Wiadomości Lekarskie
Medical Advances

Official journal of the Polish Medical Association
Wiadomości Lekarskie has been published since 1928

Volume LXXIX, Issue 7, JULY 2026 ISSN 0043-5147 E-ISSN 2719-342X

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ALUNA Publishing

Volume LXXIX, Issue 7, JULY 2026

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Wiadomości Lekarskie Medical Advances is abstracted and indexed in:
PUBMED/MEDLINE, SCOPUS, EMBASE, INDEX COPERNICUS,
MINISTRY OF SCIENCE AND HIGHER EDUCATION, POLISH MEDICAL BIBLIOGRAPHY

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Publisher:
ALUNA Publishing
29 Przesmyckiego St.,
05-510 Konstancin – Jeziorna, Poland
www.wydawnictwo-aluna.pl
www.wiadomoscilekarskie.pl
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ORIGINAL ARTICLE

CONTENTS

Carbacetam attenuates biphasic neuroendocrine dysregulation and improves neurobehavioral recovery after closedhead traumatic brain injury in rats

Sergiy V. Ziablitsev[1], Nadia O. Aleksandrenko[1], Volodymyr V. Likhodievsky[1], Danylo I. Yevstifeiev[1], Mykola S. Babenko[1], Serhey O. Khudoley[2], Liudmyla V. Molodan[3], Yuliia Ya. Fedulenkova[4], Diana G. Kiriya[3], Olena P. Sharmazanova[4], Viktoriia O. Bibichenko[3]

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ABSTRACT

Aim: To elucidate neurohormonal mechanisms underlying the development of higher-order behavioral and cognitive disturbances after traumatic brain injury and to substantiate a pathogenetic approach to their correction using carbacetam.

Materials and Methods: We examined neuroendocrine dynamics after closedhead TBI in rats and assessed pathogenetically oriented correction with carbacetam – an endogenous modulator of the Type-A γ-aminobutyric acid (GABA-A)-benzodiazepine receptor complex – compared with deproteinized calf blood hemoderivative (DCBH). One hundred seventy adult male outbred rats were used.

Results: TBI caused a 5.4-5.9fold rise in neurological deficit on Day 1 with incomplete spontaneous recovery (still 3.4fold above intact on Day 7). Carbacetam accelerated recovery (57% reduction by Day 7 vs 43% in vehicle; $p < 0.05$) and improved orienting-exploratory behavior and eightarm maze performance; DCBH improved most behavioral outcomes but was less effective for exploratory activity. Endocrine profiling revealed an acute hyperreactive response (ACTH, corticosterone and vasopressin surges on Day 1) followed by delayed central depletion at Day 14 and persistent suppression of growth hormone (to 48% of intact at Day 30; $p < 0.05$).

Conclusions: Carbacetam attenuated early hyperactivation, prevented delayed depletion and preserved somatotropic signaling, whereas DCBH primarily mitigated the delayed depletion phase. Mortality was 50% in vehicle, 16% with carbacetam and 36% with DCBH ($p = 0.002$). Immunohistochemistry on Day 7 showed reduced GFAP/S100 and increased NSE under carbacetam, consistent with attenuation of degenerative changes and preservation of neuronal metabolic activity.

KEYWORDS: Brain injuries, traumatic, cognitive dysfunction, hypothalamo-hypophyseal system, pituitary-adrenal system, vasopressins, carbolines, receptors, GABA-A, carbacetam

Wiad Lek. 2026;79(7):1451-1466. doi: 10.36740/WLek/225985 DOI

INTRODUCTION

Traumatic brain injury (TBI) is one of the most prevalent and consequential neurological conditions worldwide and remains a major driver of premature mortality and long-term disability. Contemporary syntheses emphasize that TBI should be viewed not only as an acute event but often as a chronic disorder with persistent neurocognitive and neuropsychiatric sequelae, substantial societal costs, and major unmet needs in follow-up care and rehabilitation [1, 2].

The burden of TBI is further amplified in settings of armed conflict, where blast exposure, high-energy mechanisms, and polytrauma increase both the incidence and the complexity of post-traumatic brain disorders. Since 2014 – and especially following the large-scale escalation of the war in Ukraine in 2022 – health systems have faced sustained demand for trauma care and rehabilitation, including for head injuries among both military personnel and civilians. In this context, blast-related TBI is of particular concern because it may be accompanied by long-term cognitive and neuropsychiatric consequences that can remain under-recognized in the acute phase amid competing injuries and resource constraints [3, 4].

