analysis of the dynamics of intellectual, emotional, and behavioral symptoms of stress in cadets of all training years shows that they tend to deteriorate in the course of their academic training and service activities during war. This undoubtedly has an impact on their physiology, leading to a progressive decrease in immunity, frequent illnesses, digestive disorders, etc.

Scientists [20, 21] note that stress causes many different symptoms on the mental and somatic levels. Psychological symptoms include increased anxiety, worry, fear, low mood, depression, impaired attention, perception, etc. Somatic symptoms include loss of appetite, sleep disturbances, decreased working capacity,

headaches, back pain, digestive problems, increased fatigue, decreased immunity, which is reflected in an increase in the number of diseases, etc. In our research, we conducted a comparative analysis of the dynamics of various symptoms of stress manifestation in cadets – future law enforcement officers of different training years before and after performing the tasks of their academic training and service activities (for the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets before and after their practical training sessions at training grounds, for the 3<sup>rd</sup> and 4<sup>th</sup> training year cadets before and after their internships in practical police units). The results of studies by many scientists [3, 5, 7, 8] confirm that during war, stress has more pronounced symptoms, especially in cadets who have not developed skills to cope with stressful phenomena and have a low level of stress resilience (junior cadets). The results of other scientists [10, 11, 22], who studied the level of stress in law enforcement officers under the influence of stressors of professional activities, were supplemented with information about a significant increase in the level of stress in cadets during the performance of tasks of their academic training and service activities in war conditions. The data of many scientists [2, 17, 18] on the level and dynamics of the manifestation of intellectual, behavioral, emotional and physiological symptoms of stress in cadets – future law enforcement officers of different training years during their academic training and service activities under martial law (before and after extreme conditions of activities) have been expanded.

To overcome stress and prevent it during wartime, experts [3, 14, 23] recommend taking care of sufficient rest, healthy eating, regular physical activity, communication with loved ones, finding a hobby, and developing skills to overcome stressful situations.

## CONCLUSIONS

The dynamics of the manifestation of intellectual, behavioral, emotional and physiological symptoms of stress in cadets – future law enforcement officers of different training years during their academic training and service activities under martial law is investigated. The most pronounced manifestation of all stress symptoms was found in the 1<sup>st</sup> and 2<sup>nd</sup> training year cadets. Among the most pronounced symptoms of stress in cadets after completing the tasks of their academic training and service activities were found: intellectual – predominance of negative thoughts (53.9 % and 48.6 %, respectively), constant, fruitless revolving of thoughts around the problem (56.6 % and 51.4 %), difficulties in decision-making (51.3 % and 45.8 %), passivity and desire to shift responsibility (59.2 % and 56.9 %); behavioral – low productivity (32.9 % and 27.8 %), chronic lack of time (26.3 % and 22.2 %), increased errors in performing habitual actions (19.7 % and 13.8 %); emotional – worry, anxiety (27.6 % and 25.0 %), gloomy mood (29.2 % and 30.3 %), irritability, bouts of anger (26.3 % and 26.3 %), feelings of alienation, loneliness (18.1 % and 19.7 %); physiological – increased fatigue (29.2 % and 31.6 %); decreased immunity, frequent illnesses (18.1 % and 21.1 %); digestive disorders (15.3 % and 18.4 %) and others. This is due to insufficient psycho-emotional

resilience to stressful factors of academic training and service activities among junior cadets, inadequate skills in utilizing existing means for stress prevention during task performance under martial law, and a low level of stress resilience. It has been found that among the studied cadets – future law enforcement officers of all training years, the worst level of stress, both before the start of the tasks of their academic training and service activities (8.1 and 7.5 points in the 1<sup>st</sup> and the 2<sup>nd</sup> training years, respectively) and after (11.4 and 10.2 points, respectively), was found in the junior training years.

The research demonstrates the urgent need to develop cadets' stress resilience during their academic training and service activities in the HEI with SLE to ensure the effectiveness of their academic training activities under martial law and further service activities in practical police units, as well as to develop skills in the use of available means to prevent stressful phenomena in the course of academic training and service activities.

## PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the dynamics of stress symptoms manifestation in police officers of different ages before and after performing combat missions of different duration in the combat zone.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Gingival health status and tooth wear in relation to nutritional status among primary school children in Al-Najaf city

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## ABSTRACT

**Aim:** To assess the gingival health status and tooth wear in relation to nutritional status among primary schoolchildren in Al-Najaf city.

**Materials and Methods:** In this study, 1010 children (476 males and 534 females) from primary schools were included with age range 6-14 years old. The gingival inflammation was evaluated by the application of Gingival Index of Loe and Silness. Nutritional status assessed by using body mass index for age. Data collected through clinical examination with the consent of parents and teachers.

**Results:** Prevalence of gingivitis was 47% mild gingivitis, 6.5% moderate gingivitis, and 0.8% severe gingivitis. The higher percentage of mild gingivitis was found with underweight group 46.2% for male and 60.7% for female. For total sample, the higher mean value of Gingival Index was found with underweight group 0.67, with significant relation between groups of nutritional status  $p=0.013$ . Prevalence of tooth wear was about 29.1%. The percentage of tooth wear was higher in female 32.4% than male 25.4% with significant relation  $p=0.009$ .

**Conclusions:** Mild gingivitis was higher than moderate and severe gingivitis. Gingivitis is higher in females than males. A highly significant relation was recorded between gingivitis and gender. A significant association between gingivitis and nutritional status. Gingivitis was found higher with underweight group than other groups of nutritional status. A higher percentage of tooth wear was found with underweight children.

**KEY WORDS:** Gingival Health, Gingivitis, Tooth Wear, Nutritional Status, Body Mass Index

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## ABBREVIATIONS

GI: Gingival Index

BMI: Body Mass Index

PD: Periodontal Diseases

## INTRODUCTION

One of the most prevalent chronic illnesses that have afflicted people for ages is periodontal disease (PD). Gingivitis, or localized gingival inflammation, is the first sign of Parkinson's disease. Gingivitis is a frequent oral health issue and a mild manifestation of Parkinson's disease [1]. The causative factor is dental plaque. Although gingivitis is curable, if left untreated, it frequently develops into periodontitis, a more serious illness. Because of this, prompt gingivitis treatment keeps the periodontium from being destroyed [2]. The body mass index (BMI), which is a measure of weight adjusted for height and is computed as weight (in kilograms) divided by the square of height (in meters) ( $\text{kg}/\text{m}^2$ ), is the most widely used index to assess body fat. In the realm of nutrition,

the term body mass index (BMI) is frequently used. BMI determines if a person's weight is within a healthy range [3]. An essential element of oral health is nutrition. In both health and illness, nutrition and the integrity of the mouth cavity work in constant harmony. The development of the oral cavity and the advancement of oral disorders can be impacted by malnutrition due to changes in tissue homeostasis, decreased resistance to microbial biofilms, and diminished tissue healing ability. Oral health influences nutrition, and nutrition influences oral health. Because of this mutually reinforcing link, dental health is positively correlated with nutritional health. There is a complicated relationship between diet and periodontal disease. Periodontal disease cannot be prevented or stopped by dietary consumption of micronutrients or macronutrients, but their deficiency may increase the risk of periodontal inflammation [4]. Tooth wear or tooth surface loss, also known as non-carious loss of tooth tissue, is a common occurrence that can occur on various surfaces for a variety of reasons [5]. Diet, dentition, enamel structure and hardness, jaw

muscles, and chewing habits can all affect the pattern and degree of tooth wear. Other factors that may affect this include the load placed on opposing surfaces, the length of time that opposing surfaces are in contact with one another, and the relative speed and direction of movement of opposing surfaces [6]. In the current study, we evaluated the nutritional quality of elementary kids in Najaf City in connection to their gingival health and tooth wear.

## AIM

The aim of this study is to assess the gingival health status and tooth wear in relation to nutritional status among primary schoolchildren in Al-Najaf city.

## MATERIALS AND METHODS

### STUDY DESIGN AND SETTING

This cross-sectional study was conducted in March/2024 to June/2024 in Al-Najaf city, Iraq. Using a straightforward random selection technique, five elementary schools were chosen at random from the city's school districts.

### PARTICIPANTS

A total of 1010 children, aged 6-14 years, were recruited from the selected primary schools. All children attending the selected schools within the specified age range were eligible for inclusion. Exclusion criteria (e.g., presence of systemic diseases affecting oral health, orthodontic treatment, use of medications known to affect gingival tissues), the final sample consisted of 476 males and 534 females.

### DATA COLLECTION

Data collection was performed by calibrated examiners. Clinical examinations were conducted in a standardized manner, using a dental mirror and a periodontal probe (WHO probe) under adequate lighting by dental chair light.

### GINGIVAL ASSESSMENT

Gingival inflammation was assessed using the Gingival Index (GI) of Löe and Silness (1963) [7]. The GI was recorded for six index teeth [teeth #3, #9, #12, #19, #25, #28 (Universal numbering system)]. Each tooth was divided into four gingival units (mesiobuccal, midbuccal, distobuccal, and lingual/palatal), and each unit was scored as zero for normal gingiva while mild inflammation as slight change in color, slight edema

and no bleeding on probing was given score one. Score two was given to moderate inflammation by which is characterized redness, edema, glazing and bleeding on probing. Severe inflammation manifested by marked redness and edema, ulceration, tendency to spontaneous bleeding was given score 3.

### NUTRITIONAL STATUS ASSESSMENT

The Body Mass Index (BMI) for age, which is computed as weight (in kilograms) divided by height (in meters squared) ( $\text{kg/m}^2$ ), was used to evaluate nutritional status. Weight was measured using a digital scale with children wearing light clothing and no shoes. Children were measured for height using a portable stadiometer while standing upright, barefoot, and with their upper back, buttocks, and heels touching the device. The growth charts were used to calculate BMI-for-age percentiles [8]. WHO Child Growth Standards, CDC Growth Charts categorized Underweight below the 5th percentile while healthy weight ranges from 5th to < 85th percentile.

Overweight and obesity were categorized as (85th to < 95th percentile) and ( $\geq$  95th percentile) respectively.

### TOOTH WEAR ASSESSMENT

A dichotomised scoring system based on Bardsley et al. (2004) [9] was used to evaluate tooth wear. The labial, incisal, and lingual/palatal surfaces of the six upper and lower anterior teeth as well as the occlusal surfaces of the four first permanent molars were among the forty surfaces that were inspected. Each surface was scored as follows:

- 0 = No wear or wear confined to enamel
- 1 = Wear exposing dentine (including cupping of dentine)

### CALIBRATION AND RELIABILITY

All examinations were carried out by a single trained and calibrated examiner (Dr. A.H.F) to eliminate inter-examiner variability. Dr. A.H.F re-examined 10% of the participants  $n=101$  one week after the initial examination. Intra-examiner agreement, assessed using Cohen's Kappa, was 0.92 for GI and 0.88 for tooth wear.

### STATISTICAL ANALYSIS

Microsoft Excel 2013 and SPSS version 23 (IBM Corp., Armonk, NY) were used to analyse the data. For every variable, descriptive statistics such as means, standard deviations, percentages, and frequencies were computed.

**Table 1.** Distribution and percentage of sample according to nutritional status

| Gender | Total No. | Under weight |      | Healthy weight |      | Over weight |      | Obese |      |
|--------|-----------|--------------|------|----------------|------|-------------|------|-------|------|
|        |           | No.          | [%]  | No.            | [%]  | No.         | [%]  | No.   | [%]  |
| Male   | 476       | 78           | 16.4 | 312            | 65.5 | 54          | 11.4 | 32    | 6.7  |
| Female | 534       | 61           | 11.4 | 305            | 57.1 | 87          | 16.3 | 81    | 15.2 |
| Total  | 1010      | 139          | 13.8 | 617            | 61.1 | 141         | 13.9 | 113   | 11.2 |

**Table 2.** Distribution and percentage of sample according to gingival status and gender with significant relation

| Gender | Total No. | Healthy gingiva |      | Mild gingivitis |      | Moderate gingivitis |     | Sever gingivitis |     | x <sup>2</sup> p-value |
|--------|-----------|-----------------|------|-----------------|------|---------------------|-----|------------------|-----|------------------------|
|        |           | No.             | [%]  | No.             | [%]  | No.                 | [%] | No.              | [%] |                        |
| Male   | 476       | 249             | 52.3 | 189             | 39.7 | 32                  | 6.7 | 6                | 1.3 | 0.001                  |
| Female | 534       | 212             | 39.7 | 286             | 53.5 | 34                  | 6.4 | 2                | 0.4 |                        |
| Total  | 1010      | 461             | 45.7 | 475             | 47   | 66                  | 6.5 | 8                | 0.8 |                        |

**Table 3.** Number and percentage of gingival health status according to nutritional status and gender

| Gender | Total No. | Gingival status | Under weight |      | Healthy weight |      | Over weight |      | Obese |      |
|--------|-----------|-----------------|--------------|------|----------------|------|-------------|------|-------|------|
|        |           |                 | No.          | [%]  | No.            | [%]  | No.         | [%]  | No.   | [%]  |
| Male   | 476       | Healthy         | 37           | 47.4 | 169            | 54.1 | 26          | 48.1 | 17    | 53.1 |
|        |           | Mild            | 36           | 46.2 | 116            | 37.2 | 23          | 42.6 | 14    | 43.8 |
|        |           | Mod.            | 4            | 5.1  | 23             | 7.4  | 4           | 7.4  | 1     | 3.1  |
|        |           | Sever           | 1            | 1.3  | 4              | 1.3  | 1           | 1.9  | 0     | 0    |
| Female | 534       | Healthy         | 20           | 32.8 | 116            | 38   | 39          | 44.8 | 37    | 45.7 |
|        |           | Mild            | 37           | 60.7 | 170            | 55.8 | 38          | 43.7 | 41    | 50.6 |
|        |           | Mod.            | 3            | 4.9  | 19             | 6.2  | 9           | 10.3 | 3     | 3.7  |
|        |           | Sever           | 1            | 1.6  | 0              | 0    | 1           | 1.2  | 0     | 0    |
| Total  | 1010      | Healthy         | 57           | 41   | 285            | 46.2 | 65          | 46.1 | 54    | 47.8 |
|        |           | Mild            | 73           | 52.5 | 286            | 46.4 | 61          | 43.3 | 55    | 48.7 |
|        |           | Mod.            | 7            | 5    | 42             | 6.8  | 13          | 9.2  | 4     | 3.5  |
|        |           | Sever           | 2            | 1.4  | 4              | 0.6  | 2           | 1.4  | 0     | 0    |

ed. Associations between categorical variables (such as gingival status and gender, tooth wear and nutritional status) were evaluated using the chi-square test. To compare the mean GI scores among the various nutritional status groups, a one-way ANOVA was designed.

ETHICAL APPROVAL

The University of Kufa’s College of Dentistry’s Local Ethical Committee granted ethical approval (Reference number 745). All participating children’s parents or legal guardians gave their informed consent, and the children themselves gave their assent. The study complied with the Declaration of Helsinki’s guidelines

RESULTS

Table 1 demonstrated the distribution and percentage of sample according to nutritional status. In both

male and female, the higher percentage 65.5% and 57.1% respectively was recorded with healthy weight groups. For male, the lower percentage 6.7% was found with obese groups, while foe female, the lower percentage 11.4% was recorded with underweight group.

Table 2 illustrated the distribution and percentage of sample according to gingival status and gender with significant relation. The higher percentage of gingival status was found with healthy gingiva 52.3% for male, and mild gingivitis 53.5 for female. The lower percentage of gingival status was recorded with sever gingivitis 1.3% for male and 0.4% for female. Using chi-square test, a high significant relation was recorded between gingival status and gender p=0.001. Prevalence of gingival health status was recorded about 45.7% healthy gingiva, 47% mild gingivitis, 6.5% moderate gingivitis, and 0.8% sever gingivitis as in Figure 1.



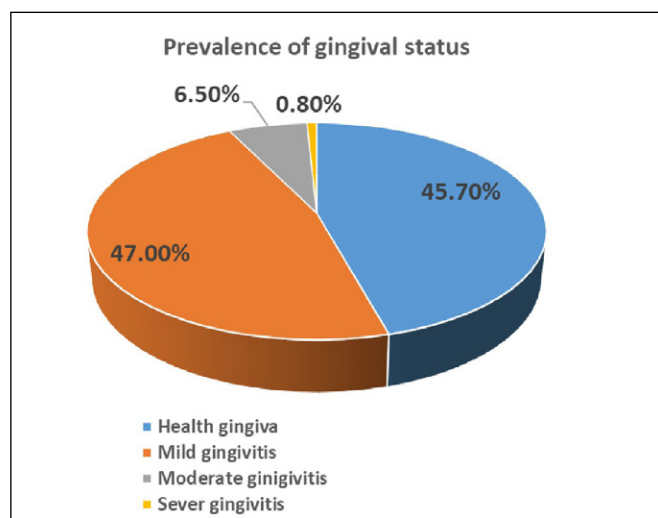


**Fig. 1.** Prevalence of gingival health status

Table 3 demonstrated the number and percentage of gingival health status according to nutritional status and gender. For healthy gingiva, the higher percentage was recorded with healthy weight group 54.1% for male, and with obese group 45.7% for female. For mild gingivitis, the higher percentage was found with underweight group 46.2% for male and 60.7% for female. For moderate gingivitis, the higher percentage was recorded with health and overweight group for male 7.4% and with overweight group for female 10.3%. Finally for severe gingivitis, the higher percentage was found with overweight group 1.9% for male and with underweight group 1.6% for female.

Table 4 illustrated mean and standard deviation of GI according to the gender with highly significant relation. For male, the higher mean value of GI was found with overweight group 0.63, with no significant relation between groups of nutritional status  $p=0.269$ . For female, the higher mean value of GI was found with underweight group 0.75, with significant relation between groups of nutritional status  $p=0.003$ . For total sample, the higher mean value of GI was found with underweight group 0.67, with significant relation between groups of nutritional status  $p=0.013$ . Prevalence of tooth wear 29.1% was recorded as in Figure 2.

Table 5 illustrated the distribution and percentage of tooth wear types according to gender with significant relation. The higher percentage of tooth wear was found with enamel 17.8% for male and 25.6% for female, while the lower percentage of tooth wear was concluded with pulp 1.9% for male and 0.2% for female. By using chi-square test, a high significant relation was found between tooth wear types and gender  $p=0.001$ .

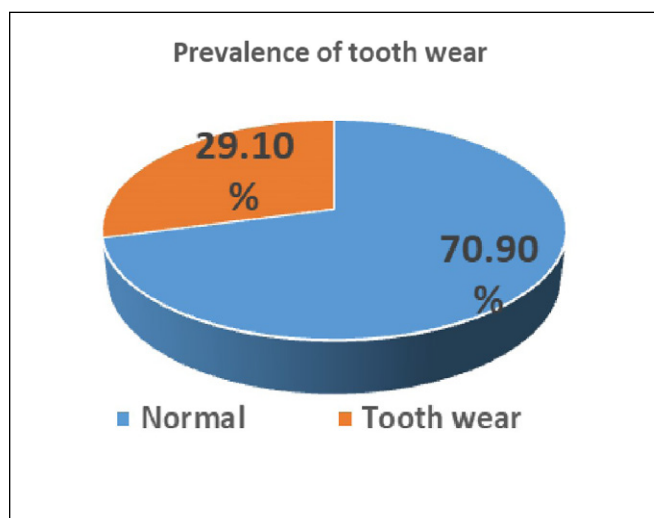


**Fig. 2.** Prevalence of tooth wear

Table 6 recorded the distribution and percentage of tooth wear according to nutritional status and gender with significant relation. For male, the higher percentage of tooth wear was found with underweight group 28.2% with no significant relation  $p=0.979$ . For female, the higher percentage of tooth wear was recorded with underweight group 37.7% with no significant relation  $p=0.637$ . The percentage of tooth wear was higher in female 32.4% than male 25.4% with significant relation  $p=0.009$ .

## DISCUSSION

In the present study prevalence of gingivitis among children was recorded at about 54.3%. Other studies had results higher than this percentage. The differences may be attributable to the difference in method of evaluation and age group and geographic area. Variable not only between races but also between different individuals of the same race [10]. In the current study, a highly significant relation was recorded between gingivitis and gender. Gingivitis is higher in female 60.3% than male 47.7%. In contrast other studies showed gingivitis in males is higher than females [11-12]. This difference could be due to the lack of proper supervised tooth brushing in young children. It can also be speculated that boys are less likely to follow oral hygiene instructions when compared to girls [13]. Other study showed no differences in gingival disease between male and female [14-15], this related to the poor oral hygiene practice among both genders. In this study, the higher mean value of GI was found with underweight group, with significant relation between GI and nutritional status. This result in line with many studies, like study by Inaam M S and Wesal A. (2103) [16], study by Harun et al (2016) [10], and study by Martens et al (2017) [17]. It is caused by the high amount of calculus in the oral cavity, and economic and social condition.

**Table 4.** Mean and standard deviation of GI according to the gender with highly significant relation

| Gender | Nutrition status | GI   |      | p-value |
|--------|------------------|------|------|---------|
|        |                  | Mean | SD   |         |
| Male   | Under            | 0.6  | 0.65 | 0.269   |
|        | Healthy          | 0.56 | 0.69 |         |
|        | Over weight      | 0.63 | 0.7  |         |
|        | Obese            | 0.5  | 0.56 |         |
| Female | Under            | 0.75 | 0.62 | 0.003   |
|        | Healthy          | 0.68 | 0.59 |         |
|        | Over weight      | 0.68 | 0.71 |         |
|        | Obese            | 0.58 | 0.57 |         |
| Total  | Under            | 0.67 | 0.64 | 0.013   |
|        | Healthy          | 0.62 | 0.64 |         |
|        | Over weight      | 0.66 | 0.71 |         |
|        | Obese            | 0.56 | 0.57 |         |

**Table 5.** Distribution and percentage of tooth wear types according to gender with significant relation

| Gender | Total No. | No tooth wear |      | Tooth wear enamel |      | Tooth wear dentin |     | Tooth wear pulp |     | x <sup>2</sup> p-value |
|--------|-----------|---------------|------|-------------------|------|-------------------|-----|-----------------|-----|------------------------|
|        |           | No.           | [%]  | No.               | [%]  | No.               | [%] | No.             | [%] |                        |
| Male   | 476       | 355           | 74.6 | 85                | 17.8 | 27                | 5.7 | 9               | 1.9 | 0.001                  |
| Female | 534       | 361           | 67.6 | 137               | 25.6 | 35                | 6.6 | 1               | 0.2 |                        |
| Total  | 1010      | 716           | 70.9 | 222               | 22   | 62                | 6.1 | 10              | 1   |                        |

**Table 6.** Distribution and percentage of tooth wear according to nutritional status and gender with significant relation

| Gender | Nutritional status | No. | Without<br>Tooth wear |      | With<br>Tooth wear |      | x <sup>2</sup><br>p-value | x <sup>2</sup><br>p-value |
|--------|--------------------|-----|-----------------------|------|--------------------|------|---------------------------|---------------------------|
|        |                    |     | No.                   | %    | No.                | %    |                           |                           |
| Male   | Underweight        | 78  | 56                    | 71.8 | 22                 | 28.2 | 0.979                     | 0.009                     |
|        | Healthy            | 312 | 234                   | 75   | 78                 | 25   |                           |                           |
|        | Overweight         | 54  | 41                    | 75.9 | 13                 | 24.1 |                           |                           |
|        | Obese              | 32  | 24                    | 75   | 8                  | 25   |                           |                           |
|        | Total              | 476 | 355                   | 74.6 | 121                | 25.4 |                           |                           |
| Female | Underweight        | 61  | 38                    | 62.3 | 23                 | 37.7 | 0.637                     |                           |
|        | Healthy            | 305 | 210                   | 68.9 | 95                 | 31.1 |                           |                           |
|        | Overweight         | 87  | 56                    | 64.4 | 31                 | 35.6 |                           |                           |
|        | Obese              | 81  | 57                    | 70.4 | 24                 | 29.6 |                           |                           |
|        | Total              | 534 | 361                   | 67.6 | 173                | 32.4 |                           |                           |

Social-economic influences plaque index because the behavior of children with bad nutrition, they don't know how to maintain their oral hygiene and bad nutrient may cause the weakness of immune system response and it may relate to the increase of dental plaque [16]. One of the most common causes of tooth wear is bruxism. It is seen as a multifactorial parafunction with a complex and contentious aetiology that is regulated centrally as opposed to peripherally [18]. The prevalence of tooth wear in this study was found to be 29.1%. Marianna et al's

investigation from 2021 revealed the similar prevalence of 29% [19]. The percentage of tooth wear prevalence was higher in other research than in this one [20–21]. There are many different reasons of tooth wear, and from a therapeutic perspective, it's crucial to distinguish between the many types [22]. A few research examined the relationship between the prevalence of tooth wear and nutritional variables. The prevalence of tooth wear and dietary status did not significantly correlate in the current investigation. These findings are consistent with

those of earlier investigations [23–24]. In the current study, children who were underweight had a higher rate of tooth wear. This finding is consistent with research by Francisco et al. (2015), which found that hunger may be one factor compromising salivary gland function in children who are underweight. Reduced salivary gland function has been linked to deficiencies in some nutrients, including protein, minerals, and vitamins. Saliva plays a crucial role in providing sufficient protection against oral illnesses, particularly tooth wear, as studies on children have demonstrated a correlation between the degree of protein-energy deficiency and the degree of decrease in stimulated salivary secretion rate [25].

## CONCLUSIONS

About 54.3% of children were found to have gingivitis. More people had mild gingivitis than moderate or severe gingivitis. Gingivitis and gender were shown to be significantly correlated. Females are more likely than guys to have gingivitis. A strong correlation between dietary status and gingivitis. Underweight groups had higher rates of gingivitis than other nutritional status groups. Among schoolchildren, the prevalence of tooth wear was 29.1%. The prevalence of tooth wear and dietary status did not significantly correlate. The proportion of tooth wear was higher in children who were underweight.

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## DATA ACCESS

*The data generated and analyzed in the present investigation can be made accessible upon a fair request.*

## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Assessment of health-related quality of life in adolescents in the conditions of war in Ukraine

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## ABSTRACT

**Aim:** The aim of our study was to assess the health-related quality of life of adolescents in the city of Myrhorod during the war in Ukraine.

**Materials and Methods:** The study involved 102 adolescents (mean age -  $14.5 \pm 1.6$  years) from the city of Myrhorod, including 58 girls and 44 boys. To study the quality of life of adolescents, the KIDSCREEN - 52 methodology was used, which is most often used in most European countries and does not require adaptation, since it includes a universal and local element. Frequency analysis with extensive indicator calculation was used to analyze the survey responses. MS Office 2016 software package, namely MS Excel module, was used to process statistical information.

**Results:** The results showed low and medium levels in such quality of life indicators as physical activity and health, psychological well-being, emotional state, autonomy, relationships with parents, social support and experiences of bullying. Sufficient levels were recorded in such quality of life indicators as financial resources, self-perception and school environment. Reduced quality of life among adolescents in relation to relationships with parents, emotional state and free time, as well as gender differences in self-perception and physical activity.

**Conclusions:** This study allowed us to analyze ten key dimensions of HRQoL and develop practical recommendations for adolescents that cover the importance of regular physical activity, supporting psychological well-being, building healthy relationships with parents and peers, and improving self-educational and organizational skills for effective use of free time.

**KEY WORDS:** health, health-related quality of life, KIDSCREEN-52 questionnaire, HRQOL measurements, adolescents

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## INTRODUCTION

Quality of life is a characteristic of a person's physical, psychological, emotional and social functioning, which is based on subjective perception [1].

In medicine, the concept of quality of life refers primarily to the state of health, which is why the term "health-related quality of life" (HRQoL) is most often used. The direction of supporting for HRQoL has become one of the important components of health care in the world [2].

The child's health is a factor and condition for its optimal further development, realization of biologically determined possibilities, long social, full and fruitful life. Methods for assessing the parameters of a child's quality of life are used in various fields of medicine: when conducting population studies, when developing medical standards and studying the effectiveness of

new methods of treatment and prevention, for individual monitoring of the condition of a sick child during treatment, etc. [3].

## AIM

The aim of our study was to assess the health-related quality of life of adolescents in the city of Myrhorod during the war in Ukraine.

## MATERIALS AND METHODS

The KIDSCREEN-52 questionnaire was used in the study to assess health-related quality of life [4]. It contains 52 items and covers 10 key aspects: physical and psychological well-being, emotional state, self-perception, level of autonomy, relationship with parents and family

**Table 1.** Level of health-related quality of life among adolescents in the city of Myrhorod

| Key aspects                                       | Level of health-related quality of life indicator, % |       |        |       |                               |       |      |       |
|---------------------------------------------------|------------------------------------------------------|-------|--------|-------|-------------------------------|-------|------|-------|
|                                                   | Low                                                  |       | Middle |       | Sufficient<br>(default value) |       | High |       |
|                                                   | Boys                                                 | Girls | Boys   | Girls | Boys                          | Girls | Boys | Girls |
| Physical activity and health                      | 10.7                                                 | 20.7  | 26.2   | 31.1  | 42.1                          | 27.6  | 21   | 20.7  |
| Psychological well-being                          | 21                                                   | 38.3  | 18     | 14.1  | 33.9                          | 23.8  | 26.3 | 23.8  |
| General condition<br>(mood and emotions)          | 36.8                                                 | 48.2  | 15.7   | 15    | 31.6                          | 33.2  | 15.8 | 3.44  |
| Self-perception                                   | 5.3                                                  | 17.2  | 27.5   | 20    | 62.7                          | 55.9  | 4.5  | 6.9   |
| Autonomy                                          | 37.4                                                 | 37    | 10.3   | 20.7  | 37                            | 35.1  | 10.3 | 17.2  |
| Relationships with parents and<br>staying at home | 36.8                                                 | 40.4  | 15.6   | 12.1  | 42.3                          | 32.7  | 5.3  | 10.3  |
| Financial resources                               | -                                                    | 6.9   | 25.3   | 20.2  | 64.3                          | 55.7  | 10.5 | 17.2  |
| Relationships with peers                          | 21                                                   | 34.5  | 23.4   | 17.9  | 50.3                          | 44.1  | 5.3  | 3.4   |
| School environment                                | 26.3                                                 | 20.7  | 10.8   | 10.1  | 21.1                          | 52    | 42.1 | 17.2  |
| Bullying                                          | -                                                    | 17.2  | 36.8   | 34.5  | 63.1                          | 48.4  | -    | -     |

Source: compiled by the authors of this study

environment, financial resources, peer support, social contacts, school environment, and bullying.

The study was conducted between May and June 2024, and the study design was cross-sectional. The KIDSCREEN questionnaire was used to assess the subjective health and psychological, mental and social well-being of adolescents aged 13 to 17 years.

A manual data processing method was used to evaluate the KIDSCREEN – 52 items [5]. The research was conducted using a questionnaire survey - the most popular method of collecting information, which makes it possible to obtain reliable information that is amenable to statistical processing. [6].

The study involved 102 adolescents (mean age - 14.5±1.6 years) from the city of Myrhorod, including 58 girls and 44 boys. To study the quality of life of adolescents, the KIDSCREEN - 52 methodology was used, which is most often used in most European countries and does not require adaptation, since it includes a universal and local element [7]..

STATISTICAL METHODS

Frequency analysis with extensive indicator calculation was used to analyze the survey responses. MS Office 2016 software package, namely MS Excel module, was used to process statistical information.

ETHICS

The research was conducted in accordance with ethical standards. The parents of the study participants were informed about the purposes of the study and gave informed consent.

RESULTS

ASSESSMENT OF THE QUALITY OF LIFE OF ADOLESCENTS BASED ON THE RESULTS OF THE KIDSCREEN-52 QUESTIONNAIRE

Assessing health-related quality of life is an important tool for identifying the physical, psychological, and social well-being of adolescents. In the context of the war in Ukraine, this problem becomes particularly important, as military actions, forced displacement, psychological stress, and loss of stability and security significantly affect the health and well-being of adolescents. The use of the KIDSCREEN-52 methodology allows for a comprehensive assessment of the main aspects of HRQoL and the development of effective recommendations for supporting the psycho-emotional state of adolescents during wartime.

The results indicate a reduced quality of life among adolescents in terms of relationships with parents, emotional state and free time, as well as gender differences in self-perception and physical activity and are generally consistent with the results of other scientists and studies (Table 1). Thus, the study reflects contemporary problems of adolescents, confirmed by global trends in science.

ANALYSIS OF QUALITY OF LIFE INDICATORS OF ADOLESCENTS USING THE KIDSCREEN-52 METHOD

Physical activity is evidence of physical development, and therefore the main indicator of human health, so the study of health-related quality of life began with it.



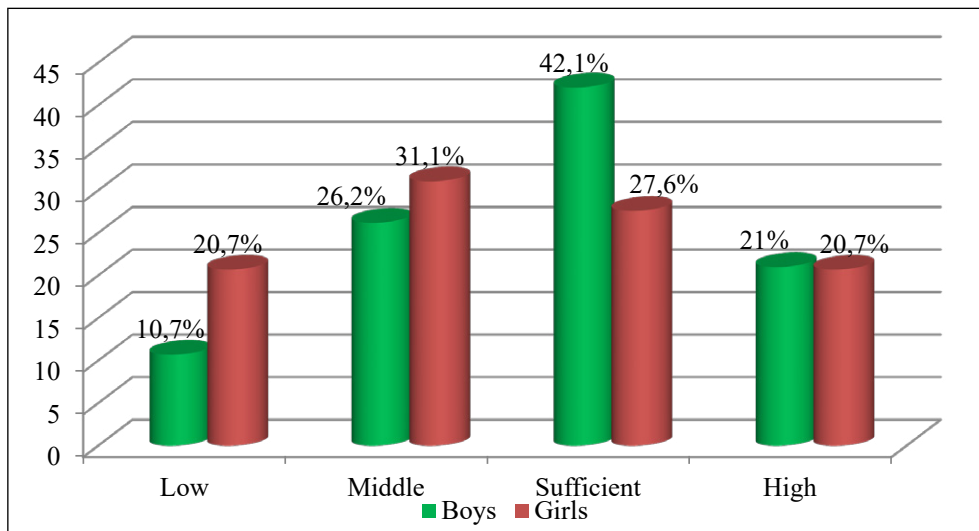


**Fig. 1.** Level of physical activity and health status among respondents  
Picture taken by the authors

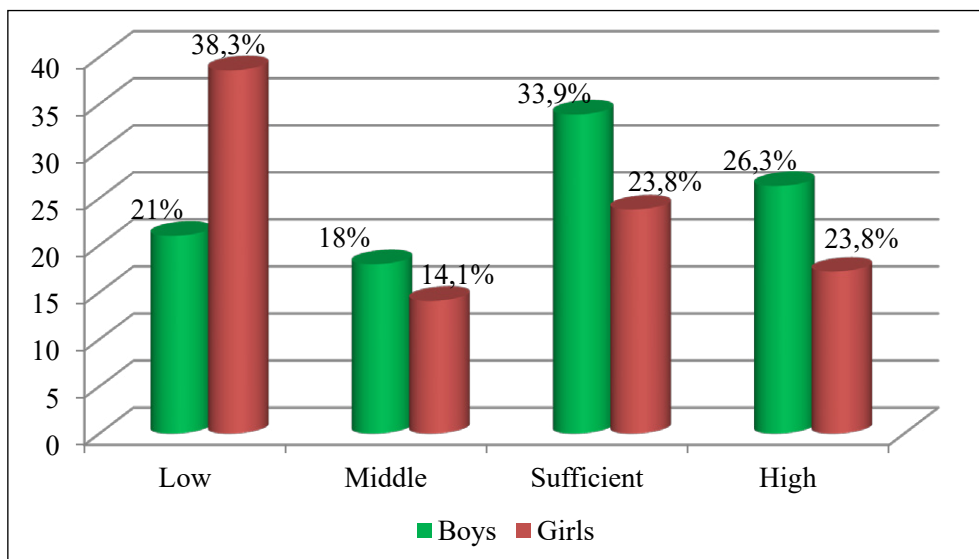


**Fig. 2.** Level of psychological well-being among respondents  
Picture taken by the authors

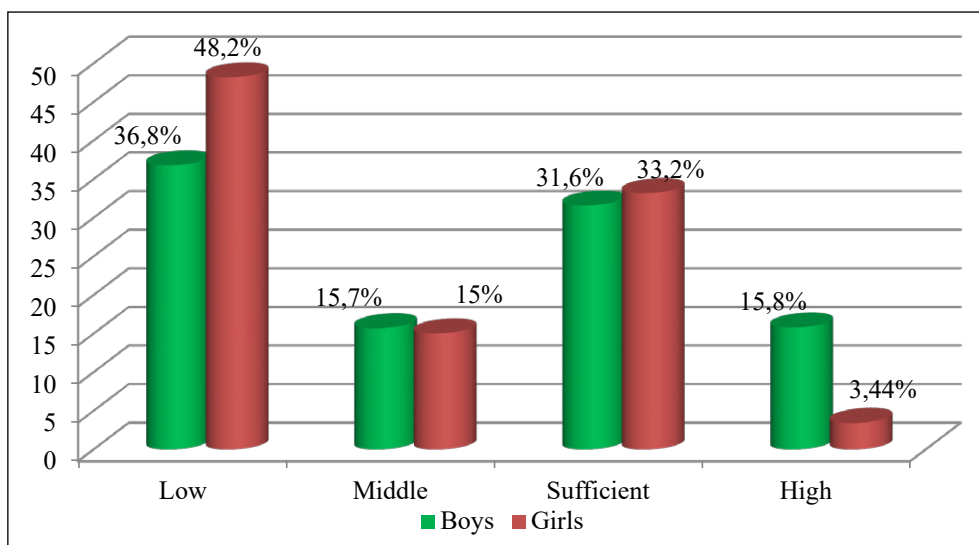


**Fig. 3.** Level of general well-being (mood and emotional state) among respondents  
Picture taken by the authors

According to the results of the analysis of the parameter "Physical activity and health", 20.7% of girls and 10.7% of boys have a low level of physical activity and health, 31.1% of girls and 26.2% of boys - average, 27.6%

of girls and 42.1% of boys - sufficient, 20.7% of girls and 21% of boys - high (Fig.1).

So, every second girl and every third boy assess their physical health as below average or weak, believe that

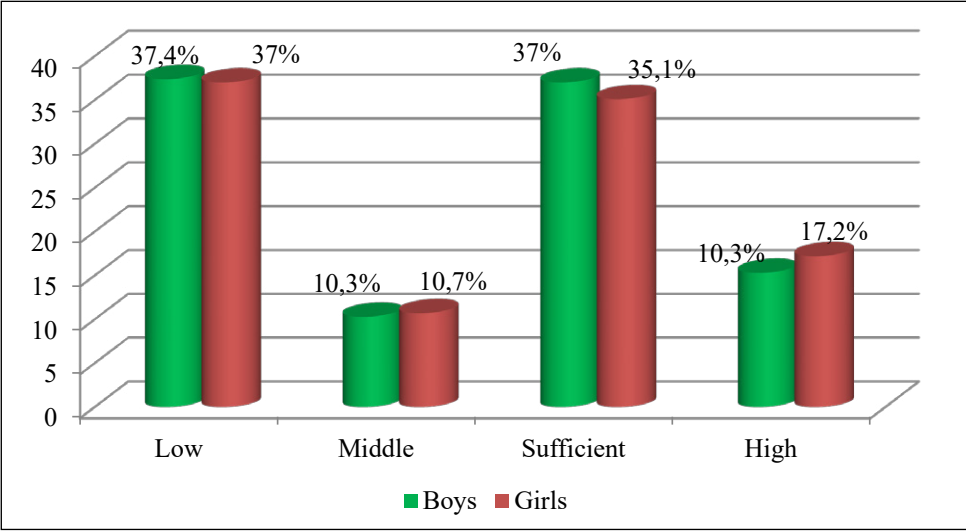


**Fig. 4.** Level of self-esteem and self-perception among respondents  
*Picture taken by the authors*

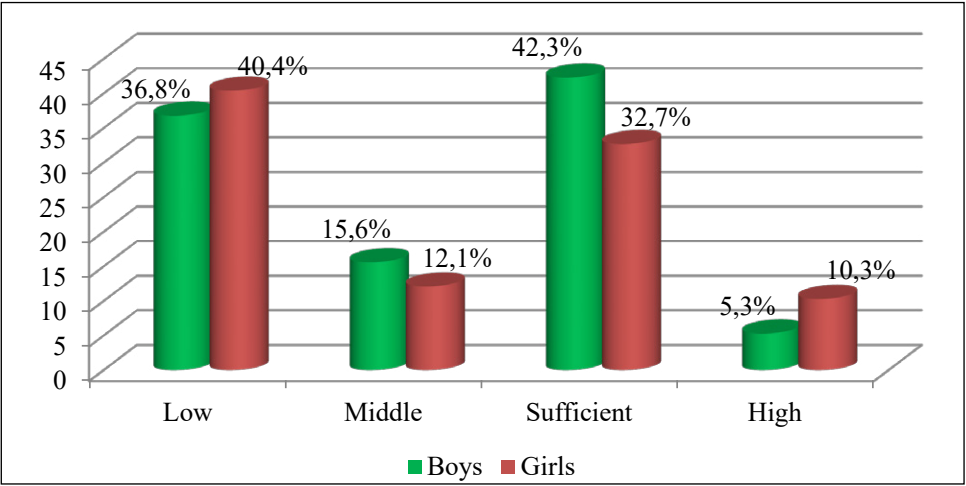


**Fig. 5.** Level of autonomy and the ability to freely manage time among respondents  
*Picture taken by the authors*

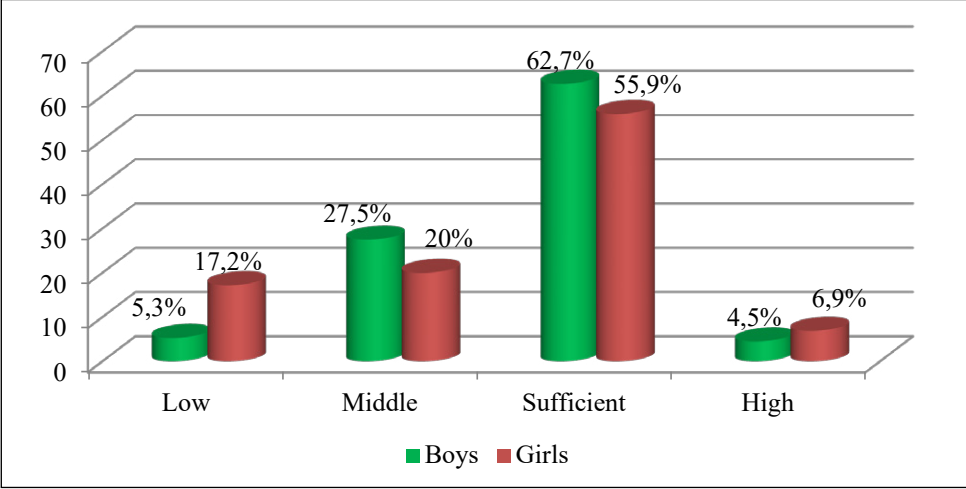


**Fig. 6.** Level of relationship with parents and staying at home among respondents  
*Picture taken by the authors*

they are not in sufficient physical shape to engage in active sports, and do not feel energetic.

According to the analysis of the parameter “Psychological well-being”, a difference in indicators was observed. 52.4% of girls and 35.1% of boys had a low and medium level of psychological well-being, were not at all or somewhat satisfied with their lives, were rarely in a good mood, had fun and enjoyed life (Fig.2).

According to the results of calculating the total scores for the “General condition” parameter, 48.2% of girls and 36.8% of boys assessed their general well-being, which included mood and emotions, as low, 15% of girls and 15.7% of boys as average, 33.2% of girls and 31.6% of boys as sufficient, 3.4% of girls and 15.8% of boys as high (Fig.3). Thus, we can conclude that every second teenager surveyed has a low level of emotional

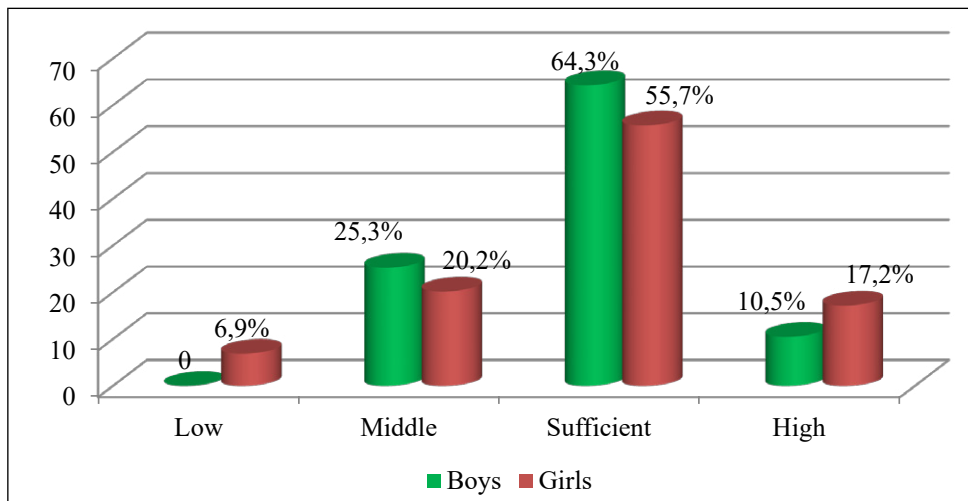


**Fig. 7.** Level of financial capabilities and available resources among respondents  
Picture taken by the authors

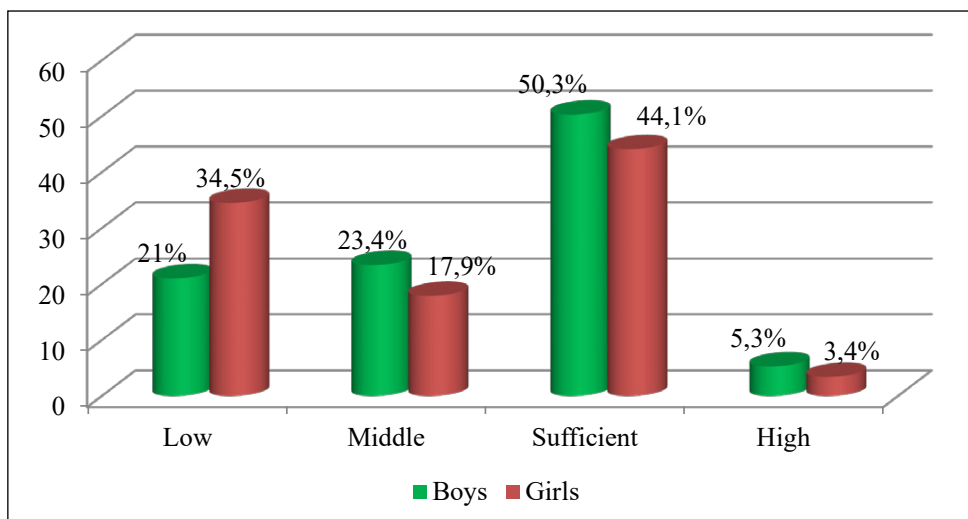


**Fig. 8.** Level of peer relationships and social support among respondents  
Picture taken by the authors

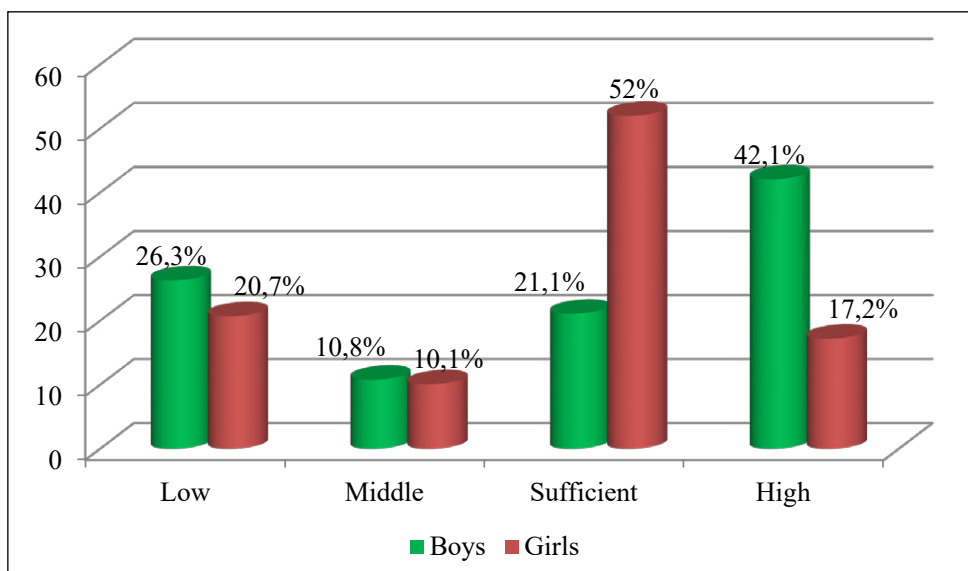


**Fig. 9.** Level of satisfaction with the school environment and learning conditions among respondents  
Picture taken by the authors

functioning: they feel that everything in life is going wrong, that they are fed up with everything, they feel lonely and feel pressure from others.

According to the results of calculating the total scores of the "Self-perception" parameter, the following indicators

were obtained: 32.8% of boys and 37.2% of girls have a low self-image, 59.7% of girls and 67.2% of boys have a sufficient quality of life associated with self-perception (Fig.4).

Regarding such an aspect of quality of life as "Autonomy", according to the responses, every second teenager

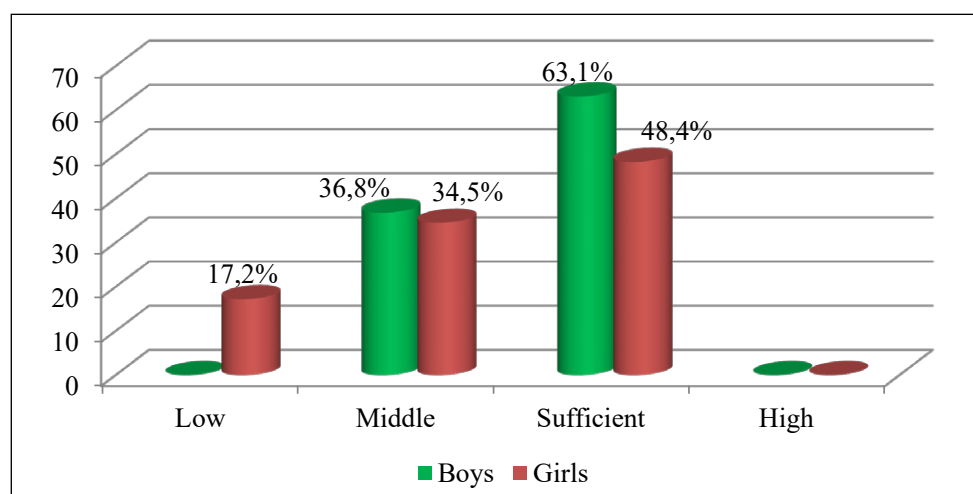


**Fig. 10.** Level of satisfaction with peer relationships among respondents

*Picture taken by the authors*

does not have enough free time for personal matters, communication with friends, walks in the fresh air and the opportunity to independently choose activities in their free time. (Fig.5).

According to the results of calculating the total score scale for the parameter "Relationships with parents and staying at home", it was found that 52.5% of girls and 52.4% of boys do not feel enough love, attention and understanding from their parents, believe that their parents are not fair enough towards them, do not have free time to talk and do not feel happy at home (Fig.6).

After calculating the total number of points for the "Financial Resources" parameter, we found out that most girls (72.9%) and boys (74.8%) have enough money for the same expenses as their friends, for their own necessary expenses, and also to spend time with friends (Fig.7).

Regarding the parameter "Relationships with peers", 52.4% of girls and 44.4% of boys noted that recently they have never or rarely spent time, done anything with their friends, and also never or rarely helped each other, could rarely talk about everything or rely on their friends (Fig.8).

The parameter "School environment" is an important part of the quality of life, because teenagers spend half of their time at school. 36.4% of boys and 31.8% of girls, that is, every third teenager, according to the total number of points for this parameter, never or rarely: felt happy at school, had good grades, could concentrate, was satisfied with their teachers and relationships with them (Fig.9). There was also an obvious difference in satisfaction with the school environment between boys and girls. 52% of girls had a sufficient level of quality of life regarding the school environment, 17.2% - high, boys - 21.1% and 42.1% respectively, which indicates a higher level of quality of this parameter among boys.

After calculating the total value of the "Bullying" subscale, we obtained the following results: 51.7% of girls and 34.5% of boys were very often or often afraid of their peers, were subjected to bullying and ridicule (Fig.10), which indicates a reduced quality of life compared to the standard value in relationships with others in every second girl and every third boy of adolescence.

## DISCUSSION

Quality of life is a relatively new term for medicine, which until recently was used only by sociologists. The definition of quality of life is based on a person's assessment of their level of well-being in terms of physical, mental (intellectual and emotional), social (professional, home, recreational activity, relationships at work, in the family, in society) and economic aspects of life; the ability to concentrate and make decisions, memory, speed of perception; visual-motor coordination, mental comfort, emotional stability and a sense of satisfaction with life are also taken into account. [8, 9].

Interest in the problem of quality of life is growing, but a unified approach to the content of the very concept of "quality of life" has not yet been formed in the scientific community, there is no generally recognized methodology and methodology for its measurement [10]. Existing conceptual approaches to assessing the quality of life can be divided into two main areas: objective approaches allow us to determine the quality of the social and physical environment; subjective approaches focus on considering value attitudes [1].

In today's world, adolescent health is an important indicator of the social and economic well-being of society. Health-related quality of life reflects not only physical condition, but also psychological comfort, social adaptation, and general well-being of young

people. The period of adolescence is accompanied by significant physiological and emotional changes that can affect the overall perception of one's own health. That is why studying of HRQoL among adolescents is extremely necessary [11,12]. Recently, there has been a trend towards creating universal questionnaires that are suitable for both healthy children and those with health problems [13].

It was previously thought that children and adolescents could not reliably assess their well-being because their understanding of health depended on age and cognitive development. However, recent research has shown that children are able to reliably assess their condition if questionnaires are adapted to their age level [13,14].

The low socio-economic level in Ukraine, when the main part of the time of many parents is spent on improving the material well-being of families by earning money mainly outside their country, as well as on the existence of double morality with low spirituality and culture of social life due to the tolerance of society to an unhealthy lifestyle (alcohol consumption, smoking, violence) create a negative microclimate for the adolescent population [12,15].

Taking into account the spread of social instability, the crisis of "social neglect" and "social isolation" of the younger generation of our country has become a pressing problem, as it worsens the health-related quality of life of adolescents [3].

It is believed that adolescents are healthier compared to other population groups, but today adolescence is recognized as a critical stage of the life cycle, during which various patterns of behavior are established, contributing to the formation of current health status and health indicators in the future [16].

There are different quality of life questionnaires for children depending on age; Youth Quality of Life Instruments - YQOL (D. Patrick); "Children's QOL Questionnaire" ("PedsQL"); Youth Questionnaire (YQOL); Youth Quality of Life Profile (QOLP-AV); Lindstrom Quality of Life Questionnaire (NQOLQ); Varniego Pediatric Quality of Life Questionnaire (PedsQL) - for adolescents aged 11 / 12-18 / 20 years; Health Questionnaire for Children and Young People KIDSCREEN-52; Children and Young People's Perception of Quality of Life Questionnaire (KPJs-DIM), etc. [17].

Important in assessing quality of life are test tasks that relate to the "perception of one's position in life". The PedsQL, QOLP-AV and NQOLQ Lindstrom methods take into account the specifics of child development, but do not contain specific topics proposed by the young people themselves. All methods, according to the WHO multidimensional concept of quality of life,

contain more than one component of quality of life from four (YQOL-R) to ten (KIDSCREEN-52). All of them capture the main indicators of quality of life listed by WHO: physical, psychological, social and environmental. Some of the methods are self-report methods (YQOL-R, QOLP-AV, KPJs-DiM), others (KIDSCREEN-52, PedsQL and NQOLQ) have similar versions for parents or extensions for younger children. The KIDSCREEN method is most commonly used in most European countries and does not require adaptation, as it includes a universal and local element. A general focus on quality of life is possible using the YQOL-R method. However, health-related quality of life can be assessed using the PedsQL 4.0 and KIDSCREEN-52 methods, a universal method that meets all methodological standards [3,18,19].

Thus, the large number of quality of life questionnaires in children allows for the optimal choice of a research tool, taking into account age, health status, and components of quality of life.

Thus, according to the results of the study, about half of adolescents report a reduced level of satisfaction with their relationships with parents and peers, which reflects a certain isolation and emotional alienation. Studies by other researchers also show that adolescents often experience difficulties in relationships with parents due to a lack of understanding or emotional distance. In studies conducted in the US and Europe, 40-50% of adolescents report feeling unsupported by their parents or experiencing conflict within the family. Peer problems are also common, and most studies indicate increased anxiety and social isolation among adolescents [10, 17].

According to a study by the World Health Organization, 20-30% of adolescents show signs of depression or anxiety disorders. At the same time, this figure is increasing every year, which indicates the general psychological pressure that adolescents are exposed to [10, 20].

Our research shows that one in two teenagers surveyed feels they don't have enough free time for personal pursuits and self-fulfillment. This is supported by research that shows that teenagers are increasingly facing pressure from school, family responsibilities and social demands, which limits their autonomy. Other studies also note that 40-50% of teenagers complain about not having enough time to relax and interact with friends, which can negatively affect their quality of life [20-22].

In our work, there is a gender difference, where girls have lower self-perception and emotional functioning scores, as well as reduced levels of physical activity compared to boys. In particular, girls are

more likely to experience emotional stress, anxiety and depression, which may be associated with an increased sense of danger. Reduced physical activity may be a consequence of limited opportunities for sports due to military operations, lack of a safe environment and changes in the usual lifestyle. Most researchers point to the difference in responses between girls and boys. Under the influence of sex hormones, girls become more emotional, sensitive to various changes in the body and to the influence of external factors, due to which their self-esteem is lower than in men [23,24].

### PRACTICAL RECOMMENDATIONS:

Based on the results of our research, practical recommendations were developed aimed at strengthening their physical and psychological health, as well as developing skills that contribute to emotional well-being and harmonious relationships. The proposed recommendations are aimed at both adolescents and teachers and can be integrated into the educational process, especially in physical education, health care, and educational hours.