Closed (non-penetrating) TBI constitutes the dominant phenotype in civilian practice and a frequent component of blast-related trauma. A key clinical challenge is the high prevalence and heterogeneity of persistent cognitive dysfunction after TBI, spanning processing speed, memory, and executive control, and only partly explained by conventional measures of injury severity. Large prospective cohorts demonstrate that, even at 6 months after injury, distinct profiles of objective cognitive impairment are common across TBI severities, underscoring the need to identify biological determinants of divergent recovery trajectories and treatment responsiveness [5].

Among the biological systems that remain insufficiently integrated into mechanistic models of cognitive recovery is neuroendocrine regulation. The hypothalamic-pituitary region is vulnerable to mechanical and vascular injury, and TBI can lead to acute and chronic disturbances of pituitary-dependent hormonal systems (including hypothalamic-pituitary-adrenal axis dysregulation) that may adversely affect metabolism, mood, and cognition – domains that critically shape functional outcomes and rehabilitation potential [6].

Despite advances in neurocritical care and rehabilitation, no disease-modifying pharmacotherapy has been approved to prevent or reverse the complex secondary injury cascades underlying long-term cognitive deficits after TBI. The persistent translational gap – including the repeated failure of Phase III neuroprotection trials – supports the need for mechanistically grounded, multi-target approaches that engage both neuronal network dysfunction and systemic stress responses [1, 7]. In this regard, inhibitory neurotransmission – particularly γ-aminobutyric acid (GABA) signaling – has been highlighted as a plausible nexus linking network-level dysfunction to neurohormonal regulation after TBI. Recent analyses argue that rationally designed GABA-A receptor modulators may represent an underexploited therapeutic direction, provided that limitations of classical benzodiazepine-site agents (e.g., sedation and cognitive adverse effects) are addressed by improved pharmacological profiles and targeted use [8].

Carbacetam is an endogenous modulator of the GABA-benzodiazepine receptor complex. Preclinical evidence suggests that carbacetam may attenuate neurodestructive processes after experimental TBI, supporting further investigation of its pathogenetically oriented potential to influence neurohormonal responses and higher-order behavioral/cognitive outcomes after closed TBI [9].

AIM

To elucidate neurohormonal mechanisms underlying the development of higher-order behavioral and cognitive disturbances after traumatic brain injury and to substantiate a pathogenetic approach to their correction using carbacetam.

MATERIALS AND METHODS

ANIMALS AND ETHICAL APPROVAL

The study was conducted in adult male outbred (non-inbred) white rats (age: 6 months; body mass: 190-210 g). Animals were housed under standard vivarium conditions (temperature 18-22 °C) with *ad libitum* access to food and water and were acclimatized for 2 weeks before experimental procedures. To reduce circadian variability, handling and all functional assessments were performed in the morning (08:00-10:00).

All animal procedures were conducted in accordance with the "General Ethical Principles of Experiments on Animals" (Ukraine, 2001), consistent with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Law of Ukraine No. 3447IV dated 21.02.2006 "On Protection of Animals from Cruelty," Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 dated 01.03.2012 "Procedure for Conducting Experiments on Animals by Scientific Institutions," and Law of Ukraine "On Protection of Animals from Cruelty" No. 440IX dated 14.01.2020. Approval for the study was obtained from the Commission on Bioethics and Academic Integrity of O.O. Bogomolets National Medical University (Kyiv, Ukraine).

STUDY DESIGN, GROUPS, AND TIMELINE

A total of 170 rats were used. Closed-head traumatic brain injury (TBI) was induced in 150 animals, which were allocated to three parallel experimental groups (n = 50 per group): Carbacetam (experimental group 1); Deproteinized calf blood hemoderivative (DCBH; experimental group 2); Vehicle (0.9% NaCl; comparison group). In addition, 15 sham-injured animals underwent identical handling and procedures without the impact, and 5 intact animals served as reference controls for baseline functional values (Table 1). Functional outcomes were assessed longitudinally at predefined time points after injury. Mortality was recorded throughout follow-up.