### PRACTICAL RECOMMENDATIONS FOR TEENAGERS TO IMPROVE THEIR QUALITY OF LIFE

1. **Physical activity and health:** regularly do physical exercises. This can be not only sports, but also ordinary walks, active games with friends, dancing. Follow a healthy diet, drink enough water and avoid bad habits (smoking, drinking alcohol).
2. **Psychological well-being:** Take time for relaxation and self-development: reading, hobbies, and creativity reduce stress. Surround yourself with people who support and inspire you. Try to find the positive even in difficult situations and appreciate the pleasant moments in everyday life.
3. **Emotional state and mood:** be attentive to your emotions, if problems arise, do not hesitate to ask your parents or friends for help.
4. **Self-esteem:** work on developing self-confidence through new achievements and small victories. Remember that each person is unique, and comparing yourself to others is not always helpful. Choose activities that increase self-esteem and help you grow.
5. **Autonomy and free time:** plan your time to be able to do things that bring you pleasure: reading, talking with friends, favorite hobbies.
6. **Relationships with parents:** try to communicate openly with your parents, share your experiences

and problems. Maintain mutual respect and understanding, find time for joint activities. If you have difficulties in communication, do not be afraid to seek support from a psychologist.

7. **Financial resources:** learn to budget and save money for important needs. Spend time with friends without spending a lot of money - this can be free activities, nature walks, or creative activities together.
8. **Peer relationships:** build healthy relationships with friends, learn to help each other, support each other, and share positive moments. It is important to have reliable friends with whom you can discuss any issues and get support.
9. **School environment:** work on your academic performance, set goals and achieve them. Communicate with teachers if you have difficulties in learning or adapting to school. Create a positive atmosphere in the classroom, help classmates and participate in extracurricular activities.
10. **Bullying:** if you are a victim or witness of bullying, be sure to seek help from adults – teachers, parents, or psychologists. Support classmates who need help, and don't stay away.

### CONCLUSIONS

Theoretical analysis of scientific literature and other sources allowed us to determine the content of the concept of "quality of life" and analyze ten main parameters of HRQoL, which include: physical activity and health, psychological well-being, emotional state, self-perception, autonomy, relationships with parents, financial resources, social support, school environment, and experience of bullying.

The study found low to moderate levels in quality of life indicators such as physical activity and health, psychological well-being, emotional state, autonomy, relationships with parents, social support, and experiences of bullying. Sufficient levels were recorded in quality of life indicators such as financial resources, self-perception, and school environment.

The results showed that girls have lower rates of psychological well-being, self-perception, and physical activity compared to boys, which may be due to increased sensitivity to stress factors and social conditions during adolescence.

Based on the results, practical recommendations for adolescents were developed. They cover the importance of regular physical activity, supporting psychological well-being, building healthy relationships with parents and peers, and improving self-education and organizational skills for effective use of free time.



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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Complications and adverse outcomes related to induced abortion in female from the Ukrainian-Russian military conflict regions: A multicentre study

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## ABSTRACT

**Aim:** To provide an overview of complications and adverse outcomes related to induced abortion in female from the Eastern and Southern Ukrainian-Russian military conflict regions.

**Material and Methods:** This are a multicentre combined retrospective and prospective cohort study. All women who underwent induced abortion in Eastern and Southern Ukrainian-Russian military conflict regions between 2022 and 2024 are included in the study.

**Results:** A total of 14,196 induced abortion were performed, 10,630 (74.9%) complications cases were observed. The most complication were infections (71.1%), cervical injury (6.6%), incomplete abortion (5.4%), hemorrhage (5.2%), and uterine perforation (4.9%). The most common infection related to induced abortions was endometritis (21.6%), cervicitis (17.8%), vaginal cuff infections (16.3%), peritonitis (12.3%) and pelvic abscess (10.7%), followed by adnexa utery (8.1%), parametritis (6.7%), salpingitis (5.1%), sepsis (1.3%) and other (0.2%) infections. The main risk factors was increased maternal age, surgical abortion approach, increased gestational age, prior cesarean delivery, presence of a bleeding disorder, fetal demise before the abortion, lack of healthcare facilities, lack of essential medication, lack of personnel and equipment, and surgical inexperience.

**Conclusions:** This study findings demonstrate the high rate of complications related to induced abortion in the Ukrainian-Russian military conflict regions. The ongoing war has led to a decline in the quality of healthcare services and main reason for the high rate of complications and adverse outcomes related to induced abortion in female from the military conflict regions.

**KEY WORDS:** induced abortion, complications and adverse outcomes, the Ukrainian-Russian military conflict regions, risk factors, Ukraine

## INTRODUCTION

According to official data, the population of Ukraine is rapidly shrinking. First of all, this is connected with the ongoing war of the Russian Federation against Ukraine.

Death rates in the country have been higher than birth rates. Many Ukrainian women fled to the European Union countries. Although emigration of women due to the war in eastern and southern Ukraine played a role, the

biggest decline was due to low birth rates. Many women are forced to terminate pregnancies through induced abortion due to the war. Induced abortion in Ukraine is legal between 12 and 28 weeks for any reason with the approval of a commission of physicians. According to the literature, between 2017-2019 the number of induced abortions has sharply increased in Ukraine [1].

Induced abortion, is a common medical procedure in world. This procedure can be performed through medication or surgery. According to the literature, worldwide, approximately 73 million induced abortions occur annually, which carry potential risks and complications [2]. Therefore, understanding induced abortion-related complications is essential for both patients and healthcare professionals. In the United States, approximately 1 million induced abortions are performed each year, and complications occur in approximately 2% of elective abortions [3-7]. According to the literature, the main complications associated with induced abortions are ongoing pregnancy, incomplete abortion, hemorrhage, ectopic pregnancy, infection, drug reaction, disseminated intravascular coagulation, hematometra, injury to the uterus or cervix and uterine rupture [2, 5-8]. The mortality rate from legally induced abortion in countries with limited access represent around 1 in 10 maternal deaths [9]. The mortality rate from legally induced abortion in the United States was 1 out of every 28 maternal deaths [3]. In Ukraine, there are no official or literary data on the frequency of complications or adverse outcomes and mortality rates associated with induced abortion.

Induced abortion rates continue to be a significant public health concern in world. However, yet the relationship between abortion events and the presence of complications and adverse outcomes remains underexplored in the existing literature. Although some studies have suggested various associations between induced abortion events and the presence of complications and adverse outcomes, gaps persist in understanding the underlying mechanisms and risk factors.

The ongoing war in Ukraine has damaged hospitals and clinics, making it difficult for women to access reproductive health services, including abortion. However, no studies have been conducted on induced abortion in women living in the zone of the Ukrainian-Russian military conflict. Knowledge of complications and adverse outcomes associated with induced abortion in these regions are unknown.

## AIM

The aims of this study are to provide an overview of complications and adverse outcomes related to in-

duced abortion in female from the Eastern and Southern Ukrainian military conflict regions.

## MATERIALS AND METHODS

### STUDY DESIGN, SETTING, AND POPULATION

This is a multicentre combined retrospective and prospective follow-up cohort study of women who had an induced abortion in Eastern and Southern Ukrainian-Russian military conflict regions between 2022 and 2024. Participants in this study were admitted to gynecologic departments at 15 hospitals located in the Kharkiv (3 hospitals), Donetsk (3 hospitals), Kherson (3 hospitals), Zaporizhzhia (3 hospitals), Dnipropetrovsk (1 hospitals) and Odessa (2 hospitals) regions. The induced abortions were managed by the gynecological clinics at all 15 hospitals. Of these, 11 are government healthcare facilities and 4 private clinics. All women who underwent induced abortion at hospitals located in the Ukrainian-Russian military conflict regions between 2022 and 2024. All women considering an induced abortion were seen by a gynecologist at the gynecological clinics. In this study, any legal induced abortion indication was acceptable. Exclusion criteria: women who did not proceed with an abortion and women who refused to sign the informed consent form.

### DEFINITION

In the present study, the length of the pregnancy in all women was established through a vaginal ultrasound and the gestational age was reported as weeks. In this study, events were defined as any abortion-related morbidity or adverse event that received an abortion-related diagnosis or treatment code at any care location. Induced abortion in women in the first trimester is defined as occurring through the 12th week of pregnancy and second trimester is defined as 13–27 weeks of pregnancy. First-trimester surgical abortion is performed with vacuum aspiration (suction curettage) and second-trimester abortion by a dilation and evacuation procedure. Induced abortion in women was classified as elective and urgent. Infection after induced abortion in women was based on criteria from the Centers for Disease Control and Prevention's National Healthcare Safety Network (CDC/NHSN).

### DATA COLLECTION

We collected the demographic and clinical data, and outcome information using structured checklist. In present study, we analyzed the inpatient data and

**Table 1.** Distribution of complications (n=10,630) related to induced abortion in female among healthcare facilities in the Eastern and Southern Ukrainian-Russian military conflict regions, 2022-2024

| Healthcare facilities | Number of women, n | Complications |      |       |      | Incidence of complications (95% CI) |
|-----------------------|--------------------|---------------|------|-------|------|-------------------------------------|
|                       |                    | Yes           |      | No    |      |                                     |
|                       |                    | n             | %    | n     | %    |                                     |
| 1                     | 1,135              | 789           | 69.5 | 346   | 30.5 | 68.1 – 70.9                         |
| 2                     | 876                | 711           | 81.2 | 165   | 18.8 | 79.9 – 82.5                         |
| 3                     | 1,422              | 1,027         | 72.2 | 395   | 27.8 | 7.1 – 73.4                          |
| 4                     | 1,143              | 874           | 76.5 | 269   | 23.5 | 75.2 – 77.9                         |
| 5                     | 987                | 741           | 75.1 | 246   | 24.9 | 73.7 – 76.5                         |
| 6                     | 518                | 427           | 82.4 | 91    | 17.6 | 80.7 – 84.1                         |
| 7                     | 1,046              | 802           | 76.7 | 244   | 23.3 | 75.4 – 78.1                         |
| 9                     | 1,207              | 996           | 82.5 | 211   | 17.5 | 81.4 – 83.6                         |
| 10                    | 1,127              | 831           | 73.7 | 296   | 26.3 | 72.4 – 75.1                         |
| 11                    | 1,401              | 987           | 70.4 | 414   | 29.6 | 69.2 – 71.6                         |
| 12                    | 931                | 681           | 73.1 | 250   | 26.9 | 71.7 – 74.6                         |
| 13                    | 836                | 597           | 71.4 | 270   | 28.6 | 69.8 – 72.9                         |
| 14                    | 755                | 576           | 76.3 | 179   | 23.7 | 74.8 – 77.8                         |
| 15                    | 812                | 591           | 72.8 | 221   | 27.2 | 71.2 – 74.4                         |
| Total                 | 14,196             | 10,630        | 74.9 | 3,566 | 25.1 | 74.5 – 75.3                         |

CI, confidence interval

Source: compiled by the authors of this study

ambulatory medical records to identify complications and adverse outcomes related to induced abortion in female from the Eastern and Southern Ukrainian military conflict regions. Checklist was used to classify specific events into 1 or more specific diagnoses: retained products of conception, failed abortion, hemorrhage, infection, uterine perforation, anesthesia reaction, symptomatic intrauterine material, postabortal hematometra, cervical injury, disseminated intravascular coagulation, missed ectopic pregnancy, and other or undetermined. Events were classified as major if they required hospital admission, others were classified as minor. The primary complications were the occurrence of at least one induced abortion -related adverse event, including hemorrhage, retained products of conception, genital tract and pelvic infection, transfusion, fistulas and neighboring lesions, local hematoma, failure of abortion, death. The secondary outcome encompassed general adverse events related to induced abortion in female. These events were monitored during the hospital stay, return to hospital, and during visits to ambulatory. In our study, interviews occurred if the woman signed an informed consent form. Abortion complications were assessed in women who came in contact with the gynecological clinics within 30 days after the procedure. Adverse outcomes related to induced abortion were assessed in women within 6 months after the procedure.

## ETHICS

This study was approved by the Ethics Committee of the Shupyk National Healthcare University of Ukraine. All eligible study participants were given detailed explanation of the study as information about confidentiality. All women signed an informed consent form. In this study all participants data were anonymized.

## STATISTICAL ANALYSIS

All complications and adverse outcomes related to induced abortion data were entered in an Excel (Microsoft Corp., Redmond, WA, USA) database for statistical analysis. In this study SPSS version 22 was used for calculating a relative risk with a 95% confidence interval (CI). Descriptive data for continuous variables are expressed as mean  $\pm$  standard deviation for continuous variables, and number and corresponding percentage for qualitative variables, and  $\chi^2$  test was used to test for statistical significance. Logistic regression analysis was used to explore associations between risk factors and occurrence of a complications and adverse outcomes related to induced abortion. In our study  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

A total the study cohort comprised 14,196 women who had an induced abortion in Eastern and Southern

**Table 2.** Distribution of specific types of abortion-related complications and adverse events (n=10,630) in female from the Eastern and Southern Ukrainian-Russian military conflict regions, 2022-2024

| Type of complications                                   | Complication cases |       | 95% CI      |
|---------------------------------------------------------|--------------------|-------|-------------|
|                                                         | n                  | %     |             |
| Infections                                              | 7,562              | 71.1  | 70.7 - 71.5 |
| Cervical trauma (injury)                                | 704                | 6.6   | 6.4 - 6.8   |
| Symptomatic intrauterine material (incomplete abortion) | 569                | 5.4   | 5.2 - 5.6   |
| Hemorrhage                                              | 557                | 5.2   | 5.0 - 5.4   |
| Uterine perforation                                     | 521                | 4.9   | 4.7 - 5.1   |
| Post-abortion hematometra                               | 279                | 2.6   | 2.5 - 2.8   |
| Missed ectopic pregnancy                                | 198                | 1.9   | 1.8 - 2.0   |
| Failed abortion (ongoing pregnancy)                     | 126                | 1.2   | 1.1 - 1.3   |
| Disseminated intravascular coagulation                  | 68                 | 0.6   | 0.5 - 0.7   |
| Anesthesia-reaction (drug reaction)                     | 32                 | 0.3   | 0.2 - 0.4   |
| Other*                                                  | 14                 | 0.1   | 0.07 - 0.13 |
| Total                                                   | 10,630             | 100.0 | -           |

CI, confidence interval  
\*Involve injury to adjacent organs, such as the bladder (11 cases) or rectum (3 cases)  
Source: compiled by the authors of this study

Ukrainian-Russian military conflict regions between 2022 and 2024. The mean age of women was 28 years (range, 13-59 years; SD, 7.3 years). Induced abortion in women was classified as elective (37.1%) and urgent (62.9%). The incidence of complications and adverse outcomes related to induced abortion among these women was 74.9% [95% confidence interval (CI), 74.5-75.3,  $p<0.002$ ]. The number of induced abortions gradually increased throughout the study. Surgical abortion in performed with vacuum aspiration (suction curettage) were the most common procedure (54.7%), followed by abortion by a dilation and evacuation procedure (27.8%), and medical abortion (6.7%). The share of induced abortions performed by a dilation and evacuation procedure increased between 2022 and 2024 while the share of abortion in performed with vacuum aspiration and medical abortions has decreased. Complications were registered in 10,630 (74.9%) of all induced abortions. The frequent of complications related to induced abortion performed by a dilation and evacuation procedure, vacuum aspiration and medical abortions were 57.9%, 35.7% and 6.4%, respectively. The incidence rates of complications related to induced abortion varied significantly between healthcare facilities (Table 1).

The highest complication rate was observed in women in government healthcare facilities, followed by private clinics (results not shown). Over time, the rate of complications for induced abortions increased. This difference was significant (RR 1.69, 95% CI 24–2.46). More than fifty percent of the patients with of com-

plications and adverse outcomes related to induced abortion required repeat hospitalization. More of thirty-five percent (35.2%) of 7,562 patients with infection required a repeat surgical procedure directly related to the wound infection.

The complication related to induced abortions were infections (7,562 [71.1%] of all cases), cervical injury (704 [6.6%]), incomplete abortion (569 [5.4%]), hemorrhage (557 [5.2%]), uterine perforation (521 [4.9%]), post-abortion hematometra (279 [2.6%]), ectopic pregnancy (198 [1.9%]), ongoing pregnancy (126 [1.2%]), disseminated intravascular coagulation (68 [0.6%]), and drug reaction (46 [0.4%]). Distribution of complication related to induced abortions in female are presented in Table 2.

In this study the most common complication related to induced abortions was infections, which occurred 7,562 times. In present study the most common infection related to induced abortions was endometritis (21.6%, 95% CI, 21.1-22.1), cervicitis (17.8%, 95% CI, 17.4-18.2), vaginal cuff infections (16.3%, 95% CI, 15.9-16.7), peritonitis (12.3%, 95% CI, 11.9-12.7) and pelvic abscess (10.7%, 95% CI, 11.9-12.7), followed by adnexa utery (8.1%, 95% CI, 7.8-8.4), parametritis (6.7%, 95% CI, 6.4-7.0), salpingitis (5.1%, 95% CI, 4.8-5.4), sepsis (1.3%, 95% CI, 1.2-1.4) and other (0.2%) infections. Distribution of specific types of abortion-related infections in female from the Eastern and Southern Ukrainian-Russian military conflict regions, 2022-2024 are presented in Table 3.

Table 4 presents factors associated with the occurrence of complications following induced abortion in female from the Eastern and Southern Ukrainian-Rus-



**Table 3.** Distribution of specific types of abortion-related infections (n=7,562) in female from the Eastern and Southern Ukrainian-Russian military conflict regions, 2022-2024

| Type of infection       | n     | %     | 95% CI      |
|-------------------------|-------|-------|-------------|
| Endometritis            | 1,567 | 21.6  | 21.1 - 22.1 |
| Cervicitis              | 1,346 | 17.8  | 17.4 - 18.2 |
| Vaginal cuff infections | 1,233 | 16.3  | 15.9 - 16.7 |
| Peritonitis             | 927   | 12.3  | 11.9 - 12.7 |
| Pelvic abscess          | 807   | 10.7  | 10.3 - 11.1 |
| Adnexa utery            | 612   | 8.1   | 7.8 - 8.4   |
| Parametritis            | 503   | 6.7   | 6.4 - 7.0   |
| Salpingitis             | 387   | 5.1   | 4.8 - 5.4   |
| Sepsis                  | 98    | 1.3   | 1.2 - 1.4   |
| Other                   | 16    | 0.2   | 0.15 - 0.25 |
| Total                   | 7,562 | 100.0 |             |

CI, confidence interval

Source: compiled by the authors of this study

sian military conflict regions, 2022-2024. The multiple logistic regression analyses showed that age  $\geq 50$  years (adjusted odds ratio (aOR)=1.97 (95% Confidence Interval (CI), 1.57-2.88), abortion approach (aOR= 4.31, 95% CI, 1.92-10.81), gestational age (aOR=2.58 (95% CI, 1.65-4.04)), prior cesarean delivery (aOR=1.53 (95% CI, 1.27-1.89)), presence of a bleeding disorder (aOR=1.84, 95% CI, 1.18-2.79), fetal demise before the abortion (aOR = 2.91, 95% CI, 2.15, 3.82), lack of healthcare facilities (aOR= 2.65, 95% CI, 1.22-5.77), lack of essential medication (aOR= 4.31, 95% CI, 1.91-10.8), lack of personnel and equipment (aOR= 4.28, 95% CI, 1.83-10.2), and surgical inexperience (aOR= 2.61, 95% CI, 1.21, 5.73) were significantly with complications related to induced abortions in female from the Eastern and Southern Ukrainian military conflict regions. Additionally, logistic regression analysis was performed to assess the association between endometriosis and induced abortion events, adjusting for confounding variables. After adjusting for confounding variables, women with a history of induced abortion had 2.27-fold increased odds of experiencing an endometriosis (OR: 2.27; 95% CI: 1.79 - 2.92;  $P < 0.001$ ). Also, infertility was associated with induced abortion in logistic regression analysis. The aOR was 5.04 (95% CI, 1.16–21.83).

## DISCUSSION

The results presented in this cohort study based on multicentre combined retrospective and prospective surveillance data for women who had an induced abortion in Eastern and Southern Ukrainian-Russian military conflict regions between 2022 and 2024. To our knowledge there are no similar studies showing the relationship between of complications and adverse

outcomes related to induced abortion in female and military conflicts. This study expands upon the previous reports on infection after induced abortion in Ukraine [1, 10-13] and is the first study to publish of complications and adverse outcomes related to induced abortion in female from the military conflict regions.

The reported incidence of complications related to induced abortion varies between studies. According to the literature, complications occur in approximately 2% of induced abortions and a minority of these require hospitalization [4-6, 14]. In our study the incidence of complications and adverse outcomes related to induced abortion among women was 74.9%.

Induced abortions in female can be performed in surgically and medically methods. However, there has been little research directly comparing dilation and evacuation (D&E) procedure, vacuum aspiration and medical methods of induced abortions. Grossman D, et al. [15] reported that women undergoing medical induction with this method were significantly more likely than those undergoing D&E to have a complication. This study found that women undergoing induced abortion with D&E were significantly more likely than undergoing medical induction method to have a complication. The frequent of complications related to induced abortion performed by a dilation and evacuation procedure, vacuum aspiration and medical abortions were 57.9%, 35.7% and 6.4%, respectively.

According to the literature, possible complications of induced abortion include ongoing pregnancy, incomplete abortion, hemorrhage, ectopic pregnancy, infection, drug reaction, cardiovascular collapse, deep vein thrombosis, disseminated intravascular coagulation, hematometra, uterine rupture, and death [2, 5, 6]. In our study the most common of complications

**Table 4.** Factors associated with the occurrence of complications following induced abortion in female from the Eastern and Southern Ukrainian-Russian military conflict regions, 2022-2024

| Variable                              | All patients<br>(n=14,196) | Complications     |      |                 |      | p-value |
|---------------------------------------|----------------------------|-------------------|------|-----------------|------|---------|
|                                       |                            | Yes<br>(n=10,630) |      | No<br>(n=3,566) |      |         |
|                                       |                            | n                 | %    | n               | %    |         |
| Age category, years                   |                            |                   |      |                 |      | <0.001  |
| 14-20                                 | 3,449                      | 1,928             | 18.1 | 1,521           | 42.7 |         |
| 21-35                                 | 3,668                      | 2,367             | 22.3 | 1,301           | 36,5 |         |
| 36-49                                 | 3,502                      | 3,054             | 28.7 | 448             | 12.6 |         |
| ≥ 50                                  | 35,77                      | 3,281             | 30.9 | 296             | 8.3  |         |
| Abortion approach                     |                            |                   |      |                 |      | <0.0012 |
| medical                               | 2,634                      | 684               | 6.4  | 1,950           | 54.7 |         |
| vacuum aspiration (suction curettage) | 4,531                      | 3,796             | 35.7 | 735             | 20.6 |         |
| dilation and evacuation procedure     | 7,031                      | 6,150             | 57.9 | 881             | 24.7 |         |
| Gestational age, weeks                |                            |                   |      |                 |      | <0.001  |
| 4-5                                   | 1,243                      | 116               | 1.1  | 1,127           | 31.6 |         |
| 6-7                                   | 3,491                      | 2,366             | 22.3 | 1,125           | 31.5 |         |
| 8-9                                   | 4,784                      | 4,026             | 37.9 | 758             | 21.3 |         |
| 10-12                                 | 4,678                      | 4,122             | 38.7 | 556             | 15.6 |         |
| Prior cesarean delivery               | 1,657                      | 1,410             | 13.3 | 247             | 6.9  | <0.001  |
| Presence of a bleeding disorder       | 2,094                      | 1,898             | 17.9 | 196             | 5.5  | <0.001  |
| Fetal demise before the abortion      | 676                        | 621               | 5.8  | 55              | 1.5  | <0.001  |
| Lack of healthcare facilities         | 4,895                      | 4,439             | 41.8 | 456             | 12.8 | <0.001  |
| Lack of essential medication          | 9,840                      | 8,796             | 82.7 | 1,044           | 29.3 | <0.001  |
| Lack of personnel and equipment       | 5,398                      | 4,765             | 44.8 | 633             | 17.8 | <0.001  |
| Surgical inexperience                 | 2,990                      | 2,401             | 22.6 | 589             | 16.5 | <0.03   |

Source: compiled by the authors of this study

related to induced abortion was infections (71.1% of all cases), cervical injury (6.6%), incomplete abortion (5.4%), hemorrhage (5.2%), uterine perforation (4.9%), post-abortion hematometra (2.6%), ectopic pregnancy (1.9%), ongoing pregnancy (1.2%), disseminated intra-vascular coagulation (0.6%), and drug reaction (0.4%). Upadhyay UD, et al [5] reported that uterine hemorrhage is often caused by retained products of conception (incomplete abortion) but can also result from uterine perforation, or cervical laceration. Injury to the uterus or cervix can occur during induced abortions [2]. Kerns JL, et al [16] and Wall LL, et al [17] may involve injury to adjacent organs, such as the bladder or rectum. Among the studies reviewed, the incidence of uterine perforation related to induced abortion in female was rare. Uterine perforation rate varied from 0.2% in US [18] and Canada [19], to 0.5% in the Viet Nam [20]. The studies with the higher rates of uterine perforation included physicians with less experience of doing surgical induced abortion. However, no cases of uterine perforation were reported in the US, despite the fact that most

of the surgical abortion procedures were performed by resident physicians in training [21]. According to the literature, the risk factors of uterine perforation include induced abortion in the second trimester and uterine scarring from prior cesarean section [16]. In our study incidence rate of uterine perforation related to induced abortion was 4.9%. In present study infections are of the most of complications related to induced abortion. According to the literature, infections related to induced abortion are most commonly caused by retained products of conception, which become a nidus for infection [17]. Aronoff DM, et al [22], Haddad LB, et al [23], and Salmanov AG, et al [24] reported that infections are especially common following unsafe abortions involving nonsterile instruments or equipment. Additionally, contamination of environmental surfaces in healthcare facilities are as a reservoir for bacteria to patients and Inadequate hand hygiene in healthcare workers may result in transmission of pathogens [24]. The reported incidence of infections related to induced abortion varies between studies. Charonis

and Larsson [25] reported a frequency of 2.4% for infectious complications from medical abortions and 4.9% from surgical abortions. A previous study in Ukraine conducted in 2021 [1] and 2024 [13] found that incidence of infection cases related to induced abortions in female were 25.9% and 27.4%, respectively. In this study incidence of infections related to induced abortions was 71.1%. The most common infection related to induced abortions was endometritis (21.6%), cervicitis (17.8%), vaginal cuff infections (16.3%), peritonitis (12.3%) and pelvic abscess (10.7%), followed by adnexa utery (8.1%), parametritis (6.7%), salpingitis (5.1%), sepsis (1.3%) and other (0.2%) infections.

Studies identified several factors that were associated with an increased risk of complications and adverse outcomes related to induced abortion. The most common risk factors of complications related to induced abortion in the literature are as follows gestational age, history of cesarean delivery, maternal age, fetal demise before the abortion, surgical inexperience, unsafe (illegal) abortion, and presence of a bleeding disorder [2, 5, 16, 17]. In our study the multiple logistic regression analyses showed that increased maternal age  $\geq 50$  years (aOR=1.97), surgical abortion approach (aOR= 4.31), increased gestational age (aOR=2.58), prior cesarean delivery (aOR=1.53), presence of a bleeding disorder (aOR=1.84), fetal demise before the abortion (aOR = 2.91), lack of healthcare facilities (aOR= 2.65), lack of essential medication (aOR= 4.31), lack of personnel and equipment (aOR= 4.28), and surgical inexperience (aOR= 2.61) were significantly with complications related to induced abortions in female from the Eastern and Southern Ukrainian military conflict regions.

Several studies have described the impact of complications and adverse outcomes related to induced abortion in female on infertility and endometriosis. In this study after adjusting for confounding variables, women with a history of induced abortion had 2.27-fold increased odds of experiencing an endometriosis (OR: 2.27,  $P < 0.001$ ). Also, infertility was associated with induced abortion in logistic regression analysis. The aOR was 5.04 (95% CI, 1.16–21.83).

The war against Ukraine has put most pregnant women in the Eastern and Southern Ukrainian-Russian military conflict regions, intended parents in impossible positions. Experience shows that access to women reproductive health services, such as safe induced abortion, remains a daily concern in a war situation. Many pregnant women in military conflict regions may need health care and assistance on a daily basis or when complications with pregnancy occur. Inaccessibility of medical facilities, shortage or absence of equipment, healthcare personnel and medical products are scarce

or unavailable, create a risk to the health of pregnant women.

## STUDY STRENGTHS AND LIMITATIONS

This is the first study that focused on complications and adverse outcomes related to induced abortion in female from the military conflict regions. One of the strengths in this study is the size of the study cohort group from the Eastern and Southern Ukrainian-Russian military conflict regions. A total of 14,196 induced abortions were included, which gave a fairly accurate depiction of the incidence of complication and adverse outcomes related to induced abortions in female.

One limitation is that some female did not contact the gynecological clinic for their post-abortion problems until after 30 days, which leads to an underestimation in the number of complications and adverse outcomes related to induced abortions. Furthermore, some patients may be missing in our statistics because they sought medical care at hospitals other than those included in our study. However, there are few private gynecological clinics in the Eastern Ukrainian military conflict regions, so it is likely that most complications are captured in this study. Another limitation our study is that various physicians were involved in categorizing the complications and adverse outcomes related to induced abortions in female. This may have resulted in misdiagnosis and might have been categorized differently.

## CONCLUSIONS

This study findings demonstrate the high rate of complications related to induced abortion in the Ukrainian-Russian military conflict regions. The most common risk factors of complications related to induced abortion was increased maternal age  $\geq 50$  years, surgical abortion approach, increased gestational age, prior cesarean delivery, presence of a bleeding disorder, fetal demise before the abortion, lack of healthcare facilities, lack of essential medication, lack of personnel and equipment, and surgical inexperience. The ongoing war in Ukraine has significantly impacted access to reproductive health services – such as safe induced abortion. Healthcare facilities across the Eastern and Southern Ukrainian-Russian military conflict regions are depleted and under immense strain, with war having led to a lack of facilities, essential medication, personnel and equipment. This has led to a decline in the quality of healthcare services and main reason for the high rate of complications and adverse outcomes related to induced abortion in female from the military conflict regions.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Design, docking study, synthesis and preliminary cytotoxic study of novel Isatin-Niflumic acid derivatives as possible VEGFR tyrosine kinase inhibitors

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## ABSTRACT

**Aim:** Design, synthesis and cytotoxic evaluation of new series of Isatin-Naiflumic acid derivatives as vascular endothelial growth factor receptor tyrosine kinase inhibitors.

**Materials and Methods:** A molecular docking study was performed to assess the binding affinities of the synthesized compounds toward the kinase domains of the vascular endothelial growth factor receptor 2. The compounds were synthesized through the esterification process of Niflumic acid, followed by treating the obtained ethyl ester compound with hydrazine monohydrate to form the Niflumic acid hydrazide compound. This compound was further reacted with six Isatin derivatives utilizing the ketone-amine condensation reaction to form the final six Schiff base compounds.

**Results:** The synthesized compounds (I-VI) showed optimum binding affinity (*S. score*) values ranging from -9.1782 to -9.5120 Kcal/mol and precise binding mode (RMSD) values ranging from 1.0608 to 1.8914 with the active kinase site of the vascular endothelial growth factor receptor 2 (PDB code: 4AG8). At the same time, the cytotoxicity results showed an optimum inhibitory effect (20.87  $\mu$ M to 78.48  $\mu$ M) against the A549 (non-small cell lung cancer) and a safer effect (175.49  $\mu$ M to 280.27  $\mu$ M) against the MRC-5 (normal lung tissue cells). In contrast to the reference drug (Sunitinib).

**Conclusions:** The new synthesized compounds (I-VI) are potent vascular endothelial growth factor receptor 2 tyrosine kinase inhibitors, inhibiting cell proliferation, angiogenesis and exhibiting promising anticancer agents.

**KEY WORDS:** VEGFR-2 inhibitors, molecular docking, Niflumic acid, Isatin derivatives

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## INTRODUCTION

Cancer is a lethal illness that remains a major global health concern. It is the second most prevalent illness behind cardiovascular disorders. Consequently, the creation of powerful and efficacious new antineoplastic agents is a primary objective of modern medicinal chemistry [1-2]. A large percentage of clinically licensed anticancer agents have a narrow therapeutic window, mostly because of significant systemic toxicity coupled with pronounced insufficient tumor selectivity [3]. Vascular endothelial growth factor receptors are a principal tumor inducer, as the overexpression of such receptors in malignancies, especially type II triggers an impaired activation of neovascularization signaling pathways that provide adequate oxygen and nutrient demand for accelerated development and proliferation of solid tumors [4-5]. The upregulation of these receptors may serve as a biomarker for the presence of malignant tumors [6]. Consequently, suppressing their pathway would result in efficient anti-angiogenesis and tumor-

igenic response [7]. Despite the availability of several VEGFR inhibitors, clinical resistance and limited efficacy remain major challenges, emphasizing the importance of exploring new scaffolds capable of selectively inhibiting VEGFR-2 tyrosine kinases. Structure-based drug design approach, driven by both literature evidence and the unmet medical need for effective VEGFR-2 inhibitors in cancer therapy. This provided the foundation for docking-guided synthesis and subsequent biological evaluation. Vascular endothelial growth factor receptor 2 (VEGFR-2) is a member of the receptor protein tyrosine kinase (PTK) family that may be categorized into three components: an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic tyrosine kinase domain [8-9]. At the extracellular part, the vascular endothelial growth factor-A (VEGF-A) binds to VEGFR-2, prompting the development of a homodimer complex of VEGFR-2, modifying the conformation of its intracellular domain. The conformational changes occur induce auto-phosphorylation of the tyrosine residues, leading



to downstream signal transmission and subsequently activating RAS/Raf/MAPK and PI3K/Akt pathways that enhance endothelial cells' proliferation, migration and durability [10]. Inhibitors that compete with adenosine triphosphate (ATP) upon binding to the tyrosine kinases and hindering phosphorylation cascade are considered a crucial chemotherapeutic agent for many types of cancers that show VEGFR-2 overexpression such as in breast cancer renal carcinoma, hepatocellular carcinoma and non-small lung cancer (NSCLC) [11]. In the case of NSCLC, studies show that there is overexpression of VEGFR-2, reaching a value of 54.2% compared to normal tissues [12]. This study involves the design, molecular docking, synthesizing, and toxicity evaluation of compounds derived from the linkage of Isatin. Scaffold with Niflumic acid. Isatin and its derivatives exhibit a wide range of biological actions. The cytotoxic and antineoplastic characteristics have been the most extensively documented among them. The great Isatin scaffold resulted in the production of numerous structurally different derivatives, including analogues derived from many substitutions on the aryl ring, as well as those obtained through derivatization of the Isatin nitrogen and Carbon 2 and 3. These chemicals impede cancer cells multiply and tumor development by interaction with several intracellular targets, including DNA, telomerase, tubulin, P. glycoprotein, protein kinases, and phosphatases [13]. A series of VEGFR-2 inhibitory activity were synthesized and assessed for anticancer potential of Benzofuran-Isatin conjugates against MCF-7, MCF-7/DOX, DU-145, and MDR DU-145 cancer cell lines by MTT assay. The presented results suggested that seven analogues could display better potency with  $IC_{50}$  values of 47.6–96.7  $\mu$ M against all tested cell lines compared to the positive control sunitinib. SAR results illustrate the carbon spacer between isatin and the benzofuran ring, the isatin motif substituted at C-3 and C-5 positions, and the incorporation of the *para*-substituted phenyl ring at the C-2 position of the benzofuran moiety as required features for significant VEGFR-2 inhibitory and anticancer potential [14]. Niflumic acid is a non-corticosteroid inflammatory suppressor agent often employed in the medical management of rheumatoid arthritis it has lately been recognized as an inhibitor of several malformation cell types, including cancers of the lungs, ovarian, renal, and liver [15].

## AIM

The present study aims to design, synthesis and cytotoxic evaluation of new series of Isatin-Niflumic acid derivatives as vascular endothelial growth factor receptor tyrosine kinase inhibitors.

## MATERIALS AND METHODS

The reagents as well as solvents used during the synthesis processes were at the laboratory level. Solvents were bought from Central Drug House Ltd., India, and all were dried before being used, while Niflumic acid and Isatin derivatives were bought from Macklin Biochemical Co. Ltd., China. The estimated cost of preparing the proposed Schiff base derivatives is relatively modest when compared to many existing tyrosine kinase inhibitors. The synthetic pathway relies on commercially available starting materials (Isatin and Niflumic acid), which are easily accessible. The conjugation reactions involve acylation and condensation steps that require no costly reagents or specialized equipment, allowing scalability at a reasonable expense. Stuart (SMP30) Melting Point Instrumentation, also was employed to ascertain melting points. Employing one sided open capillary method, the melting points of compounds were determined at the University of Kufa, Faculty of Pharmacy. The melting point describes the point in temperature at which a substance changes from solid to liquid. It's one of the most efficient methods to check compound purity. Thin-layer chromatography in ascending mode was employed to assess how the reaction progressed and determine the degree of its purity, utilizing 0.2 mm silica gel 60F<sub>254</sub> aluminum plates and a mobile phase composed of hexane and acetone in a proportion (7:3) [16]. Following exposure of silica paper to UV light, a solitary circular spot emerged, demonstrating a complete reaction and a pure yield [17], also, at the University of Kufa Shimadzu (Japan) spectrophotometer, which offers stable Fourier transform infrared spectroscopy analysis (FTIR), was used to obtain compounds spectra. Regarding the magnetic resonance spectra, a Bruker 400 MHz instrument was used to record the <sup>1</sup>H-NMR spectra at the College of Science, University of Basrah. Deuterated solvent (DMSO-d<sub>6</sub>) was used to avoid the appearance of misleading or overlapping peaks during the spectra-measuring process [18]. For esterification purposes, Niflumic acid was dissolved in a suitable amount of ethanol, and then the mixture was chilled to before adding an excess amount of thionyl chloride. After that the mixture was refluxed (using a hot plate magnetic stirrer and a glass condenser that was attached to a round-bottomed flask for several hours to obtain the desired Niflumic acid ethyl ester (compound Ia). Then after, the ester aminolysis method is used to convert the obtained ester into amide by reacting (compound Ia) with an excess amount of hydrazine monohydrate 99% in reflux conditions to obtain the desired Niflumic acid hydrazide (compound Ib). Finally, the condensation reaction is utilized by reacting the amine-containing compound (compound Ib) with different types of ketones (Isatin derivatives) under slightly acidic conditions to obtain the final six acyl hydrazone compounds (Schiff bases). The overall synthesis procedure is shown in Figure 1 [19-20].

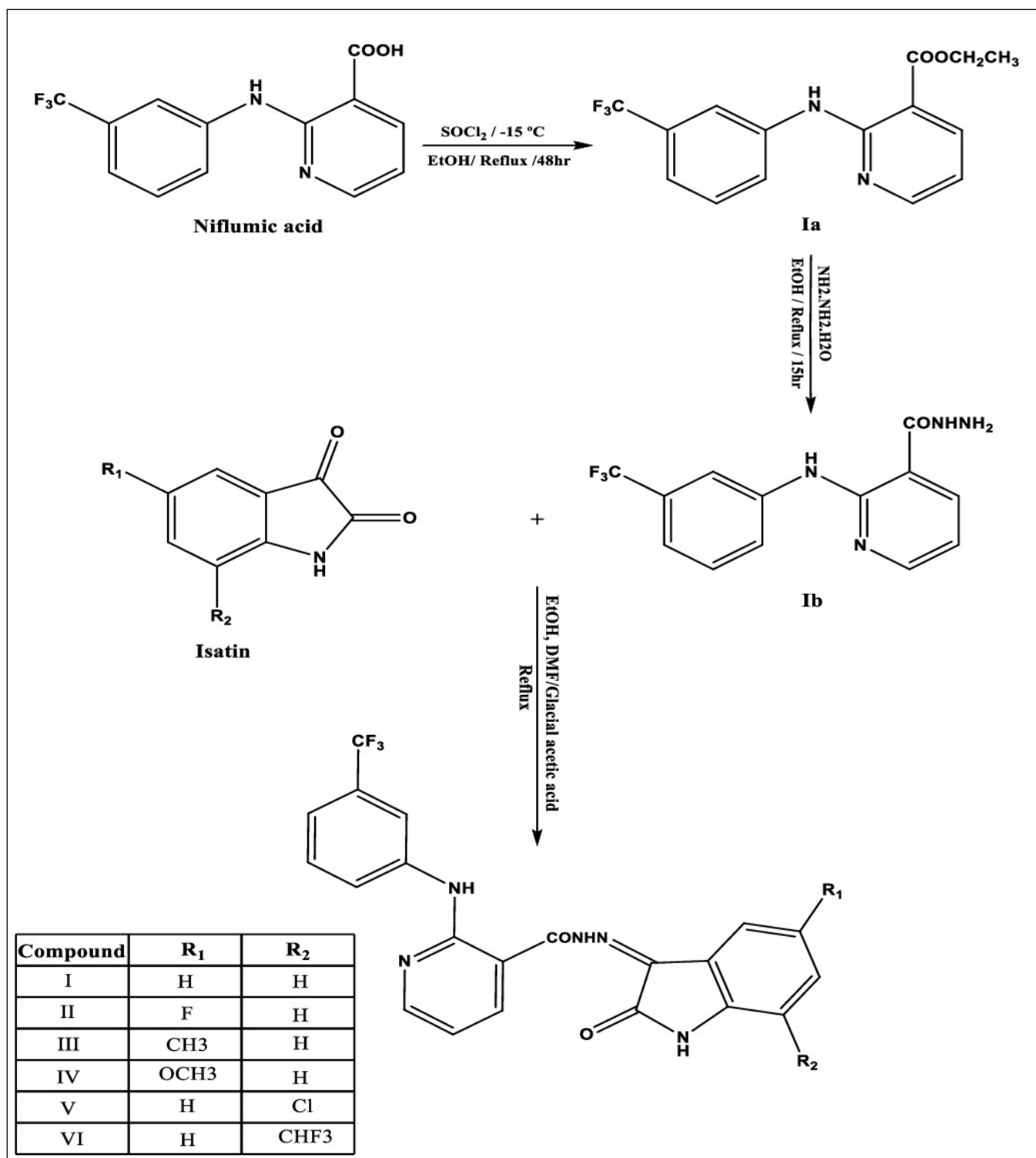


**Fig. 1.** Synthesis procedure of intermediates and final compounds

Source: Based on [19-20]

## CHEMISTRY

### SYNTHESIS OF NIFLUMIC ACID ETHYL ESTER (COMPOUND IA)

20 ml of dried ethanol was used to dissolve 2g (7.086 mmol) of Niflumic acid employing a 100 ml round-bottomed flask, the liquid was chilled to -15 °C, and an

excess of thionyl chloride (1 ml, 13.78 mmol) was gradually added. The mixing process is initiated and the resulted mixture is allowed to gradually reach room temperature before refluxing at 78°C for 48 hours using a hot plate magnetic stirrer. Upon completion of the reaction, which was confirmed using the TLC identification technique, 3 g of sodium bicarbonate dissolved in

**Table 1.** Physical and chemical characteristics of the intermediates and final compounds

| Compound | Empirical formula                                                              | Molecular weight (g/mol) | Appearance                  | Yield [%] | Melting point [°C] | Rf value |
|----------|--------------------------------------------------------------------------------|--------------------------|-----------------------------|-----------|--------------------|----------|
| Ia       | C <sub>15</sub> H <sub>13</sub> F <sub>3</sub> N <sub>2</sub> O <sub>2</sub>   | 310.28                   | Brown oil                   | 65        | -----              | 0.94     |
| Ib       | C <sub>13</sub> H <sub>11</sub> F <sub>3</sub> N <sub>4</sub> O                | 296.25                   | White to off-white crystals | 80        | 144-145            | 0.36     |
| I        | C <sub>21</sub> H <sub>14</sub> F <sub>3</sub> N <sub>5</sub> O <sub>2</sub>   | 425.37                   | Yellow powder               | 72        | 295-297            | 0.60     |
| II       | C <sub>21</sub> H <sub>13</sub> F <sub>4</sub> N <sub>5</sub> O <sub>2</sub>   | 443.36                   | Brown powder                | 75        | 311-312            | 0.50     |
| III      | C <sub>22</sub> H <sub>16</sub> F <sub>3</sub> N <sub>5</sub> O <sub>2</sub>   | 439.40                   | Orange powder               | 65        | 310-311            | 0.63     |
| IV       | C <sub>22</sub> H <sub>16</sub> F <sub>3</sub> N <sub>5</sub> O <sub>3</sub>   | 455.40                   | Dark brown powder           | 60        | 289-291            | 0.56     |
| V        | C <sub>21</sub> H <sub>13</sub> ClF <sub>3</sub> N <sub>5</sub> O <sub>2</sub> | 459.81                   | Brown powder                | 79        | 311-312            | 0.52     |
| VI       | C <sub>22</sub> H <sub>13</sub> F <sub>6</sub> N <sub>5</sub> O <sub>2</sub>   | 493.37                   | Light brown powder          | 77        | 263-264            | 0.45     |

Source: Based on [25]

30 ml of distillate water was incrementally introduced to the resultant mixture to neutralize the acidity resulting from HCl emission. The extraction technique is performed three times to ensure complete extraction of the oily ester layer using 30 ml ethyl acetate each time. The final organic layer is even more dried using an adequate amount of anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) and then filtered [21-22].

### SYNTHESIS OF NIFLUMIC ACID HYDRAZIDE (COMPOUND IB)

1.50 ml (8.89 mmol) of Niflumic acid ethyl ester (compound Ia) is dissolved in 15 ml of absolute ethanol. Subsequently, 0.6 ml (12.34 mmol) of 99% hydrazine hydrate is added gradually. The mixture is maintained at room temperature with gentle stirring for 3 hours before refluxing for 15 hours at 78°C using a hot plate magnetic stirrer. Following the confirmation of reaction completion via TLC observation, the solvent is evaporated under vacuum conditions, yielding a brown hydrazide powder. Recrystallization with a combination of ethanol and n-hexane is conducted to isolate the pure brown crystals of Niflumic acid hydrazide [23].

### SYNTHESIS OF THE FINAL ACYL HYDRAZONE COMPOUNDS (COMPOUNDS I-VI)

Niflumic acid hydrazide 0.75g (2.53 mmol) was dissolved in a solvent combination of absolute ethanol and DMF in a 1:1 ratio. In a separate round-bottomed flask, the identical solvent combination was employed to individually dissolve 3.79 mmol of each Isatin derivative accompanied by a catalytic amount of glacial acetic acid (1-2 drops) [24]. Each Isatin derivative was combined separately with the hydrazide-containing solution. The resultant mixtures were subjected to reflux at 90°C for varying periods (20-48) hours (monitored by TLC technique) to produce the final acyl hydrazone com-

pounds. After that, the solution was evaporated under a reduced pressure technique, and the six resultant compounds were recrystallized using DCM and n-hexane, washed with diethyl ether, and allowed to dry. Table (1) shows the physical and chemical properties, along with the yield percent, of the obtained intermediates and compounds [25].

## SPECTROSCOPIC EXAMINATION OF INTERMEDIATES AND FINAL COMPOUNDS

### COMPOUND IA

FTIR (cm<sup>-1</sup>): 3363 (N-H) strch of 2.nd amine, 2947, 2875 (CH) strch of aliph. (CH<sub>2</sub>, CH<sub>3</sub>), 1691 (C=O) strch. of conjugate ester, 1614 (C=N) strch, 1587, 1531, 1446 (aromatic C=C) strch, 1330 (C-F) strch, 1288 (C-O) strch, 1255 (C-N) strch.

### COMPOUND IB

FTIR (cm<sup>-1</sup>): 3415, 3309 (N-H) strch of 1.prim amine, 3041 (aromatic C-H) strch, 1608(C=O) strch of amide, 1587 (C=N) strch, 1525, 1462 (aromatic C=C) strch, (1336 C-F) strch 1255 (C-N) strch.

### COMPOUND I

FTIR (cm<sup>-1</sup>): 3236 (N-H) strch of 2.nd amine, 3041 (aromatic C-H) strch, 1695 (C=O) strch of amide, 1656 (C=N) strch of imine, 1622, 1591, 1463 (C=C) strch of aromatic, 1327 (C-F) strch, 1249 (C-N) strch.

<sup>1</sup>H-NMR (400MHz, DMSO-d<sub>6</sub>, ppm): 13.92 (singlet, 1H of amide N-H), 11.40 (singlet, 1H of N-H), 10.48 (singlet, 1H of indole N-H), 8.47-6.93 (multiplet, 11H of H-Ar).

### COMPOUND II

FTIR (cm<sup>-1</sup>): 3251 (N-H) strch of 2.nd amine, 3089 (aromatic C-H) strch, 1699 (C=O) strch of amide, 1653 (C=N)

**Table 2.** Docking outcomes of the reference drug and the synthesized compounds with the VEGF-R2 tyrosine kinase site (PDB code: 4AG8)

| Compound  | S. score (Kcal/mol) | RMSD   | No. of binding sites | Binding amino acids                           |
|-----------|---------------------|--------|----------------------|-----------------------------------------------|
| Sunitinib | -8.7276             | 2.0611 | 2                    | Asp 1046, Leu 840                             |
| I         | -9.1782             | 1.5147 | 5                    | Asp 1046, Glu 885, Lys 868, Leu 889, Phe 1047 |
| II        | -9.2425             | 1.1745 | 4                    | Asp 1046, Glu 885, Lys 868, Leu 889           |
| III       | -9.4254             | 1.0608 | 4                    | Asp 1046, Glu 885, Phe 1047, Leu 889          |
| IV        | -9.2709             | 1.8914 | 4                    | Asp 1046, Glu 885, Lys 868, Leu 889           |
| V         | -9.2731             | 1.5234 | 4                    | Asp 1046, Glu 885, Lys 868, Leu 889           |
| VI        | -9.5120             | 1.3227 | 4                    | Asp 1046, Glu 885, Lys 868, Leu 889           |

Source: Own materials

strch vibration of imine, 1608, 1585, 1479 (C=C) strch, 1328 (C-F) strch, 1255 (C=N) strch.

<sup>1</sup>H-NMR (400MHz, DMSO.d<sub>6</sub>, ppm): 13.91 (singlet, 1H of amide N-H), 11.42 (singlet, 1H of N-H), 10.43 (singlet, 1H of indole N-H), 8.47-6.88 (multiplet, 10H of H-Ar).

### COMPOUND III

FTIR (cm<sup>-1</sup>): 3176 (N-H) strch of 2.nd amine, 3059 (aromatic C-H) strch, 2818 (aliph. CH<sub>3</sub>) strch, 1695 (C=O) strch of amide, 1666 (C=N) strch of imine, 1593, 1533, 1444 (C=C) strch, 1327 (C-F) strch, 1251 (C-N) strch.

<sup>1</sup>H-NMR (400MHz, DMSO.d<sub>6</sub>, ppm): 13.93 (singlet, 1H of amide N-H), 11.31 (singlet, 1H of N-H), 10.48 (singlet, 1H of indole N-H), 8.48-6.82 (multiplet, 10H of H-Ar), 2.29 (singlet, 3H of CH<sub>3</sub>).

### COMPOUND IV

FTIR (cm<sup>-1</sup>): 3170 (N-H) strch of 2.nd amine, 3055 (aromatic C-H) strch, 2835 (aliph. CH<sub>3</sub>) strch, 1695 (C=O) strch of amide, 1666 (C=N) strch of imine, 1597, 1531, 1442 (C=C) strch, 1327 (C-F) strch, 1284 (C-O) strch, 1251 (C-N) strch.

<sup>1</sup>H-NMR (400MHz, DMSO.d<sub>6</sub>, ppm): 13.97 (singlet, 1H of amide N-H), 11.19 (singlet, 1H of N-H), 10.52

(Singlet, 1H of indole N-H), 8.48-6.81 (multiplet, 10H of H-Ar), 3.75 (singlet, 3H of CH<sub>3</sub>).

### COMPOUND V

FTIR (cm<sup>-1</sup>): 3238 (N-H) strch of 2.nd amine, 3055 (aromatic C-H) strch, 1687 (C=O) strch of amide, 1664 (C=N) strch, 1612, 1583, 1446 (C=C) strch, 1332 (C-F) strch, 1249 (C-N) strch, 759 (C-Cl) strch.

<sup>1</sup>H-NMR (400MHz, DMSO.d<sub>6</sub>, ppm): 13.88 (singlet, 1H of amide N-H), 11.87 (singlet, 1H of N-H), 10.43 (singlet, 1H of indole N-H), 8.54-7.12 (multiplet, 10H of H-Ar).

### COMPOUND VI

FTIR (cm<sup>-1</sup>): 3205 (N-H) strch of 2.nd amine, 3082 (aromatic C-H) strch, 1701 (C=O) strch of amide, 1676 (C=N) strch, 1614, 1583, 1444 (C=C) strch, 1328 (C-F) strch, 1249 (C-N) strch.

<sup>1</sup>H-NMR (400MHz, DMSO.d<sub>6</sub>, ppm): 13.79 (singlet, 1H of amide N-H), 11.82 (singlet, 1H of N-H), 10.35 (singlet, 1H of indole N-H), 8.49-7.08 (multiplet, 10H of H-Ar).

### DOCKING STUDY

The molecular docking program helps identify the ideal structural conformation and orientation using a specific algorithm, followed by a scoring function to predict binding affinity and assess the interaction mode. The primary protein structural database is the publicly accessible Protein Data Bank (PDB) [26]. The docking investigation comprises two primary parts. The first part deals with ligand preparation, which involves building the structure of the necessary derivatives using Chem Draw professional software version 12.0, followed by atoms protonation, charges, energy optimization, and structural energy minimization using the Molecular Operating Environment (MOE 2015) software [27-28]. The second part deals with target macromolecule or enzyme preparation, which requires importing the crystal structure of the VEGFR-2 kinase active site (PDB code: 4AG8) into the MOE software, subsequently removing water, ligand previously attached to the co-crystallized structure, active site protonation [29]. Following the preparation of the requisite active site, the receptor docking study commences to evaluate the binding energy values (S. score) and root mean square deviation (RMSD). Derivatives exhibiting an optimum S. score and a low RMSD will demonstrate optimal binding and compatibility with the target receptor site.

### CYTOTOXIC STUDY

The National Cell Bank of Iran (Pasteur Institute, Iran) provided the human lung cancer cell line A549 and

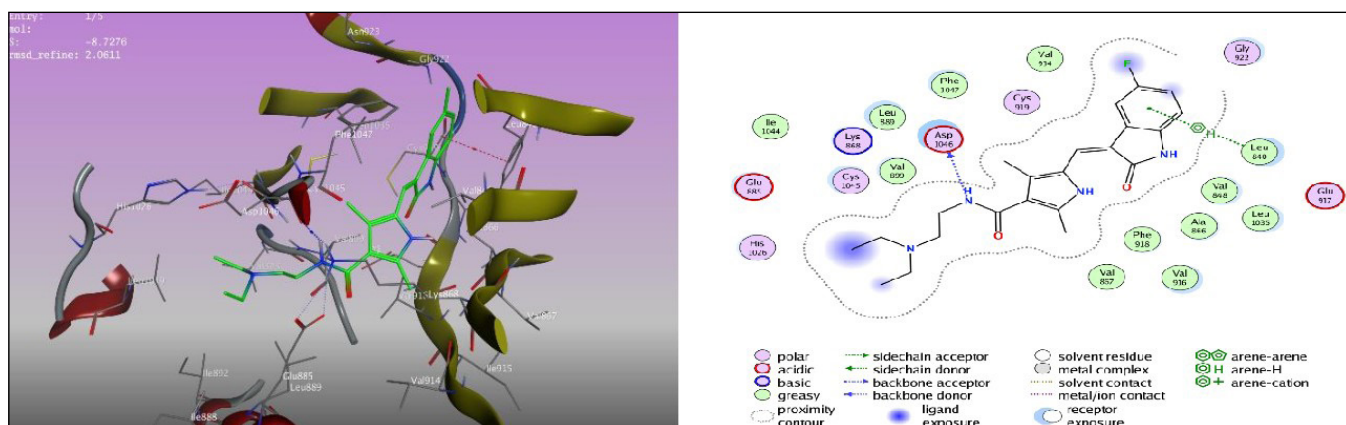


**Fig. 2** Docking result of sunitinib with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)

Source: Own materials

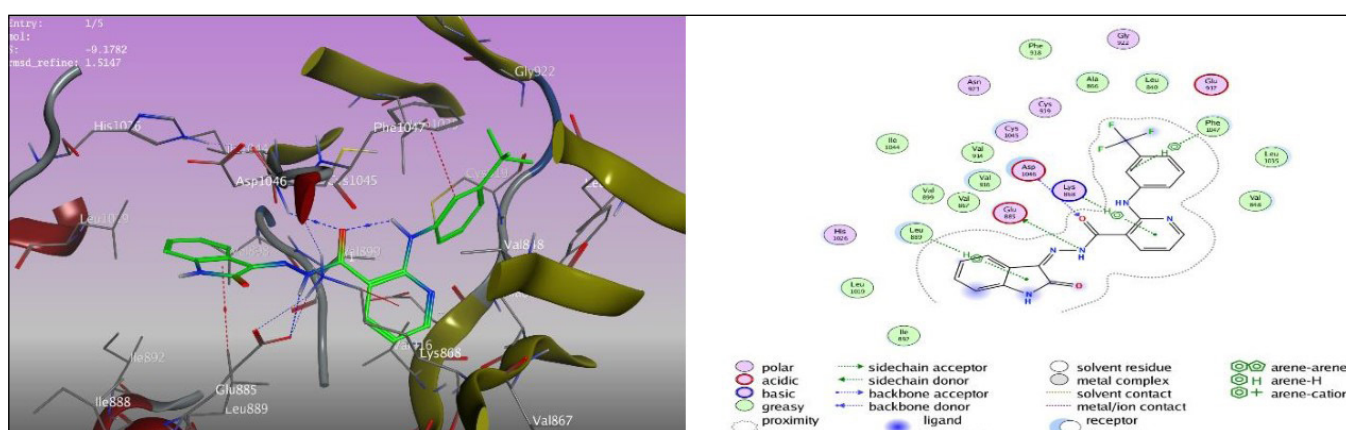


**Fig. 3.** Docking result of compound I with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)

Source: Own materials

the normal lung fibroblast cell line MRC-5. Cells had been grow in RPMI-1640 media (Gibco) with 10% fetal bovine serum (FBS) and antibiotics, such as 100 U/mL penicillin and 100 µg/mL streptomycin. Cells were kept alive in a humidified incubator at 37 °C with 5% CO<sub>2</sub>. We used a trypsin-EDTA solution to separate the cells and then rinsed them with phosphate-buffered saline (PBS)) [30].