CLOSED-HEAD TBI MODEL

Closed-head TBI was induced using a weight-drop paradigm in which a 66.7 g weight impacted the head fixed in a holder, producing an impact energy of 0.425 J and resulting in a moderate-severity injury. In previous

Table 1. Experimental groups, interventions, and sample sizes

Group	Injury status	Treatment	Dose and regimen	Allocated n
Intact reference	No TBI	None	–	5
Sham-injured	Sham (no impact)	Vehicle	0.9% NaCl, i.p.	15
Vehicle (comparison)	Closed-head TBI	Vehicle	0.9% NaCl, 1 mL i.p., once daily for 10 days	50
Carbacetam	Closed-head TBI	Carbacetam	5 mg/kg i.p., once daily for 10 days	50
Deproteinized calf blood hemoderivative (DCBH)	Closed-head TBI	DCBH	16.5 mg/kg i.p., once daily for 10 days	50
Total				**170**

Note: Closed-head TBI was induced in 150 animals (three parallel treatment arms; n=50 per arm). Additional controls comprised sham-injured animals (n=15) and intact reference animals (n=5). Longitudinal neurological and behavioral assessments were performed at predefined post-procedure time points; due to mortality and scheduled terminal sampling for endocrine analyses, the number of animals contributing to later time points may vary (exact n is reported alongside Results where applicable).
Source: compiled by the authors of this study

calibrations of this model, this injury severity is associated with 30-50% mortality. Sham-injured animals underwent the same procedure sequence without delivery of the impact.

DRUG ADMINISTRATION

Carbacetam (an endogenous modulator of the GABA-benzodiazepine receptor complex; β-carboline derivative) was administered intraperitoneally (i.p.) at 5 mg/kg once daily for 10 consecutive days after TBI (in 0.9% NaCl; injection volume 1 mL per animal). The comparator drug was deproteinized calf blood hemoderivative (Actovegin®, Nycomed, Switzerland), administered i.p. at 16.5 mg/kg once daily for 10 consecutive days after TBI (in 0.9% NaCl; injection volume 1 mL per animal). Vehicle-treated animals received 1 mL i.p. 0.9% NaCl on the same schedule (Table 1).

NEUROLOGICAL ASSESSMENT

Neurological deficit was assessed using a 100-point neurological deficit score structured across five domains: consciousness (20 points), motor function (30), cranial nerve function (20), sensorimotor integration (20), and autonomic signs (10). Each component included predefined operational criteria. Higher total scores indicated more severe neurological deficit. Neurological scoring was performed at baseline (pre-injury) and at prespecified post-injury time points (3 h, days 1, 3, 7, 14, and 30). Assessments were performed at the same time each day by a blinded investigator. Inter-rater reliability was tested in a subset of animals (ICC >0.85). All animals were randomized using a computer-generated sequence.

BEHAVIORAL TESTING

Animals were habituated to the experimental environment and test handling prior to injury (≥10 habituation sessions). Behavioral testing was performed at the same predefined time points as neurological scoring.

Open-field test was used to quantify spontaneous locomotion and exploratory activity, recorded as number of squares crossed and number of holes explored. Light-dark transition/"burrow reflex" latency (latency to enter the dark compartment from the light compartment) was recorded: longer latency interpreted as altered avoidance/anxiety-like behavior and/or impaired adaptive responding, depending on the overall behavioral context. Eight-arm radial maze was used to assess spatial learning and memory. The primary recorded endpoint was the number of correct entries into baited arms without repetition, reflecting working memory performance and task acquisition.

BLOOD SAMPLING AND HORMONE MEASUREMENTS

Endocrine measurements were performed in pre-specified subsets of animals withdrawn at each time point. On post-injury days 1, 3, 7, 14, and 30, five animals per group per time point were euthanized for blood sampling (total n=75 across the three TBI treatment groups). Animals were anesthetized with ether and euthanized by decapitation; trunk blood was collected into pre-labelled tubes containing Na-EDTA anticoagulant.

Plasma hormone concentrations were determined by ELISA using commercial rat-specific assay kits for: adrenocorticotropic hormone (ACTH), corticosterone, vasopressin, and growth hormone.

Absorbance was measured using a microplate reader (RT2100C; Rayto Life and Analytical Sciences, China) and concentrations were calculated according to kit protocols.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

For morphological and immunohistochemical analyses, brains were collected on post-injury day 7 from a subset of animals in the carbacetam group (n=5) and the vehicle comparison group (n=5) after deep thiopental anesthesia (50 mg/kg). Brain regions of interest included the hypothalamus (supraoptic and paraventricular nuclei) and hippocampus (CA3 region). Tissues were fixed, processed routinely, paraffin embedded, and sectioned at 3-4 μm. Hematoxylin and eosin staining was used for general morphology.

For immunohistochemistry, sections were treated with proteinase K (DAKO) and incubated with primary antibodies against: S100 (code Z0311), neuron-specific enolase (NSE) (code N1557), glial fibrillary acidic protein (GFAP) (code IS524).

Signal detection used a polymer HRP/DAB system. Slides were evaluated by light microscopy with digital image acquisition.

STATISTICAL ANALYSIS

Statistical processing was performed using licensed software (SPSS 11.0, MedStat, and MedCalc). Data are presented as mean ± SEM unless stated otherwise. Prior to inferential testing, distributions were evaluated using the Shapiro–Wilk test and Q–Q plots; homogeneity of variance was examined using Levene's test. All tests were twosided, and statistical significance was set at $p < 0.05$.

Relative values were calculated as follows: % of intact reference = (individual value / mean intact value) × 100; % of sham (for behavioral outcomes) = (individual value / timematched sham mean) × 100; % of Day 1 = (value at a given time point / value on Day 1) × 100; % of previous measurement = (value at a given time point / value at the preceding time point) × 100. Intact reference values were obtained from a separate intact cohort (n = 5) and were used as a timeinvariant baseline for normalization and "vs intact" comparisons throughout the study.

Cumulative mortality across injured groups was compared using the χ^2 test of independence (or Fisher's exact test when expected cell counts were <5). Where pairwise comparisons were performed, pvalues were adjusted for multiple testing using the Holm-Šidák procedure.

Neurological deficit scores and behavioral endpoints (openfield activity, hole exploration, light-dark transition latency, and radialmaze successful entries) were measured serially over time. Group and time effects were evaluated using a mixedeffects model (fixed effects: group, time, and group × time interaction; random effect: animal as a random intercept), which permits analysis in the presence of missing observations arising from mortality and scheduled terminal sampling without imputation. When a significant group × time interaction was detected, prespecified post hoc comparisons were performed using Šidákadjusted pairwise tests. Planned contrasts included: each injured group vs sham at the same time point (behavioral outcomes), and carbacetam vs vehicle and DCBH vs vehicle at the same time point.

Hormone concentrations were measured using terminal sampling (independent animals at each time point; n = 5 per group per time point). Therefore, hormone data were analyzed crosssectionally at each time point using oneway ANOVA with treatment group as the factor. Comparisons vs intact reference were performed using Dunnett's test (vs intact), and pairwise comparisons among injured groups were performed using Tukey's HSD (or Šidákadjusted pairwise tests). If assumptions for parametric analysis were violated, the Kruskal-Wallis test with Dunn's post hoc correction was used.

Histology and immunohistochemistry were evaluated descriptively from representative sections (Day 7; n = 5 per group) and are presented qualitatively as representative micrographs.

RESULTS AND DISCUSSION

IN SILICO RATIONALE AND TARGET PROFILING

βCarboline derivatives represent a "privileged" neuroactive scaffold with broad CNS pharmacology. Their tricyclic heteroaromatic core has been exploited to design ligands that engage multiple CNS-relevant targets, including the benzodiazepine site of the GABA-A receptor, and several βcarbolines have demonstrated cognitive- and neuroprotectionrelevant effects in preclinical settings. Such properties make the scaffold particularly attractive for drug development in conditions where anxiety, stress-reactivity, and cognitive impairment coexist – limitations that remain clinically relevant for classical benzodiazepines because of tolerability and cognitionrelated adverse effects during sustained use [10-12].

Carbacetam is a βcarboline isostere positioned as an endogenous modulator of the GABA-benzodiazepine receptor complex. In prior behavioral profiling in rodent models of CNS dysfunction, carbacetam was associated with improved performance in tasks reflecting higher

Table 2. Docking-derived binding energies (kcal/mol) of carbacetam and reference ligands across CNS targets

Target	PDB ID	Ligand	Binding energy (kcal/mol)
Type-A γ-aminobutyric acid (GABA-A) receptor (α1β3γ2)	6HUO	Carbacetam	-10,513
		Alprazolam (reference)	-10,663
		Diazepam (reference)	-8,253
Type-B γ-aminobutyric acid (GABA-B) receptor (GBR1, GBR2)	4MS4	Carbacetam	-8,325
		Baclofen (reference)	-7,602
Translocator protein (TSPO)	N/A	Carbacetam	-9,053
		Diazepam (reference)	-6,804
AMPA-subtype GluA2 receptor	5L1F	Carbacetam	-8,258
		Perampanel (reference)	-9,069
The voltage-gated sodium channel Nav1.2	6J8E	Carbacetam	-9,420
		Lamotrigine (reference)	-6,841
Orexin-1 Receptor (Site 1)	6TOT	Carbacetam	-8,190
		Lemborexant (reference)	-9,615
Orexin-1 Receptor (Site 2)	6TOT	Carbacetam	-8,464
		Lemborexant (reference)	-9,646

Note: Values represent Docking-derived binding energies for the bestranked pose (more negative values indicate more favorable predicted binding within the applied scoring function). "Reference" ligands are benchmark compounds used for contextual comparison within each target system.
Source: compiled by the authors of this study

nervous activity and memory retention, supporting the concept that its activity profile may extend beyond "pure" anxiolysis toward cognitionsupporting effects [13].