### MTT VIABILITY ASSAY

The MTT assay was carried out to assess the capacity for survival of the cells. A density of  $1.4 \times 10^4$  cells/well was used to seed cells in 96-well plates, and the cells were incubated for 24 hours. MTT solution (0.5 mg/mL) was introduced and incubated for 4 hours after the test compounds were treated with a range of concentrations (6.25–100 µg/mL) for an additional 24 hours. The formazan crystals that were produced were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. The dose-response data was used to calculate the IC<sub>50</sub> values [31].

## RESULTS

### CHEMISTRY

The esterification of Niflumic acid was accomplished using ethanol as solvent, with thionyl chloride added under chilled conditions to generate the acid chloride, which rapidly interacts with ethanol to produce the ethyl ester of Niflumic acid, which was observed by the disappearance of broad OH peak of carboxylic acid and appearance of C=O group of conjugate esters at 1691 cm<sup>-1</sup>. The synthesized ester was subsequently reacted with hydrazine monohydrate 99% to produce the hydrazide. The carbonyl of the ester vanished, whereas the amide C=O band appeared at 1608 cm<sup>-1</sup>, with the primary amine group at 3415, 3309 cm<sup>-1</sup>. The obtained hydrazide compound then reacted with ketone containing compound to form the final acyl hydrazone compounds, which is observed utilizing FTIR and HNMR by replacement of primary amine group bands with secondary amine single band near 3200 cm<sup>-1</sup> and the appearance of sharp imine group band at the range 1653-1676 cm<sup>-1</sup> while the <sup>1</sup>HNMR showed three singlet hydrogen peaks of secondary amine containing compounds above 10.40 ppm.



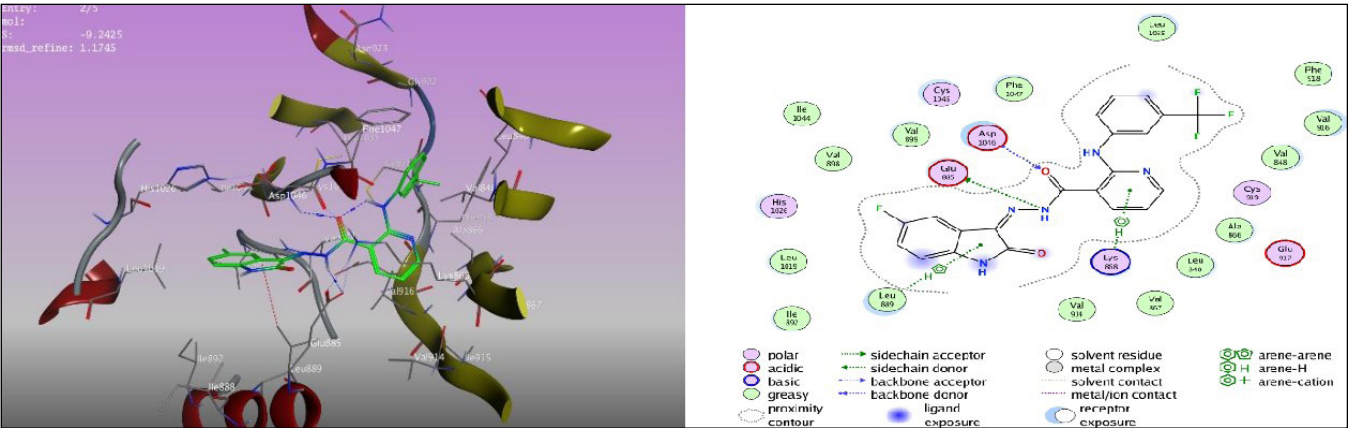


**Fig. 4.** Docking result of compound II with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)  
Source: Own materials

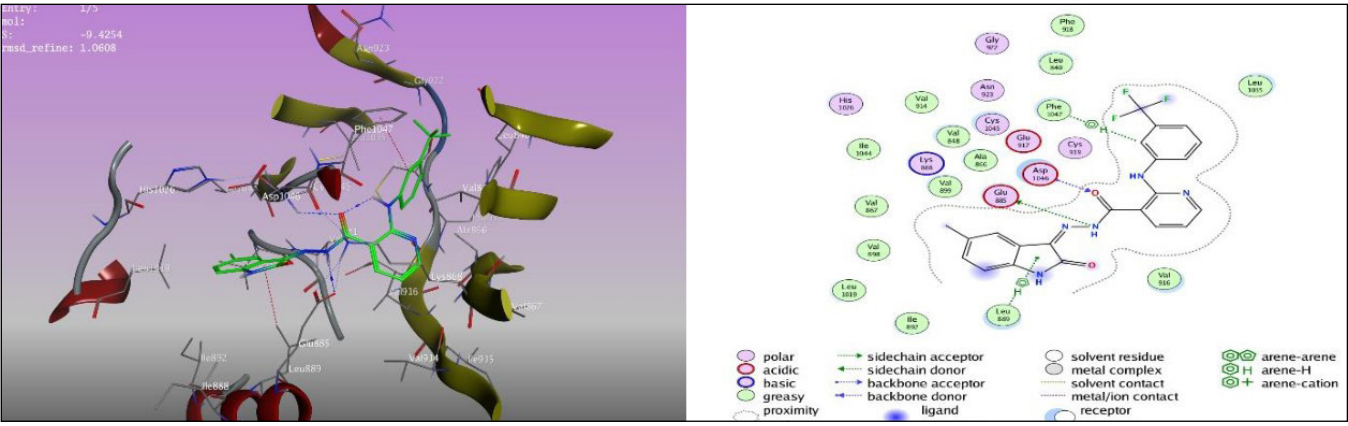


**Fig. 5:** Docking result of compound III with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)  
Source: Own materials

DOCKING STUDY

The docking results show enhanced binding energy (S. score) equal -9.1782 to -9.5120 Kcal/mol and optimum binding mode (RMSD) values equal 1.0608 to 1.8914 with the target VEGFR-2 kinase domain (PDB code: 4AG8) as a compared with the reference drug (Sunitinib) which shows S. score value equal -8.7276 Kcal/mol and RMSD value equal 2.0611. The synthesized six compounds show an additional four to five interactions with crucial amino acids involved in the gatekeeper region (Lys 868), the catalytic DFG motif (Glu885, Asp1046) and the allosteric hydrophobic pocket. As illustrated in Figures )2–8(, these interactions demonstrate the strong binding affinity and stability of the synthesized ligands within the VEGFR-2 active site. Table (2) displays the docking outcomes of the final compounds with their first to second poses with the target VEGFR-2 tyrosine kinase active compared with the reference ligand (Sunitinib) interactions at the same site.

CYTOTOXIC STUDY

In this study, six novel compounds were synthesized, and their cytotoxic effects were evaluated using adenocarci-

nomic human alveolar basal epithelial cell (A549) and a normal diploid fibroblast from human fetal lung tissue (MRC-5). The MTT cell viability assay was employed to assess the effect of the synthesized compounds and the reference drug (Sunitinib) on the two cell lines; each IC50 value was determined using different concertations of reference and synthesized compounds. The IC50 values represent the compound concentration at which 50% of cells become non-viable. The results demonstrated very promising cytotoxic results for the synthesized compounds and a safer effect on normal cells as compared with the reference drug (Sunitinib). The IC50 levels of the produced compounds against the cell lines varied from 20.87µM to 78.48µM in the A549 cell line and from 175.49 µM 280.27 µM in the MRC-5 cell line. The most pronounced inhibitory effect was noted with compound II. Both Figures (9-10) displays the relationship between concentration and impact by plotting the viability data of A549 and MRC-5 cells against the respective concentrations of the reference and synthesized compounds. The Statistical Analysis System (SAS) application version 2018 was utilized to identify the impact of various vari-



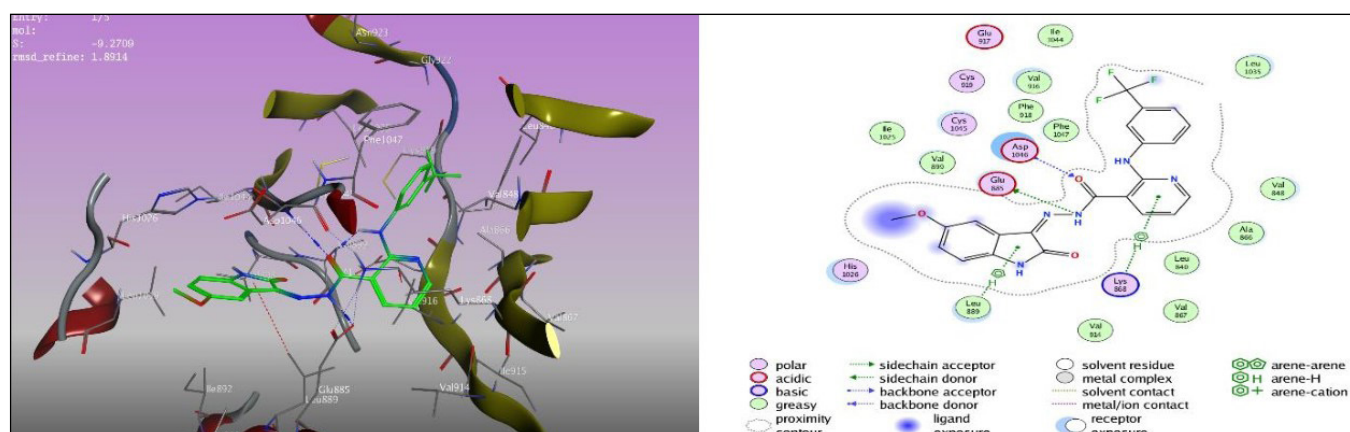


**Fig. 6.** Docking result of compound IV with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)

Source: Own materials

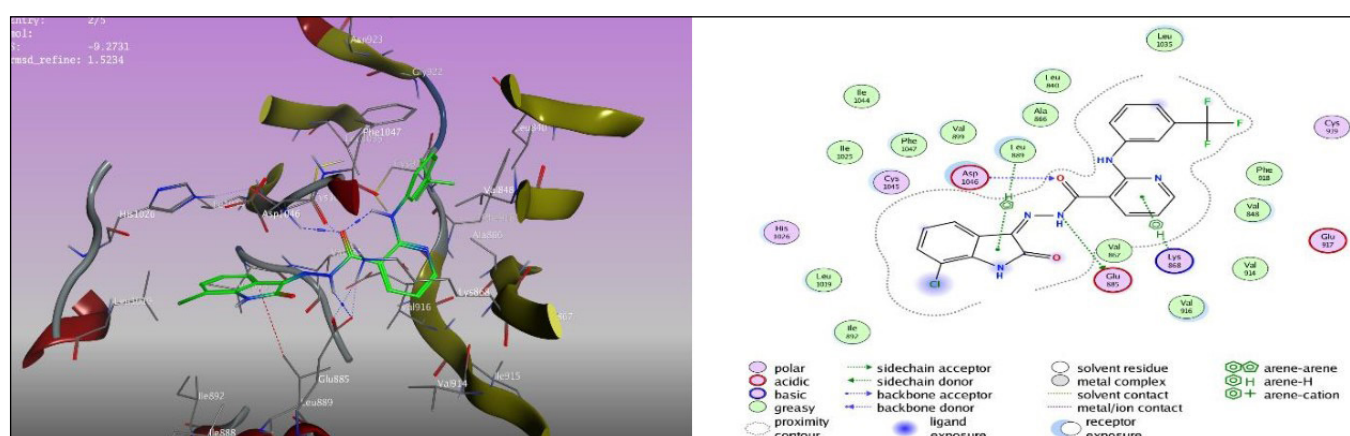


**Fig. 7.** Docking result of compound V with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)

Source: Own materials

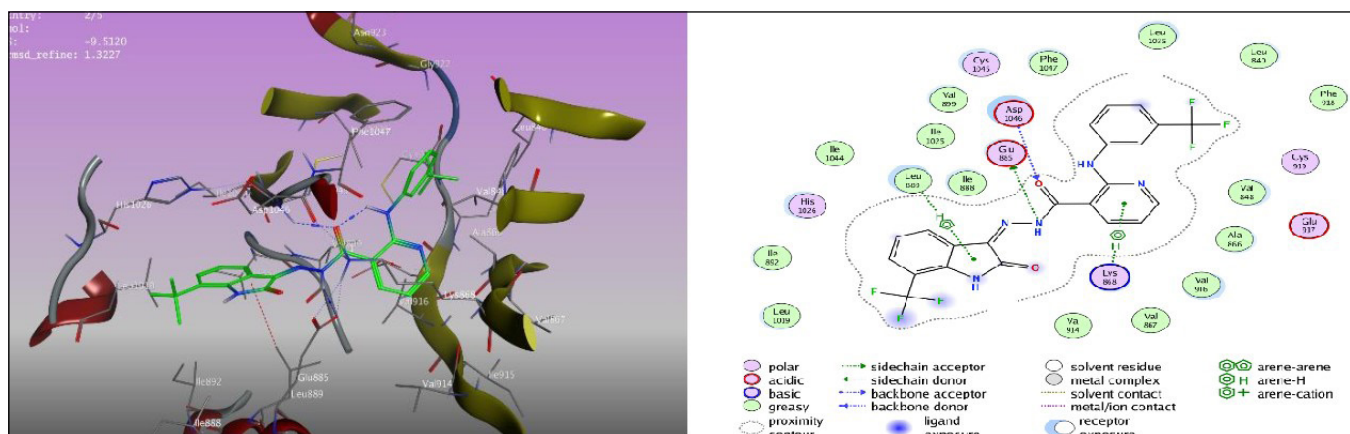


**Fig. 8.** Docking result of compound VI with the VEGFR-2 tyrosine kinase (PDB code: 4AG8)

Source: Own materials

ables on research parameters. The T-test was employed to significantly compare the means in this investigation as shown in table (3).

## DISCUSSION

The docking results indicate that the synthesized compounds have stronger binding affinities and

more stable binding conformations than the reference drug Sunitinib, as reflected by their more negative S. score values and smaller RMSD values. The observed interactions with crucial amino acids in the VEGFR-2 kinase active site, such as those in the gatekeeper region, the DFG motif, and the allosteric site, suggest that the designed compounds provide enhanced inhibition of VEGFR-2 kinase activity. The

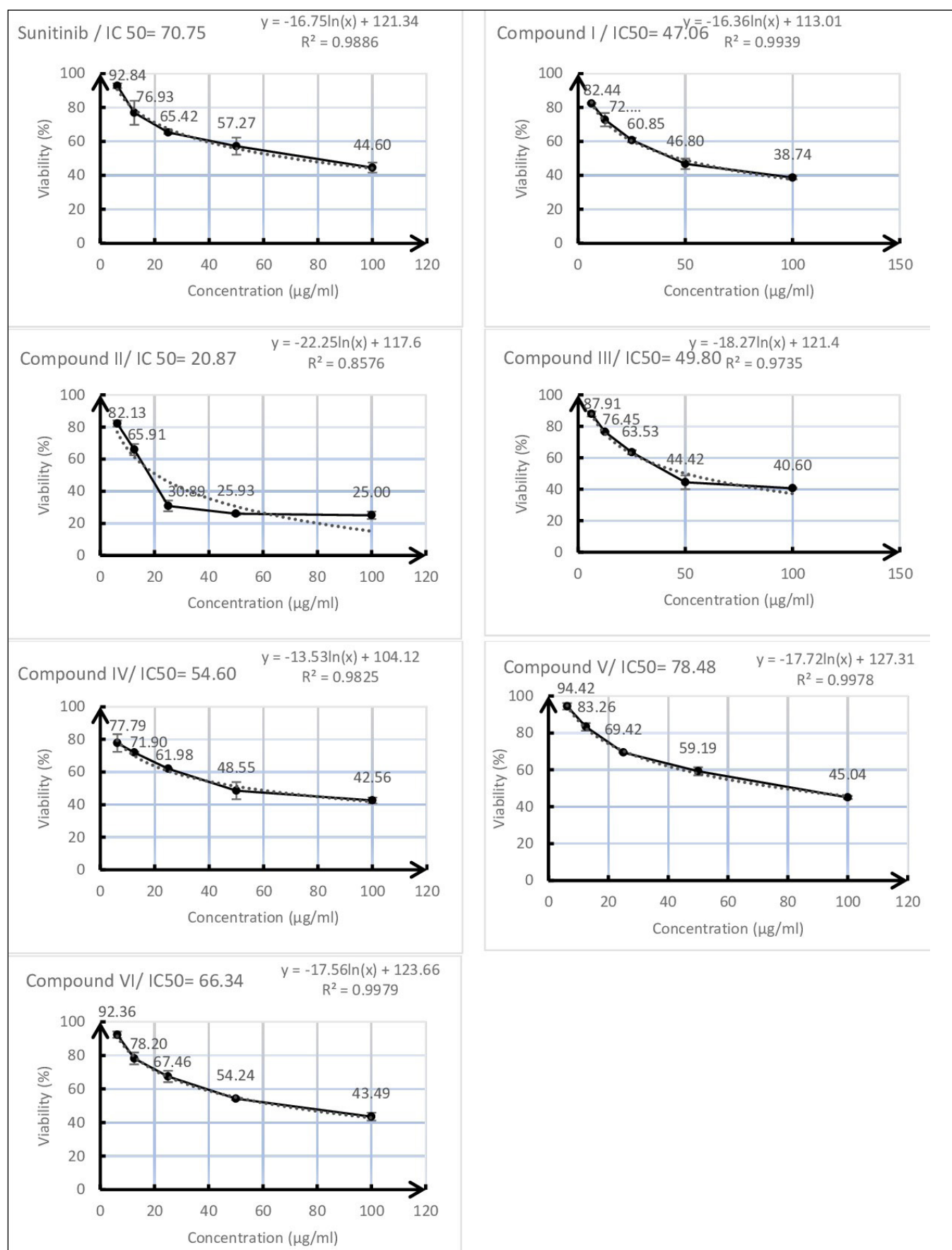
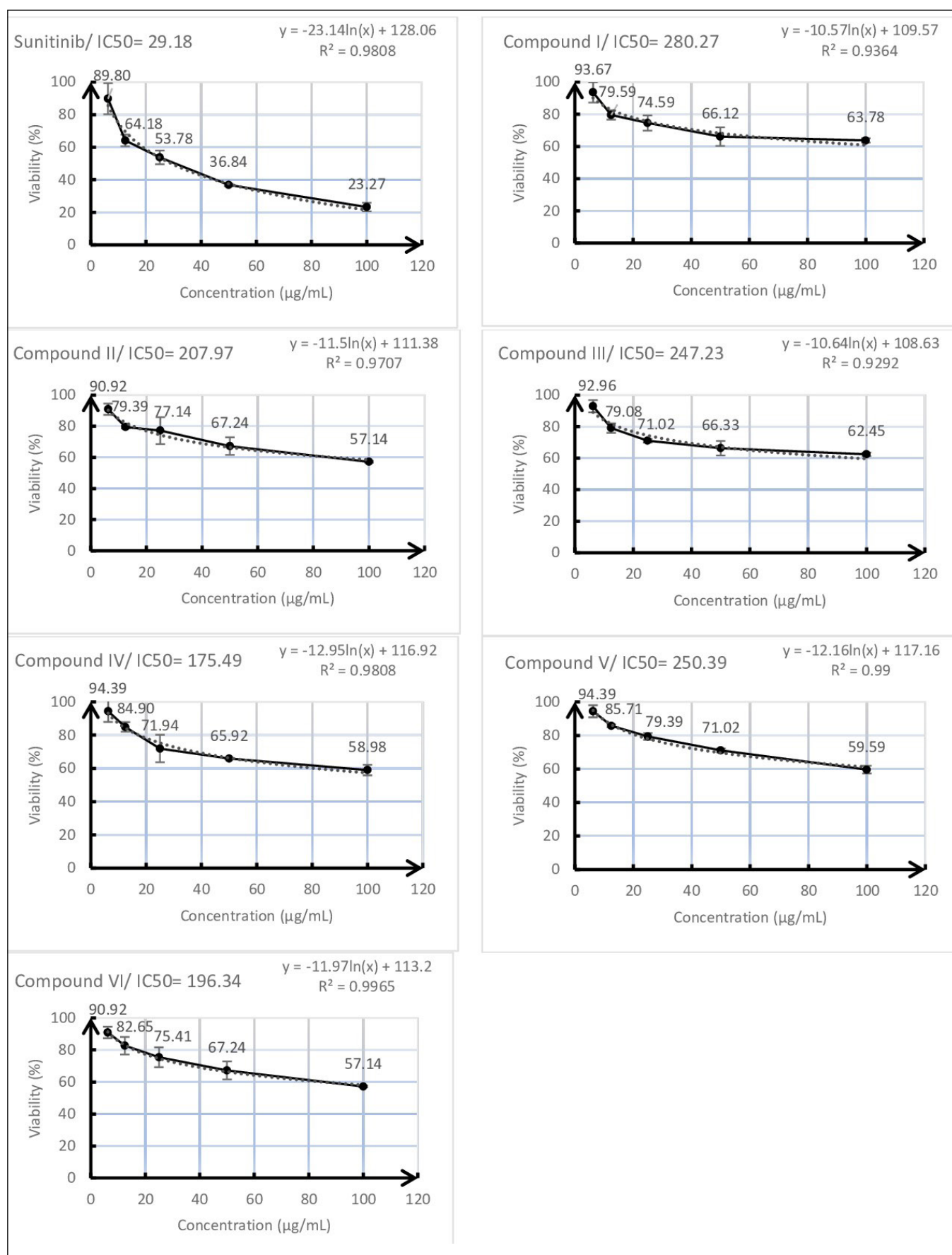


**Fig. 9.** Cytotoxicity result of the standard and synthesized compound on A549 cell line  
Source: Own materials

**Fig. 10.** Cytotoxicity result of the standard and synthesized compound on MRC-5 cell line

Source: Own materials

**Table 3.** IC50 values for the tested compounds and standard. The values are represented by the mean ± SD of triplicate measurements in A549 and MRC-5

| Compound  | A549        |             | MRC-5         |             |
|-----------|-------------|-------------|---------------|-------------|
|           | IC50 ±SD    | P-value     | IC50 ±SD      | P-value     |
| Sunitinib | 70.75 ±2.81 | Standard    | 29.18 ±1.55   | Standard    |
| I         | 47.06 ±2.07 | 0.0004 ** a | 280.27 ±26.03 | 0.0001 ** a |
| II        | 20.87 ±1.29 | 0.0001 ** a | 207.97 ±17.94 | 0.0001 ** a |
| III       | 49.80 ±3.16 | 0.0005 ** a | 247.23 ±22.41 | 0.0001 ** a |
| IV        | 54.60 ±2.78 | 0.0017 ** a | 175.49 ±9.76  | 0.0001 ** a |
| V         | 78.48 ±4.52 | 0.0381 * a  | 250.39 ±13.60 | 0.0001 ** a |
| VI        | 66.34 ±3.70 | 0.237 NS    | 196.34 ±9.96  | 0.0001 ** a |

Notes: a - Significant difference that the comparison with Sunitinib, NS - Non-significant; \* (P<0.05), \*\* (P<0.01), NS: Non-significant  
Source: Own materials

presence of additional interactions compared to Sunitinib implies improved specificity and potential biological activity. The synthetic pathway demonstrates a successful stepwise conversion from Niflumic acid to its ester, then to hydrazide, and finally to the acyl hydrazone derivatives. The use of thionyl chloride effectively activated the carboxylic acid group, facilitating ester formation. The disappearance of the hydroxyl group and the appearance of the aliphatic ethyl carbonates in FTIR confirmed the esterification. The subsequent formation of hydrazide was evident from the frequency downshifting of the amide carbonyl and appearance of 2<sup>nd</sup> amine bands. Finally, the formation of the acyl hydrazones was supported by both FTIR and <sup>1</sup>HNMR data, particularly the imine band and the downfield-shifted singlet peaks in the <sup>1</sup>HNMR spectrum. These spectral changes confirm the success of each reaction step and the formation of the final compounds along with their intermediates. The cytotoxic data suggest that the synthesized compounds exhibit promising tumor-selective cytotoxicity, showing enhanced inhibition of cancer cell viability while maintaining reduced toxicity toward normal cells compared to the reference drug and previously reported studies. This selective effect may

be attributed to the overexpression of VEGFR-2 in the A549 cancer cells, increasing compound uptake or binding. Compound II high activity is likely due to its strong electron-withdrawing substituents, which reduce electron density, and the low steric hindrance of the fluorine atom, enabling more efficient interaction with the kinase binding site. These structural features may contribute to improved binding affinity and inhibitory efficacy, supporting its potential as a lead compound for further development.

CONCLUSIONS

A set of isatin-niflumic acid derivatives (I-VI) have been successfully synthesized, and their chemical structures were confirmed using FTIR and <sup>1</sup>HNMR methods. The molecular docking studies and MTT assays for the assessed drugs revealed a robust correlation between the predicted results from molecular docking and the biological evaluations, demonstrating notable selectivity towards the VEGFR-2 kinase active site. In summary, the newly synthesized niflumic acid derivatives serve as potent anticancer medicines by blocking VEGFR tyrosine kinase, hence restricting cell development and possesses promise as targets for additional studies on anticancer treatments.

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### CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# The influence of sex hormones and the menstrual cycle on women's athletic performance: A literature review

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## ABSTRACT

The cyclical nature of female sex hormones - primarily estradiol and progesterone - exerts a multidimensional influence on physiological processes relevant to sports performance. This narrative review summarizes the current understanding of how different phases of the menstrual cycle affect energy metabolism, cardiovascular dynamics, neuromuscular function, thermoregulation, and psychological responses in physically active women. Estrogen-dominant phases, especially the late follicular stage, are generally associated with enhanced aerobic capacity, improved vasodilation, mood stabilization, and more efficient motor control. Conversely, progesterone-dominant phases - most notably the mid-to-late luteal stage - are characterized by increased core temperature, fluid retention, fatigue, and greater reliance on carbohydrate metabolism. The review also evaluates the effects of hormonal contraceptives, particularly oral contraceptives, on athletic performance and training adaptations. While individual responses vary, hormonal fluctuations create predictable patterns of performance variability that can be leveraged through phase-aware training. Based on the reviewed literature, the paper suggests practical training strategies, including phase-specific adaptations, nutritional support, and attention to menstrual symptoms. The review further highlights methodological limitations in the literature, including small sample sizes, inconsistent hormone validation, and underrepresentation of elite athletes. A personalized understanding of menstrual physiology enables more effective planning of training loads and recovery strategies, benefiting both professional and recreationally active women.

**KEY WORDS:** menstrual cycle, estradiol, progesterone, athletic performance, exercise physiology

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## INTRODUCTION

### SCIENTIFIC CONTEXT AND RATIONALE

The growing participation of women in various forms of physical activity - from recreational exercise to elite-level sport - has highlighted the necessity for an in-depth understanding of sex-specific physiological processes, particularly the impact of cyclical hormonal fluctuations. The female endocrine system is characterized by cyclical fluctuations in estradiol and progesterone, regulated by the hypothalamic-pituitary-ovarian (HPO) axis. These sex hormones exert influence beyond reproductive physiology, affecting cardiovascular, muscular, respiratory, metabolic, and neuroendocrine systems. In contrast to men, women exhibit considerable intra-cycle variability in physiological parameters such as maximal oxygen uptake ( $\text{VO}_2\text{max}$ ), lactate threshold, neuromuscular coordination, and recovery dynamics [5].

Despite a growing body of evidence, training protocols often remain standardized and fail to incorporate individual hormonal profiles. This lack of personalization may increase the risk of injuries and endocrine dysfunctions, including relative energy deficiency in sport (RED-S), polycystic ovary syndrome (PCOS), and functional hypothalamic amenorrhea (FHA) [1].

### AIM

The objective of this review is to synthesize current knowledge on the influence of estradiol, progesterone, and distinct phases of the menstrual cycle on women's physical performance. Additionally, the review examines the physiological consequences of hormonal contraceptive use in the context of athletic adaptation. Specific areas addressed include:

- Hormonal characteristics and phases of the menstrual cycle;

- Mechanisms of action of sex hormones on major physiological systems;
- Effects of both endogenous and pharmacologically induced hormonal fluctuations on athletic performance variables;
- Practical applications for optimizing training regimens and minimizing injury risk.

## MATERIALS AND METHODS

This review summarizes peer-reviewed literature published between 1993 and 2023, focusing on hormonal physiology and exercise science. The selected time frame reflects key methodological advances, including improved endocrine assays, wearable technologies for training load monitoring, and an increased focus on sex-based physiological variability. Earlier studies were excluded due to outdated protocols, methodological limitations, or lack of hormonal phase verification.

Although a scoping review was initially considered, substantial heterogeneity in study aims, populations, and outcomes led to the adoption of a narrative format. This allowed for flexible synthesis across diverse research types, including observational studies, clinical trials, and mechanistic investigations relevant to female athlete care.

Searches were conducted in PubMed, Scopus, and Google Scholar, using Boolean logic and phrase matching with the following string: (“menstrual cycle” OR “estradiol” OR “progesterone” OR “sex hormones”) AND (“exercise” OR “athletic performance” OR “training”) AND (“women” OR “female athletes”). Filters were applied to include English-language, peer-reviewed studies on human subjects. Backward citation tracking was used to identify additional seminal works.

Search and selection summary:

- ~480 records from PubMed
  - ~600 from Scopus
  - ~200 manually screened entries from Google Scholar
- After deduplication and abstract screening (~750 unique records), 160 full texts were reviewed, and 92 studies were included in the final synthesis.

### Inclusion criteria:

- Studies on eumenorrheic women (with or without hormonal contraceptive use);
- Assessment of exercise performance metrics (e.g.,  $\text{VO}_2\text{max}$ , strength, thermoregulation);
- Preferably included biochemical verification of hormonal phase.

### Exclusion criteria:

- Studies involving postmenopausal, adolescent, or pregnant populations;
- Absence of hormonal phase confirmation;

- Irrelevance to exercise physiology or athletic performance.

Both elite and recreational athletes were considered. Final inclusion required methodological transparency, scientific rigor, and relevance to individualized training.

To improve interpretive clarity, studies were classified by evidence level where applicable. Most fell under Level 3–4 (observational or non-randomized designs), with a smaller number classified as Level 2 (e.g., randomized or crossover trials with hormonal confirmation). Meta-analyses and systematic reviews were included where available. Formal risk-of-bias tools were not applied, consistent with the narrative design.

Given the evolving nature of this field, future systematic reviews are warranted. Nonetheless, this synthesis consolidates current high-quality evidence to offer cycle-specific insights and practical guidance for optimizing female athlete performance and health. Due to the heterogeneity of study designs, populations, and outcome measures, formal risk-of-bias assessment tools (e.g., ROBIS or Cochrane criteria) were not applied, as they are not universally compatible with narrative synthesis frameworks.

## REVIEW AND DISCUSSION

### PHYSIOLOGY OF THE MENSTRUAL CYCLE

#### HORMONAL PHASES

The menstrual cycle is traditionally divided into three distinct phases, each characterized by specific hormonal profiles and physiological processes:

- **Follicular phase (days 1–14):** This phase is marked by a progressive increase in follicle-stimulating hormone (FSH), which promotes follicular development and stimulates estradiol synthesis. Elevated estradiol levels contribute to improved muscle perfusion, increased glucose uptake via GLUT-4 transporters, enhanced lipid oxidation, and greater metabolic flexibility [9].
- **Ovulation (~day 14):** Ovulation is induced by a mid-cycle surge in luteinizing hormone (LH), leading to the release of the oocyte. At this time, peak estradiol concentrations enhance cardiovascular function and neuromuscular performance. However, elevated estradiol may concurrently reduce the structural integrity of connective tissue, thereby increasing the risk of musculoskeletal injury [14].
- **Luteal phase (days 15–28):** This phase is dominated by progesterone, which elevates core body temperature, promotes fluid retention, reduces insulin sensitivity, and shifts metabolic reliance toward glycogen. These physiological changes may negatively impact endurance capacity and thermoregulatory efficiency [5].

**Table 1.** Summary of estradiol and progesterone effects on female physiology and athletic performance

| Hormone           | Primary physiological effects                                                                                                              | Performance impact                                                                |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Estradiol (E2)    | ↑ Fat oxidation, ↑ glycogen sparing, ↑ mitochondrial efficiency, ↑ nitric oxide (NO) synthesis, ↑ angiogenesis, ↑ muscle protein synthesis | ↑ Aerobic capacity, ↑ endurance, ↑ coordination, ↑ recovery, ↑ mood stability     |
| Progesterone (P4) | ↑ Core temperature, ↑ fluid retention, ↑ ventilation, ↓ insulin sensitivity, ↑ GABAergic tone, ↓ concentration, ↓ neuromuscular activation | ↓ Endurance, ↓ thermoregulation, ↑ fatigue, ↓ cognitive sharpness, ↓ coordination |

Sources: adapted from McNulty et al. (2020) [2] Elliott-Sale et al. (2020) [3] Hackney (2016) [5] Constantini et al. (2005) [10] Romero-Moraleda et al. (2019) [11] Martin et al. (2018) [12]

Abbreviations: ↑ increase, ↓ decrease, E2 – estradiol, P4 – progesterone, NO – nitric oxide, GABA – gamma-aminobutyric acid

**Table 2.** Phase-specific hormonal profiles and their effects on female athletic performance

| Cycle Phase      | Hormonal Profile     | Performance Impact                                      | Risks and Limitations                                 |
|------------------|----------------------|---------------------------------------------------------|-------------------------------------------------------|
| Early follicular | Low E2, low P4       | Supports high-intensity, anaerobic efforts              | Menstrual discomfort may reduce motivation            |
| Late follicular  | High E2, low P4      | Peak aerobic/neuromuscular capacity, fast recovery      | Increased ligament laxity; soft tissue vulnerability  |
| Ovulation        | E2 peak, LH surge    | Maximal focus, reaction time, CNS activation            | Higher ACL injury risk in pivot-based sports          |
| Luteal           | High P4, moderate E2 | Decreased endurance, thermoregulation, mood variability | PMS symptoms, impaired cognition, and fluid retention |

Sources: Adapted from Julian et al. (2017) [9] Constantini et al. (2005) [10] Hackney (2016) [5] McNulty et al. (2020) [2] Elliott-Sale et al. (2020) [3] Bruinvels et al. (2017) [20]

## HORMONAL INFLUENCE ON PHYSIOLOGY AND FUNCTIONAL IMPLICATIONS

Estradiol and progesterone - key ovarian hormones - modulate a wide range of physiological systems relevant to exercise performance. Among its effects, estradiol has been shown to enhance mitochondrial efficiency and lipid oxidation, while also promoting angiogenesis and better muscle perfusion. It also promotes muscle protein synthesis and neuromuscular coordination, thereby enhancing aerobic capacity, endurance, post-exercise recovery, and psychological resilience.

Conversely, progesterone - dominant in the luteal phase - raises core body temperature, promotes fluid retention and ventilation, reduces insulin sensitivity, and suppresses neuromuscular activation. It may also affect mood, concentration, and thermoregulatory control, particularly under heat stress or psychological load. These opposing hormonal effects contribute to phase-specific performance variability across the menstrual cycle.

Hormonal fluctuations regulated by FSH and LH influence several key systems:

- **Cardiovascular function:** via changes in vascular tone and plasma volume;
- **Muscular adaptation:** through modulation of protein synthesis and satellite cell activity;
- **Neurocognitive performance:** including mood, fatigue, and coordination.

A detailed summary of these hormone-specific physiological mechanisms and their athletic implications is presented in Table 1 (Section 2.3). These concepts are further expanded in subsequent sections, particularly in the context of performance metrics (Section 3) and training strategies (Section 4).

## PHYSICAL PERFORMANCE – DEFINITIONS AND COMPONENTS

### FUNCTIONAL COMPONENTS OF PHYSICAL PERFORMANCE

Physical performance is a multifactorial trait shaped by the coordinated function of the cardiovascular, muscular, metabolic, and endocrine systems. It enables sustained physical effort across intensities while maintaining energy efficiency, homeostasis, and neuromuscular control.

Key components include:

- **Aerobic capacity**, influenced by  $\text{VO}_2\text{max}$ , cardiac output, and mitochondrial function, tends to improve during the late follicular phase due to estradiol-driven adaptations (Table 1, Section 2.3);
- **Anaerobic capacity**, driven by phosphagen and glycolytic pathways, supports high-intensity bursts but is sensitive to hormonal modulation, particularly under contraceptives with antiandrogenic activity [3];
- **Muscular strength**, shaped by fiber type, cross-sectional area, and protein synthesis pathways, may benefit

from estradiol-facilitated anabolic signaling (Table 1, Section 2.3);

- **Speed and neuromuscular control**, dependent on CNS activation and motor unit recruitment, also vary with hormonal fluctuations - especially around ovulation;
- **Endurance**, integrating metabolic and psychological resilience, is modulated by substrate use and thermoregulatory capacity across the cycle.

Given these hormonal influences, phase-specific training adjustments may enhance adaptation and reduce risk in female athletes.

### FEMALE-SPECIFIC BIOLOGICAL FACTORS

Female physical performance is influenced by distinct sex-based anatomical and physiological characteristics. In comparison to males, women generally exhibit lower skeletal muscle mass, a higher percentage of body fat, reduced cardiac chamber dimensions, and decreased hemoglobin concentrations. These factors collectively contribute to a lower  $\text{VO}_2\text{max}$  and diminished absolute power output in female individuals [13, 15, 16].

Furthermore, women tend to possess a higher proportion of type I (oxidative) muscle fibers, which supports superior fatigue resistance and aerobic endurance but may simultaneously constrain maximal strength and power capacity. These sex-specific traits must be considered in the design and interpretation of performance assessments and training interventions.

## THE IMPACT OF THE MENSTRUAL CYCLE ON ATHLETIC PERFORMANCE

### FOLLICULAR AND OVULATORY PHASES

Consider this: many elite athletes report feeling their strongest mid-cycle. This aligns with estradiol dominance during the follicular and ovulatory phases - periods linked to better aerobic performance and coordination. (see Table 1, Section 2.3). The late follicular phase supports optimal aerobic capacity, neuromuscular efficiency, and recovery, making it suitable for endurance and strength-based training (Table 2, Section 4.3).

In contrast, the early follicular phase - despite low hormone concentrations - may benefit high-intensity and anaerobic efforts, as the absence of progesterone avoids thermogenic and cognitive impairments.

The ovulatory phase, marked by an LH surge and estradiol peak, enhances central nervous system activation, neuromuscular coordination, and psychological readiness. This makes it optimal for speed, power, and skill-based training. However, transient increases in es-

tradiol may elevate ligament laxity and musculoskeletal injury risk, particularly ACL ruptures in multidirectional sports [14].

A summary of performance-relevant hormonal profiles and recommendations is provided in Table 2 (Section 4.3).

### LUTEAL PHASE AND PREMENSTRUAL SYNDROME (PMS)

During the luteal phase (days 15–28), the progressive rise in progesterone exerts both thermogenic and catabolic effects. The associated increase in core body temperature may impair thermoregulation, while reductions in lipid oxidation and insulin sensitivity contribute to diminished endurance capacity [10][5]. Additionally, progesterone promotes fluid retention and exacerbates cardiovascular load through activation of the renin–angiotensin–aldosterone system (RAAS). Its modulatory effect on the central nervous system via GABA-A receptors may lead to reduced motivation and impaired cognitive performance [3].

In the late luteal phase, the withdrawal of both estradiol and progesterone can precipitate premenstrual syndrome (PMS), manifesting as mood disturbances, irritability, sleep dysregulation, and reduced adherence to training regimens [16]. PMS may further increase the risk of injury and impede recovery, particularly through its disruption of circadian regulation [17]. Implementing individualized adjustments - such as reducing training intensity or incorporating structured recovery protocols - may support adherence and mitigate physiological stress responses [20].

### INDIVIDUAL VARIABILITY AND MONITORING

Physiological and performance-related responses to menstrual cycle phases exhibit considerable interindividual variability. These differences are modulated by factors such as ovulatory function, severity of PMS symptoms, hormonal sensitivity, training status, and genetic predispositions [2][19]. For example, elite endurance athletes with consistent ovulatory cycles may report stable performance across all phases, whereas strength-oriented athletes may experience pronounced impairments in power output, thermoregulatory control, or motivation during the luteal phase.

This variability reveals the limitations of generic training models and emphasizes the need for personalized, hormone-informed planning. Various monitoring strategies - including digital cycle-tracking applications, urinary LH surge detection, and serum hormone assays - enable precise identification of menstrual phases, early

detection of cycle disturbances (e.g., anovulation, luteal phase deficiency), and support hormone-informed training periodization.

Improving athletes' awareness of their individual hormonal profiles may enhance self-regulation, psychological resilience, and long-term training consistency. Such approaches contribute to more effective and safer performance optimization throughout the menstrual cycle. Could this explain why two equally trained women respond so differently to the same workout during the luteal phase? Possibly - but more data is needed.

## MENSTRUAL CYCLE DISTURBANCES AND ATHLETIC HEALTH

Relative Energy Deficiency in Sport (RED-S) is a clinical syndrome arising from low energy availability (LEA), wherein caloric intake is insufficient to meet the combined demands of exercise and basic physiological functions. This energy imbalance impairs multiple bodily systems, including metabolism, reproductive function, bone health, and cardiovascular regulation. Mechanistically, RED-S disrupts the pulsatile secretion of gonadotropin-releasing hormone (GnRH), leading to suppressed levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This hormonal suppression can result in luteal phase defects, anovulation, or functional hypothalamic amenorrhea (FHA) [1, 8].

Clinical manifestations of RED-S include reduced bone mineral density (BMD), impaired muscle protein synthesis, heightened risk of mood disorders, immune system suppression, and cardiovascular dysregulation.

• **Functional Hypothalamic Amenorrhea (FHA)** represents the most severe form of RED-S-related menstrual dysfunction. FHA is characterized by prolonged hypoestrogenism, resulting in accelerated bone loss, cognitive impairments, and compromised immune function [7]. Therapeutic strategies focus on restoring energy availability through nutritional rehabilitation, psychological support, and modulation of training intensity and volume.

• **Luteal Phase Deficiency (LPD)** is defined by inadequate progesterone production during the luteal phase, impairing post-exercise recovery and extracellular matrix remodeling. Clinical symptoms include chronic fatigue, mood instability, and disrupted thermoregulation [6].

**Monitoring implications:** The presence of menstrual disturbances should prompt comprehensive endocrine evaluation, including serum levels of estradiol (E2), progesterone (P4), FSH, LH, and thyroid-stimulating hormone (TSH). Additionally, dual-energy X-ray absorptiometry (DEXA) is recommended for assessing bone

density, and standardized screening tools should be employed to detect disordered eating behaviors. Early identification and targeted intervention are essential to mitigate long-term health consequences and preserve athletic performance.

## HORMONAL CONTRACEPTION AND PHYSICAL PERFORMANCE

### TYPES AND MECHANISMS OF ACTION

Hormonal contraceptives modulate female physiology primarily by suppressing the hypothalamic–pituitary–ovarian (HPO) axis, thereby inhibiting ovulation and stabilizing endogenous hormone levels.

- **Combined Hormonal Contraceptives (CHCs):** Contain synthetic estrogen and progestin (e.g., ethinyl-estradiol and levonorgestrel), delivered via oral tablets, patches, or vaginal rings. They suppress ovulation, stabilize the endometrium, and thicken cervical mucus to prevent fertilization [4].
- **Progestin-Only Contraceptives (POPs):** Include mini-pills, implants, injections (e.g., DMPA), and hormonal IUDs. These primarily thicken cervical mucus and, depending on formulation and dose, may variably suppress ovulation [3].
- **Emergency Contraceptives:** Temporarily inhibit ovulation but are not suitable for long-term use.

### PHYSIOLOGICAL AND PERFORMANCE IMPLICATIONS

By flattening natural hormonal fluctuations, hormonal contraceptives can impact performance-related physiology in both beneficial and adverse ways:

- **Positive effects:** Improved menstrual regularity and reduced PMS symptoms may enhance training consistency and psychological readiness.
- **Adverse effects:** Suppressed endogenous estradiol and progesterone can reduce bone mineral density (BMD), impair lean mass development, and affect metabolic flexibility [5].
- **Thermoregulatory changes:** Contraceptive-induced hormonal profiles may mimic luteal-phase conditions, potentially reducing heat tolerance during prolonged exertion in warm environments [5].

Responses vary by progestin type:

- **Androgenic progestins** (e.g., levonorgestrel, norethindrone) may increase insulin resistance and promote unfavorable body composition;
- **Antiandrogenic progestins** (e.g., drospirenone, dienogest) are associated with improved fluid balance and more favorable metabolic outcomes;

**Table 3.** Performance-related effects of common hormonal contraceptives

| Contraceptive type       | Mechanism                                 | Hormonal sup-pression     | Performance effects                   | Drawbacks                                            |
|--------------------------|-------------------------------------------|---------------------------|---------------------------------------|------------------------------------------------------|
| CHCs                     | Suppress FSH/LH; inhibit ovulation        | Strong (E2, P4)           | Cycle stabilization; re-duced PMS     | ↓ Lean mass, ↑ body temp, altered glucose metabolism |
| POPs                     | Mucus thickening; partial ovulation block | Moderate (dose-dependent) | Lower clot risk, flexible use         | Variable cycle control; shorter half-life            |
| Hormonal IUD             | Local progestin release                   | Minimal systemic          | Fewer systemic side effects           | Irregular bleeding; limited perfor-mance data        |
| DMPA Injection           | Long-acting ovulation suppression         | Strong                    | Long-term cycle sup-pression          | ↓ BMD, lean mass loss, possible weight gain          |
| Emergency Contra-ception | Temporary ovulation sup-pression          | Transient                 | No long-term impact if used correctly | Hormonal disruption if misused                       |
| Contraceptive Type       | Mechanism                                 | Hormonal Sup-pression     | Performance Effects                   | Drawbacks                                            |

Sources: Adapted from Elliott-Sale et al. (2020) [3] Ekenros et al. (2022) [4] Hackney (2016) [5] Martin et al. (2018) [12]

• **Depot medroxyprogesterone acetate (DMPA):** Long-acting and often suppresses estrogen markedly, leading to measurable BMD reduction with long-term use, particularly concerning for athletic females.

EVIDENCE OVERVIEW AND PRACTICAL RECOMMENDATIONS

The current body of research presents mixed findings, largely due to methodological heterogeneity across studies. Notable observations include:

- CHCs may contribute to performance stabilization by minimizing hormonal variability throughout the cycle [4];
- CHCs containing antiandrogenic progestins (e.g., drospirenone) have been linked to reduced strength gains and attenuated muscle hypertrophy, likely due to diminished androgen receptor activity in skeletal muscle [12];
- In contrast, CHCs with androgenic progestins (e.g., levonorgestrel) may better preserve anabolic signaling, but can also increase the likelihood of negative mood and metabolic effects;
- Long-term DMPA use is associated with significant decreases in BMD, warranting caution in endurance and strength-trained populations;
- Certain contraceptive formulations may impair insulin sensitivity, with potential consequences for exercise recovery and high-intensity performance capacity [3]. Due to high individual variability in response to hormonal contraceptives, it is recommended that athletes undergo regular monitoring of general well-being, training response patterns, and bone health status. Contraceptive choice should be personalized and aligned with the athlete’s sport-specific demands, hormonal tolerance, and overall health objectives.

REVIEW OF SCIENTIFIC EVIDENCE

The influence of the menstrual cycle and hormonal contraceptive use on athletic performance has been explored in cross-sectional, longitudinal, and limited randomized controlled trials (RCTs). Key performance indicators include VO<sub>2</sub>max, time to exhaustion, lactate threshold, muscular strength, and perceived exertion.

Multiple studies, including those by Romero-Moraleda et al. [11] and McNulty et al. [17], suggest that the late follicular phase - marked by elevated estradiol - supports improved aerobic capacity and neuromuscular efficiency. However, not all athletes experience these benefits consistently. In fact, Oosthuysen and Bosch [19] reported VO<sub>2</sub>max and time-to-exhaustion reductions of 6–8% in some individuals during this same phase. This discrepancy highlights the gap between objective performance metrics and subjective experience. Moreover, premenstrual symptoms may further compromise training adherence and recovery.

However, findings remain inconsistent due to methodological heterogeneity - small sample sizes, varied designs, participant variability, and frequent lack of biochemical phase confirmation. Consequently, most studies are categorized as Level 3–4 evidence (observational or non-randomized), with few robust RCTs or within-subject crossover designs. Underrepresentation of non-Western, adolescent, and perimenopausal cohorts limits broader applicability.

Hormonal contraceptives introduce additional complexity. While some CHCs help stabilize hormonal fluctuations and improve training regularity, others may interfere with anabolic signaling or thermoregulatory responses. In a meta-analysis involving over 40 trials, Elliott-Sale et al. [3] reported an average ~40% reduction in strength adaptation among CHC users compared to eumenorrheic controls, particularly in resistance-based



training protocols. Moreover, CHC-related increases in core temperature ( $\sim 0.3\text{--}0.5^\circ\text{C}$ ) may further reduce heat tolerance and recovery capacity.

Recent studies show improved methodological rigor [2][4], with growing use of hormonal assays (E2, P4, LH) and multidomain physiological metrics. Meta-analyses [3] support phase-specific trends, though interindividual variability continues to obscure definitive conclusions.

Collectively, these findings highlight the need for precision-based, hormone-informed research to inform individualized strategies in female athlete management.

## PRACTICAL IMPLICATIONS FOR FEMALE ATHLETES AND ACTIVE WOMEN

Optimizing performance and preserving health in female athletes requires awareness of cyclical hormonal variability. Menstrual cycle monitoring - via digital apps, basal temperature, HRV, or urinary LH testing - facilitates phase identification and informed training decisions. For clinical accuracy, hormonal assays may be used. Menstrual irregularities such as amenorrhea or luteal insufficiency can signal underlying issues like RED-S or overtraining and should prompt medical evaluation [1].

Training plans should be aligned with hormonal patterns:

- **Late follicular phase** (high estradiol): favorable for high-intensity, strength, and endurance sessions;
- **Luteal phase** (high progesterone): benefits from reduced load, increased recovery, and avoidance of heat stress.

Supportive strategies - such as tailored nutrition (e.g., magnesium, omega-3s), hydration, and PMS symptom management through scheduled deload phases - can improve resilience and consistency [17].

These principles are equally relevant for recreationally active women, especially those over 30 who may face heightened PMS symptoms, irregular cycles, or early perimenopausal changes [18]. Even moderate activity levels may be influenced by phase-specific variations in mood, fatigue, or thermoregulation. Adapting training - for instance, prioritizing restorative work during the late luteal phase and more demanding sessions mid-cycle - can enhance adherence, reduce injury risk, and support well-being.

### Key recommendations for coaches and practitioners:

- Normalize open dialogue about menstrual health;
- Use cycle tracking to inform periodized training;
- Refer persistent menstrual dysfunction to healthcare professionals.

Integrating hormonal awareness into training and recovery protocols promotes both peak performance and sustainable athletic development across all activity levels.

## CONCLUSIONS

### KEY OBSERVATIONS

This review underscores the fundamental role of sex hormones - particularly estradiol and progesterone - in shaping female exercise physiology across multiple systems. As detailed in Table 1 (Section 2.3), estradiol enhances aerobic performance, vascular perfusion, metabolic efficiency, and neuromuscular coordination, while progesterone - predominant during the luteal phase - elevates core temperature, promotes glucose dependence, and may impair both thermoregulation and cognitive performance under physiological stress.

Although cyclical trends in exercise performance are observable, the degree and direction of these changes vary considerably between individuals. Genetic predispositions, hormone receptor sensitivity, training background, and environmental conditions all contribute to this variability, which complicates the formulation of universally applicable training guidelines.

Despite growing interest in this field, current evidence remains limited by small sample sizes, inconsistent performance metrics, and inadequate verification of menstrual phases, underscoring the need for more methodologically robust research [2, 3].

### PERSONALIZED AND PHASE-AWARE APPROACHES

Standard training models, often designed with male physiology in mind, rarely account for hormonal cycles. But for women, timing matters. Aligning training with hormone fluctuations may not only improve recovery and performance - it might also reduce injuries. For instance, high-intensity or strength-based sessions may be optimally scheduled during periods of peak estradiol, while regenerative strategies are better suited to the progesterone-dominant luteal phase [17].

Moreover, systematic cycle monitoring enables early identification of dysfunctions such as RED-S, thereby facilitating proactive, individualized interventions. These approaches are consistent with the principles of precision medicine and support a paradigm shift toward athlete-centered coaching frameworks.

### LIMITATIONS OF THE REVIEW

As a narrative synthesis, this review provides a comprehensive yet interpretive overview of the existing

literature. However, it is subject to several limitations. The selection of studies was based on thematic relevance rather than formal bias assessment, and no meta-analytic techniques were applied. This may limit the generalizability of the findings. Furthermore, the exclusion of grey literature and non-English language publications may have resulted in the omission of relevant data.

## RESEARCH AGENDA

To advance the scientific foundation for hormone-informed training in female athletes, future research should prioritize the following:

- Conducting large-scale, controlled studies with biochemical confirmation of menstrual cycle phases;
  - Implementing stratification by contraceptive type, sport, and level of athletic performance;
  - Integrating psychosocial, nutritional, and chronobiological variables into study designs;
  - Investigating the long-term physiological consequences of hormonal manipulation;
  - Developing artificial intelligence-driven platforms for real-time, adaptive training personalization.
- Pursuing these research directions is essential for translating hormonal science into equitable, evidence-based practices in the context of women's sport and exercise performance.

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## DATA AVAILABILITY STATEMENT

*Data supporting the findings are available from the corresponding author upon request.*

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Orthofacial harmonisation of dental patients' faces: A review of research

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## ABSTRACT

**Aim:** To conduct an analytical review of modern scientific publications and research on the development of new directions of interdisciplinary approaches to the problems of orthofacial harmonization of the face.

**Materials and Methods:** Analytical-synthetic theoretical methods of analysis of scientific research and registers of professional associations were used. The results of analysis were synthesized and interpreted in the conclusions. Information sources and time frame. Searches were run in PubMed/MEDLINE, Scopus, Web of Science Core Collection, SciELO and LILACS; official registers of professional bodies were consulted for normative documents (Conselho Federal de Odontologia; Ordem dos Médicos Dentistas). Coverage: 1 January 2000 to 1 September 2025 (last search: 1 September 2025).

**Conclusions:** Orthofacial harmonisation is a promising direction that integrates minimally invasive cosmetic procedures into dentistry. Interest in orthofacial harmonization is driven by increasing patient demand. The advantages lie in the comprehensive restoration of the functionality and aesthetics of organs of the maxillofacial region, in the minimisation of the risks of medical interventions, and in socio-psychological comfort in the interaction between doctor and patient. The prospects for the development of orthofacial harmonisation include: conducting clinical trials, the validation of a scale for assessing the results of the application of orthofacial harmonization, improvement of ethical-legal foundations of physicians' activity, standardization of educational-professional training programs for specialists, and the unification of medical protocols on orthofacial harmonization.

**KEY WORDS:** interdisciplinary approach, rehabilitation, dentofacial esthetics, facial cosmetic procedures, dentistry

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## INTRODUCTION

At the present stage of the development of dentistry, traditional treatment is combined with innovative aesthetic techniques. Under the influence of social networks, dental patients have become more exacting with regard to treatment outcomes; the demand is growing for a holistic approach to the harmonisation of the smile and of the face as a whole. Today, an aesthetic smile is far from being only the beautiful shape of the teeth; it is also the harmonious proportions and functionality of the facial complex. Dentists are compelled to expand their professional functionality: aesthetic procedures are now becoming an integral part of dental treatment planning in the restoration of teeth or in the enhancement of clinical outcomes.

The complex of technologies aimed at restoring the balance between the hard and soft tissues of the organs of the maxillofacial region is outlined by the term orthofacial (orofacial) harmonisation. In contrast to classical orthodontic treatment, whose principal goal is the alignment of the dental arches, the concept

of orthofacial harmonisation is oriented towards the aesthetics of the entire face. Orthofacial harmonisation envisages the correction of the nasolabial folds, lips, chin, zygomatic areas and the contours of the middle and lower thirds of the face.

The synergy of dental and cosmetological interventions for achieving a balanced and attractive appearance of patients is developing dynamically in Brazil and the USA, where orthofacial harmonisation has been recognised as a distinct speciality for dentists. The legal precedent for the clinical practice of orthofacial harmonisation in dental treatment facilities is enshrined in the document "Reconhece a Harmonização Orofacial como especialidade odontológica Nº 198/201" [1]. Thus, the legal status has been consolidated for the use by dentists in Brazil of botulinum toxin injections, fillers and other cosmetic procedures.

The concept of orthofacial harmonisation is gaining in popularity, which in practical terms reflects the growing interest in rapid, non-surgical methods: for example, since 2000 a 144% increase in minimally

invasive aesthetic procedures has been recorded [2]. Among the principal factors of the heightened demand for orthofacial harmonisation we shall cite the peremptory ones: (1) the formation of new aesthetic canons of proportion in social networks and a reduction of the stigma regarding aesthetic interventions; (2) the mass imitation of the appearance of stars of show business, the modelling industry, politics and others; (3) public recognition of the aesthetic appearance as a characteristic of health and an individual's resources. Taken together, these factors indicate that the problem of orthofacial harmonisation goes beyond a fashion trend and is acquiring the features of a stable clinical subsystem with its own standards and boundaries of competence.

Ethical, legal and educational aspects remain critical for the sustainable development of the sphere of orthofacial harmonisation: sources on legal issues record gaps in training, competence boundaries and the readiness of dentists to manage complications, which requires the standardisation of training, the unification of informed consent and the specification of communication practices with patients and the public reporting of results.

The demand for services that combine aesthetics with functionality highlights scientific research into the interdisciplinary problem of orthofacial harmonisation. At the same time, team interaction among different specialists—dentists, maxillofacial and plastic surgeons, dermatologists—appears as a necessary condition for a safe trajectory in complex clinical cases and for improving the quality of care.

## AIM

To conduct an analytical review of modern scientific publications and research on the development of new directions of interdisciplinary approaches to the problems of orthofacial harmonization of the face.

## MATERIALS AND METHODS

**Design and reporting.** Narrative review with systematic-search elements, reported with reference to PRISMA and PRISMA-S positions (protocol not registered, as this is a non-interventional review).

**Information sources and time frame.** Searches were run in PubMed/MEDLINE, Scopus, Web of Science Core Collection, SciELO and LILACS; official registers of professional bodies were consulted for normative documents (Conselho Federal de Odontologia; Ordem dos Médicos Dentistas). Coverage: 1 January 2000 to 1 September 2025 (last search: 1 September

2025). Reference lists of included papers were hand-searched.

**Search strategy and keywords.** Controlled vocabulary and free-text terms were combined with Boolean operators and spelling variants. Core string for PubMed/MEDLINE: («orofacial harmonization» or «orofacial harmonization» or «orthofacial harmonization» or «facial harmonization» or «facial harmonisation») and (dentistry or dental or odontolog) and ((«botulinum toxin» or BoNT-A) or «hyaluronic acid» or «filler» or «digital smile design» or «PDO thread» or «biostimulator» or «collagen»). For Scopus/Web of Science the same terms were searched in Title/Abstract/Keywords; for SciELO/LILACS Portuguese/Spanish synonyms were added: «harmonização orofacial», «harmonización orofacial». Database filters (document type, year limits) were applied where supported.

**Eligibility criteria.** Population/Context: dental patients or dental/odontology settings, including interdisciplinary teams (dentists, orthodontists, prosthodontists, maxillofacial and plastic surgeons, dermatologists). Concept/Interventions: orofacial/orthofacial/facial harmonisation procedures relevant to dental practice (hyaluronic-acid fillers, botulinum toxin type A, collagen biostimulators, PDO threads, digital smile design and other digital planning tools), plus legal/ethical frameworks regulating their use by dentists. Study types: peer-reviewed clinical trials, observational studies, case series/case reports with explicit methods and outcomes; systematic, narrative or integrative reviews; doctoral theses from accredited repositories when providing unique data on ethics/legislation or outcomes; official regulations/standards of professional bodies. Language: English, Portuguese, Spanish, Ukrainian. Exclusion: non-medical blogs, promotional/training materials without methods or data; purely dermatological/cosmetology reports with no dental relevance; letters/comments without primary data. No restriction by direction of results was applied to avoid positivity bias.

## SELECTION PROCESS AND DATA ITEMS

Two reviewers independently screened titles/abstracts and full texts; disagreements were resolved by discussion. Extracted items: study design, population, intervention(s), outcomes (aesthetic/satisfaction scales, morphometric changes, safety/complications), digital-planning use, and—where applicable—ethical-legal scope and training requirements. Due to clinical and methodological heterogeneity, findings were synthesised narratively without meta-analysis.

**Study yield.** The final sample comprised 30 sources (see References).

## LIMITATIONS

Heterogeneity of designs and outcomes precluded quantitative pooling; inclusion of grey literature (theses, regulations) was necessary given the emerging status of the field; language limited to four languages; database indexing differences (especially in LILACS/SciELO) mean that some non-indexed works may have been missed.

## REVIEW AND DISCUSSION

The analysis of research and publications showed that orthofacial harmonisation is taking shape as a new line of scientific inquiry and as a distinct, interdisciplinary subsystem of modern dentistry, within which aesthetic minimally invasive interventions are integrated with orthodontic, prosthetic and surgical approaches to achieve holistic facial symmetry and proportion. The practice of orthofacial harmonisation has been implemented in the specialisation of dentists.

Orthofacial harmonisation is a modern interdisciplinary field of medicine that combines aesthetic and functional dental interventions to improve facial symmetry, contour and appearance. Its historical prerequisites were laid by the German artist A. Dürer in 1528 in his treatise «Vier Bücher von Menschlicher Proportion» («Four Books on the Proportions of the Human Body»), where modular ratios and geometric constructions were described for the body and the head with facial coordinate grids [3]. A. Dürer's canons of beauty became the "framework" for subsequent clinical rules: the equality of vertical thirds (forehead–nose–chin), the correspondences between the intercanthal distance and the palpebral fissure, and so on, which became the technical basis for further anatomic–anthropometric analysis of the face in the European tradition; in contemporary digital planning these canons are used as a starting model which is then individualised to the patient's specific morphological data [4]. Sociocultural determinants such as demographic ageing and the "pro-ageing" paradigm, the post-pandemic «Zoom effect», and the cults of social networks and AR filters are forming new standards of proportion and lowering the stigma with regard to aesthetic interventions. However, A. Dürer's canons are used in diagnostics and communication with patients [5].

To date, the integration of dentistry and cosmetology has been legally enshrined in a number of states. For example, in Brazil dentists, as early as 1966, under national legislation, were entitled to perform limited «extra-oral» aesthetic procedures, H.M. Alharkan (2024) [6]. In 2011, however, Resolution CFO-112/2011 restricted dentists' use of hyaluronic acid injections and botulinum toxin [7]. In 2016 Resolution CFO-176/2016 rescinded the

earlier acts and permitted the use of botulinum toxin and dermal fillers within dental procedures [8]. In 2019 the dental council recognised "Orofacial Harmonization" as a distinct specialisation in dentistry and outlined the professional requirements for a dentist with the new specialisation [1].

In Portugal it is legally established that rejuvenation of the perioral area belongs to the professional sphere of the dentist, and the concept of «facial harmonization» has been included in the inventory of skills of a dental practitioner [9]. The interdisciplinary approach to facial harmonisation initiated in Brazil and Portugal is rapidly gaining popularity in different countries of the world as the concept of orofacial harmonisation [10].

The publication by R. Tran Cao (2020) reports that in the USA the integration of dentistry with aesthetic medicine is denoted in the scientific literature by the term «facial esthetics/aesthetic dentistry»: injections of botulinum toxin and dermal fillers are regarded as a logical extension of aesthetic dental practice provided that the state's legal regulation is complied with and the procedures are performed by licensed clinicians. It is emphasised that legal access and the qualification requirements for the doctor vary between states and continue to evolve, Tran Cao (Dent Clin North Am, 2020) [11]. Researchers S. Davison et al. (2019) note that the expansion of cosmetic interventions in dentistry necessitates the development of a single federal standard for specialist training and the elaboration of safety protocols [12].

In the context of clinical verification of aesthetic reference points it is appropriate to cite the results of the study by A. Yakushenko et al. (2019), in which, on a sample of 40 female patients aged 20–25 years with Angle Class I occlusion, macro-, mini- and micro-analysis of the face was performed using a photometric method applying the «1/5» rule; it was found that this classical rule held true in only 15% of cases, the parallelism of the key horizontals (inter-pupillary, supra-ciliary, commissural) was recorded in 65%, overall symmetry in 70%, with exposure of the incisal edge of the upper incisors within 2–3 mm observed in 50%, a «harmonious smile» (when the upper arch follows the contour of the lower lip) in 35%, and a wide buccal corridor, indicative of narrowing of the dental arch in the premolar area, in 75%. The presented empirical results underscore the variability of aesthetic parameters and substantiate the need for individualised planning of orofacial harmonisation during the rehabilitation of dental patients [13].

The problematics of orofacial harmonisation of the face today are developing at the interface of distinct specialties: dentists, orthodontists, prosthodontists, maxillofacial surgeons, dermatologists and cosme-



tologists pool their knowledge to adjust the shape of the dental arches, the position of the jaws, the volume of the soft tissues and the features of the skin. Orofacial harmonisation includes various minimally invasive procedures: injections of botulinum toxin to reduce muscular activity, fillers based on hyaluronic acid, collagen biostimulators, polydioxanone threads, surgical liposuction of the chin, bichectomy (removal of Bichat's fat pads), non-surgical rhinoplasty, and others. Accordingly, the interdisciplinary approach encompasses prosthetics, orthognathic methods, the use of hyaluronic-acid (HA) fillers, botulinum toxin type A (BoNTA), digital technologies for smile planning, and the assessment of psychosocial outcomes.

Analysis of the source base of the research showed that orofacial harmonisation of the face is a relatively new area of scientific inquiry; therefore, full doctoral studies are still sporadic. Among them, the dissertation by L. Rodrigues (2021) is of scientific value; it focuses on legal and ethical aspects and demonstrates a low level of preparedness of dentists to eliminate complications [14].

In the doctoral study by B. Ferreira Silva (2022) the «medicalization» of beauty is explored through the prism of Michel Foucault; the author points to the risk of losing the individuality of human appearance due to counter-narratives on social media. The researcher demonstrates the influence of the commercialisation of aesthetics on medical ethics and identifies organisational measures needed for the legitimisation of the new specialisation. B. Ferreira Silva indicates that some training courses are more business-oriented than evidence-based, and therefore it is important to introduce regulatory mechanisms and educational standards. B. Ferreira Silva compares harmonisation with mass production, which gives rise to standardised faces, and therefore demands the development of ethical norms and a more considered attitude to procedures [15].

Defining the limits of professional competence is a key aspect of the dissertation by L. Rodrigues (2021), where it is shown that Brazilian dentists recognise their competence in administering botulinum toxin and performing bichectomy, but are sceptical about carrying out nasal remodelling and more complex plastic interventions; in the event of necrosis more than 72% of doctors would transfer the patient to a doctor of another speciality. This indicates the need for a clear delineation of responsibility between dentists, dermatologists and plastic surgeons [14].

The review by J. Pereira et al. (2024) summarises the outcomes of clinical cases using hyaluronic acid and botulinum toxin to correct Class III prognathism, scars after cheiloplasty, chin deformities and infraorbital

defects: in all cases improvements in aesthetics and in patient satisfaction were observed without serious complications. In patients with prognathism an increase in premaxillary volume and improved nasal projection were noted, and in patients with post-cheiloplasty scarring, satisfaction on a visual analogue scale amounted to 8 points out of 10 [16].

Patients with congenital or acquired deformities often experience psychological difficulties associated with body image, self-esteem and social interaction. Successful harmonisation can improve social integration, reduce the fear of smiling and contribute to overall well-being. However, doctors must recognise that aesthetic correction does not resolve all psychological problems; psychotherapeutic support is sometimes necessary to adapt to changes. Of interest are the social studies by D. Fangueiro (2023), which evaluated the outcomes of aesthetic rehabilitation of patients after orthodontic treatment. Injections of hyaluronic acid into the middle third of the face were administered to 18 patients with a unilateral complete cleft lip and palate. The results of assessment by non-professionals indicated improved attractiveness, whereas orthodontists did not note significant changes [17].

Objective measurement of the results of harmonisation requires validated instruments. The Brazilian adaptation of the FACE-Q Satisfaction with Facial Appearance Overall Scale (FACE-Q SFAOS) was tested on 25 specialists in harmonisation; its internal consistency was high ( $\alpha = 0.927$ ) and acceptability good. Specialists with more than two years' experience and those who apply lip fillers rated the scale higher than beginners. The use of such questionnaires makes it possible to align objective indices (shape, symmetry, proportions) with subjective satisfaction, which helps to adjust the protocol.

In more complex clinical cases dentists usually prefer manually created digital smile designs, whereas in symmetrical or less complex cases the share of choices in favour of AI-generated options increases, particularly among non-professionals; however, overall the preference still lies with manual designs [18].

Digital technologies have radically changed the approach to the analysis and modelling of the smile. The Digital Smile Design (DSD) system, developed by F. Reviansyah et al. (2025), has become a key tool in dentistry. Specialists in orofacial harmonisation actively use programmes (Adobe Photoshop, Smile Designer Pro, Planmeca Romexis Smile Design, VisagiSMile, etc.) that make it possible to combine 2D photographs with 3D digital impressions to plan the individual proportions of the face. The patient can see the result before the start of treatment, which allows the creation of smile

templates and the consideration of the anatomical parameters and aesthetic preferences of the specific person [19].

The study by G. Ceylan et al. (2023) compared designs created by professional dentists with designs generated by AI in the Smile Designer programme. Four clinical cases with symmetrical and asymmetrical features were analysed; 628 participants (dentists, students and others) evaluated the options using online questionnaires. The results showed that dentists preferred manually created designs in three of the four cases, whereas students and laypersons demonstrated a higher preference for AI-generated variants; women and orthodontists more often tended towards AI designs when assessing the third case, in which there were symmetrical features. The researchers concluded that AI may be useful for rapid generation of smile options, especially in symmetrical cases, but manual planning is more appropriate when proportions are complex [18].

A systematic review by F. Reviansyah et al. (2025) analysed 12 randomised and experimental studies to compare Digital Smile Design with traditional planning methods. The authors concluded that DSD improves aesthetics, saves time, ensures environmental friendliness by dispensing with plaster models, silicone impressions and hydrocolloid materials, and increases convenience for patients [19].

Methodological studies also reveal the standards of aesthetic proportions that are embedded in digital programmes. The ideal width of the central incisor is approximately 75–80% of its length; the occlusal plane should be parallel to the smile line of the lower lip; the dental midline should coincide with the facial midline and be perpendicular to the inter-pupillary line. The recommended ratio of contact points between the incisors–50:40:30–moves apically from the centre towards the canine, forming a natural gradation of the interdental space. These parameters, embedded in DSD software, help dentists to create a smile that harmoniously fits the face and minimises the need for invasive preparation [19].

The COVID-19 pandemic accelerated the development of telemedicine in dentistry. Platforms that allow patients to upload photographs and videos have become widely used for preliminary analysis. For example, the service *odontoangela.com* makes it possible to assess the upper, middle and lower thirds of the face using photographs sent by the patient, and to gather information about their expectations and psychological state [20]. Specialists receive materials prior to an in-person consultation, which helps quickly to determine the need for orthodontic treatment, fillers or surgery. The study by A. Herrera (2023) emphasises that digital consultations significantly reduce time in

the clinic, allow the maintenance of contact with the patient and improve adherence to recommendations [20]. Telemedicine is particularly useful for patients with somatic deformities who live in remote areas or have limited mobility.

An integrative review by J. Borges (2022) showed that the most common preparations for facial correction remain hyaluronic-acid-based fillers (HA) and botulinum toxin type A (BoNT-A). Hyaluronic acid, a natural component of the extracellular matrix, attracts water, stimulates collagen synthesis and provides volume, which makes it suitable for the correction of the lips, nasolabial folds, nose, chin and the upper part of the face [21].

Botulinum toxin type A acts by blocking the release of acetylcholine at the neuromuscular synapse, which leads to relaxation of hyperactive muscles. In orofacial practice it is used to correct wrinkles and asymmetries, to treat bruxism and myofascial pain. A narrative review by T. Gutierrez et al. (2023) emphasises that BoNT-A effectively reduces the tone of the masticatory muscles and decreases the load on the teeth in patients with bruxism; the agent also helps in temporomandibular dysfunction and migraine. However, the success of the procedure depends on precise dosing and on the professionalism of the doctor [22].

The necessity of careful dosing, appropriate intervals and radiological monitoring when incorporating BoNT-A into OFH protocols is emphasised in the *Journal of Oral Rehabilitation* [23]. In addition, the results of studies of partial loss of vision after injections of hyaluronic-acid products demonstrate the need to develop clear requirements for safety protocols, training and informed consent in OFH [24]. A publication in the journal *Drugs* recommends considering BoNT-A as a “last-line” treatment, which is important for prudent use of aesthetic injections in dental practice [25].

Alongside traditional injections, contemporary orofacial harmonisation uses biostimulators, threads and other materials aimed at stimulating intrinsic regenerative processes. One such method is the use of polydioxanone (PDO) threads. A narrative review by V. Marinho et al. (2023) sets out the specifics of the procedure for creating a facial scaffold [26]. Another promising direction is collagen biostimulators. An integrative review by M. Nascimento et al. (2025) summarised the results of 11 studies and demonstrated that biomaterials–polylactic acid (PLLA), calcium hydroxyapatite (CaHA) and polycaprolactone (PCL)–promote polarisation of macrophages towards the M2 type and stimulate neocollagenesis [27].

Thus, current trends in orofacial harmonisation are oriented towards combining various minimally invasive methods.

For example, the combination of hyaluronic acid with bio-stimulators provides simultaneous restoration of volume and stimulation of endogenous collagen; the addition of PDO threads enhances the lifting effect. Such strategies make it possible to individualise treatment depending on anatomical features, age and patient expectations. At the same time, doctors must understand the potential complications of minimally invasive interventions. A systematic review by N. Manganaro and colleagues (2022) presents the results of an analysis of 33 studies in which complications after procedures of orofacial harmonisation were reported. Most frequently, complications arose as a result of injections of botulinum toxin (19 studies), hyaluronic acid (21 studies) and other fillers; procedures of microneedling, intense pulsed light (IPL) and ultrasound were also mentioned [28]. Complications most often occurred in the eye and peri-orbital region (dry eyes, diplopia, eyelid ptosis), as well as in the forehead and nose (oedema, pain, erythema, necrosis) [28]. The study by M. Maci et al. (2024), devoted to the clinical applications of HA and BoNT-A in dentistry, also draws attention to safety. The authors note that in most included studies adverse effects were mild or moderate, but to improve the evidentiary base the authors call for randomised studies with prolonged follow-up in which both clinical outcomes and patients' quality of life are evaluated [29].

Despite the fact that most complications indicated in the studies by N. Manganaro et al. (2022) and M. Maci et al. (2024) proved to be temporary and without severe consequences, according to J. Souza da Silva (2024) patients with neurofibromatosis may experience specific complications: impaired bone growth, impaired healing, an increased risk of bleeding and fibromas. Therefore, the interaction involved in multidisciplinary work by doctors for the orofacial harmonisation of patients becomes particularly relevant [30].

The foregoing provides grounds for distinguishing directions for further scientific explorations in the field

of orofacial harmonisation in which further research is required, in particular:

- 1) the development of digital protocols for orofacial harmonisation that integrate orthodontic, prosthodontic and surgical treatment;
- 2) validation of a scale for assessing quality of life, psychological impact and social integration of the dental patient after orofacial harmonisation;
- 3) development of mechanisms for minimising risks in the performance of orofacial harmonisation procedures;
- 4) research into the specifics of managing medical institutions that provide services in orofacial harmonisation, etc.;
- 5) clinical trials that would compare the results of using different types of cosmetic agents (fillers, bio-stimulators, botulinum toxins, etc.) and methods of their application.

## CONCLUSIONS

Orthofacial harmonisation is a promising direction that integrates minimally invasive cosmetic procedures into dentistry. Interest in orthofacial harmonization is driven by increasing patient demand. The advantages lie in the comprehensive restoration of the functionality and aesthetics of the organs of the maxillofacial region, in the minimisation of the risks of medical interventions, and in socio-psychological comfort in the interaction between doctor and patient. The prospects for the development of orthofacial harmonisation include: conducting clinical trials, the validation of a scale for assessing the results of the application of orthofacial harmonization, improvement of the ethical-legal foundations of physicians' activity, standardisation of educational-professional training programmes for specialists, and the unification of medical protocols on orthofacial harmonisation.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Compliance and due diligence in healthcare: international experience and implementation challenges

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## ABSTRACT

**Aim:** To analyse the international experience of applying compliance and due diligence procedures in healthcare and to identify the main challenges to their implementation.

**Materials and Methods:** The study is based on the systematisation and comparative analysis of regulatory and empirical data on the implementation of compliance and due diligence procedures in healthcare. The empirical base includes data from the U.S. Sentencing Commission and the U.S. Department of Justice Fraud Section (2019–2025), illustrating the influence of compliance and ethics programmes on organisational accountability, supplemented by legislative frameworks and case law from the United States, the European Union, and Ukraine. The assessment covered eight indicators. Dialectical, hermeneutic, comparative, analytical, synthetic, and systems analysis methods were used.

**Conclusions:** The main challenges to implementing compliance and due diligence procedures include the absence of a legislative mandate for healthcare institutions and a low level of corporate culture and ethical awareness.

**KEY WORDS:** compliance, risk, criminal offence, liability, healthcare

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## INTRODUCTION

At a time when population health indicators tend to deteriorate, healthcare plays an extremely important role in society. In international practice, compliance and due diligence procedures are attracting increasing attention, as they have proven to be effective elements of healthcare management [1, 2]. They are capable not only of improving the reputation of healthcare institutions and pharmaceutical companies but also of reducing the severity of penalties in cases of criminal offences, as is the practice in some countries (particularly the United States) [3].

## AIM

The purpose of the article is to analyze the international experience of applying compliance and due diligence procedures in healthcare and to identify the main challenges and prospects for their implementation.

## MATERIALS AND METHODS

This study is based on the author's original systematization and comparative analysis of data on the implementation of compliance and due diligence procedures in healthcare. This study is based on the systematization and comparative analysis of regulatory and empirical data on the implementation of compliance and due diligence procedures in healthcare. The empirical base includes data from the U.S. Sentencing Commission and the U.S. Department of Justice Fraud Section (2019 – 2025), illustrating the influence of compliance and ethics programs on organizational accountability, supplemented by legislative frameworks and case law from the United States, the European Union, and Ukraine. The assessment covered eight indicators – legal framework, internal policies, anti-corruption, data protection, ethics, monitoring, whistleblower protection, and risk management – allowing a comparative evaluation of the effectiveness of healthcare compliance systems



across the three jurisdictions. Dialectical, hermeneutic, comparative, analytical, synthetic, and systems analysis research methods were used.

## ETHICS

The authors declare that in preparing this article all ethical principles and rules of academic integrity were observed. All sources used in this literature review are publicly available. A citation agreement was obtained from the copyright holder.

## FRAMEWORK

The study was conducted as a fragment of the complex scientific project of the Educational and Scientific Institute of Law Taras Shevchenko National University of Kyiv «Legal support for the sustainable development of the economy of Ukraine in the context of European integration» (state registration number 0124U003297; term: 2024-2029).

## REVIEW AND DISCUSSION

The implementation of compliance and due diligence procedures in healthcare develops unevenly and progressively across different countries. This study covers the following groups of entities: 1) state and local governments, 2) healthcare institutions of various forms of ownership, 3) pharmaceutical companies, 4) distributors of medicinal products, and 5) other entities involved in the healthcare sector (such as insurance companies providing health insurance services, research institutions and organizations, professional associations of doctors and pharmacists, suppliers of medical equipment, consumables, and IT solutions in the field of eHealth, as well as international organizations and donor institutions implementing programs in healthcare, etc.). Hereinafter, the generalized term “compliance entities and due diligence procedures” is used to refer to these entities.

One of the most successful models of compliance and due diligence procedures operates in the United States, a country with one of the highest levels of corporate culture and business ethics. Compliance entities are de facto obliged to implement compliance and due diligence procedures in their activities. This requirement arose following the adoption in 1991 of the Federal Sentencing Guidelines for Organizations (FSGO) [3], which define the principles and approaches to imposing penalties on organizations. An important component of these principles is the introduction of effective compliance and ethics programs [4]. Such programs include at least eight elements: 1) standards and procedures: the organization shall establish standards and procedures to prevent and detect criminal conduct; 2)

management responsibility; 3) promotion of a corporate culture that encourages ethical conduct and a commitment to compliance with the law; 4) prudence in the delegation of authority: due care must be exercised when granting discretionary authority to avoid delegating it to individuals with a history of illegal or unethical conduct; 5) training, information, and control; 6) disciplinary liability; 7) response and improvement of the program: after detecting a violation, the organization must take all reasonable measures to eliminate its consequences and prevent similar cases in the future by improving the compliance program.

Based on the original systematization and comparative analysis of data on the implementation of compliance and due diligence procedures in healthcare, the impact of compliance and ethics programs on organizational accountability is shown to be significant, as evidenced by data from the U.S. Sentencing Commission (Fig. 1).

The data demonstrate that an overwhelming majority of organizational offenders (89,6%) lacked a formal compliance and ethics program at the time of misconduct, indicating a strong correlation between the absence of structured compliance measures and organizational wrongdoing. Only a negligible share (0,2%) received a culpability score reduction for maintaining an effective compliance program, while 58,3% experienced increased culpability due to demonstrated tolerance of criminal activity. The findings further show that proactive disclosure and cooperation remain rare (1,5%), and court-mandated compliance reform is still a frequent corrective intervention (19,5%). These results suggest that the implementation of meaningful compliance systems is both underutilized and crucial for mitigating organizational liability.

The presented assessment reflects the authors' expert judgment grounded in an analysis of regulatory provisions, compliance standards, and practical implementation mechanisms within each healthcare system. As shown in Fig. 2, the United States demonstrates the highest overall level of implementation of compliance and due diligence elements in healthcare. All assessed components are evaluated at the maximum or near-maximum level (4–5 points). This indicates that compliance is regulated by regulations and supported by an appropriate level of corporate culture with well-developed monitoring and control systems combined with ethics training and whistleblower protection measures.

In contrast, the European Union presents a more mixed picture, with high scores in data protection (5 points) due to the implementation of the General Data Protection Regulation (GDPR) and significant progress in whistleblower protection (4 points) under Directive (EU) 2019/1937. However, other elements, such as internal policies, monitoring systems, and corporate culture, remain at a moderately high but not yet fully institutionalised level (4 points). This suggests that, while the EU maintains a robust regulatory



**Fig. 1.** The impact of compliance and ethics programs on organizational accountability (systematized based on United States Sentencing Commission data)  
*Source: compiled by the authors based on [3]*

framework, compliance practices vary across Member States, reflecting differences in national governance models and healthcare management.

Ukraine, by comparison, demonstrates an initial and uneven stage of compliance and due diligence implementation. Most indicators remain at the 2–3 point level, indicating the predominance of declarative norms over practical application. The legal framework and anti-corruption measures show moderate progress (3 points), mainly due to alignment with international obligations. However, whistleblower protection (1 point) and corporate ethics training (2 points) in healthcare remain underdeveloped (Fig. 2).

A broader application of other compliance and due diligence procedures is observed in:

a) Private medical institutions focused on international investors or patients; they are interested in implementing quality risk management. The process is composed of the assessment, control, communication, and review of quality risks. Such entities are usually interested in obtaining international accreditations. For example, one of the most prestigious accreditations is the Joint Commission International (JCI), which requires the creation of internal policies on compliance and risk management, continuous monitoring of incidents, protection of patients' rights, and strict control of clinical procedures [5]. The presence of JCI accreditation significantly increases the reputation of the institution and simplifies entry into the foreign market;

b) pharmaceutical companies. Foreign pharmaceutical companies operating in Ukraine comply with the requirements of the Foreign Corrupt Practices Act, USA, or the UK Bribery Act, which set standards for anti-corruption activities.

The due diligence procedure is also widespread in healthcare. The application of such a procedure is aimed at avoiding or minimizing potential risks when carrying out investment or other economic activities in order to verify compliance with legal norms or regulatory requirements [6].

It is not enough to formally prescribe such programs; it is necessary to ensure their implementation. If entities subject to compliance and due diligence procedures do not implement such elements in their daily work (for example, if they do not conduct training for employees, do not establish and/or provide an internal control or monitoring system), this creates risks for both the entity and investors. For example, in the case of criminal proceedings against such an entity, failure to comply with the requirements may be considered an aggravating circumstance when imposing a penalty. Such behavior is considered as liability based on reckless negligence, since the legal entity intentionally did not implement compliance programs or did not conduct due diligence procedures. This practice has already proven its effectiveness in the USA [7].

Regarding individual areas of implementation of such mechanisms in Ukraine, the following should be noted.

Firstly, compliance practices and due diligence procedures are important in the processes of digital transfor-

| Compliance / Due Diligence Element  | USA (1–5) | European Union (1–5)       | Ukraine (1–5) |
|-------------------------------------|-----------|----------------------------|---------------|
| Legal framework                     | 5         | 5                          | 3             |
| Internal policies & procedures      | 5         | 4                          | 2             |
| Anti-corruption mechanisms          | 5         | 4                          | 3             |
| Data protection                     | 4         | 5 (GDPR)                   | 3             |
| Corporate culture / ethics training | 5         | 4                          | 2             |
| Monitoring and control systems      | 4         | 4                          | 2             |
| Whistleblower protection            | 4         | 4 (EU Directive 2019/1937) | 1             |
| Risk management / audits            | 5         | 4                          | 3             |

**Fig. 2.** Level of Implementation of Compliance and Due Diligence Elements in Healthcare of USA, EU and Ukraine

*Picture taken by the authors*

mation of the healthcare system [8, 9]. For healthcare institutions that cooperate with European partners or process data of EU citizens, it is necessary to comply with the provisions of the General Data Protection Regulation (GDPR), which guarantees the patient's right to access, correct, and delete their personal data, implement the principle of data minimization, and the obligation to notify about data leaks within 72 hours [10].

Secondly, compliance practices are implemented in the implementation of the state's anti-corruption policy in the healthcare. To some extent, the implementation of compliance and due diligence programs is influenced by international donors and partners that finance projects aimed at developing internal policies for controlling and preventing abuse, providing grant support or attracting foreign investment.

Thirdly, an indicator of the effectiveness of compliance and due diligence procedures is greater transparency and oversight of procurement, personnel appointments and reporting (such as unified electronic portals for job vacancies and procurement), which will reduce opportunities for abuse.

The term "compliance" is defined in the Cambridge Dictionary as "behavior that obeys an order, rule, or request" [11]. In healthcare, this concept encompasses a wide range of measures aimed at ensuring adherence to legal norms, professional standards, and ethical principles. At the same time, compliance is closely related to risk management, since failure to comply with rules or standards in healthcare can endanger patients' lives and health.

According to scholars, this concept is based on the following criteria: 1) the introduction of new standards and programs that are objectively capable of reducing the likelihood of illegal behavior in the future; 2) systematic monitoring of the level of compliance with legal requirements by corporate management; 3) unrestricted exchange of information between all structural units; and

4) adequate responses to violations that have already been committed, as well as their prevention in the future [12].

Compliance helps organizations develop a systematic approach to adherence to norms and standards, thereby reducing the risk of violations and contributing to business reputation and market stability [13].

The concept of due diligence in the dictionary sense means "action that is considered reasonable for people to be expected to take in order to keep themselves or others and their property safe" [14]. In Ukraine, due diligence is mostly equated with legal audit, however, it is much narrower in scope and essentially means legal examination of activities regarding compliance with legislation [15]. In the USA, compliance programs in healthcare have received strong development after the adoption of FSGO [16], which defined the principles of corporate responsibility and encouraged companies to implement control systems to reduce the risks of criminal liability. An additional incentive was provided by numerous federal laws, in particular the Health Insurance Portability and Accountability Act, which protects medical data; the Anti-Kickback Statute, which prohibits improper remuneration for patient referrals; Stark Law, which provides for restrictions on financial relationships between doctors and medical institutions [17].

Legal regulation of compliance and due diligence in healthcare in Ukraine is almost non-existent. There are no laws or subordinate regulations that would oblige healthcare institutions or pharmaceutical companies to implement such mechanisms. While, for example, the USA demonstrates a comprehensive approach to protecting healthcare: strict and clear criminal legislation is in force, regular raids and investigations are carried out, and a preventive compliance system is also in place (according to the standards recommended by the Office of Inspector General of the U.S. Department of Health and Human Services).

For example, in 2009, Pfizer Inc., one of the world's largest drug manufacturers, was charged with violating

two US federal laws: the Food, Drug, and Cosmetic Act (FDCA), regarding the illegal marketing of drugs, and the False Claims Act, regarding the submission of false data and claims for referrals to federal health care programs, in particular Medicare and Medicaid [18]. The charges were based on the fact that Pfizer and its subsidiaries marketed drugs by promoting them for off-label use, that is, for the treatment of diseases and conditions that were not officially approved by the FDA in the drug product instructions. The company employed aggressive marketing strategies that led to a significant increase in drug prescriptions, resulting in substantial financial losses for government healthcare programs such as Medicare and Medicaid, while also posing risks to patient health. In response to the accusations, Pfizer began a large-scale reform of its compliance system: to prove that the company's actions were not intentional, the company proved that it had problems with controlling individual divisions; demonstrated due diligence and a desire to eliminate the consequences of violations, and thereby reduced the size of the criminal penalty (by concluding a deferred prosecution agreement).

In most EU countries the healthcare system is focused on implementing compliance and due diligence in line with EU regulatory requirements, quality management systems ISO 9001 (quality management systems), ISO 31000 (risk management), JCI accreditation and the EFQM (European Foundation for Quality Management) excellence model. Standards that set guidelines for implementing compliance and due diligence programs include GDPR, EU Directive 2011/24/EU on the application of patients' rights in cross-border healthcare, European Medicines Agency standards and recommendations, etc. In addition, there are additional requirements in national systems.

## CONCLUSIONS

1. The analysis of international and national practices in implementing compliance and due diligence procedures in healthcare demonstrates that they constitute an integral component of risk management. Due diligence is primarily a one-time comprehensive assessment, usually conducted before investment activities or the acquisition of a healthcare institution or pharmaceutical company, aimed at identifying legal, financial, and reputational risks that may affect a company's future operations and investment attractiveness. Compliance, by contrast, is a continuous process carried out through a set of actions designed to verify adherence to legislative, ethical, and corporate standards in the daily activities of entities subject to compliance and due diligence procedures.
2. In Ukraine, compliance and due diligence procedures in healthcare are currently at the stage of forming an understanding of their necessity and feasibility of implementation. In this context, the study of best global practices and recommendations of the WHO, EFPIA, and other international bodies appears promising. Such programmes should cover licensing and accreditation procedures for healthcare institutions, ethical standards of medical care and patient interaction, personal data protection, and the implementation of anti-corruption policies, including the prevention of conflicts of interest and the definition of "legitimate interactions" with pharmaceutical companies and insurance organisations. Equally important is the creation of training programmes to enhance the qualifications of healthcare institution managers and medical professionals in risk management. These measures will contribute to building a transparent and ethical healthcare system that meets European and global standards.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Criminalisation of fraud and misconduct in clinical trials on medicinal products

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## ABSTRACT

**Aim:** To develop a scientifically grounded approach to defining the basis and limits of criminalizing fraud and misconduct in clinical trials of medicinal products, while also raising awareness and encouraging serious discussion of these issues.

**Materials and Methods:** The study relies on the *Acquis Communautaire* and the legislation of Ukraine, Germany, and Poland. It also uses judicial practices from the United States, France, and Ukraine, as well as legal statistics and draft legislation from Ukraine. Scientific research methods such as induction, deduction, comparison, analysis, case studies, and systems analysis were employed.

**Conclusions:** Criminal penalties for unlawful actions during clinical trials of medicinal products, guided by the *ultima ratio* principle, should be applied only to violations that threaten the lives or health of research subjects, infringe upon their rights, or involve falsification of clinical trial results regarding the efficacy and safety of the products, rather than to any violations alone.

**KEY WORDS:** clinical trials of medicinal products, criminal punishment, fraud, crimes against life and health, crimes against public health

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## INTRODUCTION

Clinical trials of medicinal products are a necessary stage of their development. In accordance with paragraph 1 of part 2 of Article 2 Regulation (EU) No 536/2014 of the European parliament and of the council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC [1] 'Clinical study' means any investigation in relation to humans intended:

- (a) to discover or verify the clinical, pharmacological or other pharmacodynamic effects of one or more medicinal products;
- (b) to identify any adverse reactions to one or more medicinal products; or
- (c) to study the absorption, distribution, metabolism and excretion of one or more medicinal products; with the objective of ascertaining the safety and/or efficacy of those medicinal products.

Since, according to paragraph 2 of Part 2 of Article 2 of this Regulation, clinical trials are a type of clinical study, they are conducted to ascertain the safety and/or efficacy of those medicinal products. Clearly, clinical

trials always pose a risk to the research subject—an individual participating in a clinical trial, either as a recipient of an investigational medicinal product or as a control. This risk stems from the fact that the medicinal product being studied may be either unsafe or insufficiently effective, and its use may lead to a significant deterioration in the subject's health or even death.

The potential risk to the life and health of clinical trial subjects requires adherence to clinical trial regulations designed to minimize possible negative outcomes for the participants and to protect their rights and legitimate interests. Violating these regulations can lead to legal liability for those responsible. The most severe form of liability is criminal, which imposes strict restrictions on the rights and freedoms of individuals convicted of a criminal offense. However, debates continue over which specific violations of clinical trial rules should be subject to criminal punishment and what penalties should be applied to those found guilty. It is essential to balance legislation so that it, on one hand, safeguards the right to life and health of research participants, and on the other, does not create unreason-



able barriers to conducting research. Presently, laws in various countries approach this issue differently – some impose criminal penalties for any violations during clinical trials, while others punish only violations that result in death or serious harm to a participant. The ongoing scientific effort to find the best model for defining the grounds and limits of criminalization in this area keeps this topic highly relevant.

## AIM

This article aims to develop a scientifically grounded approach to defining the basis and limits of criminalizing fraud and misconduct in clinical trials of medicinal products, while also raising awareness and encouraging serious discussion of these issues.

## MATERIALS AND METHODS

The study was conducted in 2025 and based on the *Acquis Communautaire*, the legal acts of Ukraine, Germany, and Poland. It used legal practices, legal statistics, drafts of Ukrainian legal acts, and media publications from the USA, France, and Ukraine. In total, 8 laws, 2 drafts of laws, other documents, and 12 court decisions were analyzed.

Given the subject and purpose of the study, it was limited to examining the criminalization of violations during clinical trials on human subjects.

Research methods included induction, deduction, comparative, analytical, case study, and system analysis.

In the first stage of the study, induction and case study methods were used to analyze individual cases of criminal liability for violations of clinical trial procedures for medicinal products in the United States, France, and Ukraine. Following this, an analytical approach was employed to examine international documents, EU regulations (*Aqui Communitare*), Ukrainian legislation, and draft Ukrainian laws concerning liability for violations during clinical trials, as well as judicial statistics related to these criminal offenses. This facilitated deduction, comparative analysis, and systems analysis to highlight the advantages and disadvantages of different approaches to criminalizing violations of clinical trial procedures, and to develop scientifically grounded proposals for establishing the criteria and scope of such criminalization.

## REVIEW AND DISCUSSION

Fraud and misconduct in clinical trials involving medicinal products pose a potential threat to the lives and health of both study subjects and patients who will be

treated with these medicinal products. An analysis of cases involving criminal penalties for violating clinical trial procedures demonstrates that criminal law is applied in cases of: (a) harm to the life or health of subjects in a clinical trial of medicinal products during its conduct; (b) harm to the life or health of patients during the use of a medicinal product whose registration was preceded by distorted clinical trial data regarding its safety and/or efficacy.

So, in December 2023, a French court ruled that Drugmaker Servier must pay more than 430 million euros (\$471 million) in connection with weight-loss pill Mediator, saying that the company was guilty of fraud and other charges because it knew the drug was potentially harmful when selling it. At least 500 people died of heart valve problems in one of France's worst health scandals because of exposure to the active ingredient in Mediator, which was widely prescribed as an appetite suppressant. The court found the company concealed the risk of harm and heart problems from patients and doctors and ruled the initial 1974 approval and its subsequent renewals until 2007 had been obtained illegally. [2] "It has been proven that in order to obtain marketing authorisation for Mediator<sup>o</sup> in 1974, Servier took the risk of developing and promoting a new amphetamine drug, concealing its appetite-suppressing effect and misleading people about its metabolism to norfenfluramine, the cause of the drug's adverse effects on heart valves in particular. Over the decades that followed, Servier not only denied that the drug had serious adverse effects, but sought to expand its indications, even though it would have been withdrawn from the market had the pharmaceutical company not hidden what it knew from patients, health professionals and the regulatory authorities (2-4). The appeal court found that Servier's actions illustrated a peculiar concept of the harm-benefit balance: "financial benefit for the company, deadly harm to patients" (our translation) (2,4). It went further than the original court in its judgement, finding Servier guilty on all counts (2-5). Jean-Philippe Seta was given a 4-year suspended prison sentence (with 1 year of house arrest under electronic monitoring), and Servier was ordered to pay a fine of over €9 million. It also ordered the pharmaceutical company to pay €420 million in reimbursement to health insurance providers (2,3)." [3] Therefore, Drugmaker Servier's concealment of information gathered, including during clinical trials, about the side effects of Mediator<sup>o</sup>, and the failure to share this information during the drug's registration and re-registration processes, resulted in the deaths of at least 500 patients.

US judicial practice indicates that violations during clinical trials are regarded as serious crimes. Thus, on

May 11, 2021, a federal grand jury in Miami, Florida, returned an indictment charging Jessica Palacio with conspiring to falsify clinical trial data regarding an asthma medication. According to court documents Palacio worked as a study coordinator at a clinical trial firm in Miami called Unlimited Medical Research. Unlimited Medical Research was one of many companies hired to conduct a clinical trial designed to investigate the safety and efficacy of an asthma medication in children. The indictment alleges that Palacio participated in a scheme to falsify medical records to make it appear as though pediatric subjects made scheduled visits to Unlimited Medical Research, received physical exams from a clinical investigator, and took study drugs as required, when in fact these things had not occurred. The indictment also alleges that when Palacio was confronted by a Food and Drug Administration (FDA) regulatory investigator about her conduct, she made a false statement to that investigator.[4] В дальнейшем суд присяжных признал Palacio виновной лишь в for making a false statement to a government investigator related to a clinical trial that studied the effectiveness of asthma drugs in children. A federal judge sentenced Palacio to 36 months in prison and three years of supervised release [5].

Four co-conspirators previously pleaded guilty and were sentenced for their roles in the scheme at UMR. Yvelice Villaman Bencosme, M.D., 66, of Miami, was sentenced to 36 months' imprisonment, and Lisett Raventos, 48, also of Miami, was sentenced to 30 months' imprisonment. In addition, Maytee Lledo, 52, of Hialeah, Florida, was sentenced to 14 months' imprisonment, which the court later modified to time served, and Olga Torres, 50, of Miami, was sentenced to 3 years' probation [5].

On February 21, 2025 a federal jury convicted Naheed Mangi, 66, a former employee of Stanford University, of accessing a clinical research database for a multisite breast cancer study after her authorization was revoked and altering patient records in the database. As said Acting United States Attorney Patrick D. Robbins, "Naheed Mangi intentionally tampered with a breast cancer research database by entering false information and personal insults. Her senseless actions undermined a study into the safety and efficacy of a new treatment for breast cancer patients". She faces a maximum penalty of 10 years in prison as to each conviction for Intentional Damage to a Protected Computer and one year in prison as to the conviction for Accessing a Protected Computer Without Authorization.[6] However, as of now, there is no official information about whether Naheed Mangi has been sentenced or what type of punishment he has received.

A comparison of the cited decisions on criminal penalties for violations of clinical trial procedures shows that the US response to such violations is more severe. Penalties imposed for violations that merely posed a potential risk to patients are more severe than those imposed by the French court for violations that resulted in the deaths of at least 500 patients.

An analysis of Ukrainian judicial practice shows that over the past 25 years, no convictions have been issued for violating clinical trial procedures. Between 2018 and 2025, pre-trial investigations and court hearings occurred in cases involving violations of drug registration procedures by officials of the State Enterprise "State Expert Center of the Ministry of Health of Ukraine." According to the prosecution, these violations involved registering generic drugs without completing pre-clinical studies and clinical trials. However, the prosecution was unable to prove the guilt of the officials from this state-owned enterprise, resulting in acquittals. For example, on November 20, 2020, the Holosiivskyi District Court of Kyiv found an expert from the Department for Registration of Medicines not guilty of the charge under Part 1 of Article 321-2 of the Criminal Code of Ukraine, which pertains to "Violation of the established procedure for pre-clinical studies, clinical trials, and state registration of medicines" [7]; similarly, on July 7, 2025, the same court found the acting director of the Department for Expertise of Registration Materials not guilty of the same charge [8]. Notably, the court decisions did not dispute that violations occurred during the medicine registration process, specifically related to pre-clinical studies and the failure to conduct clinical trials.

Therefore, the use of case studies has shown that fraud and misconduct in clinical trials involving medicinal products pose a serious threat to human life and health. However, detecting and investigating such violations is highly complicated and requires experts with extensive professional knowledge in this area. In many cases, even after confirming violations, it is difficult to establish the guilt of specific individuals in committing the crimes.

An analysis of the European Union's regulatory framework (*Acquis communautaire*) reveals that harm caused during clinical trials may result in civil and criminal liability. However, determining the precise nature of liability, its basis, and its limits is a matter for national legislation. Thus, according to Article 95 of the Regulation (EU) No. 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC, "This Regulation is without prejudice to national and Union law on the civil and criminal liability of a sponsor or an investigator." [1]

An analysis of national legislation on fraud and misconduct in clinical trials involving medicinal products reveals two methods for establishing grounds for criminal liability. The first method asserts that only specific, clearly defined violations during clinical trials of medicinal products are punishable by law (Germany, Poland). The second method states that any violation of clinical trial procedures is formally punishable by criminal law (Ukraine).

Thus, in Germany, the Law on the Trade in Medicinal Products (Medical Products Act - AMG) establishes criminal liability for violations during clinical trials in Section 17 of this Law. Intentional violations of this Law, as listed in this section, including those committed during the clinical trials of medical products, are punishable by up to three years of imprisonment or a fine; in severe cases, imprisonment for one to three years may be imposed. If the offender acts negligently in the cases referred to in paragraph 1, the penalty shall be imprisonment for a maximum of one year or a fine. [9]

Criminal liability for violations during clinical trials on humans in Poland is governed by the Law on Clinical Trials on Medicinal Products for Human Use [10]. Section 11 of this Law, "Criminal Provisions," provides an exhaustive list of violations of clinical trial procedures, the commission of which shall be subject to a fine, restriction of liberty, or imprisonment for up to two years (Article 77). Harm to the life or health of clinical trial subjects or, later, patients, is not covered by the provisions of this Law and is punishable under the general provisions of the Polish Criminal Code regarding crimes against human life and health (Section XIX).

Unlike Germany and Poland, Ukraine provides criminal liability for any violation of the procedure for conducting clinical trials of medical products. Since Ukrainian criminal Law is fully codified, provisions on criminal liability for such violations are contained not in laws regulating pharmaceutical activity but in the Criminal Code of Ukraine (hereinafter, the CC of Ukraine) [12].

Unfortunately, the provisions of the Criminal Code of Ukraine regarding violations of the procedure for conducting clinical trials are contradictory. Article 141 of the Criminal Code of Ukraine, "Violation of the Rights of a Patient," establishes liability for clinical trials conducted without the written consent of the patient or their legal representative, or those performed on minors or legally incapable persons, where such actions lead to death or other serious consequences. This article handles only one type of violation: conducting a clinical trial without informed consent that results in death or other grave outcomes. In 2012, Article 321-2, "Violation of the Established Procedure for Pre-Clinical Studies, Clinical Trials, and State Registration of Medicines," was added

to the Criminal Code of Ukraine. This article criminalizes intentional violations of the established procedures for pre-clinical studies and clinical trials of medicines, including falsification of their findings. It applies solely to clinical trials of medicines and does not encompass violations during clinical trials of other medical products. All violations during such clinical trials, including falsification of results, fall under this provision. If such violations result in the death of a research subject or other serious consequences, those found guilty under Part 3 of this article face deprivation of liberty for eight to ten years and may be deprived of the right to hold certain positions or engage in specific activities for two to three years.

Despite the widespread criminalization of violations during clinical trials of medicines in Ukraine, judicial statistics show the ineffectiveness of this approach. Over the past five years (2020-2024), law enforcement agencies have recorded 16 criminal procedures under Article 321-2 - "Violation of the established procedure for preclinical studies, clinical trials, and the state registration of medicines." Of these proceedings, only one was sent to court with a charge [13], but an acquittal was issued in that case [8]. A negative aspect of such broad criminalization in the field of clinical trials is the potential for unfounded pressure from law enforcement agencies on the conduct of these trials. The European Business Association also pointed out the excessive criminalization of violations in clinical trials of medicines in Ukraine [14]. This situation, along with contradictions in Ukraine's criminal-legal regulation of clinical trials, prompted the development and submission of draft laws to Parliament that aim to amend the Criminal Code of Ukraine to improve criminal liability for violating the established procedures for preclinical studies, clinical trials, and market authorization of medicinal products (registration numbers 11181 and 11181-1) [15; 16]. These drafts aim to address the inconsistencies in criminal-legal regulation and to establish specific violations of clinical trial procedures that are criminally punishable.

An analysis of the scientific literature shows that researchers who have examined the criminalization of fraud and misconduct in clinical trials involving medicinal products confirm that such acts are widespread and pose potential dangers to human life and health. For example, Ashwaria Gupta states that «Research fraud is a reality which nobody can shy away from. Furthermore, clinical research is very vulnerable to fraud due to no effective mechanism in place for detecting, investigating and prosecuting fraud in most of the countries.» [17] Joseph M. Gabriel and Bennett Holman suggest that one possible origin point for the widespread fraudulent practices in clinical trials that

now characterize the pharmaceutical industry is the monopoly status of the ethical framework in which medical science took place, which was later replaced by clinical utility as the primary arbiter of pharmaceutical legitimacy [18].

The widespread presence of fraud and misconduct in clinical trials involving medicinal products, along with the high risk of harm to human life and health (both research subjects and patients) when using such products, calls for criminalization. However, excessive criminalization in this area does not lead to positive outcomes but instead obstructs the normal conduct of clinical trials for medical products [19]. The basis for criminalizing fraud and misconduct in clinical trials involving medicinal products is harm that has been or could be caused to: 1) the life and health of the research subject; 2) public health and the life and health of individual patients during future use of marketed medicinal products.

Violations that endanger the life and health of research subjects should include: (a) enrolling a person in a clinical trial without that person or, in cases provided by law, another person providing informed consent; (c) failure to take legally required measures to ensure the safety of a research subject during temporary or permanent termination of a clinical trial.

The spectrum of violations that endanger public health and pose a potential threat to the life and health of a patient should be limited to the following illegal actions: (a) conducting a clinical trial without permission obtained in the prescribed manner; (c) fraud, i.e., falsifying data obtained during a clinical trial or the results of a clinical trial.

Reasonable criminalization of fraud and misconduct in clinical trials involving medicinal products is a necessary tool for ensuring that those guilty of such violations face criminal penalties. Proper criminal regulation, in turn, aims to help prevent new crimes in this area. However, as Ukrainian judicial practice demonstrates in the application of criminal law provisions addressing violations during clinical trials, the lack of necessary mechanisms for identifying offenders hampers their criminal prosecution.

Researchers rightly raise the issue of the need to utilize modern technologies to develop monitoring

systems that detect fraud in clinical trials of medical products. For example, William J. Cragg, Caroline Hurley, Victoria Yorke-Edwards, and Sally P. Stenning presented a scoping review of dynamic methods for ongoing assessment of site-level risk in risk-based monitoring of clinical trials [20]. The study "Detection of Fraud in a Clinical Trial Using Unsupervised Statistical Monitoring" concludes that "an unsupervised approach to central monitoring, using mixed-effects statistical models, is effective at detecting centers with fraud or other data anomalies in clinical trials." [21].

## CONCLUSIONS

The study revealed that fraud and misconduct in clinical trials of medicinal products are widespread and can harm human life and health. Such harm includes damage to the research subject's life or health, as well as risks to public health and the safety of future patients who will use the medical product.

In European criminal law, there are two types of regulation concerning the criminal aspects of clinical trials of medicinal products: 1) defining specific violations related to clinical trials in criminal law; 2) establishing criminal penalties for any violations of clinical trials. The first approach is more justified because it prevents undue pressure from law enforcement agencies and avoids overcriminalization.

Criminal regulation of clinical trials should be limited to attacks on the life or health of the research participants, as well as on public health and the safety of potential patients. Violations against the research subject's life or health include: (a) enrolling a person in a clinical trial without their informed consent or, where legally required, without consent from another authorized person; (b) failing to implement legally mandated safety measures during the temporary or complete halt of a clinical trial. Violations affecting public health and future patients include: (a) conducting a clinical trial without the proper authorization; (b) fraud, such as falsifying data or results obtained during a trial.

The effectiveness of criminal law in regulating clinical trials depends both on its quality and on using modern monitoring methods that help detect fraud and misconduct in clinical trials involving medicinal products.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Retrospective analysis of the effectiveness of modern bone plastic methods in dental implantation (literature review)

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## ABSTRACT

**Aim:** The aim of our study is a retrospective analysis of modern methods of osteoplasty in dental implantation using various combinations of autogenous and allogeneic grafts.

**Materials and Methods:** The selection of publications related to the research topic was carried out in the scientific databases Scopus, PubMed, BVS and Scielo using the following keywords: dental implantation, bone grafting, alveolar bone remodeling, dentin graft, autotransplantation. When searching for publications relevant to the research topic using, it was possible to identify 187 relevant articles. The search depth parameter was 7 years in order to ensure the analysis of the most relevant data related to the research goal. The review included original scientific articles, research results and official recommendations of medical associations, only articles with positive results in the study group. The collected materials were analyzed according to the principles of content analysis with further systematization and classification of data in CADIMA software.

**Conclusions:** In reconstructive surgery, autogenous bone is still considered the cornerstone in solving the most important tasks in bone grafting. Autografts from the lower jaw are successfully used, in particular, in cases of significant atrophy of bone structures, as they have predictable and reproducible results. Three-dimensional plastic surgery, which consists in transplanting thin cortical blocks in the form of overlays, provides a long-lasting clinical result and is more effective than the use of thick bone blocks.

**KEY WORDS:** dental implantation, bone grafting, alveolar bone remodeling, dentin graft, autotransplantation

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## INTRODUCTION

Partial or complete absence of teeth is always accompanied by atrophy of the jaw bone tissue, which, when planning dental implantation, in at least 30% of cases necessitates the elimination of the alveolar bone volume deficit [1-4]. Augmentation using autogenous block graft is one of the most common and effective methods of bone tissue regeneration. In addition to its reliable fixation and optimization of the surface structure, a differentiated approach to the selection of material depending on the clinical situation also allows reducing the risk of graft "settling" [2]. Taking into account the availability of various methods of osteoplasty of defects of the alveolar processes of the jaws and a wide range of osteoplastic materials, there is currently no unambiguous solution to the problem of atrophy. Planning a specific osteoplasty technique and the selection of a graft depend on an accurate assessment of the degree of bone resorption, as well as the nature and size of the defect [3,4]. An important diagnostic value in this process

is computed tomography of the jaws, which, in addition to determining anatomical features, allows you to establish the type of bone tissue, choose the optimal surgical protocol and predict the timing of osseointegration [3-6].

Prosthetics on dental implants is considered the most modern method of restoring the dentition in case of edentia. At the same time, the main problem that specialists increasingly face at the stage of planning implantation is the insufficient volume and poor quality of bone tissue in the area of the intended implantation [5]. To solve this problem, numerous methods have been proposed at different times: installation of implants bypassing the maxillary sinus [6], the use of subperiosteal implants, implantation with perforation of the floor of the maxillary sinus and others [7-9].

According to many authors, the most effective way to restore the volume of alveolar bone in the vertical and horizontal directions after tooth loss is to use the patient's own bone tissue [10]. Autotransplantation is considered the "gold standard" for restoring organs and

tissues due to structural compliance, lack of immune response, and presence of progenitor cells in the transplants [7,8,10]. The success of such manipulation largely depends on the choice of technique and type of osteoplastic material [10]. An important factor when planning autotransplantation is the morphospecificity of tissues, i.e. the fact that at the cellular level the same tissue in different organs differs in structure, cellular composition, physiological activity, and reactivity [11,12].

## AIM

To conduct a retrospective analysis of modern osteoplasty methods in dental implantation using various combinations of autogenous and allogeneic grafts.

## MATERIALS AND METHODS

The selection of publications related to the research topic was carried out in the scientific databases Scopus, PubMed, BVS and Scielo using the following keywords: dental implantation, bone grafting, alveolar bone remodeling, dentin graft, autotransplantation. When searching for publications relevant to the research topic using, it was possible to identify 187 relevant articles. The search depth parameter was 7 years in order to ensure the analysis of the most relevant data related to the research goal. The review included original scientific articles, research results and official recommendations of medical associations, only articles with positive results in the study group. The collected materials were analyzed according to the principles of content analysis with further systematization and classification of data in CADIMA software.

## REVIEW AND DISCUSSION

The lack of functional load in the area of lost or missing teeth leads to atrophy of the jaw bone tissue. Healing of the post-extraction socket, which occurs under a blood clot, is also accompanied by natural atrophy of the alveolar bone tissue both in width and height and reaches 16-30% of the initial size with the formation of a saddle-shaped ridge [10,12]. This complicates the further implementation of dental implantation surgery and, as a result, subsequent prosthetics.

Healing of the socket of an extracted tooth is accompanied by significant spatial changes in the alveolar bone in the form of its resorption and deformation, which creates unfavorable conditions for future dental implantation and prosthetic rehabilitation. The results

of the analysis of a number of literature publications indicate that autologous dentin graft, due to its morphological similarity to the cortical membranous matrix, biocompatibility, bioactivity, excellent osteoconductive and osteoinductive properties, ability to ankylose and slow resorption, is an extremely promising material for bone-plastic substitution in the practice of surgical dentistry and maxillofacial surgery [11,12].

According to researchers, bone tissue deficiency is observed in more than half of the cases [8,10]. Alveolar bone loss can occur even before tooth extraction and complicate the course of reparative regeneration at the implantation stage. In the process of preparation for dental implantation, the structure of the bone is of decisive importance, therefore it is extremely important to determine the morphological quality of bone tissue, its architectonics and density. In the clinical practice of a dental surgeon, there are various methods of increasing the volume of bone tissue, including autogenous bone block plastic, localized barrier technique, use of autogenous bone chips, and others. To eliminate horizontal and vertical atrophy and reconstruct bone tissue, the method of expanding a narrow alveolar ridge using a bone spreader, alveolar ridge splitting technique are used [10,13]. However, even with complete well-being in the postoperative period and a favorable clinical prognosis, there is a possibility of lack of complete integration in the area of the installed implants. Autogenous block plastic is a classic of bone tissue restoration, as it combines osteoinductive and osteoconductive properties. At the same time, the basis of any regeneration is timely vascularization of the graft, which should also be given great attention. Therefore, the issue of restoring the required volume of bone tissue is one of the most important and key in dental implantology [6,9,13].

Today, there are various osteoplasty techniques that solve the problem of atrophy. Some authors [14,15] describe successful implantation based on the adaptation of the intraosseous parts of the implants to real anatomical conditions by using short screw, subperiosteal implants, basal and zygomatic implant systems [7, 8,15]. Other researchers recommend performing reconstructive osteoplastic operations to create the necessary anatomical conditions [9–15]. However, with large volumes of bone grafting, it is not always possible to obtain the proper volume of bone graft without additional surgical interventions, and in some cases there is a lack of intraoral sources of cortical bone tissue to perform such an operation. The use of extraoral bone sources (tibial tuberosity, skull, iliac crest) is not always justified and involves significant invasion, after which patients temporarily lose their ability to work and are forced to be hospitalized [8,10,13,16].

The use of alternative sources of bone biomaterials could contribute to the implementation of a less invasive osteoplasty protocol. As some authors note, the most optimal biomaterial in terms of the combination of osteoinductive and osteoconductive properties after autogenous bone grafts are allogeneic biomaterials [11-16].

Intraosseous implantation occupies an important place in the comprehensive rehabilitation of patients with dentition defects [15,17]. It is well known that dental implantation is a multi-stage approach that includes bone grafting, dental implantation and possible soft tissue grafting, which, in turn, can cause a number of additional complications for the patient:

- the undesirability of using removable temporary orthopedic structures throughout the entire stage of surgical treatment, which can deepen atrophic changes and additionally overload the functional center;
- frequent use of antibacterial drugs at the stage of maintenance conservative therapy, which can lead to the development of antibiotic resistance;
- the need for repeated traumatic interventions in the surgical field, which increases the risk of intra- and postoperative complications.

In the process of preparing for dental implantation, the structure of the jaw bone is of decisive importance, therefore it is necessary to assess the morphological quality of bone tissue, its architectonics and density, as well as to identify the degree of resorption. At the stage of planning and choosing the method of surgical intervention, one of the most frequent limitations for dental implantation is insufficient thickness and height of the alveolar process [17,18]. According to a number of authors, more than 50% of patients require additional bone-plastic operations - both previously and directly at the stage of dental implantation [18]. The presence of atrophy of the jaw bone tissue significantly complicates the traditional stages of implantation, and in most cases makes it impossible without prior surgical preparation of the alveolar ridge [13,17].

In recent years, there has been a tendency to increase the number of patients with bone pathology. Among the most common etiological factors of jaw atrophy, it is necessary to single out pathology of the endocrine system, in particular diseases of the thyroid and parathyroid glands, as well as pathology of the bone tissue itself. Loss of alveolar bone can occur even before tooth extraction - as a result of unsuccessful endodontic treatment, periapical pathology or progressive periodontitis, as well as as a natural physiological process associated with periodontal rupture [4,5,11].

Damage to the periodontal ligament during tooth extraction directly triggers bone tissue atrophy due

to disruption of the functional and anatomical connections between the periodontium and the walls of the alveolus, which worsens the vascularization of the area [19]. According to a number of studies, during the first 12 months after tooth extraction, the width of the alveolar ridge decreases by 50%, with two-thirds of this bone loss occurring in the first 3 months [18].

Faced with such problems, the professional community has developed a number of alternative treatment methods that involve simultaneous dental implantation and bone grafting [18,19]. However, the results of bone grafting are not always predictable. There are several key factors that determine a positive result: the absence of an infectious agent, the condition of the soft tissue segment, the reliability and stability of the fixation of the bone augmentation, adequate blood supply to the intervention area, as well as the presence of cellular growth factors and morphogenetic proteins of the graft, which initiate osteogenesis [20]. According to the literature, bone grafting is indicated in 30–80% of cases [6,12,15,17,20]. Various techniques are used to eliminate bone tissue deficiency, including the use of xenogeneic materials, homogeneous bone tissue and allografts.

## METHODS OF INCREASING THE VOLUME OF ALVEOLAR BONE

To increase the thickness of the alveolar ridge, the technique of directed bone regeneration or “fencing” proposed by Professor Mauro Merli can be used. The essence of the technique is to create a space for the formation of bone tissue using a resorbed plate for osteosynthesis, previously prepared on a stereolithographic model. Prof. M. Merli used deproteinized xenogeneic bone material and autogenous bone chips in his technique. The fencing of the bone material was carried out with a bioresorbable membrane. Thus, the technique of three-dimensional augmentation allows you to create a promising form of the future alveolar ridge, restored with mixed bone material, covered with a collagen membrane that forms a protective barrier. According to the results of this study, the volume of newly formed bone tissue after 6 months was 3.39 cm<sup>3</sup> [15,20].

Also Prof. M. Merli and A. Mazzoni popularized the localized barrier technique, which consists in the reconstruction of small three-dimensional bone defects in two stages. In the first stage, based on computed tomography data using a stereolithograph, a titanium plate is manufactured. It is installed at a planned distance from the native bone, forming a physical barrier that holds the biomaterial necessary for regeneration, providing vertical and horizontal augmentation. The

incision is made along the lingual surface of the crest with a vestibular slope, which allows for exposure of the crest with minimal elevation of the lingual flap. Then, the titanium plate is fixed and the defect is filled with demineralized bone material (DBM) in a reverse phase medium (RPM) in a ratio of 1:1 with autogenous shavings obtained from the external oblique line of the mandible [20].

As is known, demineralized bone material in a reverse phase medium stimulates the natural processes of bone formation, in which mesenchymal cells differentiate into osteoblasts. Since the RPM medium becomes more viscous at warm temperatures, this allows the material to be plastic at room temperature and harden already in the operating field [15]. Today, the following bone grafting methods are most popular for eliminating horizontal and vertical atrophy and reconstructing bone tissue: autogenous bone block grafting, narrow alveolar ridge expansion technique using a bone spreader, alveolar ridge splitting technique, etc. [15, 16, 18, 20]. However, even with a successful postoperative period and a favorable clinical prognosis, there is still a possibility of complete or partial bone integration in the area of the installed implants.

## AUTOGENOUS BONE BLOCK RECONSTRUCTION

Autografting is considered the "gold standard" of bone tissue restoration. Autografting allows for the restoration of bone tissue both vertically and horizontally, combining osteoinductive and osteoconductive properties. Early vascularization of the graft is extremely important to ensure a positive clinical outcome. The mechanism of bone tissue remodeling goes through three consecutive phases. Osteocytes of the cancellous bone of the graft remain viable, producing osteoid plates for 3–4 weeks, feeding diffusely from the surrounding vascular system. However, after 2 weeks, only osteocytes located within a radius of up to 300 µm from blood vessels survive, which determines the first phase of neoosteogenesis. The second phase begins approximately 6 weeks after transplantation and lasts up to 6 months; it is characterized by the destruction of graft cells and the release of growth factors and inductive proteins, which trigger remodeling of the graft by newly formed bone tissue cells. The third phase is osteoconductive, when the inorganic hydroxyapatite skeleton of the graft acts as a passive matrix for remodeling of new bone tissue with subsequent resorption of the graft. In addition, the cortical plate of the graft acts as a barrier that prevents the infiltration of connective and epithelial tissue into the recipient area [7, 12, 21, 22].

The indisputable advantage of the autotransplantation method is complete biocompatibility, lack of immunogenicity, and the formation of organotypic bone tissue. Autografts can be harvested from both intraoral and extraoral donor sites. However, in order to avoid additional trauma, it is more expedient to use intraoral sources - the chin of the lower jaw and the projection of the external oblique line. Cortical blocks are obtained from the external oblique area, and cortical-cancellous blocks are obtained from the submental area. The difference between them is the presence of a cancellous layer, rich in cells, which promotes faster revascularization and neoosteogenesis. As already noted, adequate vascularization of the graft is a prerequisite for successful osteogenesis [23, 24].

## METHODS OF BONE AUTOGRAFT HARVESTING

Methods of graft harvesting from the submental area. The first stage is to provide adequate anesthetic support. This intervention is performed under local anesthesia in the area of the right and left submental foramina. Next, a vestibular incision is made apical to the mucogingival junction, placing the blade at a right angle. The flap is peeled to the lower edge of the chin, which creates conditions for wider wound edges and better mobilization of the mucogingival flap [18, 20, 24]. Then, two horizontal cuts are made with a microsaw, which are connected by vertical ones, determining the shape and size of the future autograft. A safe zone of 3–5 mm from the apices of the tooth roots should be taken into account. As a rule, the diamond disc used to perform the cuts completely dissects the vestibular cortical plate. Additional holes are made along the line of the cuts. The graft thus selected is separated with a thin chisel. Then, a scraper or chisel can be used to remove the volume of spongy tissue up to the lingual cortical plate [23–25]. To facilitate the graft collection, its fragmentation in the middle part is allowed. Closing the defect of the donor site is advisable to be carried out using a collagen membrane, which acts as an inner lining for bone chips, bone-plastic material and a non-resorbable membrane (for example, titanium foil or a membrane with a titanium reinforcement), since the main task is to preserve the contour of the chin and stabilize the material. After that, layer-by-layer suturing of soft tissues without tension is performed. Among the possible complications when using this technique (in the presence of anterior teeth) is damage to sensitive nerve fibers and blood vessels that innervate and vascularize the anterior group of teeth of the lower jaw. Transplantation of a graft from the chin area is par-

ticularly relevant in the reconstruction of the anterior part of the mandible, as it reduces the duration of the operation and provides a single access to the donor and recipient areas [23,25-27].

Method of graft harvesting from the external oblique line. This method does not require local anesthesia, as the doctor can focus on the patient's subjective feelings regarding the approach to the mandibular nerve. Infiltration anesthesia is performed from the vestibular and lingual sides. Then a trapezoidal incision is made and the mucoperiosteal flap is peeled off. The size of the graft depends on the length of the external oblique line and the volume of bone tissue [8,13,19,24,27]. Two vertical and one upper-horizontal cuts are made with a microsaw, using a milling cutter. An important task is to avoid damage to the mandibular nerve. Its exposure is possible with a weakly expressed external oblique line, during preparation below the level of the nerve or when performing a vertical cut on the ascending branch of the jaw. Point holes up to 3 mm deep are drilled between the vertical cuts. The block is separated by hammer blows on a thin chisel. The resulting graft consists of a thick cortical layer and a thin spongy layer [18,20,27,28]. Closure of the donor site defect is usually performed with a collagen sponge, which stabilizes the blood clot and accelerates healing. It is also possible to use bone-plastic materials. In this case, the collagen membrane is laid tightly to prevent migration of the material, which can irritate and damage the nerve, and only then is bone material added. The soft tissues are sutured in layers with knotted sutures [28,29]. The literature also describes a "veneer" technique of autoplasty, which is characterized by the presence of osteoperforations in the area of the receptive bed. Also important is the maximum correspondence of the graft configuration to the recipient bed. The inner surface of the graft is carefully polished, which is necessary for tight contact with the bone tissue of the recipient, as well as to avoid the smallest cavities that prevent graft engraftment. Graft fixation is performed with compression mini-screws, which provide conditions for healing and reconstruction of the autograft [29]. Adaptation of the obtained autograft is performed at some distance from the receiving area with titanium screws followed by filling with bone chips. There are methods of using platelet mass with an increased fibrin content (FRP), obtained by centrifugation, as membranes. In this case, after installing the implants in the bone bed and laying the osteoplastic material, FRP (Fibrine Riche en Plaquette) is used. According to the results of the study, a lower rate of hematomas and severity of soft tissue edema is noted in the postoperative stage compared to the control group [26,29].

The use of piezo instruments in bone surgery is a proven method [27]. Implant bed preparation by splitting and expanding the bone is relevant even with a small thickness

of the alveolar crest. One of the important advantages of this method is the preservation of the available volume of bone tissue. Since the end of the last century, researchers have used the technique of extension plastic surgery, which consists of osteotomy along the middle of the alveolar crest in the distal-medial direction and filling the defect formed with hydroxyapatite or autogenous bone tissue [30]. During this surgical intervention, the crest is split along its apex along the length of the defect with a thin bur or microsaw with displacement of the vestibular wall. The state of blood supply and the activity of remodeling of the displaced vestibular cortical wall are of great importance. In case of violation of the listed processes, resorption of the vestibular cortical plate is noted. When the bone is expanded, the implant bed is prepared with drills and osteotomes of increasing diameter. A bed is gradually formed that corresponds to the shape of the implant.

Due to the layer-by-layer exfoliation of the vestibular mucoperiosteal angle, the vestibular plate displaced by the chisel is held by the periosteum, which ensures its vascularization and prevents chipping. With sufficient primary stability, a single-stage installation of dental implants is performed, and the free space is filled with autogenous bone chips with subsequent suturing of the soft tissue flap. [27,30,31]. The protocol for the operation of splitting the alveolar ridge with a single-stage installation of dental implants includes an incision of the mucous membrane in the area of the dentition defect along the center of the alveolar ridge. Then, using microsaws and Lindemann burs, a vertical osteotomy is performed along the middle of the ridge within the defect and to the depth of the planned implant placement. In the area of the distal and medial borders of the defect, vertical osteotomy lines are performed in the vestibulo-oral direction to the middle of the ridge. At the base of the potential bone flap within the borders of the defect, a compact osteotomy is performed with a spherical bur or Lindemann bur. Next, a microscrew is installed in the base of the bone bed of the implant to the depth of the bone flap, taking into account immersion in the fixed part of the defect area.

Then, the space between the installed implants is filled with deproteinized osteoplastic material with a membrane overlap and differentiated, hermetic wound suturing [32-34]. In cases of small alveolar ridge volume, optimal mobilization of the flap during the bone plastic stage is difficult. Prof. Marius Steigmann, Maurice Salama proposed the method of subperiosteal envelope flap. The technique consists in forming a mucous flap on the vestibular surface without violating the integrity of the periosteum. Then the periosteum is peeled off in the apical direction to the depth of the planned dental implants with the formation of a pocket into which the bone material is hermetically placed (Tutodent, Bio-Oss).

Then a resorbable collagen membrane is sutured to the periosteum with absorbable suture material, ensuring the tightness of the subperiosteal space and preventing the germination of soft tissue structures deep into the surgical intervention. The edge of the membrane is placed under the thickness of the lingual flap, thereby preventing the migration of bone material. Then a full-layer suturing of the soft tissues is performed. According to the results of the study, an increase in the thickness of the alveolar crest is noted after 6 months, on average by 6.71 mm [32,33,35].

## CONCLUSIONS

Thus, our content analysis of scientific publications indicates that in case of bone deficiency, it is possible to use various methods of restoring the volume of lost bone tissue. The combination of bone-plastic materials,

in particular autologous dentin graft, with platelet-enriched fibrin, may be a promising therapeutic option for replacing defects after tooth extraction due to acute or chronic periodontal pathology. The composition of autologous dentin graft with platelet-enriched fibrin proves its effectiveness in replacing periodontally compromised teeth, periapical granulomas, and radicular cysts.

In reconstructive surgery, autogenous bone is still considered the cornerstone in solving the most important tasks in bone plastic surgery. Autografts from the lower jaw are successfully used, in particular, in case of significant atrophy of bone structures, since they have predictable and reproducible results. Three-dimensional plastic surgery, which consists of transplanting thin cortical blocks in the form of overlays, provides a long-lasting clinical result and is more effective than the use of thick bone blocks.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Significance of the $\gamma$ -aminobutyric acid and FKBP5 genes polymorphisms in the risk of post-traumatic stress disorder developing

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## ABSTRACT

**Aim:** To analyse and interpret international and domestic literature regarding the potential relationship between the risk of developing post-traumatic stress disorder (PTSD) and the presence of single-nucleotide polymorphisms (SNPs) in the  $\gamma$ -aminobutyric acid (GABA) and FKBP5 genes.

**Materials and Methods:** The literature search was conducted in the following databases: PubMed, Web of Science, DOAJ, Scopus, Medline, Google Scholar, as well as on the official websites of the Ministries of Ukraine, libraries of higher medical institutions, the World Health Organization (WHO), and general Google search resources. The search period covered the years 2015–2025. To more comprehensively achieve the research objective, several historical scientific publications were also taken into account. The search keywords included PTSD, SNP, GABA gene, and FKBP5. The search encompassed scientific sources in English, Ukrainian, German, and French addressing issues related to PTSD and SNPs. Studies concerning the treatment of PTSD or single-nucleotide polymorphisms other than those of the FKBP5 and GABA genes were excluded. The initial article pool comprised 100 publications, of which 40 were included in the final analysis.

**Conclusions:** Early detection of PTSD within specific population groups allows the implementation of timely preventive measures aimed at reducing the incidence, severity, and duration of the disorder, thereby accelerating the restoration of psychological balance and resilience. The combination of genetic and epigenetic factors with neurotransmitter dysfunction constitutes the basis for the development of stress-related disorders. In particular, determining the frequency of GABA and FKBP5 gene SNPs may serve as a diagnostically significant biomarker of the pathophysiological mechanisms underlying PTSD.

**KEY WORDS:** post-traumatic stress disorder, single-nucleotide polymorphism

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## INTRODUCTION

The consequence of the Russian Federation's full-scale invasion of Ukraine and the ensuing large-scale military operations is a complex set of significant negative social, economic, environmental, sanitary-epidemiological, and psychological impacts. The combined effect of these factors is the main cause of stress-associated mental disorders, in particular post-traumatic stress disorder (PTSD) [1].

Traumatic events such as military conflict, accidents, natural disasters, or sexual violence, when witnessed or experienced by psychologically unprepared individuals, can cause profound and often critical negative changes in their psycho-emotional state [2]. Most people who experience such events suffer from

considerable psychological distress but eventually recover without external assistance. However, there are individuals who, in similar situations, develop a wide range of mental health disorders—such as PTSD, depressive, and anxiety disorders—that may persist for several months or even years. Each of these disorders alone can significantly affect a person's health and quality of life, and their combination amplifies these negative effects [3].

Under direct and prolonged exposure to traumatic events, especially in wartime, PTSD is considered the most critical disorder because it is directly associated with traumatic experiences, characterised by the chronic nature of its symptoms, its impact on a person's ability to function, and the combination of

specific manifestations (flashbacks, nightmares, severe anxiety, emotional detachment, etc.), social isolation, and the possibility of collective trauma [4].

When referring to war as a traumatic event, mental trauma is common among a large portion of the population, and PTSD can become a “norm” for many, as numerous individuals may experience similar symptoms due to shared experiences. This increases the significance of PTSD as a social phenomenon. In such circumstances, it is essential to recognise that not only individuals but also entire communities may require psychological support and treatment. This underscores the importance of educating the Ukrainian population about the manifestations of PTSD [1–4].

Among other aspects, the relationship between genetics and PTSD is also of particular importance, especially in facilitating timely diagnosis through genetic testing aimed at determining the frequency of single nucleotide polymorphisms (SNPs) in the  $\gamma$ -aminobutyric acid (GABA) and FKBP5 genes. Such testing may support the early implementation of social and preventive programmes directed at restoring mental health [5].

## AIM

The analysis and interpretation of international and domestic literature sources regarding the potential relationship between the risk of developing PTSD and the presence of single-nucleotide polymorphisms (SNPs) in the  $\gamma$ -aminobutyric acid (GABA) and FKBP5 genes.

## MATERIALS AND METHODS

The literature search was conducted in the following databases: PubMed, Web of Science, DOAJ, Scopus, Medline, Google Scholar, as well as on the official websites of the Ministries of Ukraine, libraries of higher medical institutions, the World Health Organization (WHO), and general Google search resources. The search period covered the years 2015–2025. To more comprehensively achieve the research objective, several historical scientific publications were also taken into account.

The search keywords included PTSD, SNP, GABA gene, and FKBP5. The search encompassed scientific sources in English, Ukrainian, German, and French addressing issues related to PTSD and SNPs. Studies concerning the treatment of PTSD or single-nucleotide polymorphisms other than those of the FKBP5 and GABA genes were excluded. The initial article pool comprised 100 publications, of which 40 were included in the final analysis.

## ETHICS

Publicly available sources were used. The authors adhered to the ethical principles of the World Medical Association Declaration of Helsinki and international standards for publications in medical journals, including the ICMJE guidelines. Plagiarism and data fabrication are excluded. All sources of information are appropriately cited and properly formatted.

## FRAMEWORK

The study was conducted as a fragment of the scientific project of the Department of Occupational Epidemiology, Laboratory of occupational hygiene and physiology of State Institution «Kundiiev Institute of Occupational Health of the National Academy of Medical Science of Ukraine» under the R&D direction «Analysis of the frequency of distribution of single nucleotide polymorphisms in the FKBP5 and gamma-aminobutyric acid genes for the detection of PTSD in military personnel and workers in hazardous professions» (state registration number 0124U003776; term: 2025–2027).

## REVIEW AND DISCUSSION

### THE HISTORY OF PTSD BEFORE THE OFFICIAL TERM WAS RECOGNIZED

Concerning the symptoms of PTSD, individual observations were recorded by Herodotus, Hippocrates, Xenophon, and other notable figures of antiquity, as well as in even earlier reports describing combat-related mental disorders from the era of the Assyrian Empire (circa 1300 BC) [6]. However, the specific symptoms of the disorder have been more clearly described over the past few centuries. For instance, during the American Civil War (1861–1865), surgeon Jacob Mendes da Costa identified a syndrome later referred to as somatoform autonomic dysfunction (also known as Da Costa’s syndrome or soldier’s heart) [7]. In 1888, the neurologist Hermann Oppenheim proposed the term traumatic neurosis [8], and around the same time, Pierre Marie Félix Janet observed that victims of psychological trauma repeatedly re-enacted the behaviours, emotions, and bodily sensations experienced at the moment of trauma [9].

During the First World War, the recognition of military post-traumatic stress disorder as a disease allowed veterans in England, France, and Germany to claim pensions and receive treatment. Approximately 80,000 cases of post-traumatic disorders were registered, including soldiers who lost their memory, sight, hearing, smell, taste, or ability to walk. At that time, the principal diagnosis became the so-called shell shock (Fig. 1), a term introduced by psychiatrist Charles Samuel Myers [10].



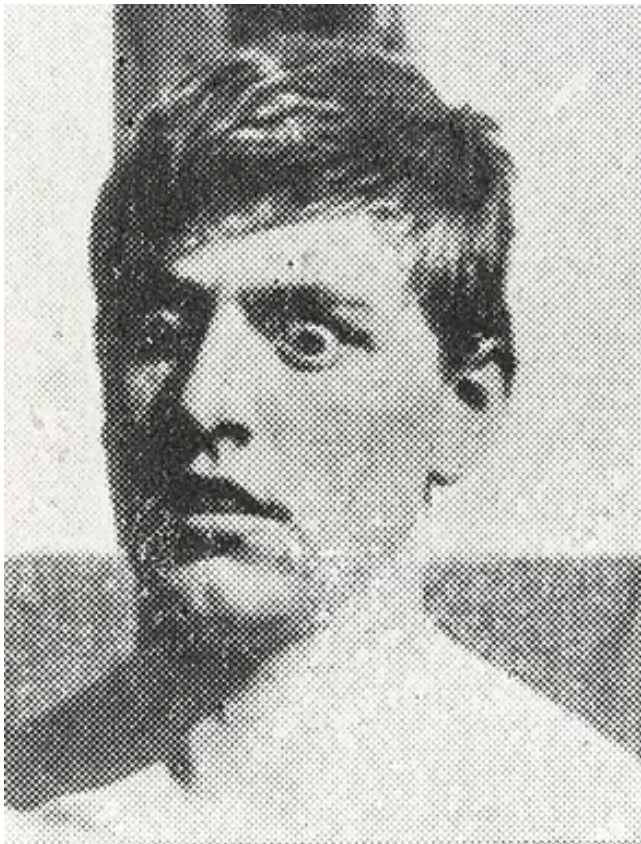


**Fig. 1.** Characteristic signs of shell shock: stunned facial expression and an immobile, frozen gaze

*Source: compiled by the authors based on [10]*

In 1940, psychologist Abram Kardiner studied World War I veterans and concluded that post-traumatic psychological disorder has distinct symptoms, with the development of “war neurosis” largely resulting from the maladaptation of combatants to post-war conditions [11].

During the Second World War (1939–1945), Soviet psychiatrists and neurologists used the term concussion (mild traumatic brain injury) to describe servicemen exhibiting various manifestations of combat stress [12]. Moreover, during World War II, post-traumatic symptoms were identified not only among military personnel but also among concentration camp prisoners. These individuals developed a form of PTSD characterised by an overwhelming sense of guilt for having survived extreme events (e.g., accidents, terrorist attacks, riots, natural disasters, epidemics, wars, genocides, or concentration camps) while many others perished. This condition became known as concentration camp syndrome (KZ-syndrom, from Konzentrationslager in German), survivor guilt syndrome (Das Überlebenden-Syndrom in German), or Holocaust syndrome. The extreme psychological stress endured in the camps resulted in the syndrome developing in more than 75% of victims [4, 13, 14].



**Fig. 2.** “A Look at Two Thousand Yards”. The thousand-yard stare

*Source: compiled by the authors based on [16]*

Similar symptoms were observed among survivors of the atomic bombings of Hiroshima and Nagasaki on August 9, 1945. Approximately one-third of the survivors of the Hiroshima attack (Japanese: hibakusha – “those who survived the explosions”) experienced profound feelings of guilt that developed into a psychological complex. Around 10% of the victims of the bombings suffered from post-traumatic stress disorders [4]. In studies of World War II veterans, a syndrome resembling PTSD was referred to as gross stress reaction [15]. In the United States in 1945, the colloquial expression “the thousand-yard stare” (Fig. 2) was used to describe the vacant, unfocused gaze commonly seen in soldiers who had suffered psychological trauma in combat [16].

During the Vietnam War (1959–1975), the development of dependencies in those who were in combat was added to the list of already known symptoms. According to statistics, 700,000 American veterans were diagnosed with post-traumatic symptoms. Mental disorders with these characteristics were previously undescribed in psychological literature and, thus, were named “Vietnam syndrome.” Approximately 25% of participants in combat operations in Vietnam suffered personality changes due to psychological trauma [4]. Research of the problem led to the introduction of the term “traumatic neurosis” in a new version of the Diagnostic and Statistical Manual of Mental Disorders (DSM). In 1968, this term was replaced by “post-traumatic stress disorder” (PTSD). This term

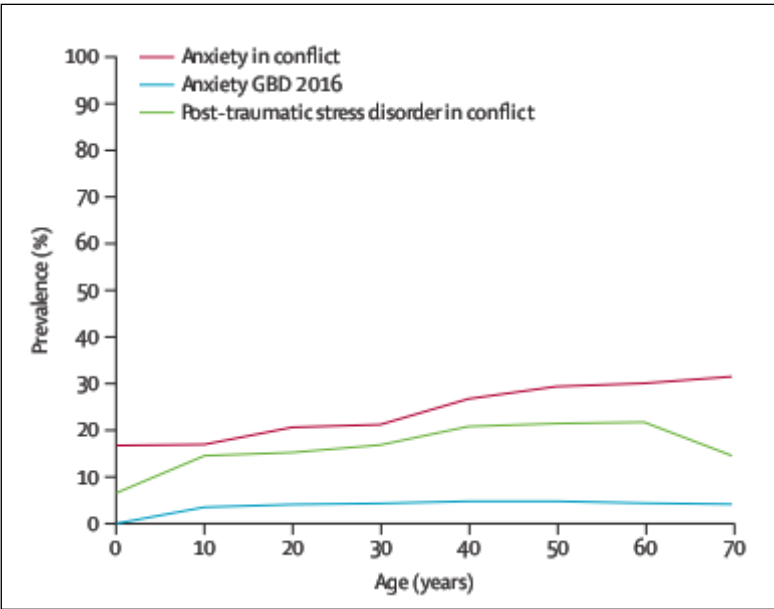


**Fig. 3.** Age prevalence (average) of depression, any anxiety, and PTSD in conflict-affected populations GBD –Global Burden of Disease  
*Source: compiled by the authors based on [1]*



**Fig. 4.** The map of the number of global scientific studies on PTSD from 1980 – 2017  
*Source: compiled by the authors based on [1]*

was officially recognized by the American Psychiatric Association in 1980 in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) [17].

CONTEMPORARY INSIGHTS INTO PTSD AND THE IMPORTANCE OF IDENTIFYING THE PROBLEM, ESPECIALLY IN UKRAINE

According to WHO estimates, as of May 2024, 3,9% of the world’s population suffers from PTSD [3]. In conflict situations, tendencies towards depression and anxiety increase with age, and conversely, the average prevalence of post-traumatic stress disorder in older age groups decreases (Fig. 3).

The likelihood of developing PTSD varies depending on the type of traumatic event experienced. According to Fiona Charlson, one in five people living in post-conflict environments suffer from depression, anxiety disorder, PTSD, bipolar disorder, or schizophrenia. Moreover, PTSD rates are more than three times higher

(15,3%) among individuals who have experienced conflict accompanied by violence or war [1]. The highest prevalence of disaster-related PTSD is observed among survivors (30%–40%) and first responders (10%–20%), compared to the general population (5%–10%) [2].

According to WHO data, the number of psychiatric epidemiological studies has increased significantly in countries affected by military conflicts and other emergencies. Between 2000 and 2017, comprehensive scientific studies on PTSD were conducted in only 34 countries worldwide, with almost none performed in Ukraine (Fig. 4).

However, just after the start of the full-scale invasion of Ukraine, there has been a significant increase in the diagnosis of PTSD among Ukrainians. According to the National Health Service of Ukraine (NHSU), in 2021, 3167 patients were officially diagnosed with PTSD, in 2022 – 7051 people, in 2023 the number of Ukrainians diagnosed with PTSD increased to 12494, and in just the first two months of 2024 (as of March 6, 2024), 3292 cases have already been diagnosed



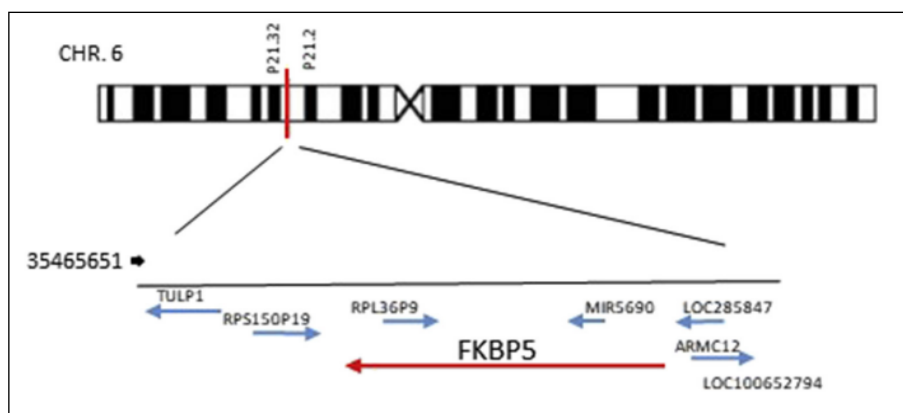


**Fig. 5.** The schematic diagram of FKBP5 location on chromosome 6 (6p21.31). The FKBP5 gene spans 154 kilobase pairs (kbp) from 35541362 to 35696360 on the reverse strand

Source: compiled by the authors based on [36]

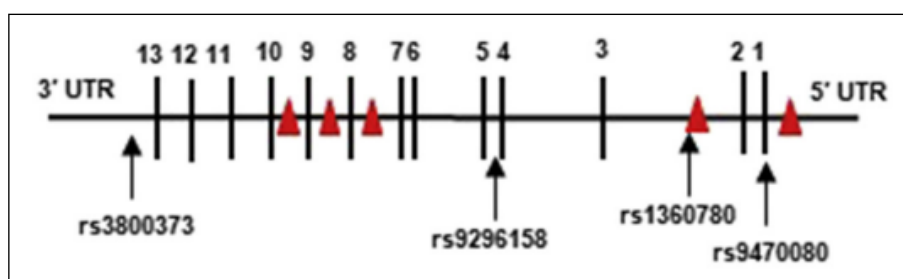


**Fig. 6.** Schematic diagram of polymorphism locations around the FKBP5 gene and glucocorticoid receptor sites

Label: ▲ – hormonal response element

Source: compiled by the authors based on [36]

[5]. In Ukraine, the prevalence of PTSD diagnosis varies depending on many factors, but undoubtedly the greatest risk of developing the disorder is present in individuals who are directly exposed to a stressor, and there is an interdependence between the probability of PTSD occurrence and the severity, duration, and scale of the traumatic event. This correlation can be traced by comparing the prevalence of PTSD before and after the full-scale invasion of Ukraine – if during the Anti-Terrorist Operation (2014-2018 years) PTSD was diagnosed in 27,7% of the military personnel who participated in it [18], then as of the end of 2024, that increased to 53,3% [19].

According to WHO data, the prevalence of mental disorders, including PTSD, is significantly higher for displaced persons and other war-affected individuals than for the general population [1]. In Ukraine, according to the Ministry of Social Policy, as of April 23, 2025, there were more than 4,5 million (4 594 270 people) officially registered internally displaced persons (IDPs) [20]. According to N. Masik et al., signs of PTSD were more often found in IDPs (83,33%) compared to civilians (10,0%). Moreover, IDPs predominantly exhibited symptoms of avoidance (76,67%), negative cognition (76,67%), and hyperarousal (88,33%), which emphasized the significant role of emotional reactions and possible influence on other PTSD symptoms [30].

## THE DIAGNOSTIC CRITERIA FOR GENETIC MARKERS FOR INCREASED RISK OF DEVELOPING PTSD

The influence of genetics on PTSD has been insufficiently studied due to the inherent limitations of genetic

research on mental disorders. Nevertheless, it is well established that genetic factors play a significant role in the development of PTSD [21].

Analysis of the frequency of single-nucleotide polymorphisms (SNPs) in various genes is an important tool in exploring the genetic basis of PTSD, as it enables a deeper understanding of the primary genetic and subsequent biological mechanisms underlying this disorder. An SNP is a variation in deoxyribonucleic acid (DNA) in which a single nucleotide – the basic unit of DNA (adenine, thymine, cytosine, or guanine) – is substituted for another at a specific location in the genome. In other words, it represents a point polymorphism, where different bases occur at a given locus of the genome across individuals or populations. SNPs constitute the foundation of population diversity and are the most common type of polymorphism in the human genome, occurring on average once every 1,000 nucleotides in human DNA [22, 23].

Studies of specific nucleotide polymorphisms in genes associated with central nervous system metabolism make it possible to identify various risks and mechanisms of PTSD development, which, in turn, may inform the creation of targeted preventive measures.

## THE ROLE OF SNPS IN THE GABA AND FKBP5 GENES IN THE RISK OF DEVELOPING PTSD

**THE ROLE OF GABA GENE POLYMORPHISMS**  
Changes in the activity of GABA gene are of particular importance in the neurobiological mechanisms that

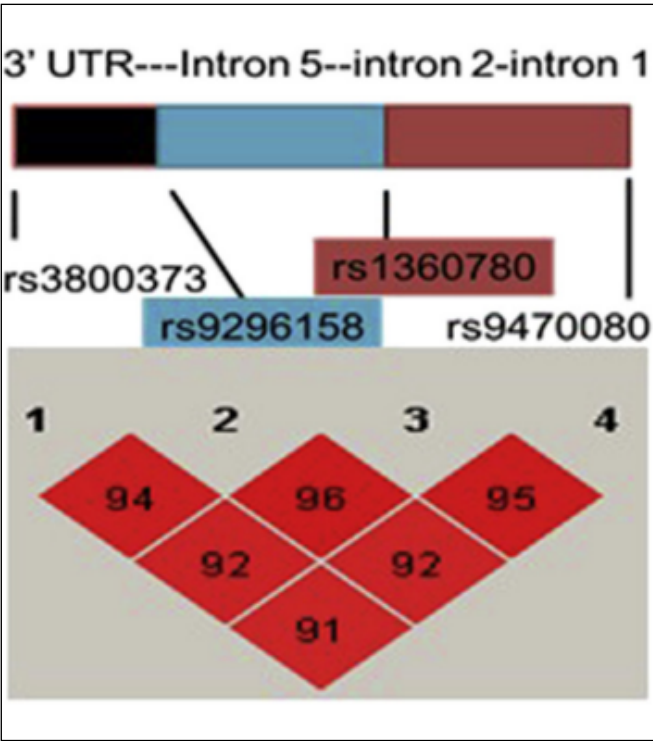


**Fig. 7.** Relative position of FKBP5 polymorphisms and their haplotype block structure. Numbers in squares refer to one block of strong pairwise linkage disequilibrium ( $r = 0,91-0,96$ )  
*Source: compiled by the authors based on [36]*

determine the body's response to stress and traumatic events. GABA is the main inhibitory neurotransmitter in the central nervous system. When GABA is released into the synaptic cleft, it activates the ion channels of GABAA- and GABAC-receptors, leading to the inhibition of nerve impulses. Under the influence of GABA, brain energy processes are also activated, tissue respiratory activity increases, glucose utilization by the brain improves, and blood supply improves. In extreme conditions with a large energy deficit, GABA is oxidized in the brain by an oxygen-free pathway, releasing a lot of energy and normalizing the content of histamine and serotonin in the brain. Since the main physiological role of GABA is to ensure a stable balance between excitatory and inhibitory systems, normalize sleep, and provide the brain with energy and its resistance to hypoxia, as well as other harmful influences, including distress, a decrease in GABA levels disrupts the balance of excitatory and inhibitory neurons, leading to neurotransmission dissonance, impaired neuroendocrine regulation, and, as a consequence, hypothalamic dysfunction. Stress-limiting systems are capable of limiting the activity of the stress system and excessive excitatory reaction at the central and peripheral levels of regulation [24]. At the same time, P. Hepsomali's research has shown that the consumption of natural and biosynthetic GABA can positively affect stress levels and sleep [25].

However, after prolonged stress, there is a decrease in synthesis and depletion of GABA due to the activation of GABA-transaminase, and nerve impulse transmission in neurons is disrupted with an increase in glutamate (an excitotoxic amino acid), leading to cell apoptosis and neuronal death, and increased excitation with impaired Krebs cycle and worsening cognitive functions of the body. Against the background of chronic stress and decreased GABA synthesis, the main physiological role of GABA – the formation of a stable balance between excitatory and inhibitory systems – is disrupted. Thus, with a deficiency or blockade of GABA synthesis, thinking productivity decreases and memory worsens, other cognitive functions are impaired, anxiety and irritability increase, aggression appears, resistance to hypoxia, stressors, and body endurance decreases, the sleep cycle is disrupted, and, consequentially, the circadian rhythm of hormones [24]. GABA receptor ligands are considered potential agents for the treatment of various mental and central nervous system disorders, including sleep disorders (insomnia, narcolepsy), epilepsy, Parkinson's and Alzheimer's diseases [26]. Genes associated with its synthesis, receptors, and metabolism may have SNPs that affect neurotransmission and neuroplasticity. Such variations may be associated with the risk of developing mental disorders such as depression, anxiety disorders, and schizophrenia. A study by K. Skelton et al. showed a significant correlation between three polymorphisms in the gene of the GABA receptor alpha-2 and the severity of childhood trauma, which is important for predicting PTSD in adults. According to the study, people with a certain genotype of G-protein signaling 2 (RGS2), a protein that reduces G-protein-coupled receptor signaling, experience high levels of stress due to environmental factors in adulthood and have a significant predisposition to PTSD throughout their life. This was especially true for adults with previous trauma and low social support [21].

THE ROLE OF FKBP5 GENE POLYMORPHISMS

The FKBP5 gene (FK506 binding protein 5) (Fig. 5) encodes a protein that regulates the activity of glucocorticoid receptors (GR), which are an important component of cellular stress response mechanisms, regulate numerous physiological processes, including metabolism, immune reactivity, and affect the body's stress response [27-29]. Assuming that one of the key systems mediating the long-term consequences of stress and PTSD is the glucocorticoid receptor (GR) system of the hypotha-

lamic–pituitary–adrenocortical (HPA) axis, individuals with PTSD may exhibit either heightened sensitivity to stress or, conversely, less severe symptoms, depending on molecular anomalies in the signaling pathways involved in GR stimulation. As a transcription factor – that is, a protein that binds to specific DNA regions and regulates the expression of target genes – the glucocorticoid receptor is activated upon binding to its ligand (e.g., cortisol) and translocates from the cytosol to the cell nucleus, where it interacts with DNA sequences to modulate gene expression. This process is regulated by a large molecular complex that includes the *FKBP5* protein [30, 31].

It is known that SNPs in the *FKBP5* gene (Fig. 6) can alter stress sensitivity and affect the risk of developing mental disorders such as depression, anxiety, and PTSD, as well as other adverse psychological outcomes in which stress hormone regulation plays a critical role [32]. Moreover, identifying specific polymorphisms in this gene can inform personalised approaches to therapy and stress management strategies [30, 33].

Since FKBP5 is part of the FKBP/GR complex, which directly regulates GR sensitivity, its polymorphisms are associated with its functionality, which is caused by glucocorticoid action and GR sensitivity. According to P.D. Reynolds, FKBP5 plays a key role in intracellular ultrashort negative feedback with GR activity [34], which indicates the extremely important role of FKBP5 activity in the development of PTSD, given the cortisol-induced higher level of FKBP5 associated with PTSD [30]. K. Skelton's research points to the main polymorphisms of the FKBP5 gene, each of which has a special influence. The correlation between gene polymorphisms and the severity of childhood abuse predicts the severity of PTSD symptoms in adults. For example, the TT genotype of the FKBP5 gene is associated with the highest risk of PTSD among those who experienced abuse or other adversity in childhood, however, individuals with a similar genotype who did not experience childhood difficulties had the lowest risk of PTSD. Alcohol dependence interacts with FKBP5 polymorphism and stressful events in childhood, contributing to an increased risk of PTSD in these individuals. It has been proven that lower FKBP5 mRNA expression, which additionally occurs after trauma, including combat trauma, indicates greater severity of post-traumatic stress disorder symptoms [21].

A direct link is observed between parental mental trauma and the mental health of offspring; a greater intensity of reparative adaptive influences in parents increases the risk of developing stress disorders in

offspring due to changes in FKBP5 gene methylation [35]. Childhood trauma is also a prognostic criterion for the risk of development and severity of PTSD in adults in the presence of four SNPs in the FKBP5 gene (rs9296158, rs3800373, rs1360780, and rs9470080) [30, 36, 37], especially rs9470080 [38]. Separate analysis of individual markers is less effective compared to statistical analysis based on haplotypes [39]. According to L. Zhang's study, probable PTSD subjects were significantly more likely to carry the A-allele of rs3800373, the G-allele of rs9296158, the C-allele of rs1360780, and the C-allele of rs9470080. In addition, the four SNPs were in one block of strong linkage disequilibrium ( $r = 0.91-0.96$ ) (Fig. 7). Within the block, there were two main haplotypes CATT and AGCC (rs3800373-rs9296158-rs1360780-rs9470080), accounting for 99% of haplotype diversity. The distribution of the AGCC haplotype was significantly higher in patients with probable PTSD compared to patients without PTSD. Analysis based on diplotypes showed that AGCC carriers have a significant risk of developing PTSD [36].

*FKBP5* expression levels are also associated with the size and functional capacity of several key brain structures, including the amygdala, hippocampus, and prefrontal cortex, and may influence therapeutic responses in PTSD. The amygdala plays a central role in the formation and persistence of fear, amplifying negative emotional reactions to traumatic cues. Stress hormones, in concert with neurotransmitters, modulate the functional activity of the amygdala, directly affecting the development and maintenance of PTSD [40].

## CONCLUSIONS

1. Early identification of individuals or population groups at high risk of developing PTSD based on specific genetic variations enables the timely implementation of comprehensive preventive measures aimed at preventing the widespread occurrence of stress-related disorders, reducing their severity and duration, and accelerating the restoration of psychological balance and resilience.
2. The combination of genetic and epigenetic factors, along with neurotransmitter dysfunction, forms the basis for the development of PTSD. Determining the frequency of single-nucleotide polymorphisms (SNPs) in the GABA and FKBP5 genes represents a diagnostically significant approach for identifying pathophysiological changes underlying stress-related disorders.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Artificial intelligence in the training system of future doctors

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## ABSTRACT

**Aim:** To analyze the potential of artificial intelligence in the process of solving innovative ideas in the system of training future doctors, improving the educational process in medical universities, and analyzing the concept of "health and quality" as a key term in professional activity. In order to achieve the stated goal, we plan to examine the importance of this concept for regulatory documents and propose an original (authorial) solution to these issues.

**Materials and Methods:** A systematic literature search was carried out in the following databases: PubMed, Scopus, Web of Science, Google Scholar. Additional grey literature was identified through institutional repositories, conference proceedings, and relevant policy documents of the Ukrainian government and the European Union. Such keywords and their combinations as "artificial intelligence", "medical education", "empathy", "anamnesis", "academic integrity", "iatrogenesis", "digital transformation", "higher medical school", "information culture", "AI in healthcare", "Poland", "Ukraine" were used. Inclusion criteria encompassed peer-reviewed articles published between 2018 and 2025 in English, Ukrainian, or Polish that focused on the use of AI in medical education, clinical training, and healthcare organization, as well as studies analyzing its advantages, limitations, ethical considerations, and offering comparative or practical recommendations. Exclusion criteria included non-scientific sources, articles unrelated to medical education or healthcare, and studies lacking clear methodology or outcome measures.

**Conclusions:** Artificial intelligence in organizing the administrative work of medical institutions has the significant potential. This refers to information about staff and patients, organizing communication schedules with specialists of the relevant profile, and optimizing schedules.

**KEY WORDS:** education, medicine, university, personality, creativity, artificial intelligence, communication

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## INTRODUCTION

Artificial intelligence is becoming an everyday reality. We should note the rapid pace at which new technological advancements are being implemented. At the same time, a review of the literature indicates that the number of publications on the threats and benefits of artificial intelligence significantly exceeds the number of specific recommendations for its effective practical use. In training future doctors, attention is drawn to potential violations of academic integrity. At the same time, there is a need for a series of studies on the use of AI in the educational process, particularly in preparing for high-quality history taking: the culture of questioning, empathy, the ability to speak and listen to the interlocutor, and avoiding iatrogenesis.

## AIM

To analyze the potential of artificial intelligence in the process of solving innovative ideas in the system of training future doctors, improving the educational pro-

cess in medical universities, and analyzing the concept of "health and quality" as a key term in professional activity. In order to achieve the stated goal, we plan to examine the importance of this concept for regulatory documents and propose an original (authorial) solution to these issues.

## MATERIALS AND METHODS

The study was conducted over the past five years (2020-2025) at I. Horbachevsky Ternopil National Medical University. On the basis of comparative studies, the experience of Polish colleagues was taken into account. Methods of comparison, analytical generalization, expert assessments, creative essays were used. To achieve the goal of the study, the capabilities of artificial intelligence were used, data from computer systems were compared with the conclusions of teachers and young people, and their own experience in solving complex social issues was summarized. Special attention was paid to correctness, creative use of the capabilities of

artificial intelligence, and consideration of the requirements of academic integrity. A systematic literature search was carried out in the following databases: PubMed, Scopus, Web of Science, Google Scholar, and the Polski Mercury Lekarski and Wiadomości Lekarskie journal archives. Additional grey literature was identified through institutional repositories, conference proceedings, and relevant policy documents of the Ukrainian government and the European Union. Such keywords and their combinations as “artificial intelligence”, “medical education”, “empathy”, “anamnesis”, “academic integrity”, “iatrogenesis”, “digital transformation”, “higher medical school”, “information culture”, “AI in healthcare”, “Poland”, “Ukraine” were used. Inclusion criteria encompassed peer-reviewed articles published between 2018 and 2025 in English, Ukrainian, or Polish that focused on the use of AI in medical education, clinical training, and healthcare organization, as well as studies analyzing its advantages, limitations, ethical considerations, and offering comparative or practical recommendations. Exclusion criteria included non-scientific sources, articles unrelated to medical education or healthcare, and studies lacking clear methodology or outcome measures.

## REVIEW AND DISCUSSION

For In modern medicine, it is important to respond promptly to new challenges, changes in public life, to assess the benefits and threats posed by new information technologies. One of the current tasks is to understand the opportunities and threats of artificial intelligence, which has become an everyday reality in a few years. The authors of the article «The impact of the introduction of artificial intelligence technologies on the current human rights and freedom as a concept» (Vitalii M. Pashko, Andrii O. Harkusha, Oleksii S. Soloviov), which was published in the journal “Polski Mercury Lekarski” rightly emphasized the significant advantages of artificial intelligence in healthcare, but also made valid comments regarding possible threats, the need to protect patients’ rights, and compliance with the requirements of confidentiality of medical information [1].

The authors of the articles constantly emphasize the possibilities of artificial intelligence in management, rationalization of document flow, interaction with patients. Changes in the work of pharmacy employees, doctors’ search for drugs available for a given region have been repeatedly noted. At the same time, doctors and teachers of medical universities note a growing trend towards self-medication, self-diagnosis of diseases, and patients’ attempts to influence the choice

of treatment strategy and tactics. Some students are excessively fond of using information technologies, which reduces the level of creativity, finding a way out of complex, ambiguous situations, and increases communication deficiencies, which is the basis of the anamnesis, and the problem of iatrogenesis becomes relevant. We also recorded a gradual decrease in the level of empathy when solving specific situations of suicidal tendencies. Suicide attempts are recorded among students. These issues require additional study. The war in Ukraine and mass emigration have only exacerbated these problems.

It is time to modernize higher medical education. As rightly noted in the article “Artificial Intelligence in Medical Education: Promise, Pitfalls, and Practical Pathways” (Sarup Saroha) educational institutions should use clear criteria for evaluating the effectiveness of integrating artificial intelligence into curricula, use modeling, portfolios, adhere to the principles of ethics, correctly use the capabilities of computer systems during clinical practice, diagnostics, and the development of critical thinking. Medical education institutions, preparing students for thoughtful and collaborative work with artificial intelligence “By preparing students this engage thoughtfully and collaboratively with AI, medical schools have an unparalleled opportunity this shape the future of healthcare, one that is smarter, fairer, and more effective for both patients and practitioners. This includes equipping graduates with the skills this navigate ethical and clinical complexities and encouraging awareness of broader issues such as algorithmic bias and global disparities in access to AI tools. With thoughtful integration into medical curricula, AI can enhance care, reduce inefficiencies, and serve as a force for innovation without compromising the human connection at the heart of medicine. By embedding thoughtful AI education today, medical schools can ensure that the doctors of tomorrow lead a healthcare system that is both technologically advanced and deeply human” [2].

Other researches [3, 4] deserve attention as well. Based on a review of a significant number of sources, the authors of the article came to an important conclusion: “In summary, the integration of AI into healthcare education has been widely embraced due this its capacity this enhance diagnostic precision and simplify intricate medical procedures. Nevertheless, it is important to note the existing gaps in the literature that can guide future research endeavors. Firstly, it is critical to develop long-term training protocols that not only evaluate the lasting impacts of AI on healthcare training but also ensure continuous updates in the curriculum this reflect fast technological changes.

Secondly, integrating the patient's perspective into AI applications is essential for preserving a patient-centered approach in healthcare. Future investigations should focus on measuring patient satisfaction and the direct impacts of AI-trained professionals on patient outcomes. Thirdly, the advancement in explainable AI (XAI) models is also crucial. Improving the transparency of AI decision-making processes will help build trust and acceptance among healthcare providers, ensuring AI is used ethically in clinical settings. Finally, the question of accountability in medical decisions involving AI cannot be overlooked. Establishing clear legal and ethical guidelines is imperative to navigate the complexities and maintain trust in AI-enhanced healthcare settings. Moving forward, addressing these challenges with thoroughly research and strategic policy making will be pivotal in maximizing the benefits of AI for training healthcare providers and enhancing patient care outcomes" [5].

The editorial staff of the magazine "Wiadomości Lekarskie" it will be interesting to know how the GPT-4 artificial intelligence was evaluated by specialists who read articles from this popular journal on the topic of AI. Upon our request, we received information that these are mainly: «Medical specialists and doctors who are interested in applying new technologies to improve clinical decisions and medical practice. Scientists and researchers in the field of medicine, healthcare and medical technologies who follow new trends and research in artificial intelligence. Lawyers and ethicists who analyze the legal and ethical aspects of introducing AI into the medical field. Medical teachers and educators in higher medical educational institutions who introduce innovations into educational programs. Administrative and managerial employees of medical institutions who plan to use AI to manage and optimize processes.»

In 2025, we decided to propose twenty paragraphs for the textbook «Medical Pedagogy». The study of European experience allowed us to conclude that this is one of the promising areas for improving the quality of education for students of medical universities. Summarizing many years of teaching experience, we were able to substantiate the structure of the textbook, comparing the results later with the proposals of artificial intelligence. The results are presented in Table 1. For comparison, exploring the possibilities of artificial (computer) intelligence, we asked the GPT chat to prepare twenty paragraphs for the textbook. In a second, the computer system proposed its option. The results are presented in Comparative Table 1.

Draw your own conclusions about the competition between a human and a computer system. Pay attention to similar topics and differences. These topics cover

key aspects of medical pedagogy according to modern standards and the structure of leading textbooks.

We will not search winner of the competition human and artificial intelligence. Faster language it's about interaction and rational using information. It should be noted another feature. We spent our native testing capabilities of artificial intelligence [6, 7]. The results will be of interest to specialists. Artificial intelligence coped well with the definition of concepts, review of literary sources, and originally reviewed the author's articles. Difficulties were evident in solving creative, non-standard tasks, giving advice when suicidal thoughts arise, and responding to text with elements of humor and irony. Among students and teachers of medical universities, the expert assessment of the advantages and disadvantages of loneliness [8] attracted particular attention. These areas require further study.

To understand the capabilities of artificial intelligence, it is worth paying attention to how the computer system searches for information about the popularity of topics when studying AI in Ukraine. The only requirement is the accuracy of the formulation of questions and an assessment of the objectivity of the results. We received the following answer, which will be interesting for a wide range of specialists: "A review of educational programs, courses and research in recent years shows that the greatest interest among Ukrainians is in the following areas and topics in the field of artificial intelligence: 1. *Machine learning (ML) and working with data.* The greatest emphasis in schools, universities and private educational centers is on data analysis, designing machine learning models and products based on them. More than 60% of AI/ML specialists in Ukraine work in this area. 2. *Generative AI*, working with modern large-scale language models (Chat GPT, Gemini, Claude), creating texts, images, code - one of the most demanded educational competencies. 3. *Using computer models* for object recognition in images, application in medical diagnostics, agribusiness, protection. 4. *Ethical and legal aspects of AI.*

A significant part of educational courses, especially government and partnership courses with Google and Diia, contain modules on ethics, privacy, security and regulation in the field of AI. 5. *AI in business, education and management.*

The emphasis is on the practical use of AI technologies for automating business processes, analyzing markets, implementing in public administration, education, medicine, and the agricultural sector. 6. *Automation of routine tasks and digital transformation.* 7. *Courses from Google, Diia and other platforms* focus on skills that can significantly increase efficiency by automating work processes. 8. *Using AI in cybersecurity and defense.*

**Table 1.** Comparative characteristics of topics for the textbook «Medical Pedagogy»

| Teacher's suggestions (A. Vykhreshch )                               | Artificial intelligence suggestions                                                                 |
|----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| 1. System of basic concepts. Lexicography.                           | 1. Introduction to medical pedagogy: modern teaching and role in the training of medical personnel. |
| 2. Regularities of development of educational systems.               | 2. History of the development of medical pedagogy and medical education.                            |
| 3. The formation and development of medical pedagogy. Periodization. | 3. Subject, tasks and methods of medical pedagogy.                                                  |
| 4. Medical rhetoric: past and future.                                | 4. Basic categories and concepts of medical pedagogy.                                               |
| 5. Pedagogical culture of medical university teachers.               | 5. Pedagogical process in higher medical school: structure and features.                            |
| 6. Student personal development.                                     | 6. Features of educational and cognitive activities of medical students.                            |
| 7. Medical pedagogy in the context of comparative studies.           | 7. Principles and patterns of learning in medical education.                                        |
| 8. Content, forms, and methods of teaching.                          | 8. Didactics of medical disciplines: content, forms and methods.                                    |
| 9. Psychological characteristics of patients.                        | 9. Innovative technologies of education in medicine.                                                |
| 10. Praxeology in the educational process.                           | 10. Monitoring and evaluation of academic achievements of medical students.                         |
| 11. Fundamentals of acmeology.                                       | 11. Theory of education in medical education: main directions and tasks.                            |
| 12. Pedagogical personology.                                         | 12. Formation of professional communicative competence of a doctor.                                 |
| 13. Communication in medicine.                                       | 13. Medical ethics and deontology in the pedagogical process.                                       |
| 14. Integral thinking of the individual.                             | 14. Interdisciplinary integration in medical pedagogy.                                              |
| 15. Information culture of a professional.                           | 15. Organization and implementation of preventive work using medical pedagogy.                      |
| 16. Development of creative abilities of the individual.             | 16. Corrective and practical medical pedagogy.                                                      |
| 17. Anamnesis: theory and practice.                                  | 17. Inclusive education in medical pedagogy.                                                        |
| 18. Iatrogenicity: causes and consequences.                          | 18. Socio-psychological aspects of teaching and educating student youth.                            |
| 19. Psychological and pedagogical culture of a professional.         | 19. Pedagogical competencies of a higher medical education teacher.                                 |
| 20. Prospects for the development of medical pedagogy.               | 20. Prospects for the development of medical pedagogy in the context of educational reform.         |

Source: compiled by the authors of this study

Ukraine is actively integrating AI into digital security and the defense sector, which is reflected in thematic courses and projects. It should be noted that AI not only offered generalized information from researchers, but also named scientific conferences whose materials were used and published in 2025 [9, 10].

It is worth noting that one general tendency inherent in book authors. A sufficiently professional theoretical base has been created in monographs and manuals on the possibilities of AI [11 - 18]. Now necessary move to system examples with the transition from medicine to specific subareas i.e. pediatrics, surgery, therapy etc. Let us emphasize importance exactly systemic assessments, not individual ones examples. It is no coincidence experts The Club of Rome in the anniversary reports "Come on" in December 2017 set the development of integral thinking as a priority task.

In our opinion, a promising task is to compare state documents on the use of artificial intelligence in dif-

ferent countries. For example, in December 2020, the Ukrainian government approved the "Concept for the Development of Artificial Intelligence in Ukraine". This document emphasizes that Ukraine is "a member of the Special Committee on Artificial Intelligence at the Council of Europe, in October 2019 it joined the "Organization for Economic Cooperation and Development, Recommendation of the Council on Artificial Intelligence, OECD/LEGAL/0449", and artificial intelligence is considered as "an organized set of information technologies, with the use of which it is possible to perform complex complex tasks by using a system of scientific research methods and algorithms for processing information obtained or independently created during work, as well as create and use their own knowledge bases, decision-making models, algorithms for working with information and determine ways to achieve the set tasks"[19]. As a positive fact, we note the objective recognition of existing problems. According to the

**Table 2.** Definition of the concept of «health» by medical university scientists

| Structure                                                                                                                                                         | scientists | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|---|---|---|---|---|---|---|---|---|----|----|----|
| Human greatest treasure                                                                                                                                           |            |   | + |   |   |   |   |   |   |   |    |    |    |
| The state of organs and systems in which they perform their functions well                                                                                        |            |   | + |   |   |   |   | + |   |   |    |    |    |
| The state of organs and systems in which the full functioning of the body is ensured                                                                              |            |   | + | + |   |   | + |   |   |   |    |    |    |
| A state of complete emotional, mental, physical well-being, balance                                                                                               |            | + |   |   | + | + |   | + | + | + | +  | +  |    |
| Social integration of the individual                                                                                                                              |            |   |   |   |   |   | + |   |   |   |    |    |    |
| Multidimensional dynamic state that develops in the process of realization of genetic potential in the conditions of a specific social and ecological environment |            |   |   |   |   |   |   |   |   | + |    |    |    |
| A condition that allows a person to perform their biological and social functions to varying degrees                                                              |            |   |   |   |   |   |   |   |   | + |    |    |    |
| The process of preserving and developing physiological properties and potentials                                                                                  |            |   |   |   |   |   |   |   |   | + |    |    |    |
| The process of maximizing life expectancy with optimal work capacity and social activity                                                                          |            |   |   |   |   |   |   |   |   | + |    |    |    |
| Absence of diseases and defects                                                                                                                                   |            |   |   |   |   |   |   |   |   |   |    |    | +  |

Source: compiled by the authors of this study

authors of the order, there are as many as 17 of them: from the low level of digital literacy, awareness of the population regarding the general aspects, opportunities, risks and safety of using artificial intelligence to the lack of modern advanced training programs for teachers of higher education institutions.

The experience of Polish colleagues deserves special attention. We mean a special portal dedicated to AI [20], the results of a unique sociological survey of Poles' opinions on artificial intelligence [21, 22], analytical books of the level [23], good examples of medical care for the elderly using AI [24].

In scientific research, insufficient attention is paid to keywords. In our works (A. Vykhreshch and O. Khrystenko) we studied the opinion of future doctors regarding keywords in medicine and received eloquent data. According to students, these are primarily "mutual understanding, responsibility, faith, diagnosis, trust, help, experience, life, health, knowledge, doctor, treatment, humanity, medicine, peace, skills, organism, professionalism, prevention, mind, sterility, resilience, stress resistance, accuracy, care, help, "do no harm", empathy, improvement" [25]. With a similar task for artificial intelligence, we received the following list: doctor, patient, diagnosis, disease, symptom, therapy, treatment, prevention, research, organism. This is an interesting topic for further research, discussion in student classrooms, and modeling a successful doctor.

In addition, we asked first-year students to choose one word that characterizes a medical university graduate.

The answers are quite interesting: "adaptation, endurance, courage, responsibility, discipline, enthusiasm, motivation, learning, perseverance, memory, cognition, hard work, confusion, diligence, curiosity, purposefulness" [25]. To a similar question about artificial intelligence, we received no fewer interesting answers: 1. *High level of emotional intelligence* - the ability to recognize and manage one's own emotions and show empathy to the point of satisfaction. 2. *Communication skills* - the ability to communicate effectively with patients, parents, and colleagues, which is important for medical practice. 3. *The desire for self-development and learning* is the motivation to constantly improve one's qualifications, master new knowledge and skills. 4. *Responsibility and professionalism* - a serious attitude towards training and clinical practice. 5. *Ability to adapt to change* - skills for working in the dynamic and stressful conditions of modern medicine. 6. *Practical orientation* - active use of simulation centers and stages to consolidate theoretical knowledge. 7. *Teamwork* is the ability to collaborate effectively within medical teams. 8. *Mobility and readiness for academic exchanges* - willingness to participate in mobility and exchange of experience. 9. *A holistic view of health* is an understanding of the biomedical, social, and cultural aspects of medicine. 10. *Psychological resilience* - the ability to manage stress and emotional burnout.

Meanwhile, the keywords regarding the negative qualities of modern youth are interesting: apathy, irresponsibility, sedentary lifestyle, inflated self-esteem,

self-confidence, frivolity, laziness, arrogance, imperfection, inconstancy, oversaturation" [25]. How artificial intelligence reacted to a similar question: "Stress vulnerability - difficulties in managing emotions in stressful situations. Lack of practical experience at the initial stages of training - sometimes theoretical training prevails over practice. Overload - a large amount of educational material, which can lead to burnout. Sometimes insufficient motivation for independent learning - dependence on teachers and help. Problems with time organization and self-discipline - difficulties in balancing study and personal life. Tendency to perfectionism - which can lead to internal pressure and anxiety. Limited conflict management skills - difficulties in solving problems in a team. Insufficient development of leadership qualities at the beginning of training. Heterogeneity of the level of initial training among students - difference in knowledge of basic sciences. Some inertia in the use of modern digital technologies at the start of training - the need for additional training.

In our opinion, it is advisable to continue these studies in different countries for comparative characteristics and identification of priorities in the youth environment.

In the folk culture of many nations there is an interesting saying «I don't have a clue». In fact, this is a very important problem. The outstanding Polish educator Wincenty Okon argued that the level of lexicography determines the level of science [26]. In one of the articles, we highlighted the problem of defining the term «health» (Table 2). It would be advisable to conduct a comparative characterization of the essence of this concept among representatives of different cultures. This is important for several reasons: «First, teachers use the concept of health in the process of teaching students and thus «replicate» their position. Second, the correlation of theoretical generalizations, regulatory documents, recommendations of the World Health Organization and the position of practitioners is important, which, by the way, can become the topic of a separate study» [27].

## CONCLUSIONS

To understand the patterns of development of new phenomena, it is necessary to study the periodization of their formation, identify stages and define prospects. When it comes to artificial intelligence, four main stages can be distinguished. First, these are the works of mathematicians and philosophers who prepared the theoretical basis of programming. It is obvious that this preparatory stage can be divided into separate parts. The second stage coincides with the emergence and spread of computers. The beginning of the third stage was the availability of the Internet. Currently, we are witnesses

of the rapid development of artificial intelligence. Note that each subsequent stage required less time. The need to continue theoretical generalizations is obvious. At the same time, a dynamic process of assessing the possibilities of using artificial intelligence in various types of human activity is underway. Medicine is no exception.

Researchers underline the significant potential of artificial intelligence in organizing the administrative work of medical institutions. This refers to information about staff and patients, organizing communication schedules with specialists of the relevant profile, and optimizing schedules.

The next higher stage is information about the course of the disease, comparison with similar cases, recommendation of the computer system regarding the search, availability, and choice of medical drugs. It is worth emphasizing that this information can only be of a recommendatory nature, only the doctor in the process of anamnesis can take into account the individual characteristics of the patient, the dynamics of changes in health, and make a final decision on the treatment strategy.

Studying the features of the use of artificial intelligence, especially among young people, an alarming trend has been recorded regarding the spread of self-medication, and in the case of a positive result, the promotion of its advantages among peers. We note the possibility of manipulative influence. The authors of computer programs have worked so that in the process of communicating with artificial intelligence, the user not only receives answers to questions, but also receives information on an emotional level, with elements of empathy, support, and compassion.

The possibilities of using artificial intelligence in medical universities are promising. Teachers have the opportunity to access generalized information of world level, the preparation of illustrative materials is significantly facilitated, with the help of simulation training it is possible to show the course of the disease, the dynamics of changes in the body, the functioning of organs and the body as a whole. The work of postgraduate and doctoral students is significantly facilitated. It is through the intelligent use of artificial intelligence in the process of training future doctors that it is possible to achieve optimal use of its capabilities in the future.

When used correctly, artificial intelligence has many positive opportunities for self-improvement and professional development of students. Interesting examples are the comparison of the results of a brainstorming session, a student group, and solving a similar problem with artificial intelligence. The threat of violating the norms of academic integrity, substituting creative search, and one's own efforts for the help of artificial intelligence is also obvious.



The idea of testing the capabilities of artificial intelligence by practitioners of various levels and promptly summarizing the results and discussing them on the pages of periodicals is promising.

Nowadays, we are witnessing a transition to a qualitatively higher level of information culture. This is another civilizational challenge of the globalized world, which representatives of science and practice will have to take into account.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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 – Work concept and design,  – Data collection and analysis,  – Responsibility for statistical analysis,  – Writing the article,  – Critical review,  – Final approval of the article

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# Correlation between skeletal maturity assessed by spheno-occipital synchondrosis (SOS) fusion and cervical vertebrae maturation index (CVMI): A systematic review and meta-analysis

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## ABSTRACT

This review aims to systematically collect and analyze studies investigating the correlation between the skeletal age assessed by spheno-occipital synchondrosis (SOS) fusion and CVMI (cervical vertebrae maturation index). Based on PECO, a research question was framed as “Is there a correlation between skeletal maturity assessed by spheno-occipital synchondrosis (SOS) fusion and cervical vertebrae maturation index (CVMI)?” The review was submitted for registration in PROSPERO (receipt 550152). Keywords: skeletal maturity; spheno-occipital synchondrosis; and cervical vertebrae maturation were used to search data in the search engines: PubMed Central, Lilac, EBSCOhost, Science Direct, and Google Scholar. The search question was formulated as “skeletal maturity” AND “spheno-occipital synchondrosis” (OR “SOS”) AND “cervical vertebrae maturation” (OR “CVMI”). The data extracted from the articles was tabulated and a meta-analysis of correlation was performed. Overall, a strong positive correlation ( $r = 0.876$ ) between SOS fusion and CVMI was found, with significant statistical support ( $p < 0.01$ ). Gender-specific analyses also showed strong correlations for both males ( $r = 0.898$ ) and females ( $r = 0.877$ ), however, high heterogeneity was observed, suggesting variability among studies. This systematic review demonstrates a strong positive correlation between SOS fusion and CVMI, suggesting that SOS fusion can be a reliable indicator of skeletal maturity. However, the considerable heterogeneity observed, particularly in gender-specific analyses, warrants further investigation with a homogenous population and larger sample size.

**KEY WORDS:** skeletal maturity, spheno-occipital synchondrosis, cervical vertebrae maturation index

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## INTRODUCTION

In orthodontics and orthopedics assessment of skeletal maturity is crucial for determining growth status and planning appropriate treatment strategies. The gold standard for assessing skeletal maturity is the hand-wrist (HW) maturation method. However, HWM assessment exposed patients to an unnecessary dose of radiation hence, the cervical vertebral maturation (CVM) in the lateral cephalometric radiographs was evaluated for its correlation to the skeletal maturity as an alternative [1, 2]. The lateral cephalometric radiograph is routinely required for orthodontic diagnosis and treatment planning and, therefore, no extra radiograph is needed. But, this method is not sensitive in detecting the growth maturity in periods away from the growth spurt [3].

The spheno-occipital synchondrosis (SOS) located in the midline between the sphenoid and occipital bones and is considered the most important growth center in the cra-

nial base because of its late ossification and contribution to post-natal cranial base growth [1, 2]. The cranial base is the template for facial development; therefore, it is directly related to the maxillary and mandibular growth. Understanding the correlation between SOS-assessed skeletal age and cervical vertebrae maturation index (CVMI) is thus, crucial for refining clinical assessments and treatment planning in orthodontics and orthopedics. While both methods aim to assess skeletal maturity, variations in their application and interpretation exist. Therefore, a systematic review and meta-analysis was deemed essential to synthesize existing evidence and evaluate the strength of correlation between these two measures.

## AIM

This review systematically collects and analyzes studies investigating the correlation between the

skeletal age assessed by SOS fusion and CVMI. By synthesizing these findings, we aim to provide clinicians with a comprehensive understanding of the relationship between these two methods of skeletal maturity assessment.

MATERIALS AND METHODS

The review was submitted for registration in PROSPERO (Registration Id: CRD42024550152). PRISMA guidelines were followed for conducting this review [4].

RESEARCH QUESTION: PECO

P (Problem): Healthy participants  
E (Exposure): SOS  
C (Comparison): CVMI  
O (Outcome): Correlation between skeletal maturity assessed by SOS and CVMI

On the basis of PECO, research question was reframed as “Is there a correlation between skeletal maturity assessed by spheno-occipital synchondrosis (SOS) fusion and cervical vertebrae maturation index (CVMI) among healthy participants?”

SEARCH STRATEGY

Using keywords: skeletal maturity; spheno-occipital synchondrosis; cervical vertebrae maturation, search engines: PubMed Central, Lilac, EBSCOhost, Google Scholar, and Science Direct were used by applying the Boolean operator “AND”. The search question was formulated as “skeletal maturity” AND “spheno-occipital synchondrosis” (OR “SOS”) AND “cervical vertebrae maturation” (OR “CVMI”). Cross-sectional or longitudinal studies that compared the skeletal maturation assessed by SOS fusion and CVMI in healthy patients were included. Studies using data of syndromic patients, cleft lip/palate, scoliosis, or other skeletal abnormalities, and those with a history of fracture in craniofacial base were excluded. Abstracts, dissertation, theses, patents, convention abstracts, opinion articles, commentaries, article not in English language, and animal study were also excluded.

The literature was reviewed in the entirety up to 31<sup>st</sup> May 2024 for clinical studies evaluating correlation between skeletal maturity assessed between SOS fusion and CVMI, irrespective of the method used. Title and abstract screening was done by two authors independently (A.S and S.V), followed by retrieval of full text reports of the eligible articles. All the available full text was compiled and thoroughly scrutinized.

Table 1. Summary of studies evaluating the correlation between SOS fusion and CVMI

| Author    | Year | Popula-<br>tion   | Sample<br>size<br>(n) | n<br>(M) | n<br>(F) | mean<br>age<br>(M) | mean<br>age<br>(F) | Age<br>range<br>(year) | Test used               | r     | r (M) | r (F) | SOS<br>method          | SOS<br>stages | CVMI<br>method            | CVMI<br>stages |
|-----------|------|-------------------|-----------------------|----------|----------|--------------------|--------------------|------------------------|-------------------------|-------|-------|-------|------------------------|---------------|---------------------------|----------------|
| Kocasarac | 2016 | Turkish           | 116                   | 43       | 73       | 15.13              | 16.39              | 8 to 28                | Spearman<br>correlation |       | 0.851 | 0.618 | Franklin<br>and Flavel | 4             | Hassel<br>and Far-<br>man | 6              |
| Perez     | 2016 | North<br>American | 315                   | 167      | 148      | 15.6+<br>_7.3      |                    | 6 to 23                | Spearman<br>correlation | 0.89  | 0.89  | 0.88  | Bassed<br>et al        | 5             | Baccetti                  | 6              |
| Fayad     | 2020 | Lebanese          | 117                   | 55       | 62       | 13.7               | 12.6               | 8 to 18                | Spearman<br>correlation | 0.852 | 0.839 | 0.868 | Franklin<br>and Flavel | 4             | Baccetti                  | 6              |
| Kim SM    | 2023 | Korean            | 630                   | 308      | 322      | 6.0-18.0           |                    |                        | Spearman<br>correlation |       | 0.955 | 0.964 | Bassed<br>et al        | 5             | Baccetti                  | 6              |

Source: Authors’ own work

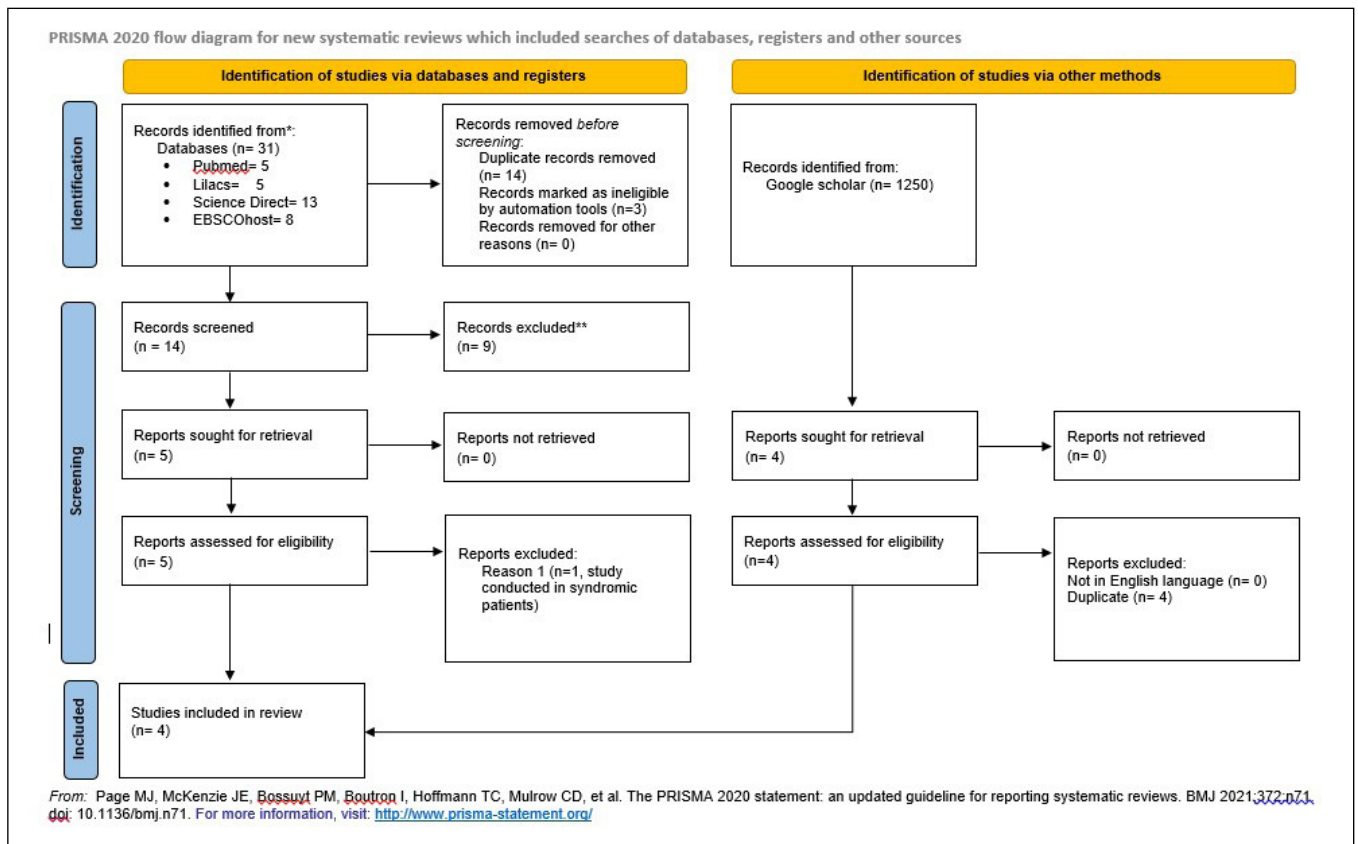


**Fig. 1.** Search strategy

Source: [4]

## DATA EXTRACTION

Two authors (A.S and S.V) independently searched the data. A third author (S.A.M) resolved any conflict arising in case of disagreement amongst the two authors regarding eligibility of an article. The data was entered in the specially designed excel sheet. The characteristics of the studies: author, year, country, sample size (n), age (range/ mean, standard deviation), gender, method used for skeletal maturity assessment (SOS and CVM), spearman correlation value (r) both combined and separately for males and female, and any other reported outcome measures were recorded (Table 1). RevMan (Review Manager Computer program version 5.4.1 The Cochrane Collaboration, 2020) was used for risk of bias assessment of individual studies [5]. Quality assessment was done using QUADAS-2 tool [6].

## DATA ANALYSIS

Meta-analysis of correlation coefficient obtained was performed in the Medcalc software v 12.7.8 (Medcalc software, Ostend, Belgium). Hedges-Olkin method was used as it reduces the risk of Type 1 error as compared to Schmidt-Hunter method if the studies are homogenous. Heterogeneity was assessed using  $I^2$  statistic.

## REVIEW

### LITERATURE SEARCH

After applying the aforementioned filters and removing duplicates, five articles were left for screening. From these, one article was removed after reading the title and abstract. Four articles were assessed for eligibility. Thus, a total of four articles were found suitable for the review (Fig. 1).

Quality assessment of the selected articles was done using RevMan software [5]. The studies were critically appraised under four categories: patient selection, index test, reference standard, and flow and timing. Each category was further evaluated under the following domains: description, signaling questions ("yes", "no", or "unclear"), risk of bias ("high", "low" or "unclear"), and concerns about applicability ("high", "low" or "unclear"). All studies showed low risk of bias and low applicability concerns suggesting that the result of the studies are reliable and valid (Fig. 2-3).

For SOS assessment, two studies (Kim and Perez) [7, 8] used Frankel and Flavin's [9] method, and two (Fayad and Kocasarac) [10, 11] used Bassed et al's method [12]. Both these methods determine skeletal maturity, but differ in their level of details for assessment. Franklin and Flavel used a three- point scoring method (unfused, partly fused, completely fused) providing a broad

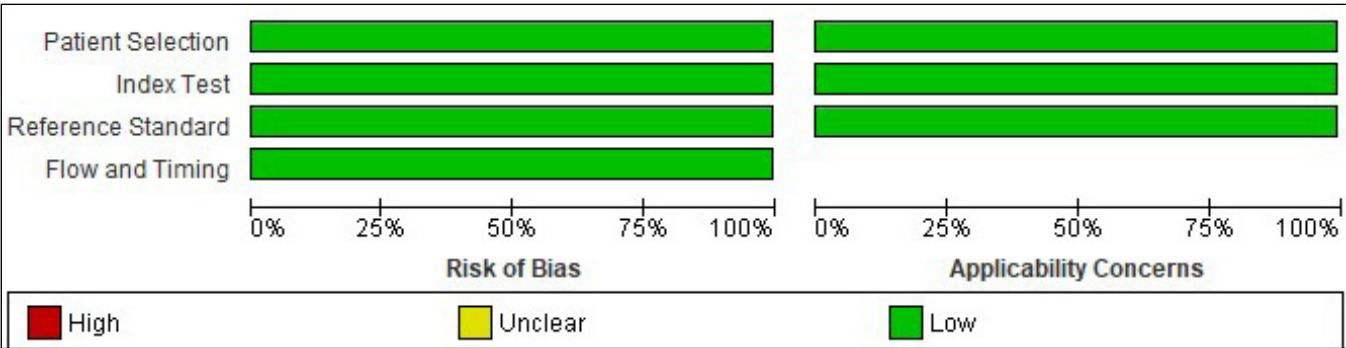


**Fig. 2.** Risk of bias and applicability concerns graph: review authors’ judgements about each domain presented as percentages across included studies  
*Source: Authors’ own work*

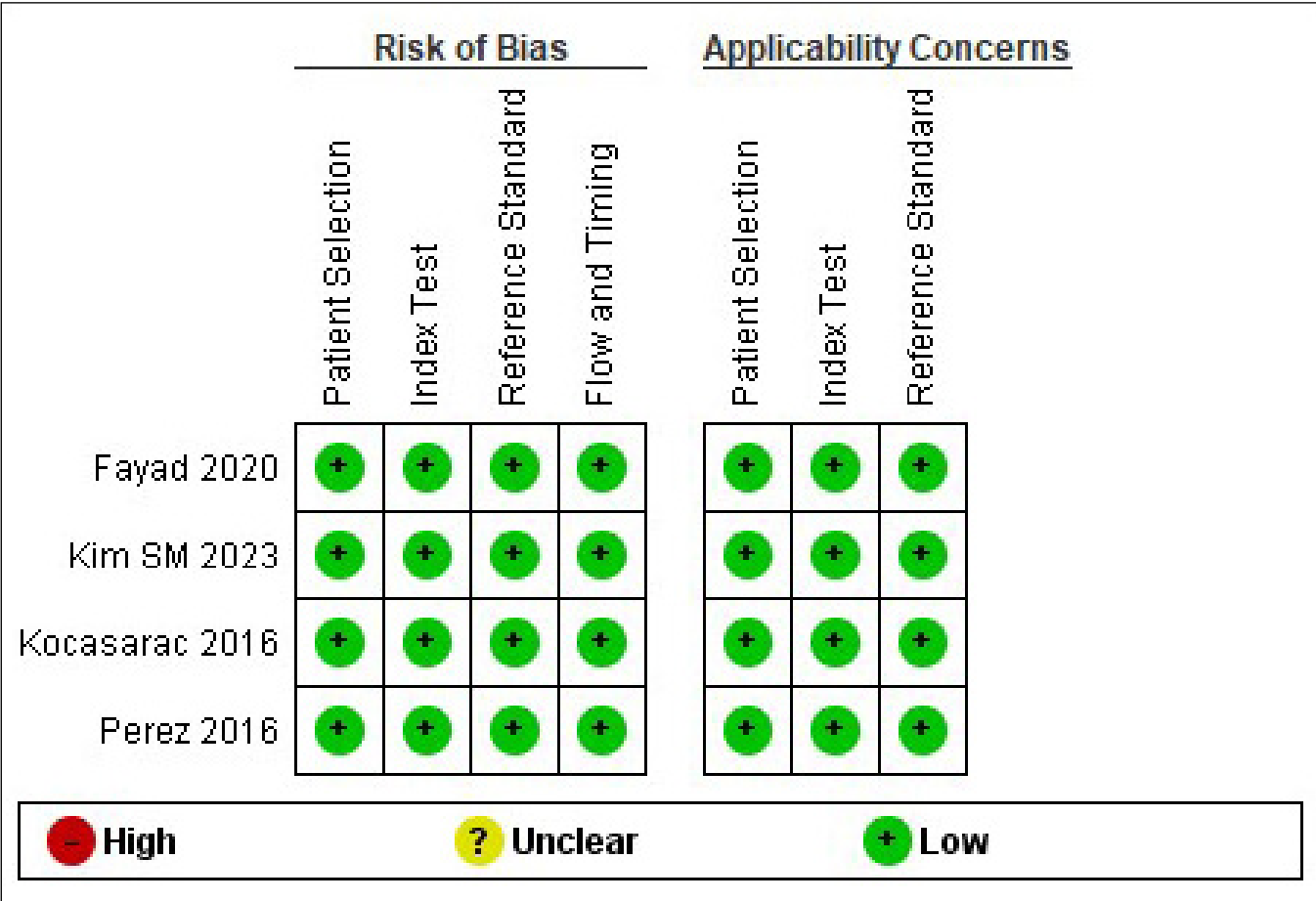


**Fig. 3.** Risk of bias and applicability concerns summary: review authors’ judgements about each domain for each included study  
*Source: Authors’ own work*

classification of fusion stages. In contrast, Bassed et al’s system used a detailed five stage scoring system (completely open, initial ossification, partial fusion, nearly complete fusion, and complete fusion). For CVMI, three studies [7, 8, 10] used Baccetti et al’s method [13] while one (Kocasarac) [11] used Hassal and Farman’s method [2]. As compared to Hassel Farman, Baccetti et al’s method uses more detailed description of morphology of vertebrae and can be considered superior to former.

Of the four studies, correlation between SOS fusion and CVMI was higher in males [8,10,11], except in the study by Kim SM [7]. They also found that SOS fusion stages were more advanced in females as compared to males. The correlation coefficient from the combined studies was 0.876 with 95% confidence interval, suggesting a strong positive relationship between SOS and CVMI (Figure 4). The p value was <0.01 which is statistically significant (Table 2a). The I<sup>2</sup> value is 52.34% implicating moderate heterogeneity (Table 2b).



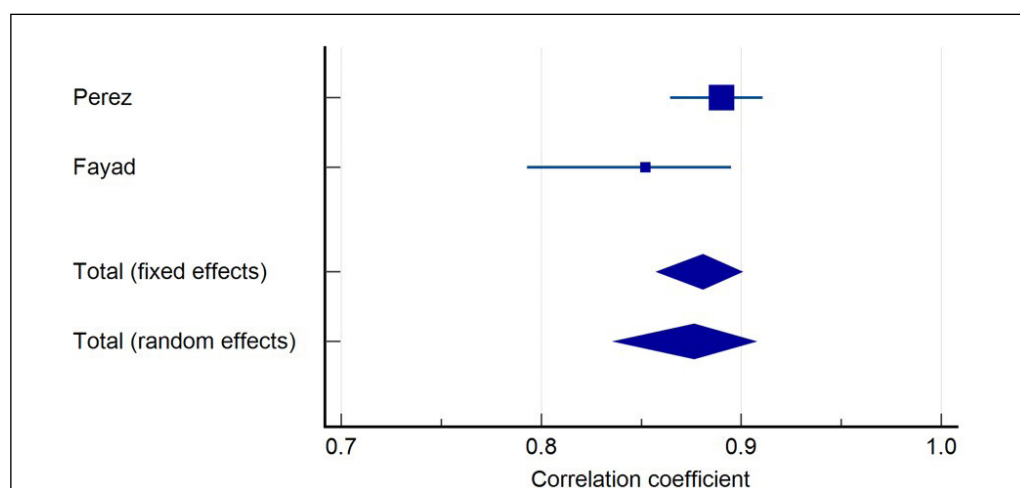


**Fig. 4.** Forest plot without gender specification

Source: Authors' own work

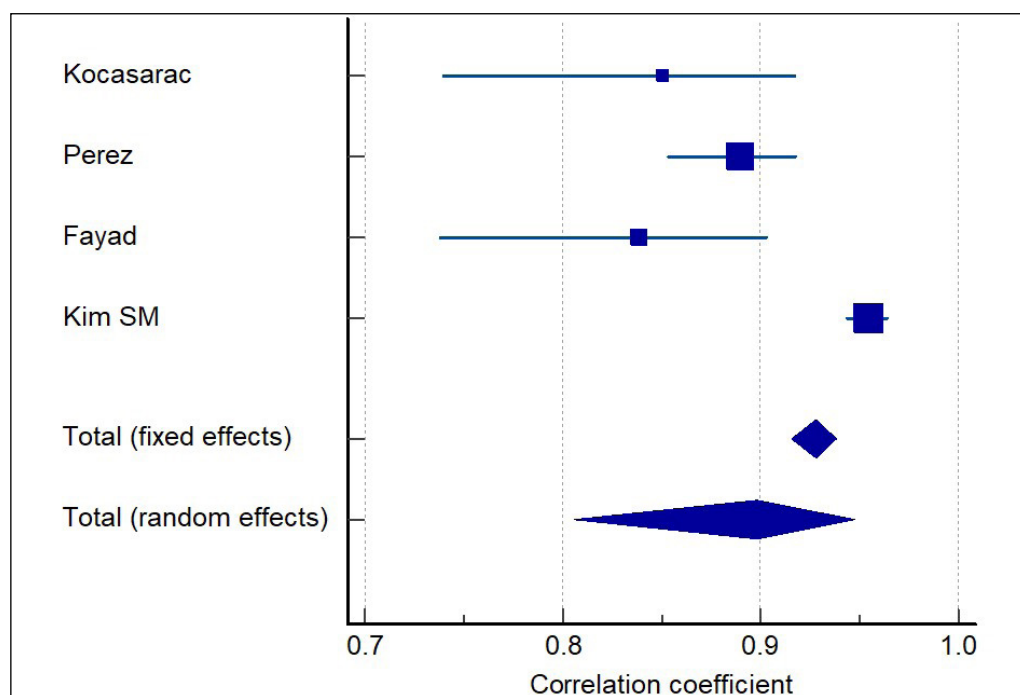


**Fig. 5.** Forest plot for males

Source: Own materials

The meta-analysis was also conducted separately for males and females. For males, the meta-analysis yielded a correlation coefficient ( $r$  value) of 0.898 with 95% confidence interval, suggesting a strong positive correlation (Fig. 5). The  $p$  value was  $<0.01$  which is statistically significant (Table 3a). The  $I^2$  value of 92.73% suggests considerable heterogeneity among the studies included in the meta-analysis (Table 3b).

For females, the meta-analysis yielded a correlation coefficient ( $r$  value) of 0.877 with 95% confidence interval, suggesting a strong positive correlation (Fig. 6). The  $p$  value was  $<0.01$  which is statistically significant (Table 4a). The  $I^2$  value of 97.42% suggests considerable heterogeneity among the studies included in the meta-analysis (Table 4b).

Kocasarac et al., [11] in addition to determining correlation between SOS and CVMI, also found a strong correlation between chronological age and third molar

mineralization, age and SOS fusion, age and CVMI fusion, TMM and SOS fusion, and TMM and CVMI.

## DISCUSSION

Lateral cephalometric radiographs have been an integral part of orthodontic treatment. Therefore, CVMI gets seamlessly integrated into the clinical set up, eliminating the need for additional mode of investigation for skeletal age assessment [13]. Previous studies have established its simplicity and reliability in assessing skeletal maturity [2, 14].

However, CVMI has its own limitations. Since CVMI is based on visual assessment of morphological changes in vertebrae, it is at risk for subjective variability [15]. Also, it is inadequate in determining small changes in skeletal maturity over short periods of time, hence cannot be used in conditions where precise growth

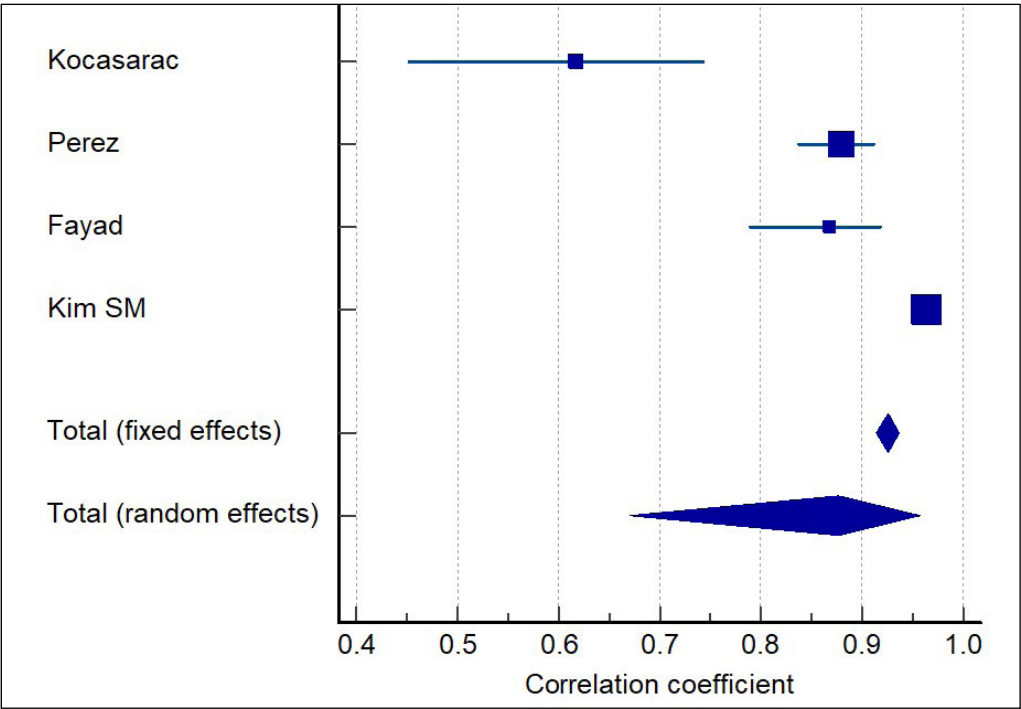


**Fig. 6.** Forest plot for females  
Source: Own materials

assessment is needed. Since CVMI varies with age and gender, it cannot be used in patients who do not follow typical growth patterns [14].

SOS located in the base of the skull is the nidus of craniofacial growth. SOS undergoes distinct sequential changes corresponding closely to somatic changes hence, is considered a reliable indicator of pubertal growth spurt [1]. SOS fusion is more of an objective assessment as compared to CVMI which is subjective in nature. The assessment of SOS not only provides an insight of skeletal maturity but also the craniofacial growth making it more comprehensive as compared to other skeletal assessment markers [16].

The present systematic review aimed to evaluate the correlation between SOS and CVMI in assessing skeletal maturity.

**CORRELATION BETWEEN SOS AND CVMI**

The overall analysis indicated a strong correlation between SOS fusion and CVMI. The combined correlation coefficient value was 0.876. The 95% confidence interval and a p value of <0.01 reinforce the statistical significance and strength of this relationship. The moderate heterogeneity (I<sup>2</sup> value of 52.34%) is considered acceptable, suggesting that the variability among the studies does not significantly impact the overall findings.

**GENDER SPECIFIC META-ANALYSIS**

For males, the correlation coefficient 0.898 with a 95% confidence interval and a p value of <0.01, indicating a

strong positive correlation. However, the high I<sup>2</sup> value of 92.73% suggests considerable heterogeneity, indicating significant variability among the studies.

For females, the correlation coefficient was 0.877 with a 95% confidence interval and a p value of <0.01, also indicating a strong positive correlation. Similarly, the I<sup>2</sup> value of 97.42% suggests considerable heterogeneity among the studies.

The strong positive correlations in both the overall and gender-specific analyses underscore the potential utility of SOS fusion as an indicator of skeletal maturity alongside CVMI. However, the considerable heterogeneity in the gender-specific analyses highlights the need for cautious interpretation. The high heterogeneity could be attributed to variations in study designs, population demographics, or assessment methodologies.

Given the strong positive correlation between the two methods, for routine cases, clinicians especially orthodontists can consider CVMI over SOS fusion for assessing skeletal maturity. In cases requiring precise measurement of craniofacial growth or cases where CBCT is indicated for other concomitant factors, the clinicians can rely on SOS fusion.

**CLINICAL IMPLICATIONS**

The assessment of skeletal maturity is crucial for timing growth-dependent therapies. In orthodontics, functional appliances for mandibular retrognathia, rapid maxillary expansion (RME), headgear for Class III malocclusion, and vertical growth control procedures are

**Table 2a.** Spearman correlation (r) without gender specification

| Study                  | Sample size | Correlation coefficient | 95% CI         | z      | P      | Weight (%) |        |
|------------------------|-------------|-------------------------|----------------|--------|--------|------------|--------|
|                        |             |                         |                |        |        | Fixed      | Random |
| Perez                  | 315         | 0.890                   | 0.865 to 0.911 |        |        | 73.24      | 61.08  |
| Fayad                  | 117         | 0.852                   | 0.793 to 0.895 |        |        | 26.76      | 38.92  |
| Total (fixed effects)  | 432         | 0.881                   | 0.858 to 0.900 | 28.473 | <0.001 | 100.00     | 100.00 |
| Total (random effects) | 432         | 0.876                   | 0.836 to 0.907 | 17.599 | <0.001 | 100.00     | 100.00 |

Source: Authors' own work

**Table 2b.** Test for heterogeneity

|                                |               |
|--------------------------------|---------------|
| Q                              | 2.0981        |
| DF                             | 1             |
| Significance level             | P = 0.1475    |
| I <sup>2</sup> (inconsistency) | 52.34%        |
| 95% CI for I <sup>2</sup>      | 0.00 to 88.07 |

Source: Authors' own work

**Table 3a.** Spearman correlation (r) for males

| Study                  | Sample size | Correlation coefficient | 95% CI         | z      | P      | Weight (%) |        |
|------------------------|-------------|-------------------------|----------------|--------|--------|------------|--------|
|                        |             |                         |                |        |        | Fixed      | Random |
| Kocasarac              | 43          | 0.851                   | 0.740 to 0.917 |        |        | 7.13       | 22.78  |
| Perez                  | 167         | 0.890                   | 0.853 to 0.918 |        |        | 29.23      | 26.40  |
| Fayad                  | 55          | 0.839                   | 0.738 to 0.903 |        |        | 9.27       | 23.77  |
| Kim SM                 | 308         | 0.955                   | 0.944 to 0.964 |        |        | 54.37      | 27.04  |
| Total (fixed effects)  | 573         | 0.928                   | 0.916 to 0.939 | 38.930 | <0.001 | 100.00     | 100.00 |
| Total (random effects) | 573         | 0.898                   | 0.806 to 0.948 | 8.255  | <0.001 | 100.00     | 100.00 |

Source: Authors' own work

**Table 3b.** Test for heterogeneity

|                                |                |
|--------------------------------|----------------|
| Q                              | 41.2634        |
| DF                             | 3              |
| Significance level             | P < 0.0001     |
| I <sup>2</sup> (inconsistency) | 92.73%         |
| 95% CI for I <sup>2</sup>      | 84.61 to 96.57 |

Source: Authors' own work

**Table 4a.** Spearman correlation (r) for females

| Study                  | Sample size | Correlation coefficient | 95% CI         | z      | P      | Weight (%) |        |
|------------------------|-------------|-------------------------|----------------|--------|--------|------------|--------|
|                        |             |                         |                |        |        | Fixed      | Random |
| Kocasarac              | 73          | 0.618                   | 0.452 to 0.742 |        |        | 11.80      | 24.68  |
| Perez                  | 148         | 0.880                   | 0.838 to 0.912 |        |        | 24.45      | 25.27  |
| Fayad                  | 62          | 0.868                   | 0.789 to 0.919 |        |        | 9.95       | 24.48  |
| Kim SM                 | 322         | 0.964                   | 0.955 to 0.971 |        |        | 53.79      | 25.58  |
| Total (fixed effects)  | 605         | 0.926                   | 0.914 to 0.937 | 39.671 | <0.001 | 100.00     | 100.00 |
| Total (random effects) | 605         | 0.877                   | 0.671 to 0.957 | 4.854  | <0.001 | 100.00     | 100.00 |

Source: Authors' own work

**Table 4b.** Test for heterogeneity (females)

|                                |                |
|--------------------------------|----------------|
| Q                              | 116.1848       |
| DF                             | 3              |
| Significance level             | P < 0.0001     |
| I <sup>2</sup> (inconsistency) | 97.42%         |
| 95% CI for I <sup>2</sup>      | 95.52 to 98.51 |

Source: Authors' own work

most effective when carried out during pubertal growth spurt. For instance, beginning functional treatment too early in a pre-pubertal child may prolong the therapy with limited skeletal effect, whereas delaying treatment until after growth cessation may eliminate the possibility of orthopedic correction altogether. In pediatric dentistry and craniofacial anomaly management, understanding the growth status aids in sequencing surgical, orthodontic, and prosthetic interventions to achieve stable long-term results. Thus, skeletal maturity indicators help clinicians decide whether to initiate, delay, or alter the treatment modality directly impacting treatment outcomes. Incorporating these tools into clinical decision-making enhances the ability of the clinician to customize therapies to individual growth potential, optimizing both functional and aesthetic outcomes

## LIMITATIONS

Despite the robust findings, the review has certain limitations. Although all the included four studies scored well in the quality assessment tool, it is to be noted that all of them had used convenience sampling, and were cross-sectional studies. None of the studies have been carried out in a longitudinal manner. All studies represented different population groups thus, generalization of the results should be done with caution. The considerable heterogeneity in the gender-specific anal-

yses suggest the need to conduct further studies with larger sample sizes. The use of different methodologies for assessment could have also introduce variability in the results.

## CONCLUSIONS

This systematic review demonstrates a strong positive correlation between SOS fusion and CVMI, suggesting that SOS fusion can be a reliable indicator of skeletal maturity. However, the considerable heterogeneity observed, particularly in gender-specific analyses, warrants further investigation. Due to its practicality and established methodology clinicians can use CVMI for general assessments. For cases requiring detailed analysis of craniofacial growth, incorporating SOS assessments can provide a more comprehensive evaluation. Also, skeletal maturity assessment must be done using the method that uses a detailed description of morphological changes.

## FUTURE DIRECTIONS

Based on the findings of this review, future research should aim to

1. Establish uniform protocols for assessing CVMI and SOS
2. Increase the sample size to enhance generalizability of the results
3. Conduct longitudinal studies to understand the temporal relationship between SOS fusion and CVMI.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# The effectiveness of biosuggestive therapy in treatment of stress-related and psychosomatic disorders

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## ABSTRACT

**Aim:** To provide a comprehensive overview of the biosuggestive therapy (BST), with a critical evaluation of its effectiveness in managing patients with stress-related and psychosomatic disorders, and to review the published research on its therapeutic applications across various clinical conditions.

**Materials and Methods:** We conducted a literature search in the PubMed, Scopus, and Google Scholar databases for articles published between the early 2000s and mid-2025 that focus on examining the effectiveness of the BST method in managing and alleviating symptoms of various stress-related and psychosomatic disorders. During the literature search, we used the following set of key words: biosuggestive therapy, stress-related disorders, psychosomatic disorders, brain–gut axis, psychological distress, functional gastrointestinal disorders, and somatoform disorders. Considering the lack of research conducted and published to date, the authors compiled all the available information into a collection. We analyzed that collection, which ultimately contained 29 publications relevant to the topic of our review, and forwarded it for further analysis.

**Conclusions:** BST is a safe and effective method for treating a wide range of stress-related and psychosomatic disorders. As a technique derived from hypnotherapy, its application in assisting individuals exposed to war-related trauma is gaining increasing attention. In addition, BST has demonstrated positive outcomes in the treatment of chronic pain, depression, anxiety, internet addiction, post-traumatic stress disorder, and logoneurosis. The presented evidence underscores the need for further research and broader implementation of this therapeutic approach.

**KEY WORDS:** anxiety, depression, suggestion, brain-gut axis

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## INTRODUCTION

Rapid scientific and economic changes, alongside the coronavirus disease (COVID-19) pandemic and the ongoing war in Ukraine, have significantly impacted the mental health of the population, contributing to the development of neuropsychological disorders that also affect physical well-being [1, 2]. Ukrainians have experienced prolonged anxiety, fear, and sleep deprivation due to years of air raids and missile strikes. Anxiety and depression influence all organs and systems, leading to a wide range of health disorders [2]. Somatic and psychological states are closely interrelated and demonstrate a direct cause-and-effect relationship, as psychological distress is frequently manifested through physical symptoms. It is estimated that approximately 15–31% of patients seek medical care for various psychosomatic disorders [3].

Under the influence of psychotraumatic factors, the body exhibits elevated levels of stress hormones and mediators, disturbances in the neural regulation of organ and system functions, and disruption of humoral

homeostasis. These processes contribute to the manifestation of disease even in the absence of identifiable organic pathology. In cases where pathology already exists, prolonged stress further exacerbates psychological distress and intensifies the severity or onset of somatic symptoms.

In the context of adjustment and neurotic disorders associated with chronic distress, the primary goal of medical and psychological interventions is to prevent mental maladaptation as well as the development of mental and psychosomatic illnesses. Psychological interventions, including psychotherapy, are essential for preventing psychosomatic and stress-related conditions, complementing pharmacological treatments [4]. Psychotherapy, in particular, has gained increasing relevance in modern clinical practice. Considering the above, the search for effective, evidence-based, and safe pharmacological and/or non-pharmacological approaches that can be applied in both structured and single-session psychotherapeutic or psychocorrective interventions remains an important and timely objective.



One of the methods of psychological interventions is a hypnotherapy-based procedure – biosuggestive therapy (BST) – developed in Ukraine by A. Strashny [5]. It combines suggestion, tactile, verbal, and auditory techniques in individual or group sessions. BST effectively alleviates stress, anxiety, pain, and fatigue, gaining recognition in Ukraine since and during the active stage of the war [6]. An eight-session BST course improves overall health by targeting psychological stress and negative emotions, which are key factors in the dysregulation of the gut–brain axis, normalization of the response to aversive visceral stimuli, and subsequent normalization of visceral sensitivity and motility [7–10]. This method does not require deep hypnosis, which provides an advantage in patient acceptance and easier training for providers [5].

The digestive system is among the organ systems most affected by chronic stress in the human body. In particular, the disruption of neurohormonal regulation leads to the development of disorders of gut–brain interaction (DGBI) [11]. DGBI, formerly termed functional gastrointestinal disorders, represents a group of chronic or recurrent gastrointestinal conditions characterized by symptoms that lack identifiable structural or biochemical abnormalities. DGBI are systematically categorized into distinct subgroups based on anatomical or functional domains, as outlined by the Rome IV framework. These include oesophageal disorders (e.g., functional heartburn, characterized by retrosternal burning without evidence of gastroesophageal reflux), gastroduodenal disorders (e.g., functional dyspepsia, marked by postprandial fullness or epigastric pain), bowel disorders (e.g., irritable bowel syndrome [IBS], defined by recurrent abdominal pain associated with altered bowel habits), and anorectal disorders (e.g., functional defecation disorders) [12, 13].

The Rome Foundation's recommendations for managing diseases of the DGBI include evidence-based approaches like cognitive-behavioural therapy (CBT), gut-directed hypnotherapy, and mindfulness [7, 14, 15]. Gut-directed hypnotherapy effectively reduces gastrointestinal symptoms in IBS and functional dyspepsia, comparable to the FODMAP diet, with group sessions showing high efficacy [16, 17]. The Rome recommendation for hypnotherapy became the justification for proposing the use of the BST method for the correction of DGBI, as BST is the successor to hypnotherapy, which allows for the restoration of impaired gut–brain connections in a state of light trance [7–10].

## AIM

The aim of this article is to provide a comprehensive overview of BST, with a critical evaluation of its effective-

ness in the management of patients with stress-related and psychosomatic disorders, and to review the published research on its therapeutic applications across various clinical conditions.

## MATERIALS AND METHODS

We conducted a literature search in the PubMed, Scopus, and Google Scholar databases for articles published between the early 2000s and mid-2025 that focus on examining the effectiveness of the BST method in managing and alleviating symptoms of various stress-related and psychosomatic disorders. During the literature search, we used the following set of key words: biosuggestive therapy, stress-related disorders, psychosomatic disorders, brain–gut axis, psychological distress, functional gastrointestinal disorders, and somatoform disorders. Considering the lack of research conducted and published to date, the authors compiled all the available information into a collection. We analyzed that collection, which ultimately contained 29 publications relevant to the topic of our review, and forwarded it for further analysis.

## ETHICS

The preparation of this article followed the principles of scientific ethics and academic integrity, maintaining a commitment to transparency, accuracy, and ethical responsibility throughout all stages of literature selection, analysis, and interpretation.

## REVIEW AND DISCUSSION

Our review includes a small number of studies because BST, while well known to Ukrainian practitioners, is only just beginning to accumulate scientific publications. This review includes studies examining the effectiveness of BST for various psychosomatic disorders, with some studies comparing it to CBT. All existing studies indicate the effectiveness of the method to varying degrees. The efficacy of BST is predictable, as it is a direct descendant of hypnotherapy, which has already proven its effectiveness. However, unlike hypnotherapy, which requires specialised training [7], the simplicity and error-proof nature of BST allow it to be used by both medical and psychological specialists after a relatively short and straightforward training course.

For example, K. Zelenska and T. Kraskovska [18] studied 86 internally displaced persons (IDPs) aged 18–55 in 2014. Among the enrolled participants, 42,5% had acute stress, 35,2% anxiety–depression, and 22,3% mixed reactions. Those treated with BST (50 patients,

8 sessions) showed a greater reduction in Hospital Anxiety and Depression Scale (HADS) anxiety/depression scores than the control group (36 patients treated according to traditional protocols), shifting symptoms to subclinical levels.

O. Venger et al. [19] enrolled 165 participants; 114 completed BST (64 IDPs and the comparator group of 50 permanent residents [PRs]). At baseline (pre-BST), severe depression according to DASS-21 was found in 15,62% of IDPs and 4,00% of PRs. After the BST course (post-BST), the authors observed an increase of 17,18% (from 23,44% to 40,62%) and 18,00% (from 20,00% to 48,00%) in the frequency of normal DASS-21 depression scores among IDPs and PRs, respectively.

Severe anxiety, detected in 17,19% of IDPs and 14,00% of PRs before BST, was not observed in either group after treatment. Moreover, a normal DASS-21 anxiety level post-BST was recorded in 28,12% of IDPs (vs. 0 % pre-BST) and 34,00% of PRs (vs. 4,00% pre-BST). Stress levels also decreased: severe cases, observed initially in 17,19% of IDPs and 18,00 % of PRs, were absent after BST; finally, 26,56% of IDPs and 32,00% of PRs demonstrated normal stress levels upon completion of therapy.

Furthermore, normal sleep quality increased by 39,06% in IDPs (from 3,13% to 42,19%) and by 40,00% in PRs (from 18,00% to 58,00%). In addition, after BST, the proportion of IDPs who considered their quality of life to be high increased by 7,81%, while in the PR group it increased by 40,00%, and another 10,00% of PR respondents began to rate their quality of life as very high [19].

The study by O. Osokina et al. [20] adapted BST for use as a psychocorrection method for pain. The authors aimed to explore the potential of BST in managing patients with acute and chronic pain of various origins and locations in psychoneurological and dental practice. The pain was assessed using the McGill Pain Questionnaire (MPQ) in 148 patients who reported pain. The MPQ evaluates two key indicators: the Number of Chosen Descriptors Index (NCDI), which reflects the number of descriptive words selected, and the Pain Rating Index (PRI), representing the sum of the scores from all chosen descriptors. The study included 72 psychoneurological patients and 76 dental patients, with pain intensity, sensory, and emotional components tracked over time. The patients from the main group (82 respondents, including 40 psychoneurological and 42 dental profile patients), underwent a session of BST after the initial psychodiagnostic examination, followed by a repeat examination two hours later. The control subjects (66 people, including 32 and 34 patients of psychoneurological and dental profiles) decided to cope with the pain syndrome on their own. In the experimental group,

the average NCDI was  $6,6 \pm 1,9$ , compared to  $6,1 \pm 1,8$  in the control group (non-significant). The average PRI was  $32,4 \pm 2,0$  in the experimental group and  $20,6 \pm 2,1$  in the control group (non-significant). The study groups were also comparable in terms of baseline mean pain score ( $3,7 \pm 2,5$  vs.  $3,6 \pm 2,6$  in the experimental and control groups, respectively;  $3,5 \pm 2,6$  for psychoneurological patients and  $3,8 \pm 2,7$  for dental ones).

The study found that patients undergoing BST reported normalized emotional states, reduced anxiety, and decreased irritability. These changes influenced pain perception, leading to reduced pain intensity. In the experimental group, MPQ scores improved significantly post-BST, from  $3,8 \pm 2,5$  to  $2,1 \pm 2,4$  for psychoneurological patients, and from  $3,6 \pm 2,6$  to  $2,2 \pm 2,5$  for dental patients (both  $p < 0,05$ ).

Conversely, the control group showed non-significant changes in both MPQ components (NCDI:  $3,2 \pm 2,6$  initially to  $5,4 \pm 2,3$  after two hours; PRI:  $13,8 \pm 2,7$  to  $19,9 \pm 2,5$ ). The authors concluded that BST effectively targets the emotional aspects of pain, modifies sensory and subjective pain components, and can be used independently or alongside pharmacological treatments for pain management [20].

Let us return to the negative impact of war on the human psychological state. Armed conflict exerts profound and multifaceted effects on mental health, influencing emotional regulation, cognitive functioning, and overall psychosocial well-being. Exposure to the chronic stressors of war—including life-threatening situations, witnessing violence, loss of comrades, and prolonged uncertainty—triggers complex neurobiological and psychological responses. These effects are most pronounced among military personnel, who experience sustained exposure to traumatic events and extreme environmental stress. Consequently, soldiers often exhibit elevated rates of post-traumatic stress disorder, depression, anxiety disorders, and psychosomatic manifestations, which collectively contribute to long-term psychological maladaptation and impaired quality of life [21]. Recent studies show that BST improves the emotional well-being of military personnel. After soldiers received BST, the HADS test revealed a significant reduction in anxiety and depression levels. None of the participants exhibited clinically significant anxiety or depressive conditions after the treatment, and the majority transitioned to normal ranges, indicating the effective resolution of anxiety and depression issues. Additionally, the Beck Depression Inventory (BDI) results demonstrated a significant reduction in depressive symptoms. The number of military personnel without depressive symptoms increased from 2 to 18, while cases of moderate and severe depression decreased

significantly. No participants exhibited severe depression before or after therapy, suggesting stabilization of their emotional state. Overall, BST reduces anxiety and alleviates depressive symptoms in combat veterans. Based on these findings, practical recommendations were developed for integrating this method into rehabilitation programs [22].

The war affects not only military personnel but also adult civilians. A quantitative study was conducted to evaluate the efficacy of BST in alleviating anxiety and depression symptoms among Ukrainian civilians under martial law. The study by L. M. Prudka [23] examined the role of BST in reducing anxiety and depression levels within this group. It involved 100 participants, split into two groups: an experimental group (50 people) undergoing BST and a control group (50 individuals) receiving routine psychological assistance.

The effectiveness of the therapy was evaluated using the Beck Anxiety Inventory (BAI) and the Beck Depression Inventory (BDI) before and after an eight-session programme. In the experimental group, the baseline average score was 30 on the BAI (indicating elevated anxiety) and 28 on the BDI (suggesting moderate depression). Following BST, these scores decreased to 18 on the BAI (moderate anxiety) and 14 on the BDI (mild depression).

For the control group, the initial scores were 29 on the BAI and 27 on the BDI. These decreased to 25 and 23, respectively, which continued to reflect high anxiety and moderate depression. Conversely, the experimental group demonstrated a significant reduction in both anxiety and depression levels compared with the control group. Overall, the experimental group experienced a 40% decrease in anxiety and a 50% decrease in depression, versus 14% and 15%, respectively, in the control group [23].

As a result of the war, children and teenagers in Ukraine experience anxiety, fear, depression, and other psychological disorders that require medical and psychological rehabilitation. The effectiveness of BST in children and adolescents is demonstrated in the work of Y. Yashchyshyna [24]. The authors administered up to eight BST sessions for 46 children and adolescents in groups of 6–7 people. Initial and final measurements were performed according to the Children's Questionnaire of Neuroses (CQN) [25].

According to quantitative data analysis, 89,5% of the examined patients showed positive dynamics on at least one scale and specific changes during treatment. Following BST, indicators on the scales «Sleep disturbance» improved by 26%, «Depression» by 22,3%, «Vegetative disorders» by 16,5%, «Asthenia» by 11%, «Violation of behaviour» by 10,4%, and «Anxiety» by 8,1%.

Additionally, before and after the session, the emotional state evaluation method "Thermometer of Feelings", with a colour scale measuring well-being (from green [good] to red [bad]), was used to assess the results. The obtained data indicated that BST favourably changed the distribution of colour patterns: the «red zone» reduced from 13% to zero cases, the «orange zone» decreased from 19% to 6%, the «yellow zone» declined from 37% to 19%, and the «green zone» showed a more than twofold increase—from 31% to 75% [24].

Addiction's development is linked to modern issues such as the impact of virtual life on socialisation. The issue of Internet addiction in children and adolescents has recently gained prominence [26]. The potential of BST in the treatment of Internet addiction was investigated in the work of O. Venher and T. Ivanitska [27]. The Kimberly Young Internet Addiction Test (IAT) scale was used; Internet addiction was detected in 61 people aged 16 to 28 years (19 [31,2%] males and 42 [68,8%] females). Outcomes were assessed in three groups using psychometric scales. The Beck Depression Inventory (BDI-1A) scores decreased by 23,2% in group I (autogenic training), 46,6% in group II (CBT), and 33,3% in group III (BST), which was divided into subgroups IIIa and IIIb depending on patients' suggestibility.

Clinical anxiety levels according to the Sheehan Anxiety Rating Scale (ShARS) decreased by 49,2% in group I, 86,7% in group II, 66,7% in group IIIa, and 100% in group IIIb. Thus, CBT and BST methods were the most impactful, especially among respondents with high levels of suggestibility [27].

Psychological and emotional stress contribute to behavioral disorders in both children and adults, which can be accompanied by the development of speech disorders. One of the most common language disorders is stuttering, which in most cases is caused by logoneurosis. T. Nesterenko [28] demonstrated the efficacy of BST psychotherapeutic component in the correction of logoneurosis in adult patients. The study included 26 adult patients with logoneurosis aged 18 to 29 years. As a result, there was a normalization of the emotional background in the patients who underwent a BST course, primarily due to a decrease in the level of anxiety and irritability. The study confirmed the effectiveness of the BST method in achieving emotional and physical calmness and relaxation, which significantly facilitated further therapeutic work with patients suffering from logoneurosis [28].

Finally, regarding the war in Ukraine, we conducted our own research (O. Gubska et al. [29]) in April–July 2022 in the de-occupied areas of the Kyiv region. The BST was applied to 15 residents (mean age  $53,14 \pm 16,95$  years). As a result of 5 BST sessions, HADS (anxiety score)

decreased, sleep normalized, and abdominal pain (assessed by VAS) decreased significantly in the majority of patients. Thus, this small pilot study demonstrated the effectiveness of BST in war-related psychological stress.

We did not find any study dedicated to researching the use of BST in the treatment of DGBI. However, given the effectiveness of the method in treating psychosomatic disorders and the essence of the approach itself, it is likely to be effective in treating DGBI as well. Moreover, its versatility and ease of use suggest that it could be a suitable method for the non-pharmacological treatment of these diseases. As we already know, DGBI disorders are chronic stress-related, and we can therefore establish a link between the effectiveness of BST for stress-induced states and its possible positive therapeutic effect on DGBI patients. Based on the above, we propose investigating the effectiveness of BST in DGBI as a promising area for further research.

## CONCLUSIONS

The evidence reviewed in this article supports the efficacy and safety of BST as a promising therapeutic modality for the management of a wide range of stress-related and psychosomatic disorders across different age groups. The analysed studies consistently demonstrate that BST contributes to the alleviation of both psychological and somatic symptoms in patients with functional and chronic conditions, emphasising its potential as an integrative mind–body intervention. In the context of the ongoing war in Ukraine, the relevance of BST becomes even more pronounced, as it offers an effective, non-pharmacological approach for individuals exposed to prolonged stress and trauma-related psychosomatic disturbances. Further large-scale, controlled studies are warranted to better elucidate its underlying mechanisms, refine its clinical protocols, and expand its implementation within integrative and psychosomatic medicine.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Modern approaches to the development of recommendations on the safety of sun protection products use and the need to control their composition and labelling

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## ABSTRACT

**Aim:** To enhance the effectiveness and safety of sun protection product use by developing recommendations based on an analysis of the Ukrainian SPF product market.

**Materials and Methods:** A literature review was conducted using publications indexed in the databases Google Scholar, Web of Science, and Scopus, and the PubMed and ToxNet search engines. A structured search was conducted using relevant keywords and their logical combinations, including: "skin cancer" and "ultraviolet radiation", "sunscreens", "SPF products", "sunscreens cosmetics", "efficacy and safety of sunscreens", and others. Information about sunscreens contained only on the official websites of the manufacturers was used. Information from unofficial sources was not considered. The review is based mainly on recent studies; however, selected scientific sources (2011–2018) were intentionally included to highlight the evolution of scientific knowledge in this field. The analysis used formal-logical and systemic-complex methods, which ensured a comprehensive and critical synthesis of the available evidence.

**Conclusions:** Ultraviolet radiation is the main factor in the development of melanoma, basal cell carcinoma, and squamous cell carcinoma of the skin. The increasing incidence of skin cancer prompts health professionals to encourage the population to use sunscreens. Sun protection factor (SPF) serves as the main criterion for choosing the necessary level of protection in conditions of insolation. International recommendations support the use of preparations with high SPF indices, but in practice, frequent cases of inaccurate information provided by cosmetic manufacturers have been recorded. At the same time, cosmetic sunscreens, even with the same SPF value, demonstrate different levels of effectiveness under insolation conditions. The Ukrainian cosmetic market offers hundreds of SPF products in the form of creams, sprays, oils, milks, or foams. In addition to well-known brands from France, the USA, Italy, Switzerland, Germany, Poland, and Ukraine, dozens of brands from lesser-known manufacturers, especially from Korea, are now available thanks to online shopping. As a rule, such resources do not indicate the exact SPF used in the product but instead declare marketing characteristics such as "lightest" or "safest." Today, their use is gaining popularity among the population; however, the need for legislative control over them must be addressed.

**KEY WORDS:** skin cancer, melanoma, sun protection products

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## INTRODUCTION

Every year in May, many countries around the world observe Skin Cancer Awareness Month, as skin cancer is one of the most common groups of oncological diseases. The World Health Organization (WHO) reports that more than 1.5 million new cases of skin cancer were detected in 2022, among which melanoma – the most dangerous malignant tumour of the skin, developing from pigment cells (melanocytes) – was diagnosed. In 2022, an estimated 330,000 new cases of melanoma were diagnosed worldwide, and almost 60,000 people died from the disease. There are large geographical variations in melanoma incidence rates across countries

and world regions. In most world regions, melanoma occurs more frequently in men than in women [1].

According to the National Cancer Registry, in Ukraine, the total number of new cases of non-melanoma skin cancer in 2021 was 11,109; incidence – 15.7 per 100,000 population (standardized rate, world standard), mortality – 0.5 per 100,000 population (standardized rate, world standard). In 2022–2023, 5,051 cases of melanoma were detected for the first time, and by the beginning of 2023, more than 31,000 people with this diagnosis were registered [2].

It is well established that solar ultraviolet (UV) radiation is the main cause of melanoma, basal cell carcinoma

ma, and squamous cell carcinoma of the skin [3]. The most noticeable increase in the incidence of melanoma occurs among the fair-skinned populations of countries with a moderate level of insolation. For example, the Danish Cancer Registry documented one case of melanoma per 100,000 population in 1950, three cases per 100,000 population in 1970, and the forecast figure for 2036 is 50–70 cases per 100,000 population [4].

A similar trend is observed in many European Union (EU) countries, the USA, the United Kingdom (UK), and others. Understanding the urgency of the issue, a working group of experts, which included clinicians and researchers from Africa, America, Asia, Australia, and Europe, together with scientific societies (European Association of Dermato-Oncology, Euromelanoma, Euroskin, European Union of Medical Specialists, and Melanoma World Society) jointly formulated scientifically based recommendations on photoprotection as a strategy for preventing skin cancer. Specifically, people with fair skin, especially children, should minimise exposure to UV radiation, and it is recommended to take protective measures when the UV index reaches 3 or higher (the UV index is a standard international indicator of UV radiation. Its values start from “0” and can exceed “10”; the higher the number, the greater the likelihood of skin and eye damage and the shorter the time required to cause harm) [5, 6].

To prevent skin cancer, international organizations support the use of sun protection products (products containing Sun Protection Factor [SPF]) with high sun protection values. However, manufacturers of such cosmetics do not always provide reliable information about the actual sun protection properties of their products or their safety for consumers, as revealed by a comparative analysis of the sun protection product market.

## AIM

The aim of the research was to enhance the effectiveness and safety of sun protection products use by developing recommendations based on an analysis of the Ukrainian market of SPF products.

## MATERIALS AND METHODS

A literature review was conducted using publications indexed in the databases Google Scholar, Web of Science, and Scopus, and the PubMed and ToxNet search engines. A structured search was conducted using relevant keywords and their logical combinations, including: “skin cancer” and “ultraviolet radiation”, “sunscreens”, “SPF products”, “sunscreen cosmetics”, “efficacy and safety of sunscreens”, and others. Information about

sunscreens contained only on the official websites of the manufacturers was used. Information from unofficial sources was not considered. The review is based mainly on recent studies; however, selected scientific sources (2011–2018) were intentionally included to highlight the evolution of scientific knowledge in this field. The analysis used formal-logical and systemic-complex methods, which ensured a comprehensive and critical synthesis of the available evidence.

## ETHICS

Since this study was conducted exclusively as a systematic review of the available scientific literature obtained from open sources, permission from the ethics committee was not required. All data included in the review were previously published and freely available, and their use was carried out exclusively for scientific purposes.

During the preparation of the article, the principles of academic integrity were observed, including proper citation of sources in accordance with the established requirements. The authors did not use unreliable or falsified data, and also avoided any manifestations of plagiarism.

When preparing the review, the provisions of international ethical guidelines were taken into account, in particular the recommendations of COPE (Committee on Publication Ethics) and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), which ensures transparency, reliability and scientific quality of the presented material.

## FRAMEWORK

The study was performed as a fragment of the Scientific Department of Scientific Basis of Chemical Factors Risk Assessment (State Enterprise «L.I. Medved's Research Center of Preventive Toxicology, Food and Chemical Safety of the Ministry of Health of Ukraine (state enterprise) «Scientific Substantiation of Measures for the Toxicological Safety of the Human Environment in the Health Care System» (state registration number 0123U102087; term: 2023–2027).

## REVIEW AND DISCUSSION

The increasing incidence of skin cancer is prompting health professionals to encourage the use of sun protection products among the population. The SPF index is the main criterion for selecting the necessary level of protection under insolation conditions: the higher the SPF, the greater the level of protection. It has been estab-

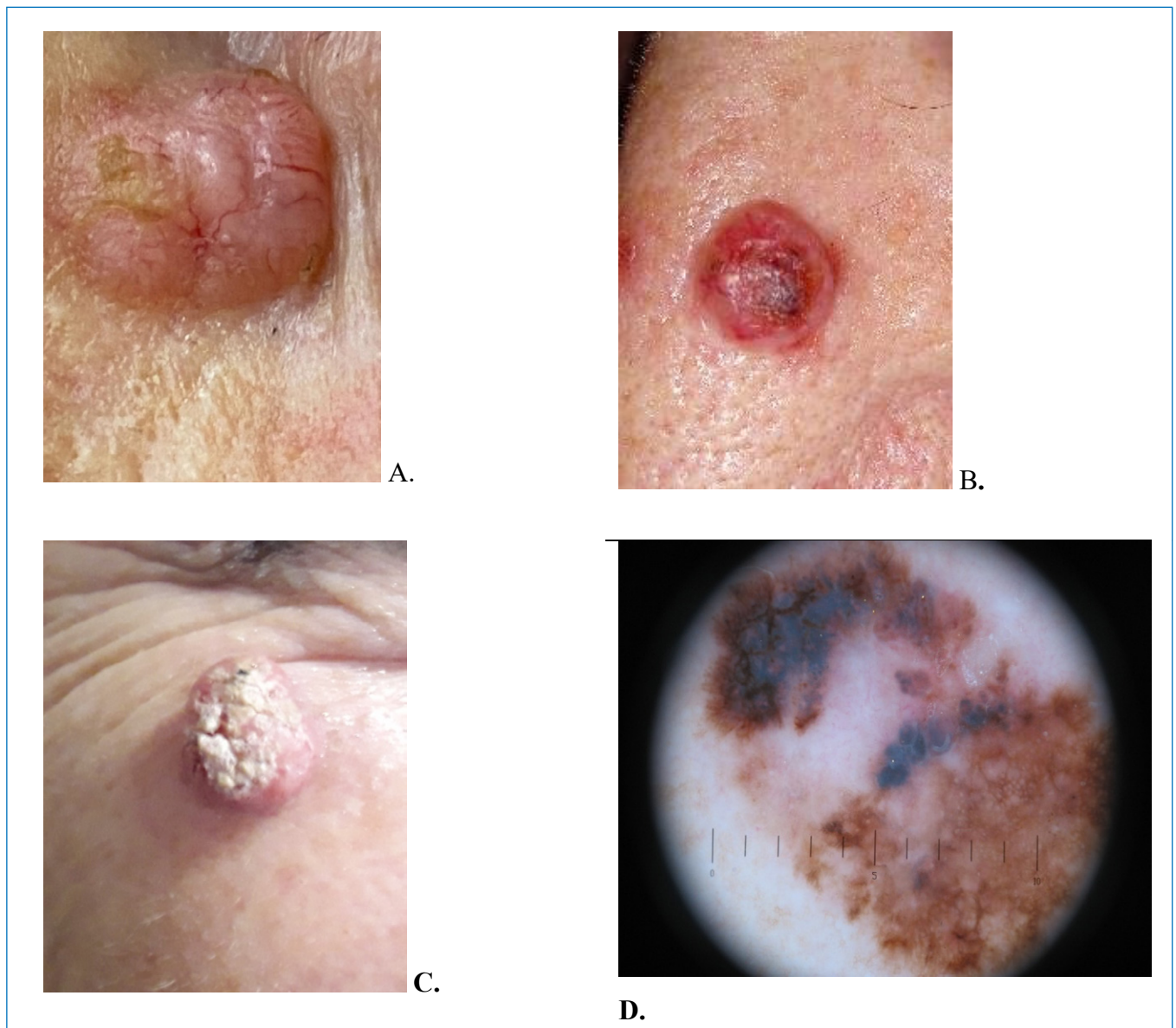


**Fig. 1.** Examples of keratocytic skin cancer (A, B, C) and melanoma (D) (photo by O. Havrylyuk): **A.** Basal cell carcinoma, nodular form periocular face skin area, woman 74 years old; **B.** Basal cell carcinoma, nodular form, wing of the nose skin, woman 68 years old; **C.** Squamous cell carcinoma, verrucous form, upper mandibular facial skin area, woman 61 year old; **D.** Melanoma of the skin. Dermatoscopy, Heyne Delta, woman, 27 years old  
*Pictures taken by the authors*

lished that the incidence and mortality from melanoma among individuals with light skin is significantly higher than among those with darker skin. The risk is classified according to the scale developed by American dermatologist Thomas B. Fitzpatrick, ranging from I to IV [7].

Thus, keratinocyte carcinoma (Fig. 1) has higher prevalence rates among individuals with light skin compared to those whose skin is less sensitive to the sun and of a darker shade. At the same time, the highest density of melanocytes per unit area of skin is observed in areas most frequently exposed to the sun, such as the face, head, and neck.

Experts estimate that in fair-skinned populations, up to 95% of keratinocyte skin cancers and 70–95% of cutaneous melanomas are caused by UV radiation. Therefore, a

significant proportion of skin cancers can be prevented by reducing excessive exposure to UV radiation through effective sun protection [6].

When characterising the effect of solar radiation on humans, it should be noted that UV radiation (100–400 nanometres, nm) accounts for only 5–10% of terrestrial sunlight. Of this, the B spectrum – UVB (280–315 nm) – is considerably more harmful, accounting for 5%, while the remaining 95% is represented by the A spectrum – UVA (315–400 nm). Both UVB and UVA spectra adversely affect the skin, but UVB is more active. Although less aggressive, the UVA spectrum is constantly present. Therefore, for optimal protection, SPF products must provide a high level of defence against both UVA and UVB rays. SPF products have also been shown to

be effective, with varying degrees of efficacy, in preventing actinic keratosis, squamous cell carcinoma, photoaging, basal cell carcinoma, and melanoma [8].

The main active components of cosmetic SPF products are compounds that absorb, scatter, or reflect UV radiation, known as sunscreens. These are divided into two categories: chemical sunscreens (chemical UV absorbers of both old and new generations) and physical sunscreens, which form a protective layer that acts like a mirror, reflecting and scattering UV rays.

Physical sunscreens are represented by titanium dioxide (Latin: *Titanium Dioxide*) and zinc oxide (Latin: *Zincum Oxide*). Titanium dioxide and zinc oxide are usually incorporated into SPF products formulations as ultra-fine (20–50 nm) particles, called micro reflectors. Particles, which are smaller than 10% of the wavelength of incident light, scatter light according to Rayleigh's law, which states that the intensity of scattered light is inversely proportional to the fourth power of the wavelength. As a result, they scatter UVB light (wavelengths between 280 and 320 nm) and UVA light (wavelengths between 315 and 400 nm) more than visible light with longer wavelengths, preventing sunburn while remaining invisible on the skin [6, 8]. Physical sunscreens use solid particles to scatter and reflect UV radiation, although some modern products with metal oxide nanoparticles are also able to absorb it. Titanium dioxide and zinc oxide are chemically inert metal oxides. Physical sunscreens protect against the UVB spectrum and to some extent against visible UVA radiation. Physical sunscreens are usually thick, opaque, can have a «whitening» effect on the face, and are cosmetically unacceptable to many people [8].

The Ukrainian cosmetic market is represented by many brands that use physical sunscreens. According to open sources and information provided directly by manufacturers, physical filters in SPF products are actively used by many companies. However, these products may have unsatisfactory organoleptic properties, cause skin whitening and unacceptable aesthetic results. Therefore, recently, formulations with micronized or nanoparticle components with a diameter of less than 100 nm have begun to appear, which improves the transparency and cosmetic acceptability of physical filters in sunscreens [9–11]. Today, there are no regulatory restrictions on the use of chemical filters in Ukraine, as cosmetic products that meet international requirements is expected to be sold on the market starting from August 3, 2026, in accordance with the Technical Regulations on Cosmetic Products, approved by the Resolution of the Cabinet of Ministers of Ukraine No. 65 of January 20, 2021 [12].

A lot of brands represented on the Ukrainian cosmetics market continue to use toxic filters in their products, which poses a potential threat to consumer health.

In contrast to the abovementioned, new generation chemical filters have been developed taking into account

modern requirements of efficiency and safety. They guarantee a wide spectrum of protection against UV radiation (UVA and UVB); have better photo stability and a lower risk of adverse reactions. Additionally, local photolyases and antioxidants (vitamin C, vitamin E, selenium and polyphenols contained in green tea extract) appear as potential agents of local photo protection [13].

Products containing such components are gradually entering the Ukrainian market, but their share remains insignificant. Instead, products manufactured in the Republic of Korea (South Korea) constitute a significant segment of cosmetics, having gained popularity due to their favourable organoleptic properties and affordability [14].

The Ukrainian cosmetic market offers hundreds of SPF products in the form of creams, sprays, oils, milks, or foams. In addition to well-known brands from France, the USA, Italy, Switzerland, Germany, Poland, and Ukraine, online shopping has made dozens of brands from lesser-known manufacturers, especially from Korea, readily available. As a rule, such products do not indicate the actual SPF value but promote «marketing» characteristics such as the lightest, safest, etc. [15].

In our opinion, this situation in Ukraine has arisen because there are currently no regulatory restrictions on the use of SPF filters. Cosmetic products that comply with European requirements are expected to be allowed on the market starting from 3 August 2026. In Ukraine, the profit from the SPF products market in 2025 is projected to reach 21,53 million US dollars, with the market expected to grow annually by 4,74% (CAGR 2025–2030), driven by increasing public awareness of the harmful effects of UV radiation and a surge in demand for SPF 50+ products [16].

The above highlights the necessity of introduction of stricter quality control for cosmetic products, in particular sun protection products, to ensure their effectiveness and safety for consumers, since among the adult population, the main sources of information about skin health are Internet resources, social networks, and to a lesser extent, people receive knowledge from medical professionals, who have more reliable data on the composition of cosmetic products.

The European Commission monitors the effectiveness and safety of sun protection products, which are classified as cosmetic products and must comply with the EU Cosmetics Regulation 1223/2009 [17].

In the United States, manufacturers must follow strict testing protocols to confirm the effectiveness and safety of SPF products before they become available to consumers. The American Academy of Dermatology recommends the widespread use of SPF products in skincare, especially during outdoor recreational activities. Although the FDA oversees and regulates the production of sun protection products, their ingredient profiles still require improvement. The FDA classifies SPF products as over-the-counter drugs,

meaning they are subject to more rigorous testing for safety, stability, compatibility, and efficacy than conventional cosmetics [18].

UV filters, which provide SPF products with their protective properties, are considered “active ingredients,” and only FDA-approved UV filters may be included in sun protection products. However, only some older-generation physical and chemical filters are permitted in the US. These filters tend to whiten the skin and can increase sebum production, making them cosmetically less acceptable. Consequently, manufacturers are limited to selling products based solely on older-generation physical or chemical filters. By comparison, the European Union allows the use of twenty-seven active ingredients to block the harmful effects of sunlight and prevent skin damage, whereas the FDA has approved only seventeen. FDA approval of UV filters lags significantly behind other countries, as no new UV filters have been added to the FDA’s approved list since 2002. The number of available ingredients is important because not all filters are easily incorporated into formulations that satisfy both skincare and organoleptic requirements [19].

The American Academy of Dermatology recommends the daily use of sun protection products with an SPF of 30 or higher for people of all skin types. The Canadian Dermatology Association also recommends the use of broad-spectrum sun protection products with an SPF of 30 or higher [6, 19]. Health Canada does not recommend the use of SPF products for infants under six months of age due to the theoretical risk of increased absorption of sunscreen ingredients, which is associated with a thinner epidermis and a higher skin surface area-to-body volume ratio [20].

SPF 50 products provide protection against approximately 98% of UV radiation and are considered the most appropriate for use. However, SPF filters with values above 50 offer virtually no additional protection against sunburn or UVB radiation [6]. It should be noted that most SPF products do not provide permanent sun protection, as organic sunscreens can degrade under the influence of light or be adversely affected by other components of the product when exposed to UV radiation. The protective effect of SPF products against skin damage caused by UV radiation has been confirmed in numerous studies, and their effectiveness against sunburn is unequivocally determined by the SPF value [8].

Following the detection of regulatory violations, the FDA ordered stricter quality control and recalled several sunscreens from the market [22]. This is why many Korean brands are not available on cosmetic markets in Europe and the United States, whereas most remain accessible in Ukraine due to the lack of proper regulation [23].

In practice, the situation can be significantly different, as many sun protection products have not been tested to meet the stated standard. In 2020, Korean-made cosmetic products were the subject of critical reports from laborato-

ries and scientific institutes. Those were related to the actual SPF level in popular products.

The Korean Institute of Dermatological Sciences released data on the product, which, after being tested, showed an average SPF of  $28.4 \pm 3.2$ , even though the packaging stated SPF 50/PA++++. Those findings had a huge impact on consumers and raised concerns about the reliability of Korean SPF products [24, 25].

It has been found that the problem is not limited to a single brand. A similar situation was observed during testing of a popular cream (SPF 50/PA++++), which demonstrated significantly lower SPF values in an *in vivo* study. According to the report on the determination of the degree of protection against UV radiation of a cosmetic product No. EU/121/10/2023 (SPF 50/PA++++), the average protection factor was 44.8. For another popular Korean product (SPF 50+ PA++++), the average SPF was 45.8. It is noteworthy that some samples showed significantly lower SPF values, down to 15.7, which does not meet international recommendations for the required level of sun protection. This finding raises concerns regarding the reliability of information provided by individual manufacturers and the quality of their products, as the study of the UV protection factor was conducted in accordance with the Recommendation of the Commission of the European Communities of 22 September 2006 on the effectiveness of sunscreen products and claims made thereon (document number C (2006) 4089, 2006/647/EC) and ISO 24444:2020/A1:2022 [14, 23–25].

The prospects for further research are driven by the relevance of identifying effective and consumer-safe methods for skin protection against ultraviolet radiation, as confirmed by the work of Ukrainian scientists [26, 27]. The continuous expansion of the cosmetic market for various sunscreens capable of mitigating the harmful effects of ultraviolet radiation on human skin underscores the need to improve measures for quality control and consumer safety. One current priority is to establish regulatory oversight of sunscreens at the legislative level [28].

## CONCLUSIONS

1. Ukraine has a disappointing statistic on the incidence of skin cancer among the population, so the availability of high-quality sun protection products on the domestic market and objective information about them is the key to increasing the effectiveness of skin protection from the harmful effects of UV radiation.
2. SPF products available on the Ukrainian market from various countries and manufacturers do not consistently indicate the SPF index, which is the primary indicator of the effectiveness of SPF products; there are also frequent cases of discrepancies between the



- data declared by the manufacturer and the indicators obtained under experimental conditions.
3. The public health service of Ukraine should be tasked with informing the population about the need to use SPF products and the rules for their selection in accordance with their availability on the existing SPF product market.
4. The State Service of Ukraine on Food Safety and Consumer Protection must carry out regular random control over the compliance of consumer qualities of SPF products with the manufacturer's information about them indicated on the product labelling.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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## Pain epistemology and treatment approaches

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### ABSTRACT

**Aim:** To investigate the epistemology of chronic pain, the methodology of approaches to its treatment, and to highlight the economic and social aspects related to the issue of chronic pain.

**Materials and Methods:** A comprehensive literature search was conducted using the following databases: Google Scholar, PubMed, Scopus, Web of Science, and SpringerLink. The search included combinations of the following key words: chronic pain, causes of pain, biopsychosocial model of pain, multimodal pain management, economics of chronic pain. The search covered publications from 1994 to 2025. Studies were selected based on the following inclusion criteria: peer-reviewed articles, meta-analyses, systematic reviews. The following were excluded from the search: conference abstracts, editorials, commentaries. The initial search yielded over 90 publications. After checking for relevance, 34 articles were included in the final review.

**Conclusions:** Pain is dualistic, both in origin and in its mechanisms of implementation. It is simultaneously a physical sensation and an emotional reaction, encompassing cognitive and social components. Convincing evidence has been obtained regarding the presence of functional, structural, and chemical changes in the brain that occur due to continued pain. Chronic pain is a complex biopsychosocial concept. Therefore, the development of a biopsychosocial model of pain is needed, both along the lines of patient–society and patient–medicine, as well as incorporating feedback. Only targeted multimodal medical care, together with a transformation in the sociocultural perceptions of patients with chronic pain, allows improvement in treatment outcomes, economic efficiency, and social adaptation of affected individuals. This requires an appropriate multidisciplinary approach and wide implementation of innovative methods and techniques of medical care for patients with pain syndromes, taking into account the biopsychosocial model of pain.

**KEY WORDS:** chronic pain, treatment

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### INTRODUCTION

Chronic pain is one of the most burdensome conditions and one of the most common chronic diseases, as its incidence reaches epidemic levels, and the economic losses associated with providing care to patients with chronic pain syndromes outweigh the costs of cardiovascular disease, cancer, and diabetes combined. In the United States, the Institute of Medicine, a non-profit non-governmental organization, recently published a report estimating that healthcare costs due to lost productivity were up to \$635 billion in 2010. This is significantly higher than the estimated costs for heart disease (\$309 billion), diabetes (\$188 billion), and cancer (\$243 billion) [1]. Chronic pain is a major challenge for healthcare systems in various countries due to its international prevalence, associated disability, economic burden, and high utilization of healthcare services by sufferers. In the latest Global Burden of Disease systematic review, pain-related conditions such as chronic back pain, headache, depression, and others are identified as ten main causes of disability in the age group 10–49 years world-

wide, representing a global cause of disability in both developed and developing countries [2]. The results of studies by many authors show a significant burden of pain in Europe and the USA [3–5]. Pain is a global medical and socio-economic problem that requires complex, comprehensive solutions at the state and administrative level, new methodologies of biological and socio-cultural orientation, and new technologies and techniques aimed not only at the patient suffering from chronic pain, but also at society. The subjective origin of pain, its duality, the lack of a clear biomarker, and insufficient understanding of its mechanisms make it difficult to determine the pathological cause of chronic pain and the effectiveness of its treatment. The biopsychosocial model of pain has not yet been clearly defined or applied to the social aspects of chronic pain, both from the patient's perspective and the sociocultural environment. As a result, social aspects have yet to be fully considered in theoretical models of chronic pain and practically implemented to benefit patients suffering from it. Consequently, a number of medical,

economic, and social problems related to providing effective care to a large number of such patients remain unresolved [6]. The biopsychosocial model of pain has the potential to shift multi-directional societal efforts toward significant improvement in the outcomes of chronic pain treatment and the reduction of suffering in these patients.

## AIM

The aim of the research was to investigate the epistemology of chronic pain, the methodology of approaches to its treatment, and to highlight the economic and social aspects related to chronic pain.

## MATERIALS AND METHODS

This review of chronic pain was conducted to clarify the biopsychosocial causes of pain, their correlation and multifaceted approaches to its treatment. To highlight the economic and social aspects associated with the problem of chronic pain.

A comprehensive literature search was conducted using the following databases: Google Scholar, PubMed, Scopus, Web of Science, SpringerLink. The search included combinations of the following key words: chronic pain, causes of pain, biopsychosocial model of pain, multimodal pain management, economics of chronic pain.

The search covered publications from 1994 to July 2025, starting from the proposed definition of chronic pain and its classification by the International Association for the Study of Pain (IASP) with coverage of the latest available literature.

Studies were selected based on the following inclusion criteria: peer-reviewed articles, meta-analyses, systematic reviews. The following were excluded from the search: conference abstracts, editorials, commentaries.

The initial search yielded over 90 publications. After checking for relevance, 34 articles were included in the final review.

## ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines, and databases. No patient-identifying data were used during the work, and no approval from an ethics committee was required, as the study did not involve new clinical interventions or primary collection of patient information.

The authors adhered to the ethical principles of the Declaration of Helsinki of the World Medical Association

and international standards for publication in medical journals, including the recommendations of the ICMJE (International Committee of Medical Journal Editors).

No element of the work contains plagiarism or fabrication of data. All sources of information are appropriately cited and properly formatted.

## FRAMEWORK

The study was conducted as a fragment of the Scientific Department of Minimally Invasive Surgery scientific project (State Institution of Science «Center of innovative healthcare technologies» State Administrative Department) «Optimization of approaches to providing specialized medical care for surgical patients using personalized anesthetic support» (state registration number 0125U000315; term: 2025-2029).

## REVIEW AND DISCUSSION

### PHILOSOPHICAL AND PHILOLOGICAL ASPECTS OF THE UNDERSTANDING OF PAIN

Pain is a sensation, which means that it is subjective, and therefore, there are no clear criteria for its measurement. Pain accompanies a person throughout life, performing, on the one hand, a functional, either protective or destructive, role, and on the other, indicating its source, whether bodily or spiritual. Already at the level of world perception and linguistic forms of pain expression, its duality is evident: external–internal, bodily–spiritual, concept–sensation. From a philosophical perspective, the cause of pain is dual: it is, on the one hand, an obstacle that a person seeks to overcome, and on the other, a universal mechanism that warns of danger and mobilizes the body's resources. Pain is both a universal physiological process and a deeply individual experience.

Analysis of ideas about pain in a sociocultural context allows for an assessment of the level of humanity, culture, and rationality within a society. European culture tends to eliminate pain as a factor of discomfort that interferes with normal life. However, such alienation of pain creates an imbalance: the direct sense of reality is lost, natural bodily self-regulation is disrupted, and the ability to orient oneself to the body and adapt to internal and external changes is weakened. This leads to a disconnection from the body, breaking the unity of body and spirit (psyche, somatics, consciousness), which in turn causes a crisis of personality and its interaction with the external world—conceived as an extended space of one's body. Civilization, in its technological overcoming of pain, ultimately deprives a person

of the right to personal choice. Pain contains a primary reflex shared by all living beings. At the same time, in the human body, the natural process of perceiving and independently experiencing pain is contested.

The analysis of linguistic forms of pain expression, which reflect the duality of its origin and its structural and functional components, is an important aspect [7]. The word “pain” in Greek is interpreted through *άλγος* and *πόνος*, which denote not only pain but also suffering, grief, sadness, trouble, longing, hard work, tension, and torment. The English word pain comes from the Latin *poena*, “punishment, torment,” which frames the perception of pain as a form of punishment for sin. Notably, the concept of “pain” is not inherently negative; it does not indicate absence or lack but rather emphasizes presence. The lexical material reflects philosophical and medical ideas about the duality of pain, encompassing both physical and psychological dimensions and combining destructive and protective aspects.

Traditionally, in the interpretation of the concept of pain, a distinction is made between physical and mental pain, the latter more often being referred to as suffering. In European culture, there exists a hierarchy of pain, according to which physical pain is considered worse than mental pain; hence, the punishment of criminals historically involved corporal punishment. It is believed that mental torment can be experienced in despair, whereas physical pain does not allow one to disengage from the present moment of being. However, the ancient Greek philosopher Epicurus, by contrast, considered mental pain to be worse, since the body suffers only from events in the present, while the soul suffers from both the past and the future. The duality of the origin of the pain sensation is implemented through complex, heterogeneous mechanisms operating in two directions: from the body to the soul and vice versa. This biological mechanism provides a dualistic perception and understanding of suffering, both physical and spiritual. In essence, these are two sides of the same biological process, which is crucial for regulating behaviour and ensuring the survival of the individual.

## DEFINITION OF PAIN, CLASSIFICATION, AND MANIFESTATIONS

The recognition of chronic pain as a distinct pathological unit is still disputed. This issue is considered in a historical context in the study of William Raffaelli and Elisa Arnaudo “Pain as a disease: an overview”; the authors draw attention to this global medical problem that requires further research and development of new specific therapeutic approaches [8]. The International Association for the Study of Pain (IASP), presented at

the first Global Day Against Pain, notes that “chronic and recurrent pain is a separate health problem, a distinct disease” [9]. This is widely supported by leading scientific societies dealing with pain issues and is still a matter of debate in the scientific community. The main question is whether chronic pain, already recognized as distinct from pain as a symptom, can be considered as an independent pathology. In 2015, the IASP Working Group on the Classification of Chronic Pain suggested a new system for categorizing pathological pain conditions for the ICD11 [10].

The IASP (1994) [11] defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. In 2016, the IASP clarified this definition: pain is an unpleasant sensation associated with actual or potential tissue damage with sensory, emotional, cognitive and social components. This is an essential addition, since it allows for interpretation and influence on the course of the disease as pain always contains a subjective component.

Pain can be classified according to mechanism, duration, aetiology, and location. By mechanism, pain is divided into nociceptive (resulting from damage to pain-sensitive structures) and non-nociceptive (neuropathic or psychogenic). Nociceptive pain can be further subdivided into somatic and visceral types, depending on which areas of the body the nociceptors are activated. Neuropathic pain is classified as peripheral or central, depending on which parts of the nervous system are responsible for maintaining pain and are damaged. Chronic pain is defined as pain that persists beyond the usual period of acute injury or illness, or that recurs over months or years [12]. The definition of chronic pain is further specified by duration: pain that continues or recurs for more than three months, exceeding the expected time for healing and tissue repair after damage [13]. High-intensity chronic pain (HICP) is also distinguished, referring to pain that limits life or work on most days, or every day, over the past three months.

Pain can be classified as somatic, visceral, or a combination of both, depending on the anatomical characteristics of the tissues involved. Somatic pain, which occurs in the joints, muscles, and skin, is typically well localized. Visceral pain, originating from internal organs, is usually poorly localized and diffuse due to the involvement of multiple spinal segments and the phenomenon of convergence. In addition, visceral pain is often accompanied by somatic pain due to the convergence of afferent pathways of visceral and somatic nerves [14]. The American Academy of Pain Medicine suggests distinguishing two types of pain using new terminology: good (eudynia) and bad (maldynia) pain.

Good pain serves as a symptom of an underlying pathology, disease, or injury, whereas bad pain represents a pathological process associated with neuropathological changes at the cellular and molecular levels [15].

Chronic pain is frequently accompanied by anxiety and depression, fatigue, sleep disturbances, functional disability, and reduced quality of life. Pain is both a physical sensation and an emotional response, encompassing cognitive and social components. Thus, this process is bidirectional: pain affects behaviour (emotional and social), and conversely, behaviour influences the manifestations and course of pain. The presence of verified structural changes in tissues that generate pain syndromes is a key factor in their development. Structural changes, for example in the spine, can act as a persistent or periodic trigger of pain, such as in cases of back pain. Pain caused by structural changes in the spine is understandable, and its mechanisms are clear. However, the presence of significant structural changes does not always correspond to persistent or severe pain. Conversely, individuals often experience long-lasting debilitating back pain with no significant structural abnormalities. Why? Pain is, on the one hand, a manifestation of tissue damage, and on the other, psychological mechanisms may also contribute to its development. This phenomenon, known as somatization, occurs when psychological disorders and conflicts are transformed into somatic complaints in a subconscious attempt to reduce mental stress. The model of disease behaviour emphasizes cognitive (evaluative) factors and considers the influence of the external environment as a primary mechanism in shaping an individual's perceptions of their health.

Muscle tension is the basis of the psychosomatic mechanism, which leads to regional discomfort, and the evidence of effectiveness of relaxation and various local blockades confirm the presence of such a mechanism. From the patient's subconscious point of view, a "benefit" is developed from the feeling of pain – the avoidance of a mental conflict by transforming it into a physical illness. A way to resolve unconscious conflicts. If the conflict is not allowed into consciousness, it is unresolved, and the constantly increasing mental tension is always ready to be discharged. The release of restraining energy causes an exacerbation of the reaction to ordinary or mild pain stimuli. People suffering from chronic pain feel a certain dissatisfaction, face both passive and active exclusion from social participation, family interaction and professional activities. The experience of communicating with patients with chronic pain shows that the forms of their misunderstanding, rejection and ostracism greatly outline their suffering. These aspects exacerbate the problem of socialization of patients; their exclusion from social life becomes real,

which is an important part of the suffering of patients with chronic pain. Chronic pain is a complex bio-psycho-social phenomenon with broad consequences not only in terms of clinical care, but also in terms of social and economic impact [16-18].

Thus, an important aspect of pain syndrome is the possible psychological mechanisms of its development, in particular through somatization and the corresponding psychosomatic processes based on muscle overstrain, which causes regional discomfort. Psychological disorders and contradictions are transformed into somatic complaints in a subconscious attempt to reduce mental stress. The manifestation of a mental disorder through physical symptoms is common in such cases. The results of psychoanalysis show that pain reduces the pleasure of a dependent position and sexual needs, dissipates aggression as well. Anger can be manifested indirectly in the form of aggressive and passive behaviour, when family members, the attending physician become hostages to endless complaints and demands for attention from the patient. In modern society, the majority believes that more attention is given to physical diseases than psychological ones that contributes to expression of complaints in organic pathologies. The behavioural choice model indicates that cultural and social factors encourage people to express complaints in somatic categories in order to avoid the social condemnation associated with mental disorders.

Convincing evidence that chronic pain is accompanied by functional, structural and chemical changes in the brain is presented in a 2009 review [19]. Pain becomes pathological when it loses its signalling function and as a result of psychophysiological changes becomes a destructive factor that is difficult to cope with using traditional methods of therapy. Recognizing chronic pain as a distinct pathological condition could significantly contribute to raising awareness of this global problem among both medical professionals and the public. The ability to modulate the pain response (enhance or suppress it) is crucial and is implemented at the cortical, brainstem, and spinal levels.

## MECHANISMS OF PAIN DEVELOPMENT

There are three primary mechanisms of pain response modulation: gate control, the descending modulatory system, and neuromodulators [14]. The gate control theory, first proposed by Wall and Melzack in 1965, explains that the transmission of nociceptive signals at the primary synapse between nociceptors (primary nociceptive fibres) in the dorsal horn of the spinal cord can be modulated or blocked. This mechanism has been described in detail in subsequent studies [20].

The pain pathway begins with the activation of nociceptors. It is a complex and heterogeneous process involving several interconnected mechanisms regulated by the nervous, endocrine, and inflammatory systems, and is accompanied by the modulation and amplification of the response through complex biochemical changes [21].

In cases of tissue damage, the local immune balance is disturbed. Damaged cells, activated mast cells, neutrophils, and macrophages release inflammatory mediators (hydrogen ions, sodium ions, serotonin, cytokines, bradykinin, histamine, prostaglandins, leukotrienes) that activate and sensitize local transient receptor potential (TRP) channels on nociceptors. Specific TRP channels play a key role in the maintenance of chronic pain [22] and are also involved in the development of hyperalgesia, as they can be sensitized by inflammatory mediators [23]. Therefore, early control of the local inflammatory response is crucial to prevent peripheral sensitization.

The descending modulatory system can exert both inhibitory effects, by reducing signal transmission at the primary synapse, and excitatory effects, by facilitating transmission at the primary synapse, which contributes to central sensitization. With repeated or prolonged nociceptive stimulation, central sensitization develops and is maintained at the level of the spinal cord. This is a critical process in the development and maintenance of chronic pain, resulting in a persistent state of pain hypersensitivity [24]. Early prevention of sensitization through optimal analgesia during the acute period of a pain syndrome is essential for preventing the development of chronic pain, regardless of the cause of tissue damage, such as trauma or inflammation.

Feedback mechanisms are also significant, as patients suffering from depression or anxiety have an increased tendency to experience more severe pain following traumatic events or medical procedures. Therefore, clinicians should always consider the importance of psychological interventions, particularly cognitive-behavioural therapy, in the comprehensive management of chronic pain, and combine these with therapy for depression and anxiety [25].

The third group of mechanisms involved in the implementation and modulation of the pain response operates through neuromodulators. Neuromodulators are molecules that modulate the efficiency of neurotransmitter signal transmission at the synapse and can be either excitatory or inhibitory. Excitatory neuromodulators that enhance nociception include cholecystokinin-8 (an endogenous anti-opioid), prostaglandins, dynorphin 1-17, orphanin FQ, and nociceptin. Inhibitory neuromodulators that suppress nociception

include opioids, cannabinoids, acetylcholine, orphanin FQ, and nociceptin [14]. Neuromodulators play a crucial role in the endogenous control of pain intensity.

It is also established that pain has a genetic component [26]. Currently, genes associated with the pathology of the afferent component of pain sensitivity have been identified. These genes encode approximately 50 neurotransmitters, their receptors and transporters; 8 ion channels; 15 anti-inflammatory cytokines and their receptors; approximately 15 neurotrophic factors and their receptors; and more than 15 intracellular messengers. This suggests that pain perception can potentially be regulated by targeting specific genes [27].

## TREATMENT APPROACHES

The world requires innovative organizational, structural, social, and medical approaches to understanding and treating chronic pain. According to the World Health Organization (WHO), approximately 50% of patients with various types of pain worldwide do not receive appropriate therapy, highlighting the significant challenge faced by professionals involved in pain management.

Given the unsatisfactory outcomes of chronic pain treatment, a biopsychosocial model of pain is necessary from both the patient–society and patient–medicine perspectives. This model frames patient and societal behaviour in a broader context, enabling a more comprehensive approach to addressing the often-overlooked aspects of suffering experienced by patients with chronic pain. Considering the exhausting struggle of these patients for recognition, attention should be given to social factors, which must be carefully considered theoretically and implemented in practice to help patients adapt and participate more fully in society [28].

The dualistic origin of pain reflects, on the one hand, possible sources of pain (damage to the soma and/or dysfunction of the nervous system) and, on the other hand, the interdependence between pain and emotions, which allows for the interpretation of various factors influencing the course of the disease. This understanding significantly affects the selection of medical treatment strategies and the development of a comprehensive recovery program for individual patients, encompassing medical devices, technologies, therapeutic methodologies, and social rehabilitation. Chronic pain also negatively impacts quality of life due to unmet needs in pain management; surveys on the quality of life of such patients indicate a high prevalence of problems related to mental health, employment status, sleep, and personal relationships.

Two primary factors should be distinguished in the development of most chronic pain syndromes: somatic



(most often inflammatory, compressive, musculoskeletal, autonomic, or vascular) and psychogenic, or a combination of both. This distinction is crucial for selecting appropriate treatment strategies, as understanding the causes and pathogenetic components of pain determines the therapeutic approach. The management of chronic pain requires the cooperation of multiple specialists and a comprehensive, multidisciplinary approach to patient care [29].

A call to action is needed for administrators and policymakers to recognize the significance of chronic pain and to invest in comprehensive strategies encompassing structural organization, prevention, education, and access to effective treatment methods. Recent advances in chronic pain management include a wide range of approaches, primarily minimally invasive techniques [30], pharmacological therapies [31], non-pharmacological interventions [32], psychological interventions [33], and complementary or unconventional treatment methods [34].

The expansion of medicine into increasingly narrow specialties is not without limitations. When each physician focuses solely on “their” pathology, a holistic perception of the patient is lost. Conversely, the multidisciplinary approach also has its constraints. The next stage in the development of effective medical care, including for patients with chronic pain, lies in the integration of medical knowledge to ensure more comprehensive and effective treatment. The future belongs to integrative medicine.

## CONCLUSIONS

The problems associated with chronic pain are a real financial burden to every country. Governments need to be aware of pain issues, and managers at different levels and politicians should necessitate the importance of strategies, both medical and social, for investment in comprehensive policies that cover structuring, prevention, education and available effective treatment methods. Taking into account the significance of this health issue and the associated economic and social burden on viability, chronic pain should be considered a priority in the health care system of states. The complexity of the mechanisms

of origin of pain from purely structural, somatic to psycho-social and combined require appropriate mechanisms for rehabilitation of pain syndrome depending on its manifestations, development and duration, starting from psychological, corrective behavioural, informational, social to minimally invasive contemporary interventions, medication, physiotherapy, and traditional medicine.

Main approaches to improvement of medical care to patients with chronic pain:

1. A bio-psycho-social approach to understanding the development of chronic pain should be chief in overcoming this suffering. Balanced multifaceted relationships of the patient with public/private regulatory authorities, medical professionals, family, professional and home environment should play an important role in improving the quality of their life.
2. A multidisciplinary approach to the treatment of patients with chronic pain using multimodal, especially minimally invasive interventions should be used; the doctors should willingly implement innovative methods and techniques for providing medical care to patients with pain syndromes.
3. Specialized medical units of various structures should be expanded to enable the rapid and effective management of vertebrogenic, vegetative, traumatic, pelvic, psychogenic, cephalic, cancer-related, and other pain syndromes. Pain syndromes should be alleviated as quickly as possible, “here and now,” through a combination of minimally invasive surgical techniques, pharmacological treatments, and psychological interventions.
4. Improving awareness of the medical personnel, their training, certification of knowledge; awareness of all social components surrounding and contacting patients suffering from pain.

Considering the alarming epidemiological data on the global prevalence of chronic pain and the unsatisfactory outcomes of its treatment, further systematic research is needed on the medical aspects of pain, including its epidemiology, diagnostics, course, and treatment methodologies. Additionally, there is a need to develop clinical examination protocols and treatment strategies for various types of pain, taking into account the biopsychosocial model of pain.

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## CONFLICT OF INTEREST

The Author declare no conflict of interest

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# Evolution of nursing education and continuing professional development: Challenges of the past and demands of the present

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## ABSTRACT

**Aim:** To review the history of nursing education development, its limitations, and the necessity of transitioning to a modern model of continuing professional development (CPD).

**Materials and Methods:** A systematic, focused review of the literature (2010–2025) was conducted using PubMed, Scopus, Web of Science, Google Scholar, and legislative portals, applying MeSH-compliant terms and a subsequent two-stage filtration process. We included original research, high-quality systematic reviews, meta-analyses, as well as binding legislative and regulatory documents (in English or Ukrainian) that provided empirical data or analytical frameworks regarding nursing education reform, CPD models, or professional competence standards.

**Conclusions:** The historical model of nursing education, predominantly focused on technical skills and passive learning, no longer meets the modern requirements of the healthcare system, which demands autonomy and critical thinking from nursing professionals. The evolution of the nurse's role, particularly in primary care and emergency settings, necessitates the immediate integration of contemporary educational standards and continuous skill enhancement. CPD is recognised as a key prerequisite for quality nursing practice in the 21st century. Effective CPD should prioritise digital competencies, evidence-based practice, and specialised simulation training. It serves as a fundamental mechanism for maintaining competence, ensuring patient safety, and promoting the professional mobility of nursing personnel through flexibility and rapid knowledge acquisition.

**KEY WORDS:** continuing education; nurses; professional competence; policy

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## INTRODUCTION

The modern healthcare system in Ukraine and worldwide is undergoing rapid transformation. The growing prevalence of chronic non-communicable diseases (NCDs), the introduction of digital technologies, integration into the European area, and the need for medical workforce mobility are among the most significant vectors of change. In this context, continuing education for nurses is a key prerequisite for ensuring patient safety, improving the quality of healthcare, and maintaining compliance with international standards. The current requirements for continuing professional development (CPD) are enshrined in Ukrainian legislation and Ministry of Health regulations, aligning with European directives and World Health Organization (WHO) recommendations [1–7].

The strain on Ukraine's healthcare system, the burden of chronic NCDs, technological modernisation, workforce mobility, and the country's European integration trajectory make CPD an imperative. Evidence indicates a

strong association between CPD and improved quality of nursing care, enhanced patient safety, and higher staff satisfaction [7–10].

## AIM

To review the history of nursing education development, its limitations, and the necessity of transitioning to a modern model of CPD.

## MATERIALS AND METHODS

The foundation of this review was a systematic, focused search performed across leading international biomedical databases and official resources, specifically PubMed, Scopus, Web of Science, Google Scholar, and key Ukrainian legislative and regulatory portals. The comprehensive review period was established from 2010 to 2025, encompassing the decade and a half of significant global healthcare and educational reforms. The information

retrieval strategy employed targeted MeSH-compliant terms and their Boolean combinations, including: “nursing education evolution” AND “continuing professional development nurses”, “CPD reforms Ukraine”, and “nursing professional competence”. We included original research, high-quality systematic reviews, meta-analyses, as well as binding legislative and regulatory documents (in English or Ukrainian) that provided empirical data or analytical frameworks regarding nursing education reform, CPD models, or professional competence standards. Sources were systematically excluded if they comprised conference abstracts, dissertations, non-peer-reviewed editorials, opinion articles, or publications lacking verifiable primary data or a direct analytical link to the objectives of this review. The initial exhaustive database search generated a “raw” pool of 284 potentially relevant sources. Following a meticulous two-stage filtration process (title/abstract screening and subsequent full-text evaluation based on the established criteria), a final set of 25 sources was selected for integration into this review.

## ETHICS

This review publication is based on the secondary analysis of publicly available scientific materials, which include peer-reviewed articles, clinical protocols, and data from scientific databases. As the work did not utilize identifiable patient data and did not involve new clinical interventions or primary data collection, ethical committee approval was not required. The authors fully adhered to the ethical standards defined by the World Medical Association’s Declaration of Helsinki, as well as the international ICMJE requirements for publishing in the medical field. The complete absence of plagiarism and fabricated data is confirmed; all utilized sources are properly cited and referenced.

## FRAMEWORK

The study was conducted as a fragment of the scientific project of the Department of Public Health of the Educational and Scientific Institute of Public Health and Preventive Medicine, Bogomolets National Medical University. The research aligns with the thematic plan “Scientific Justification for the Improvement of Organizational Principles of the Healthcare System in the Context of Modern Transformation Changes.” (state registration number 0123U101432; term: 2023-2025).

## REVIEW AND DISCUSSION

Continuing professional development (CPD) for nurses is now recognized as a key element in improving the

quality and safety of healthcare. However, such a system has not always existed. Traditional nurse training pursued different objectives that did not fully align with the needs of modern healthcare and failed to take into account variable factors such as technological progress, epidemiological trends, and international standards.

Until the late 20th century, nurse education in most countries, including Ukraine, was based on a technical model—short-term programs at hospitals or colleges lasting two to three years. The main emphasis was placed on following physicians’ orders rather than on clinical reasoning or evidence-based practice [11]. During the Soviet period, nursing education was classified as secondary-specialized, lacking a scientific component and skills in clinical data analysis. Until the 1990s, most nurses undertook refresher training only once every five years. These courses were largely formal, limited to lecture-based instruction, and did not assess acquired competencies [12]. Consequently, the quality of patient care remained confined to basic procedural tasks, while the level of professional autonomy was low or, in some cases, entirely absent.

Furthermore, the previous nurse education system had several significant drawbacks:

- Limited Content: Focus on technical skills without developing clinical reasoning [13];
- Lack of Evidence Base: Educational programs did not include the principles of evidence-based medicine (EBM);
- Static Nature: After graduation, most nurses lacked incentives or opportunities for further professional development;
- Non-recognition Abroad: Nursing training programs did not meet the requirements of the European Union (EU) [14].

With the development of society and the medical field, new challenges have emerged in Ukraine, necessitating a change in the approach to nurse preparation.

The modern healthcare system presents fundamentally new demands on the professional role of the nurse:

1. Changing Role: The nurse has become a key member of the multidisciplinary team, participates in clinical decisions, and coordinates patient care [15]. In uncomplicated situations, a nurse should independently perform physician functions, such as an obstetrician performing physiological labor delivery.
2. Technological Competencies: Digitization and other digital tools, telemedicine, data analytics, and statistics require new skills [16].
3. Evidence-Based Medicine: Knowledge updating is mandatory to maintain the quality of care and patient safety [17].
4. International Mobility: Directive 2013/55/EU obligates healthcare professionals to participate in CPD to maintain the right to practice [18].

With the onset of the war, new challenges emerged in educational and scientific processes: the disruption of research teams, the inability to plan scientific activities, risks to infrastructure access, and difficulties in maintaining research groups, all of which require flexibility in decision-making. Additional problems arose at the institutional level. State policy in education demands a clear understanding of management culture, scientific expertise, and effective coordination. The quality management system for delivering educational services in accordance with international standards requires continuous feedback between students and lecturers.

In modern healthcare systems, continuing professional development (CPD) is integrated into human resources policy, and the outcomes of this policy correlate with clinical results, including reduced mortality and complication rates. For this reason, the need for CPD in Ukraine's healthcare system is supported by the national regulatory framework. The Regulation on the CPD System (Cabinet of Ministers Resolution No. 725 of 14 July 2021) extends to all healthcare workers. In 2025, Law of Ukraine No. 4246-IX was adopted, which systematically updates specialist training, CPD, and certification. Ministry of Health Order No. 650 (16 April 2025) introduced a new certification procedure and a credit-point system for all healthcare professionals. From 2025, nurses are required to obtain 30 CPD points per year and maintain an individual educational portfolio [1–5].

At the European Union level, CPD and lifelong learning (LLL) are priorities defined in Directive 2013/55/EU and in EFN policy documents, which support the automatic recognition and mobility of nurses provided their professional competence is maintained [6]. International frameworks such as the WHO SDNM 2021–2025 and professional regulatory bodies (e.g., NMC, ANCC) also require structured CPD as a prerequisite for maintaining registration and certification [19, 20].

What is the practical CPD model for a nurse in Ukraine in 2025? The minimum is one annual cycle aiming for  $\geq 30$  points and an annual portfolio. Or several CPD activities with the final goal of  $\geq 30$  points and an annual portfolio. The portfolio structure should reflect: competence goals  $\rightarrow$  activities  $\rightarrow$  evidence  $\rightarrow$  reflection  $\rightarrow$  impact on practice. The balance of activities can include: courses, simulations, journal clubs, conference participation, mentorship, QI projects, e-courses. CPD content priorities are patient safety, infection control, digital skills, evidence-based practice, ethics, and care for vulnerable groups. Evaluation of the CPD effect occurs through the assessment of departmental Key Performance Indicators (KPIs). KPIs are used to measure and evaluate the success of achieving

strategic goals, tasks, and processes both within an individual department and the medical institution as a whole. Performance indicators assess how effectively the medical department operates and achieves set goals, such as improving the quality of medical care or optimizing resources. Based on this, management decisions are made to adjust work directions and set priorities, and in the current market conditions, this may affect final compensation. Recognition of professional qualifications in the EU is ensured by meeting the requirements of Directive 2013/55/EU and the EFN, which set rules for the recognition of professional qualifications in EU member states [6]. Although Ukraine is not currently an EU member, and this directive is not mandatory for execution in Ukraine, it is obligated under the Association Agreement with the EU, which provides for the harmonization of Ukrainian legislation with European standards in the field of professional qualifications.

The activities of university departments are aimed at establishing a solid foundation of knowledge for the training of highly qualified medical personnel among both domestic and international students, in accordance with the requirements of the European Credit Transfer and Accumulation System (ECTS). Lecturers at Bohomolets National Medical University have developed curricula, case studies, and methodological recommendations – including “Fundamentals of Cardiopulmonary Resuscitation,” “The Role of the Nurse in Providing Emergency and Urgent Medical Care” (particularly relevant during wartime), and “Infection Control” – as well as step-by-step guidelines for preparing the injured for transportation and preventing complications.

In recent years, the situation has been complicated by the widespread introduction of market relations in clinical practice and changes in the legislative framework. In this context, the development of opportunities for phantom and simulation training for students represents a necessary and promising direction in the educational process [21, 22].

With the onset of the Russian invasion, most scientific and educational events had to transition to an online format. Despite these challenges, over the past two years alone, more than thirty certified scientific events have been organised and moderated, with CPD points awarded and added to participants' personal educational portfolios based on the completion of CPD activities.

Currently, CPD training occurs in three forms: in-person learning, online learning, and blended learning. Which form of learning ensures better effectiveness? In-person learning provides intensive interaction, discussions, and team simulations. According to the Cochrane Re-



**Table 1.** A Comparison of CPD Formats

| Nº | CPD Format               | Strengths                                                      | Limitations                                              | Correlation with Quality                                  |
|----|--------------------------|----------------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------|
| 1  | In-person (face-to-face) | Intensive interaction, simulations, development of team skills | High cost, limited time availability                     | Improvement of care processes, reduction of complications |
| 2  | Online (e-learning)      | Flexibility, scalability, possibility of repetition            | Less social interaction, weaker formation of soft skills | Improvement of knowledge, protocol adherence              |
| 3  | Blended                  | Combination of the advantages of both approaches               | Requires resources for organization                      | Strongest impact on knowledge and practical skills        |

*Source: compiled by the authors of this study*

view, in-person workshops have a small to moderate positive impact on practice and clinical outcomes [23]. Online learning has shown equivalent effectiveness in improving knowledge and skills, sometimes exceeding in-person due to the flexibility of access and material repetition [24]. Regarding quality, in-person CPD is more closely associated with the development of team skills, communication, and clinical simulations, which directly impact patient safety [23]. Online learning scales more effectively and positively influences the implementation of clinical protocols [9], although it is less effective in forming practical skills (soft skills). A correlation established when studying learning outcomes through patient surveys (questionnaires) shows that in-person CPD is more strongly associated with a reduction in medication errors, pressure ulcers, and falls, while online learning promotes increased knowledge and protocol adherence, but data on the direct impact on mortality are limited [24]. When studying the effectiveness and quality of acquired knowledge and skills in blended learning, it is established that combining online courses for theory and in-person simulations for practice is the optimal model. The EFN and WHO recommend blended CPD as the most effective format (Table 1) [25].

Nurses constitute the largest proportion of healthcare professionals in the healthcare system—over 50% of the total staff, typically 75–80% of medical staff in hospitals. According to the WHO, nurses are responsible for up to 60–70% of all clinical procedures and care interventions, including patient status monitoring, medication administration, infection control, and communication with patients' relatives.

In EU countries, one nurse serves an average of 8–12 patients in a hospital, whereas in Ukraine this figure may reach 15–20, indicating a high workload. Nurses ensure the continuity of the treatment process, as they remain with patients around the clock, unlike physicians, whose involvement is often episodic. The importance of nursing work lies in its direct impact on the quality and safety of healthcare.

From the perspective of evidence-based healthcare, systematic reviews and meta-analyses demonstrate that continuing professional development (CPD) enhances

standards of care, patient safety, and interprofessional collaboration, while e-learning has proven to be an effective CPD format [7–10]. A positive organisational effect is also achieved, as the effective organisation of CPD correlates with staff retention and career progression, provided there is a supportive workplace culture and sufficient time allocation during working hours [6–9].

The necessity of substantially strengthening the practical component of nursing education, while maintaining the required level of theoretical knowledge, represents a key factor for national recovery in the post-war period. Innovations in pedagogy, including massive open online courses (MOOCs), are expected to have a significant global impact on education in the coming years [21, 22].

## PROSPECTS FOR FURTHER RESEARCH

The obtained review data clearly outline the necessity of enhancing the quality and sustainability of nursing care through the profound modernization of CPD. Future scientific endeavors should focus on overcoming identified systemic limitations and unresolved issues. Prospective research directions include:

1. Systemic multi-level analysis of the effectiveness of hybrid (blended learning) and online simulation CPD models within the Ukrainian healthcare system. This analysis must incorporate machine learning and Big Data tools for the objective evaluation of the correlation between the training format and the increased professional competence of nurses, as well as their impact on clinical indicators.
2. Development and validation of universal standardized tools for skills assessment (e.g., Objective Structured Clinical Examination – OSCE), necessary to ensure the objective inclusiveness of CPD results across various demographic (age, socioeconomic) groups.
3. Investigation of the ethical and legal aspects of integrating telemedicine solutions and novel digital devices (particularly wearables) into daily nursing practice. The emphasis should be placed on regula-

tory provisions that guarantee the accessibility and safety of utilizing such innovations. Thus, further research holds the potential to create a reliable evidence base, which is critically necessary for making well-grounded management decisions and formulating state policy aimed at the complete transition to a modern, flexible, and high-quality CPD system.

## CONCLUSIONS

1. The historical model of nursing preparation does not meet the modern requirements of the healthcare system. Today, the professional role of the nurse has significantly expanded: from a technical executor to an autonomous specialist who implements evidence-based practice, coordinates the team,

and applies digital technologies. That is why CPD is recognized as a key condition for quality nursing practice in the 21<sup>st</sup> century.

2. CPD for nurses is not a formality, but a basic mechanism for maintaining competence, safety, and mobility. Ukrainian requirements for 2025 synchronize practice with the EU; moving forward, stable funding, a digital portfolio platform, and linking CPD to quality indicators are needed.
3. In-person learning develops practical and team skills, while online learning offers flexibility and rapid knowledge acquisition. The strongest correlations between CPD and the quality of nursing work are found when using the blended format. Blended CPD is recommended to be established as the standard for Ukrainian practice.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Guarantees for protection of the right of drug addicts to confidential medical information

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## ABSTRACT

**Aim:** To study the theoretical and practical problems in implementing legal guarantees for the protection of the rights of drug addicts regarding confidential medical information, to determine the legal grounds for the disclosure of such information, and to identify the classification of legal guarantees for protecting rights in this area.

**Materials and Methods:** This study employs an interdisciplinary approach, using comparative law, as well as dialectical and systemic methods. The materials of the study serve as a basis for systematizing legislation on the implementation of legal guarantees for protecting the rights of drug addicts regarding confidential medical information. The main legislative sources include the Constitution of Ukraine (Verkhovna Rada of Ukraine, 1996), Fundamentals of Ukrainian Legislation on Healthcare (Verkhovna Rada of Ukraine, 1992), and the Law on Measures to Combat Illicit Trafficking in and Abuse of Narcotic Drugs, Psychotropic Substances, and Precursors (Verkhovna Rada of Ukraine, 1995), among others.

**Conclusions:** The problems of protecting the confidentiality of medical information of drug addicts stem from the lack of legal awareness among medical professionals, outdated medical data protection systems, insufficient state control mechanisms, and ineffective legal liability frameworks, particularly given the development of digital technologies in the healthcare sector.

**KEY WORDS:** human rights, confidentiality, drug addicts, healthcare

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## INTRODUCTION

In accordance with Article 3 of the Constitution of Ukraine, human rights and freedoms, as well as the guarantees of their protection, determine the content and direction of state activity [1]. UN experts emphasize that drug policy should aim to “ensure health, well-being, and security” and must be grounded in human rights principles [2]. The introduction and development of the electronic healthcare system in Ukraine have highlighted the need for enhanced protection of medical information [3], including specific guarantees for safeguarding the rights of drug addicts regarding such information [4]. This information holds a special status, as deficiencies in its protection can create obstacles to the exercise of other rights and freedoms [5–8]. Confidential medical information of drug addicts, if disclosed unlawfully, can have significant negative consequences for their employment, health insurance, and social relationships [6]; it can also contribute to stigmatization, discrimination, and violations of personal autonomy [9], thereby adversely affecting the

realization of their right to healthcare. Consequently, one of the key legal mechanisms for preventing the illegal disclosure of confidential medical information of drug addicts is the establishment of legal guarantees for its protection, which remains insufficiently explored in scientific literature.

## AIM

To study the theoretical and practical problems of implementing legal guarantees for the protection of the rights of drug addicts regarding confidential medical information, determining the legal grounds for the disclosure of such information, and identifying the classification of legal guarantees for the protection of rights in this area.

## MATERIALS AND METHODS

This study employs an interdisciplinary approach to the analysis of national and international legal

instruments, peer-reviewed scientific publications, and documents of international organizations relevant to addressing the implementation of legal guarantees for the protection of the rights of drug addicts regarding confidential medical information. It is based on a review of the scientific debate on concepts such as confidential medical information, the protection of the rights of drug addicts, and constitutional guarantees for the protection of individual rights.

The methodological foundation of the study includes formal-dogmatic, systemic, semantic, epistemological, axiological, comparative-legal, and systemic-structural methods, as well as methodologies encompassing both methods and techniques of scientific knowledge. In the context of legal analysis of the protection of confidential medical information, the methodology is grounded in the interpretation of this concept within the framework of human rights protection in healthcare, as considered by the doctrine of medical and administrative law. The scientific review covers the period from Ukraine's ratification of international conventions on individual rights and drug control to the present, using criteria aligned with the objectives of this study.

The materials of the study serve as a basis for systematizing legislation on the implementation of legal guarantees for protecting the rights of drug addicts regarding confidential medical information. The main legislative sources include the Constitution of Ukraine (Verkhovna Rada of Ukraine, 1996), Fundamentals of Ukrainian Legislation on Healthcare (Verkhovna Rada of Ukraine, 1992), and the Law on Measures to Combat Illicit Trafficking in and Abuse of Narcotic Drugs, Psychotropic Substances, and Precursors (Verkhovna Rada of Ukraine, 1995), among others.

## ETHICS

This article is based on an analysis of publicly available scientific data, including peer-reviewed journal articles, clinical guidelines, and databases. No data that could identify patients were used during the work, and there was no need to obtain approval from the ethics committee, as the study did not involve new clinical interventions or primary collection of patient information. The authors adhered to the ethical principles of the World Medical Association Declaration of Helsinki and international standards for publication in medical journals, including the recommendations of the ICMJE (International Committee of Medical Journal Editors). No element of the work contains plagiarism or fabrication of data. All sources of information are appropriately cited and properly formatted.

## FRAMEWORK

The work is a fragment of a comprehensive target program of the Department of Administrative Law and Administrative Activities, Yaroslav Mudryi National Law University, "Constitutional and legal problems of ensuring the rule of law in the functioning of the mechanism of public power in Ukraine" (№ state registration 0111U000966; deadline: 2022-2025).

## REVIEW AND DISCUSSION

National legislation has established a system for providing specialized care for drug addicts, which differs from basic medical care for other patients [4, 10]. The realization of the rights of drug addicts within the human rights framework is closely linked to other rights, such as access to appropriate, high-quality, and affordable medical care; rights that promote health, including social and economic rights; and the right to participate in decision-making regarding healthcare and drug policy implementation [11–13].

Within the human rights protection system, particular attention is given to legal guarantees for the protection of drug addicts' rights regarding confidential medical information. Confidential information refers to information about an individual, as well as information to which access is restricted by the individual or a legal entity, except for authorized government bodies [14], and which may only be disclosed according to the conditions set by the individual (Part 1 of Article 7) [15].

A key aspect of protecting the legitimate interests of drug addicts concerning confidential health information is the protection of personal data. For instance, under the EU General Data Protection Regulation, three types of personal data relevant to healthcare are distinguished:

(a) Health data – any information relating to a person's physical or mental health, including information about the type of care received;

(b) Genetic data – any data that may reveal details of a person's physiology or health, including laboratory test results;

(c) Biometric data – any data related to specific physical or behavioral characteristics (e.g., facial images, fingerprints, gait patterns) that can be used to identify an individual [16].

Personal data of drug addicts may be classified as confidential information by law or by individuals. Personal data related to the performance of state or local government functions, official or service powers, is not confidential information (Part 2, Article 5) [15]. Therefore, disclosure of confidential medical information of drug addicts is possible in two cases,



**Table 1.** The components of the object of protection of medical confidentiality of drug addicts

| <b>Components:</b>                                                             |
|--------------------------------------------------------------------------------|
| information about the health status of a drug-addicted patient;                |
| information about the drug addict's illness;                                   |
| information about the diagnosis of a drug addict;                              |
| information obtained during a medical examination of a drug addict;            |
| information about the fact of seeking medical help from drug addicts;          |
| information about the medical examination of drug addicts and their results;   |
| information about treatment methods for drug addicts;                          |
| information about the intimate and family aspects of the lives of drug addicts |

*Source: compiled by the authors based on [18, 19]*

**Table 2.** The main grounds for the legal disclosure of the medical data of drug addicts

| <b>Types of legal grounds:</b>                                                                                                                                                                                                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| in case of written consent of the drug-addicted patient himself;                                                                                                                                                                      |
| in the event of a motivated request from the bodies of inquiry, investigation, prosecutor's office, and court;                                                                                                                        |
| if keeping the medical secrets of drug addicts significantly threatens the health and life of such patients and/or other persons;                                                                                                     |
| Information about a person's treatment in a narcological institution may only be provided to law enforcement agencies if this person is held criminally or administratively liable;                                                   |
| in cases where it is necessary in the interests of national security, economic well-being and human rights, or in the interests of preserving territorial integrity or public order, for the purpose of preventing disorder or crime; |
| in cases where it is necessary to protect public health, to protect the reputation or rights of others, to prevent the disclosure of information received in confidence, or to ensure the authority and impartiality of justice;      |
| information that ensures the anonymity of the patient may be used in scientific and educational work (without the consent of such person);                                                                                            |
| in the case of involving other specialists in the treatment of a drug-addicted patient, for whom this information is professionally necessary.                                                                                        |

*Source: compiled by the authors based on [12,15,17,20]*

namely: (a) with consent and (b) without their consent [1, 17, 18]. It should be noted that a special legislative document in Ukraine – the Fundamentals of Ukrainian Legislation on Health Care – does not define a clear list of conditions under which a lawful violation of the principle of confidentiality of information regarding drug addicts is permissible [18], and there is no special legislative regulatory act in this area. We present the components of the object of protection of medical confidentiality of drug addicts as defined in national legislation in Table 1 [18, 19].

It should be noted that the right to access a drug addict's confidential medical information without their consent is granted to parents (or adoptive parents), guardians, or trustees (Part 2 of Article 285) [19]. Additionally, in the event of the patient's death, family members or other individuals authorized by them have the right to participate in the investigation of the causes of death and to review the conclusions regarding the cause of death (Part 5 of Article 39) [18].

In the following, we will reveal the main legal grounds for the lawful disclosure of confidential medical information regarding drug-addicted patients in Table 2 [12,15,17,20].

Thus, when treating drug-addicted patients, a health-care professional should consider the importance of the information and the grounds for its lawful disclosure, namely: (1) the patient's personal data, especially age, gender, etc.; (2) medical information, which includes complaints, diagnoses, and other information about the state of health; (3) data on heredity, illnesses of family members and loved ones; (4) information about the social environment, lifestyle, experiences, emotional state and much more related to the private life of such a person.

The category of legal guarantees of compliance with the rights of drug addicts to protect confidential medical information is not new to public law [5], however, its content and concept are still a matter of debate. This is due to both the scope and complexity of the issue. In particular, the confidential medical information of drug addicts is particularly "sensitive" among other categories of patients [7, 8]. Some scholars emphasize legal guarantees for the protection of medical information as means [8] or methods of protecting rights (for example, judicial protection) [21], while others consider them as conditions or factors (economic, political, social) [6,11] that create

favorable conditions for the realization of the rights of such individuals.

The scientific doctrine does not define uniform criteria for classifying legal guarantees of the rights of drug addicts to the protection of medical information in a general approach. Let us draw attention to one more problem - the preservation of confidential information on electronic media. We are talking about the «Electronic Patient Registry» in Ukraine. It does not have a classification of legal guarantees of the rights of drug addicts in the sphere of the circulation of confidential medical information. In our opinion, it is worth distinguishing three groups of such guarantees for the protection of rights. The first group is guarantees of the rights of drug addicts to the protection of medical information (creation of conditions for their emergence and existence). The second group is guarantees for the realization of the rights of drug addicts regarding this information (establishing rules and legal mechanisms for their implementation). The third is guarantees for the protection of the rights of drug addicts in the sphere of circulation of confidential medical information (means and institutions for the restoration of violated rights). It should be noted that the problem of forming additional guarantees aimed at protecting the confidential medical information of minors who are addicted to narcotic drugs is particularly important.

In addition, legal guarantees for the protection of medical information regarding drug addicts, depending on their role, can be divided into basic (which include legal norms, legal mechanisms, and institutional structures), specific (legal means explicitly provided for by law), and “additional” guarantees. For example, the second type of guarantee includes norms that establish a regime of medical confidentiality in this area [22]. Another example of specific guarantees for the protection of the rights of drug addicts in the sphere of circulation of confidential medical information is legal norms that prohibit unauthorized access to such information by third parties [20].

It is also appropriate to highlight “additional” guarantees for the protection of the medical information of drug addicts, particularly minors [7]. These should be considered as legal means aimed at protecting a special type of medical information, often referred to as “sensitive information.” This is because, in some cases,

society negatively perceives information regarding the presence of diseases related to addiction to psychoactive substances in individuals [9]. Hence, the need for clear procedures for the storage and recording of this type of medical information in a dedicated national law becomes particularly important.

For example, in the USA, there is a separate legislative act that regulates the protection of confidential information of drug addicts. It outlines procedures for obtaining written consent for the provision of such information, requires healthcare institutions to follow specific measures regarding access, use, and disclosure of health information, and provides patients with the right to review, amend, and manage their health records [8].

Additional guarantees for the protection of the medical information of drug addicts include the institution of legal liability. Thus, under national legislation, medical professionals are legally and ethically liable for the illegal disclosure of confidential medical information of drug addict patients. Such violations may result in criminal, civil, and disciplinary liability for the medical professional. Administrative liability may also apply to healthcare institutions and their employees in the event of illegal disclosure of confidential medical information of drug addicts. However, in most cases, the mechanism of legal liability of medical professionals in the studied area is ineffective.

## CONCLUSIONS

The problems of maintaining the confidentiality of medical information of drug addicts stem from the lack of legal awareness among medical professionals, outdated medical data protection systems, and the absence of effective mechanisms for state control and legal liability. These problems are further compounded by the use of digital technologies, artificial intelligence programs for analyzing medical data, and the implementation of electronic medical records and electronic documentation. Such factors create additional risks for protecting information about drug-addicted patients, may lead to violations of their rights, reduce trust in healthcare institutions and medical professionals, and potentially prevent these individuals from exercising their right to medical care.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Prerequisites for implementing expert medical modeling based on human digital physiology programs

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## ABSTRACT

**Aim:** To explore the components, approaches, and principles of expert medical modeling utilizing modern digital medical imaging and human digital physiology programs.

**Materials and Methods:** The research methodology involves an analysis of current regulatory documentation, including acts of the Cabinet of Ministers of Ukraine related to healthcare digitalization. It also incorporates scientific and analytical sources on medical informatics, digital medical simulation, and data visualization standardization. Comparative analysis methods were applied to medical imaging standards, along with content analysis of medical literature and an evaluation of the potential for expert assessment of the human condition using immersive technologies.

**Conclusions:** The study emphasizes the need to integrate digital media across all levels of healthcare to develop a unified expert information visualization system based on human digital physiology programs. This approach enables objective decision-making and supports the development of personalized models using 3D/4D imaging and artificial intelligence.

**KEY WORDS:** expert medical modeling, medical imaging standard

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## INTRODUCTION

Digital transformation in healthcare is a key element in modernizing clinical practice [1, 2], diagnostics, and rehabilitation. One of the leading directions is the implementation of digital medical visualization standards, which ensure the storage, processing, and exchange of visual medical data [3]. Within the framework of national digital development strategies—particularly in the context of implementing the Concept for the Development of Digital Competencies and the Electronic Healthcare System in Ukraine—the integration of digital visualization technologies is of strategic importance [4].

For the implementation of expert medical modeling, it is necessary to define the essential conditions for the circulation of digital medical content within specialized and expert networks.

First and foremost, all medical—especially expert—personal models and conclusions must be entered under blockchain conditions, a decentralized network [5] that operates on the principle of a sequential chain of blocks containing information about operations with tokens, a unit of account in the blockchain network

through which digital values can be created. Records in the blockchain are immutable and publicly accessible, thereby preventing data manipulation and ensuring their security [6, 7].

Compliance with this condition will ensure the use of non-fungible tokens (NFTs)—a type of digital asset created on the blockchain that allows ownership of a unique item in the network, such as an image, animation, or video, while verifying its authenticity [8].

## AIM

The aim of this study is to explore the components, approaches, and principles of expert medical modeling (EMM) using modern digital medical visualization tools and computer-based human digital physiology programs.

## MATERIALS AND METHODS

The methodological basis of the study is the analysis of current regulatory and legal documentation, including

acts of the Cabinet of Ministers of Ukraine regarding the digitalization of the healthcare sector, as well as scientific and analytical sources on medical informatics, digital medical simulation, and data standardization of visualization systems. Methods included comparative analysis of medical visualization standards and content analysis of medical literature addressing the possibilities of conducting expert assessments of the human body's condition.

The data analysis was conducted using open sources, predominantly covering the period 2010–2025. Some earlier sources were included to trace retrospective trends and sustainability. The main search keywords were “standards of medical imaging”, “digital examination methods”, “virtual technologies in medicine”, and “application of artificial intelligence in medicine”. The search criteria focused on contemporary approaches and practical experience in the use of digital technologies, particularly in simulation medicine. Sources primarily aimed at transforming objective digital media into subjective forms—by generating textual conclusions or reports—were excluded from consideration. The initial database comprised approximately 70 sources, of which 35 were included in the final analysis.

## ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines and databases. No patient-identifying data was used during the work, nor was there a need to obtain approval from an ethics committee, as the study did not include new clinical interventions or initial collection of patient information.

The authors adhered to the ethical principles of the Helsinki Declaration of the World Medical Association and international standards for publications in medical journals, including the recommendations of the ICMJE (International Committee of Medical Journal Editors).

No element of the work contains plagiarism or data fabrication. All sources of information are appropriately cited and properly formatted.

## REVIEW AND DISCUSSION

During the study, the authors formulated a definition of the term “expert medical modeling” (EMM) as follows: EMM is the creation of a digital model of the human body based on a computer program of digital physiology for conducting a comprehensive expert assessment of the possibility of performing a proposed functional load and for further prognosis of its condition [9, 10].

Historically, the modeling of the human body—apart from works of science fiction—has been used in medicine for quite some time [11, 12]. The introduction of the term “virtual twin” into the medical lexicon in modern Ukraine dates back to 1971, when it was primarily used by pharmaceutical companies. The term “avatar” has gained wider use. In the context of computer technology, it can be traced back to Neal Town Stephenson's novel *Snow Crash* (1992). The INACSL (International Nursing Association of Clinical Simulation and Learning) Standards Committee defines an avatar as “a graphical representation, usually three-dimensional, of a person, capable of reproducing relatively complex actions, including facial expressions and physical reactions during participation in virtual simulation training” [12].

Conditionally, such programs were mainly educational or clinical in nature [13, 14]. The most well-known programs and developments include Stanford Virtual Reality Integration, particularly Stanford Virtual Heart and Surgical Theater; Oxford Medical Simulation; SimX; and the AI BODY digital physiology program, among others. Considerable progress has been achieved in the creation of 3D models based on digital media obtained from computed tomography (CT) or magnetic resonance imaging (MRI) scans, with subsequent reconstruction of lost anatomical connections of the body through 3D printing [15, 16]. This direction has developed most actively in prosthetics and reconstructive surgery, particularly in response to the large number of casualties from combat operations.

At the same time, for stable functioning and realistic reproduction of visual effects, these programs rely on digital media generated using medical visualization tools [17, 18]. Medical visualization refers to the methods and processes used to create images of different parts of the human body for diagnostic and therapeutic purposes within digital medicine [1, 2, 4].

The use of medical visualization enables physicians to obtain more accurate diagnoses and make corresponding treatment decisions. Visualization standards and file formats play a significant role in the annotation of medical images. Standardization applies not only to the processes of archiving digital files but also to ensuring access to them and facilitating exchange between structural units of the medical and preventive care network [19, 20].

The main universally recognized standards in the field of digital medical visualization include:

- DICOM (Digital Imaging and Communications in Medicine) - provides a universal format for the exchange and archiving of medical images, supported by most modern diagnostic equipment [21];
- NifTI (Neuroimaging Informatics Technology Initiative) - solves problems in the field of neurovisualization,



**Table 1.** Comparative Implementation of DICOM and NifTI Formats in Clinic and Research

| Format     | Geography of implementation | Industry          | Example of use                                                         |
|------------|-----------------------------|-------------------|------------------------------------------------------------------------|
| DICOM [24] | USA, UK, Japan              | Clinical Medicine | PACS Archives, telemedicine, AI diagnostics at Mayo Clinic             |
| NifTI [25] | USA, EU, Canada, Australia  | Neuroscience      | Human Connectome Project, depression analysis, functional MRI modeling |

Source: compiled by the authors based on [24, 25]

**Table 2.** Comparative Analysis of DICOM and NifTI Applications

| Criterion          | DICOM [24]             | NifTI [25]                          |
|--------------------|------------------------|-------------------------------------|
| Format             | 2D image with metadata | 3D/4D Arrays                        |
| Appointment        | Clinical practice      | Neuroresearch                       |
| Image orientation  | Partially supported    | Spatially and temporally determined |
| Processing speed   | Low                    | High                                |
| Amount of metadata | High                   | Surveys                             |

Source: compiled by the authors based on [24, 25]

with an emphasis on functional magnetic resonance imaging [22, 23].

Since the study involves working with digital data of the human body under blockchain conditions, it is worth mentioning the standards and protocols of confidentiality of personalized information, namely:

- PACS (Picture Archiving and Communication System) - a system for storing and accessing images;
- HL7 (Health Level Seven) - a standard for the exchange of clinical and administrative information between medical systems;
- FHIR (Fast Healthcare Interoperability Resources) - a modern standard for the exchange of electronic medical information. FHIR is based on open web standards and provides a more flexible and easier exchange of data between different electronic medical documentation systems;
- TLS (Transport Layer Security) and HTTPS (Hypertext Transfer Protocol Secure) - protocols that provide encryption of data during its transmission over the Internet, ensuring the confidentiality of patient data;
- VPN (Virtual Private Network) - provides a secure tunnel for transmitting data between network nodes, allowing to protect data from unauthorized access during their transmission over unprotected networks, such as the Internet [23].

For a more gradual movement, it is advisable to briefly review the history of creation and directions of use of the two aforementioned main standards of digital medical visualization, namely DICOM [24] and NifTI [25].

DICOM format was developed in the 1980s by the American College of Radiology (ACR) in collaboration with the National Electrical Manufacturers Association (NEMA) in the USA. The first DICOM 1.0 specification was

released in 1985 under the name ACR/NEMA 300 [24].

Over time, the format became an international standard (ISO 12052) and was adapted in over 150 countries. Universities, hospitals, and research centers around the world use it in daily clinical practice:

In the USA, DICOM is a standard in all institutions accredited by the Joint Commission; large networks (Mayo Clinic, Cleveland Clinic) have integrated PACS systems based on DICOM [24]:

- In Great Britain, the NHS (National Health Service) has fully transitioned to DICOM images under the “National PACS Programme” back in 2008 [24];
- In Japan, over 90% of hospitals use DICOM-compatible systems, including in telemedicine consultations in rural areas;
- In Germany and France, DICOM is part of national medical informatics standards, supported by legislation on electronic medical documentation (eHealth). Hospitals around the world use DICOM not only because it allows exchanging images and annotations but also because of the simplicity of transmission, providing references to patient medical records and other valuable metadata [24].

NifTI format was developed in 2001-2003 under the auspices of the National Institute of Mental Health (NIMH) for the needs of neuroscientific research, particularly functional MRI [10]. Its main advantage is the compact storage of large arrays of 3D/4D data, with spatial and temporal orientation [25].

It has become the primary format used in the Human Connectome Project (USA), UK Biobank (United Kingdom), and the ENIGMA Consortium—a global neuroscience initiative encompassing more than 40 countries. NifTI is widely applied in scientific and medical compu-

tations (HPC/AI), including in software packages such as SPM (Statistical Parametric Mapping), FSL (FMRIB Software Library), and AFNI (Analysis of Functional NeuroImages), which are considered de facto standards for functional MRI analysis. It is also supported within the European Union as the primary format in Horizon Europe projects focused on brain research and neuropsychiatric disorders [25].

For a clearer visual representation of the global distribution of medical visualization standards and the predominant directions of their scientific and practical use, all the aforementioned characteristics of these standards are summarized in Table 1 [24, 25].

The conditions of technological application in various fields of medical practice, and peculiarities of work with different standards of medical visualization are grouped and presented in Table 2 [24, 25].

The concept of expert medical modeling (EMM) has evolved through several technological and methodological stages – from the early era of telemedicine, focused on remote consultations and data transfer, to the modern paradigm of the digital twin, which enables full-scale virtual replication of human physiology. Telemedicine primarily addressed the accessibility of healthcare and real-time communication between physicians and patients. The next stage emerged with the advancement of medical visualization technologies such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET), which allowed precise digital reconstruction of anatomical structures. The development of interoperability standards further enabled the integration of heterogeneous medical data into unified expert systems [24, 25].

Today, EMM represents a synthesis of medical informatics, big data analytics, and bioethics, forming the foundation for the creation of dynamic, data-driven digital replicas of patients – virtual clinical twins – to support diagnostic, rehabilitative, and prognostic decision-making. This transition from telemedicine to digital twin-based modeling reflects the global shift from reactive to predictive and personalized medicine, where expert judgment is grounded not only in professional experience but also in objective digital evidence [26].

Digital visualization is changing the paradigm of long-term expert clinical observation. The transition from subjective interpretations to objective digital models creates new challenges and requirements for the digital literacy of medical professionals and the overall organization of medical care. In particular, the implementation of EMM poses a specific algorithm of actions:

- obtaining a digital medium based on a specific standard of medical visualization;

- saving the digital medium based on a specific standard of medical visualization;
- integrating the specified digital data into the research program of expert modeling;
- creating a current expert model of the organism of the study subject;
- conducting virtual load tests and generalizing and saving their results;
- forming a preliminary expert conclusion with reference to the virtual file of the current virtual research model;
- further forming expert models based on fresh digital media to track trends in the development of improvements/complications in the human body over time [26-30].

This shift towards digital models necessitates a re-evaluation of training programs for medical professionals, emphasizing digital literacy and the interpretation of complex datasets. Furthermore, the implementation of expert medical modeling (EMM) requires careful consideration of data security and patient privacy, particularly when using blockchain technology [8]. As Y. Ghadi et al. [9] note, the role of blockchain in securing the Internet of Medical Things (IoMT) is crucial; however, challenges related to scalability and regulatory compliance remain to be addressed.

The virtual visualized object, formed with all its anatomical, physiological, clinical, psychological, and functional characteristics, and presented in accordance with the International Classification of Diseases (ICD) and the International Classification of Functioning, Disability, and Health (ICF), can be subjected to virtual loading tests to determine the organism's tolerance to various levels of functional stress. The virtual conclusions obtained as a result of executing the expert chain should serve as the basis for decisions regarding the level of disability, recommendations for further treatment and rehabilitation methods, and guidance on future employment [2, 4, 12].

This is particularly relevant in Ukraine today, against the backdrop of the large number of casualties resulting from combat operations. The number of people requiring rehabilitation after emergencies and specialized medical care continues to increase almost daily. The consequences of traumatic factors of various origins have led to a significant number of individuals who have partially or completely lost the ability to perform their previous professional duties. This situation hampers medical and social adaptation, affects the psycho-emotional state of injured persons, and generates negative expectations regarding their own future prospects. As a result, many demobilized individuals suffer from so-called "military syndromes", which pose significant obstacles to their full reintegration into civilian life. Further

research is needed to explore the long-term impact of these syndromes and to assess the effectiveness of EMM in facilitating rehabilitation [31, 32].

In addition, numerous scandals concerning the subjectivity of conclusions reached by medical-social and military-medical expert commissions have further undermined public confidence. The introduction of the proposed EMM, based on a computer program of digital physiology, would make it possible to achieve the most objective and transparent form of expert decision-making [2, 4, 11, 13].

The shift toward digital expert systems also brings to light an essential dimension – the ethical implications of using digital patient replicas. The implementation of expert medical modeling (EMM) inevitably raises a complex set of ethical, legal, and psychological questions. Creating and utilizing digital copies of patients – so-called virtual twins – requires a well-defined regulatory and moral framework that ensures respect for human dignity, autonomy, and privacy. The ability to simulate a person's physiological and psychological state *in silico* blurs the boundary between clinical data and personal identity [10–12, 33].

First and foremost, informed consent must explicitly include provisions for the creation, processing, and potential reuse of digital replicas. Patients should be fully aware that a digital twin may persist and evolve beyond the duration of their physical treatment. Secondly, data ownership and control become key ethical issues: who owns the digital twin – the patient, the healthcare institution, or the software developer? Thirdly, algorithmic transparency and the right to explanation must be guaranteed, particularly when AI-driven conclusions influence clinical or legal decisions [3].

It is equally important to prevent the dehumanization of medical expertise. Digital models should support – not replace – the clinician's ethical responsibility. In this regard, expert medical modeling must adhere to the principles of the Declaration of Helsinki [34], the Oviedo Convention [35], and relevant national bioethical frameworks [4], ensuring that technology serves humanity – not the reverse.

Among the promising perspectives of this approach are the creation of a national archive of digital visualizations, the expansion of medical information system functionality, and the implementation of AI algorithms for image analysis. The development of such algorithms should be oriented toward addressing the specific needs of medical professionals and ensuring that the resulting models remain clinically relevant, interpretable, and ethically compliant.

Prognostic assessment and clinical decision-making based on large arrays of integrated and generalized indicators should be performed using artificial intelligence (AI) tools. This corresponds to the broader global trend of integrating AI into healthcare.

In Ukraine, the implementation of expert medical modeling (EMM) could become a cornerstone of the national strategy for integrating medicine, science, and digital transformation within the healthcare system. At the clinical level, EMM enables the creation of patient-specific digital twins for simulating treatment processes, forecasting surgical outcomes, and planning rehabilitation programs. In rehabilitation medicine, this approach allows clinicians to assess a patient's physiological tolerance to therapeutic loads, monitor recovery dynamics, and design personalized rehabilitation trajectories for veterans, civilians affected by war, and individuals with occupational diseases [4].

At the scientific level, EMM may serve as a foundation for developing large-scale databases of digital physiology, supporting interdisciplinary research across biomechanics, neuroscience, and psychophysiology. For expert medicine – including medical-social, military-medical, and forensic expertise – EMM provides a framework for standardizing and objectifying decision-making through automated analysis of digital physiological replicas, thereby minimizing the human factor and enhancing transparency [1–4, 10–13].

From an organizational perspective, the establishment of a national platform for medical digital visualization based on EMM could become an integral component of the Ukrainian electronic healthcare system. Such a platform would ensure interoperability between clinics, research institutions, and rehabilitation centers; promote the application of blockchain technologies for data protection; and accelerate the adoption of AI-driven tools for image analysis and predictive modeling [5, 9, 33].

Ultimately, the use of EMM in Ukraine holds not only technological but also social significance – forming a new culture of digital medicine centered on the patient, their safety, and the quality of recovery.

## CONCLUSIONS

1. Modern domestic clinical practice demonstrates insufficient consistent use of digital media: images often remain in the form of printed conclusions, and digital formats are not integrated into medical information systems.
2. There is an urgent need in Ukraine to introduce digital media in all areas of healthcare and to create an integrated EMM.
3. A promising direction is the formation of personalized models of the patient based on 3D/4D images for conducting expert tests using artificial intelligence technologies.
4. Increasing the digital competence of medical professionals is a prerequisite for the effective use of digital standards.

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## CONFLICT OF INTEREST

The Authors declare no conflict of interest

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# Digital dentistry in action: Analysis of clinical outcomes of computer-aided design and manufacturing, three-dimensional printing, and artificial intelligence in tooth defect rehabilitation

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## ABSTRACT

**Aim:** To systematically evaluate the clinical outcomes of digital dentistry technologies - computer-aided design and manufacturing (CAD/CAM), three-dimensional (3D) printing, and artificial intelligence (AI) - in the rehabilitation of tooth defects, compared to conventional techniques.

**Materials and Methods:** A systematic search of PubMed, Scopus, Web of Science, and the Cochrane Library (up to mid-2025) identified clinical studies on CAD/CAM, 3D-printed dental prostheses, and AI in tooth restoration. Studies reporting survival, complications, patient outcomes, and diagnostic accuracy were included. Thirty-two studies (≈1580 patients) met inclusion criteria; 25 were included in quantitative synthesis. The five-year survival of CAD/CAM restorations was ~90%, comparable to conventional crowns. Milled and 3D-printed dentures showed similar satisfaction, with milled types offering better fit and fewer adjustments. Digital workflows shortened production time and reduced costs. AI models detected caries with ~85% sensitivity and ~90% specificity, and AI-based implant planning matched expert accuracy while cutting planning time (≈10 min vs 30 min). No safety issues were reported.

**Conclusions:** Digital dentistry (CAD/CAM, 3D printing) achieves high-quality, durable restorations (~90% five-year survival) with greater efficiency and lower cost than conventional methods. AI tools show strong potential for accurate, time-saving diagnostics and treatment planning. Overall, digital methods are safe, effective, and suitable for clinical integration. Further long-term studies, especially on AI-driven workflows and 3D-printed materials, are recommended to confirm sustained outcomes and establish evidence-based standards.

**KEY WORDS:** dental prosthesis; three-dimensional printing; artificial intelligence

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## INTRODUCTION

Digital dentistry has revolutionized modern dental practice by introducing advanced technologies for designing restorations, diagnosis, and treatment planning. Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) technology—the use of computer-based design and manufacturing for dental prostheses—offers several advantages over traditional laboratory methods. Notably, it enables faster production, improved convenience, consistent high quality, and the elimination of interim steps such as temporary crowns.

With chairside CAD/CAM systems, for example, a prepared tooth can be digitally scanned and restored with a permanent ceramic crown in a single appointment, streamlining the workflow. Clinical studies have shown that these same-day CAD/CAM restorations

can eliminate the need for provisional crowns and additional visits.

Similarly, 3D printing is transforming prosthodontics and other fields by enabling the precise, customized fabrication of devices—from dentures to surgical guides—with high accuracy and reproducibility. Additive manufacturing can reduce material waste and labour, although current challenges (equipment cost, material properties, post-processing) have tempered its universal adoption. Nonetheless, the trend is toward increasing use of 3D-printed solutions as materials improve [1].

In parallel, artificial intelligence (AI) has rapidly emerged as a valuable tool in dentistry. AI refers to computer algorithms (often based on machine learning) that perform tasks requiring human-like intelligence. In dental applications, AI systems can analyse radio-



graphs or photographs to aid in detecting diseases (e.g., caries or periapical pathology) and assist in treatment planning (such as optimal implant positioning). By learning from large datasets of expert decisions, AI can support clinicians with diagnostic suggestions and decision-making.

All major dental disciplines—restorative, prosthodontics, orthodontics, and oral surgery—are beginning to adopt AI-driven solutions for improved efficiency and accuracy. For example, AI-based image analysis can highlight incipient lesions on bitewing X-rays that a human might overlook, and predictive models can forecast the long-term success of a treatment for a given patient profile [2].

Despite the enthusiasm for these digital technologies, it is crucial to evaluate them based on evidence from clinical studies. Key questions remain: Do CAD/CAM restorations and 3D-printed prostheses last as long and fit as well as conventionally fabricated ones? Can they improve patient outcomes or clinical efficiency? Likewise, can AI tangibly enhance diagnostic accuracy or treatment outcomes compared with standard practice?

Previous research has addressed parts of these questions—for example, systematic reviews comparing digital and conventional dentures or assessing AI accuracy in detecting dental diseases—but a comprehensive synthesis encompassing CAD/CAM, 3D printing, and AI in tooth defect rehabilitation is still lacking [2].

## AIM

To systematically evaluate the clinical outcomes of digital dentistry technologies - computer-aided design and manufacturing (CAD/CAM), three-dimensional (3D) printing, and artificial intelligence (AI) - in the rehabilitation of tooth defects, compared to conventional techniques.

## MATERIALS AND METHODS

A total of 912 unique articles were identified in PubMed, Scopus, Web of Science, and the Cochrane Library. After duplicates were removed and abstracts were reviewed, 76 papers were selected for full-text screening. Finally, 32 studies published between 2010 and 2025 met the inclusion criteria, and 25 of these were included in the quantitative analysis.

The included research covered three main topics: (1) comparisons between CAD/CAM and conventional dental prostheses; (2) evaluations of 3D-printed and conventional prostheses; and (3) the use of artificial intelligence in dental diagnostics and treatment planning [3].

The studies consisted of randomised controlled trials, cohort studies, case series, and diagnostic accuracy studies. The median follow-up period for prosthetic outcomes was approximately two years, while most AI-related research was cross-sectional and assessed diagnostic accuracy at a single time point.

An exhaustive literature search (last updated August 2025) was performed in MEDLINE (PubMed), Scopus, Web of Science, and the Cochrane Library. We used combinations of keywords and MeSH terms related to digital dentistry and tooth restoration. For example, search terms included “CAD/CAM” OR “computer-aided design” OR “digital fabrication” combined with “dental prosthesis” OR “crown” OR “denture”; “3D printing” OR “three-dimensional printing” combined with “dentistry”; and “artificial intelligence” OR “machine learning” combined with “dentistry” OR “tooth”. We also included outcome-related terms such as “survival,” “complications,” “accuracy,” and “patient satisfaction” to focus on clinical results.

No language restrictions were applied (studies were included if an English abstract was available). Reference lists of relevant articles were manually screened to identify any additional studies [4].

## INCLUSION CRITERIA

We included studies meeting all of the following: (1) Population: Patients requiring restoration of tooth defects (due to caries, fracture, edentulism, etc.), in any clinical setting; (2) Interventions: Use of CAD/CAM milling or 3D printing to fabricate a restorative dental prosthesis (inlay/onlay, crown, fixed partial denture/bridge, complete denture, implant abutment, etc.), or use of an AI algorithm in diagnosis or treatment planning related to tooth rehabilitation; (3) Comparators: Ideally a conventional technique group (e.g. traditional lab-fabricated prosthesis, conventional diagnostic method) for comparison, though studies without a control group were included if they reported relevant outcomes for a digital technique; (4) Outcomes: At least one clinical outcome measure, such as restoration survival/failure rate, complication incidence, prosthesis fit (e.g. marginal gap), patient satisfaction or quality of life scores, number of appointments, or diagnostic accuracy metrics (sensitivity/specificity for AI) [5].

## DATA EXTRACTION

Two reviewers independently screened titles/abstracts for eligibility, then evaluated full texts of potentially relevant articles. From each included study, data were extracted using a standardized form. Extracted data

included: study design, sample size and patient characteristics, details of the digital technique (type of CAD/CAM system or printer and materials; type of AI algorithm and its training data if applicable), details of any conventional comparator, follow-up duration, and all reported outcomes of interest. For CAD/CAM and 3D-printed prosthesis studies, we recorded the number and type of restorations, follow-up period, any reported survival or success rates, failure or complication counts (and causes, such as secondary caries or fracture), measures of fit or accuracy (e.g. mean marginal gap in microns), patient satisfaction or functional scores, number of adjustment visits, and any cost or time data provided. For AI studies, we extracted performance metrics (sensitivity, specificity, accuracy, area under ROC curve) and any comparisons to clinician performance or conventional diagnostic methods. Discrepancies in data extraction were resolved by consensus or by involving a third reviewer.

## ETHICS

This review article is based entirely on publicly available scientific data published in peer-reviewed journals, clinical guidelines, and established databases. No patient-identifiable information was used, and approval from an ethics committee was not required because the study did not involve new clinical interventions or the primary collection of patient data.

The authors adhered to the ethical principles of the World Medical Association's Declaration of Helsinki and to international standards for medical journal publications, including the recommendations of the International Committee of Medical Journal Editors (ICMJE).

No part of this work contains plagiarism or data fabrication. All sources of information are properly cited and appropriately referenced.

## REVIEW AND DISCUSSION

This review analyzed evidence from clinical studies comparing digital and conventional methods in dental prosthetics and evaluating artificial intelligence applications in dentistry. Sample sizes across studies ranged from approximately 20 to 250 participants [6].

Most investigations on CAD/CAM systems, particularly chairside milling units such as CEREC, focused on the fabrication of ceramic (lithium disilicate, zirconia) or composite restorations. 3D-printing studies primarily employed stereolithography or digital light processing to produce denture bases or interim restorations. In contrast, conventional approaches relied on the lost-wax technique, heat-polymerized acrylic, or metal-ceramic fabrication methods.

AI-related studies covered diverse diagnostic and planning applications, including radiographic caries detection through deep learning, automated intraoral image analysis, cephalometric landmark identification, and AI-assisted implant planning. Most algorithms—predominantly convolutional neural networks—were trained on large image datasets and compared with expert evaluations using standard metrics such as sensitivity, specificity, and overall diagnostic accuracy [7].

Among the eight randomised controlled trials, five were rated as low risk of bias, while three were assessed as having some risk due to the practical impossibility of blinding. Non-randomised studies were of moderate quality, mainly limited by shorter follow-up periods and potential selection bias. AI diagnostic studies generally demonstrated a low risk of bias, though several lacked independent verifications of reference standards. Funnel plot analysis for the five-year survival of CAD/CAM crowns revealed no significant publication bias. Overall, the quality of evidence was high for short-term outcomes ( $\leq 5$  years) and diagnostic accuracy, and moderate for long-term results owing to methodological heterogeneity and limited follow-up beyond five years.

Across longitudinal research, CAD/CAM restorations consistently achieved survival rates comparable to conventional methods. The pooled five-year survival of CAD/CAM crowns was approximately 90%, while ten-year survival for all-ceramic bridges ranged between 80% and 82%, aligning with traditional benchmarks. A large six-year retrospective analysis involving around 500 crowns reported no significant survival difference between CAD/CAM and conventionally fabricated crowns (overall 96%). The most frequent causes of failure were secondary caries and ceramic fracture—similar to conventional outcomes—while monolithic zirconia restorations exhibited fewer veneer chippings [8].

Digital workflows demonstrated equal or superior marginal fit and precision. Mean marginal gaps typically ranged from 50 to 100  $\mu\text{m}$ , with lower variability in CAD/CAM restorations. Improved adaptation helps reduce plaque accumulation and secondary caries, thereby extending prosthesis longevity. In complete denture trials, patient satisfaction and oral health-related quality of life were similar between digital and conventional dentures. However, milled dentures provided a better fit, required fewer post-insertion adjustments, and offered greater material strength due to reduced porosity [9].

Studies assessing clinical efficiency consistently showed time and cost benefits of digital fabrication. Chairside, single-visit crowns eliminated the need for temporary restorations and additional appointments. For complete dentures, digital workflows shortened total production time by 20–30% and reduced labora-

tory labour costs, offsetting higher material expenses. These improvements resulted from automation, fewer remakes, and streamlined appointments, rendering CAD/CAM workflows economically advantageous, especially in high-volume clinical settings.

Patients generally reported high satisfaction with digital restorations, emphasising improved comfort during intraoral scanning and shorter treatment durations. Functional outcomes and Oral Health Impact Profile (OHIP) scores were comparable between digital and conventional groups, although convenience and patient preference clearly favoured digital methods. Complication rates were similar across fabrication types: common issues such as veneer chipping, secondary caries, and endodontic problems occurred at comparable frequencies. Monolithic CAD/CAM crowns and milled denture bases demonstrated lower fracture rates, and no new or unique complications were identified, confirming the safety and clinical reliability of digital techniques.

Beyond diagnostics, AI has shown promise in treatment planning and predictive analytics. In one comparative study, AI-generated dental implant plans differed from expert plans by less than 1 mm in position and 2° in angulation, while requiring one-third of the planning time. Surgical guides printed from AI- and human-derived plans achieved equally precise implant placement, indicating clinical reliability. Emerging predictive models for caries risk, prosthesis failure, and endodontic success suggest that AI can aid personalised treatment planning, although validation remains limited [10].

AI applications also extended to orthodontic and radiologic analysis. Systems for cephalometric landmark detection achieved localization errors under 2 mm and correlation coefficients above 0.9 with expert measurements. Models assessing periodontal bone loss or interpreting cone-beam CT scans reached accuracies of 85–90%. Experimental AI-driven crown design tools produced anatomically realistic 3D models, foreshadowing integration of AI into CAD/CAM workflows to automate design steps and enhance efficiency [11].

In summary, digital dentistry—encompassing CAD/CAM, 3D printing, and AI—achieves clinical effectiveness and safety comparable to, or exceeding, conventional techniques, with notable benefits in precision, efficiency, and standardisation. Limitations remain in the availability of long-term follow-up data and the need for continued clinical validation of AI systems [12].

This comprehensive analysis evaluated how digital dentistry technologies—CAD/CAM milling, 3D printing, and AI—are impacting clinical outcomes in the restoration of tooth defects. Across a broad range of studies, the findings consistently show that these innovations

either match or improve upon traditional methods on multiple fronts. These results carry important implications for dental practitioners considering the adoption of digital workflows [13, 14].

**CAD/CAM and 3D Printing – Proven Performance.** One of the clearest messages from our review is that CAD/CAM-fabricated restorations perform at least as well clinically as conventionally fabricated ones. Earlier concerns that the convenience of digital fabrication might come at the expense of restoration longevity or quality are not supported by the evidence. On the contrary, the precision of CAD/CAM often leads to an equal or better fit, which is a cornerstone of restoration success.

Even a decade ago, studies were hinting at parity between CAD/CAM and traditional crowns. For example, Sailer et al. [15] found similar five-year survival rates for all-ceramic CAD/CAM crowns and conventional metal–ceramic crowns. Now, with improved high-strength ceramic materials and refined milling techniques, the performance gap has essentially closed. Our pooled estimates showed approximately 90% five-year survival for CAD/CAM single crowns, which is virtually identical to established benchmarks for traditional crowns. Additionally, CAD/CAM crowns exhibit similar failure patterns (mostly caries and fractures) to traditional ones, indicating no new vulnerabilities introduced by the digital process.

In the realm of complete dentures – once thought to be too complex for full digital fabrication – digital techniques have rapidly caught up. The fact that patient satisfaction with digital dentures is comparable to conventional dentures means that, from the patient's perspective, the end-product of either workflow is acceptable given competent execution. However, the advantages of digital dentures manifest in efficiency and certain clinical outcomes: reduced adjustment visits and faster turnaround are significant benefits, especially for elderly edentulous patients who may have difficulty with multiple appointments. Our findings, supported by others, show that milled dentures often require fewer post-delivery adjustments and have better initial fit than conventional dentures [11]. This is a win-win scenario: fewer sore spots for patients and less chairside time for clinicians. Furthermore, the cost savings in lab fabrication for digital dentures (as demonstrated by Lo Russo et al. [13] and other studies) suggest that digital workflows can be more economical in the long run, especially as the digital infrastructure (software, mills, printers) becomes more common and amortized.

It should be noted that 3D printing in prosthodontics is a slightly newer approach than CAD/CAM milling. The current evidence for 3D-printed dentures is promising – clinical outcomes (fit, satisfaction) appear similar to

conventional dentures - but printed dentures did not yet outperform conventional ones in measured parameters except for reduced material waste and inventory (digital libraries of tooth shapes, etc.). This is understandable. The layer-by-layer photopolymerization used in 3D printing can introduce fine layer interfaces or slight inaccuracies that milled dentures (carved from a homogeneous puck) avoid. Milled dentures at present have an edge in terms of material properties (density, strength) and fit. However, constant improvements in printable resins and printer resolution are narrowing the gap. It is foreseeable that as new printable high-impact acrylics or even printable ceramics emerge, 3D printing will become equally competitive or even preferable due to its flexibility for complex geometries and automated production.

## PATIENT AND CLINICIAN ACCEPTANCE

Another important point is the excellent acceptance of digital methods by both patients and clinicians in the studies. Patients appreciate shorter treatment times and often comment on the increased comfort of digital impressions (no gag-inducing impression trays). Clinicians, on their side, appreciate the predictability and repeatability that CAD/CAM provides - a digital workflow can reduce variability caused by differing technical skill levels among dental lab technicians. For instance, one study concluded that digital dentures were clinically effective and also more cost-efficient than traditional ones, highlighting that investment in digital can pay off in improved efficiency without harming quality. Of course, transitioning to digital dentistry requires initial investment in equipment and training. The learning curve for using CAD/CAM design software or for interpreting AI outputs should not be underestimated. Many early-adopter studies were done in academic or specialist centers with enthusiasts in digital dentistry. A broader implementation in general practice might face practical issues like the cost of acquiring technology, the need for technical support, and modifications to clinic workflow. Nonetheless, the strong evidence of clinical equivalence (and in some cases superiority) should encourage practitioners that these investments likely yield returns in more efficient patient care. Over time, as costs come down and new graduates enter the workforce already trained in digital methods, these hurdles will continue to diminish.

In treatment planning scenarios, the positive results—such as AI planning matching expert implant plans—point towards a future in which routine planning tasks could be offloaded to AI for greater efficiency. Importantly, in the implant planning study [8], AI did

not introduce new errors, and each plan still underwent the surgeon's approval. Rather than making clinicians obsolete, AI in this role could help standardise care, reduce planning oversights (such as missing vital anatomical considerations), and ensure that even novice clinicians can achieve safe and consistent treatment plans by starting from AI-generated suggestions.

In the busy environment of dental practice, AI could free up clinicians' time to focus on complex decision-making and patient communication by handling the heavy lifting of data analysis and initial planning. One can imagine future AI-based recommendations for restorative treatment—for example, given a large lesion, an AI might recommend either a crown or an onlay based on learned success rates in similar cases—which the clinician can then evaluate alongside their own judgement [9]

## LIMITATIONS OF CURRENT EVIDENCE

Despite generally positive findings, there are limitations and areas where evidence is still maturing. Many studies have relatively short follow-ups. While 3-5 year data for CAD/CAM crowns are solid, 10-year data (though promising in isolated studies) come from fewer sources. Long-term performance, especially of newer materials like some 3D-printed resins, remains to be verified. It's possible that certain complications or late failures could emerge over a decade (for example, will the bond at the tooth-restoration interface of a resin-printed crown hold up over 10+ years?). Therefore, continued surveillance and more long-term cohort studies or RCTs are needed to monitor outcomes over time [10].

Heterogeneity within the "digital dentistry" umbrella is another important consideration—there are many different CAD/CAM machines, printer types, and AI algorithms. Not all systems produce identical outputs; results achieved by a skilled operator with one system might not generalize precisely to another system or to an average user [11]. However, the consistency of positive outcomes across multiple systems in our included studies provides reassurance that the beneficial effects are general features of the technology class rather than characteristics of a single brand [12].

For AI, a key limitation is that many tools evaluated remain proprietary or are not yet widely available commercially. The performance observed in research settings might not immediately translate to every dental practice unless those specific algorithms become both accessible and user-friendly. Moreover, AI systems require large volumes of high-quality data for training; therefore, for uncommon conditions or atypical presentations, the AI may be less reliable. For example, most

caries-detection algorithms are trained on thousands of common bitewing radiographs, but how would they perform when faced with an unusual artifact or a rare lesion? The clinician's expertise thus remains crucial as the final arbiter [12-13].

Ethical and practical considerations also accompany the adoption of AI. Some practitioners have raised liability concerns: if an AI misses a diagnosis, the dentist is still responsible; conversely, if an AI suggests an aggressive treatment that turns out to be unnecessary, who is accountable? Professional guidelines will need to evolve to address these questions, and informed consent might need to include disclosure of AI assistance in care (analogous to informing patients when a trainee is involved in their treatment). Transparent reporting of AI's role and ensuring that AI tools meet regulatory and safety standards will be important as they integrate into clinical practice [14-15].

## FUTURE DIRECTIONS

The trajectory of digital dentistry appears overwhelmingly positive, and our analysis substantiates that it is not just an experimental trend but a fundamental improvement in many aspects of care. Going forward, improvements in materials for both CAD/CAM and 3D printing will likely address the few remaining gaps (such as the slightly lower strength of some printed resins). We anticipate more hybrid workflows - for instance, printing an interim prosthesis to verify fit and occlusion, then milling the final version for ultimate strength and accuracy. This kind of dual approach is already being tested to harness the advantages of both methods (speed and low cost of printing, with the robust properties of milled acrylic or ceramic) [16-17].

For AI, as data accumulates and algorithms become more sophisticated, we might see AI contributing beyond assistance to more autonomous predictions and decisions (with oversight). One interesting area is AI in preventive dentistry: imagine AI models that sift through patient data (clinical history, diet, genetic markers) and predict which patients are at high risk of developing tooth defects (caries or cracks) so that preventive measures can be intensified for those individuals. This could personalize preventive care schedules in a way that current risk assessment tools only approximate [18-19].

To fully realize the benefits of digital dentistry, integration is key. Currently, many dental offices use a mix of digital and analog systems. In the near future, we expect a more unified digital ecosystem where a patient's intra-oral scan flows seamlessly into AI analysis, then into CAD design software, and finally to a fabrication device - all

the while updating the electronic health record. Such integration can create efficiencies and reduce manual data re-entry or errors. This also raises the possibility of "big data" analyses in dentistry: aggregated outcomes from thousands of digitally documented cases could be analyzed (likely with the help of AI) to continually refine best practices - a concept known as a learning health system [20-21].

## CLINICAL SIGNIFICANCE

For practicing dentists and specialists, the results of this analysis should build confidence that investing in digital technologies can improve practice efficiency without compromising patient outcomes. A dentist can comfortably tell a patient that a CAD/CAM crown will serve them as well as a traditional lab-fabricated crown - with the added bonus of getting it done faster. Prosthodontists managing edentulous patients can embrace digital denture workflows knowing that patient outcomes (comfort, function) will not be worse than conventional techniques, and that certain clinical steps (like border molding or multiple trial fittings) might be streamlined or eliminated. The positive evidence may also encourage dental educators to incorporate more digital training in curricula, ensuring new graduates are proficient in these tools from day one [22].

## LIMITATIONS OF THIS REVIEW

We acknowledge some limitations in our own review process. First, despite exhaustive search efforts, it is possible some relevant very recent studies (especially those published in late 2025) were not yet indexed or available to us and thus were missed. Second, due to the breadth of the topic, some of our subgroup analyses included relatively few studies (for example, only a handful directly compared printed vs milled dentures). Therefore, conclusions in those sub-areas should be interpreted with caution until more data emerges. Third, when pooling data, we sometimes had to combine outcomes reported in slightly different ways by different authors (e.g. one study's definition of "success" might differ from another's). We tried to harmonize definitions (for instance, treating "survival without complication" as equivalent to "success" in another study) to make pooling valid. Nevertheless, some statistical heterogeneity was present in a few analyses, reflecting clinical and methodological diversity across studies. We used random-effects models to account for between-study variability, but any residual heterogeneity means the pooled result is an average effect and individual patient outcomes may vary case by case [22].

## CONCLUSIONS

Within the limitations of the available evidence, this analysis supports the conclusion that digital dentistry technologies deliver clinical outcomes comparable to—and, in certain aspects, superior to—those achieved with conventional dental techniques for tooth defect rehabilitation. CAD/CAM and 3D printing enable clinicians and dental technicians to fabricate restorations and prostheses with high precision and predictability,

yielding survival rates and patient satisfaction comparable to traditional methods.

Patients benefit from the streamlined workflows of digital processes—such as single-visit crowns and faster denture deliveries—without compromising quality of care. In particular, CAD/CAM-milled prostheses have demonstrated excellent fit and durability (five-year survival around 90%), while 3D-printed prostheses are rapidly advancing and offer unique advantages, including design flexibility and reduced material waste.

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### CONFLICT OF INTEREST

The Authors declare no conflict of interest

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