COMPREHENSIVE MOLECULAR DOCKING SCREEN ACROSS CNS TARGETS

To explore a plausible polypharmacological basis for the observed neurobehavioral profile, we performed a docking-based screen of carbacetam against a panel of CNS targets implicated in inhibitory and excitatory neurotransmission, neuroinflammation/stress signaling, and arousal regulation. Docking-derived binding energies for the top-ranked poses of carbacetam and reference ligands are summarized in Table 2.

The docking screen suggested that carbacetam may engage the benzodiazepine site of the GABA-A receptor with a highly favorable predicted binding energy (-10.513 kcal/mol), comparable to alprazolam and more favorable than diazepam in this docking setup (Table 2). Importantly, the predicted interaction profile was not limited to a single target. Carbacetam also demonstrated a favorable docking score at the GABA-B receptor (-8.325 kcal/mol), exceeding the reference agonist baclofen (-7.602 kcal/mol), and at the translocator protein TSPO (-9.053 kcal/mol), with a markedly more favorable score than diazepam (-6.804 kcal/mol) (Table 2). Given the established interest in TSPO ligands as modulators of neurosteroidogenesis, stress-related neurobiology, and neuroinflammation, these findings warrant consideration as a mechanistically relevant signal rather than a definitive proof of target engagement [12].

Beyond ligand-receptor systems, carbacetam also exhibited favorable docking scores in ion channel binding sites. Notably, the predicted binding energy within the Nav1.2 pore (-9.420 kcal/mol) was more favorable than that of lamotrigine (-6.841 kcal/mol) in this model (Table 2). In addition, carbacetam showed detectable predicted affinity across both identified binding sites of the orexin receptor 1 (OX1R) (Table 2), supporting a working hypothesis of broader CNS target coverage. Collectively, the docking results support a polypharmacology-oriented interpretation; however, docking scores should be considered hypothesis-generating and require orthogonal experimental validation (e.g., binding assays and functional readouts).

IN SILICO ADMET PROFILE AND INTEGRATIVE POLYPHARMACOLOGY

The *in silico* ADMET profiling suggested that carbacetam has a generally favorable physicochemical and early safety/PK risk profile for a CNSdirected small molecule (Table 3). In particular, the compound was predicted to display high gastrointestinal absorption and blood–brain barrier permeability, while showing a low probability of mutagenicity in the Ames test, cardiotoxicity related to hERG inhibition, and drug induced

Table 3. *In silico* predicted physicochemical and ADMET properties of carbacetam

Parameter	Carbacetam	Interpretation
Physicochemical properties		
Molecular weight (g/mol)	278.36	CNSactive small molecule range
cLogP	4.18	Lipophilicity consistent with CNS exposure
Lipinski ruleoffive violations	0	Druglikeness criterion met
Synthetic accessibility score (SAscore)	0.20	Lower values indicate easier synthesis
Pharmacokinetics		
GI absorption (HIA)	High	Predicted high intestinal absorption
BBB permeability	Yes	Predicted blood-brain barrier penetration
Pglycoprotein substrate	Yes	Potential efflux liability
Toxicity		
Ames mutagenicity	No	Low predicted genotoxicity risk
hERG blockade	No	Low predicted cardiotoxicity risk (screen)
Hepatotoxicity (DILI)	No	Low predicted DILI risk (screen)
Tox-score	0.51	Lower values indicate more favorable profile

Note: ADMET – absorption, distribution, metabolism, excretion, toxicity; HIA – human intestinal absorption; BBB – blood-brain barrier; Pgp – Pglycoprotein; DILI – druginduced liver injury. *In silico* predictions are hypothesissupporting and should be interpreted qualitatively.
Source: compiled by the authors of this study

liver injury liability (Table 3). These outputs collectively support the feasibility of systemic administration and CNS exposure in vivo, while also emphasizing that experimental confirmation (PK and targetengagement studies) remains necessary before translational conclusions can be drawn.

When interpreted alongside the docking screen (Table 2), the data support a working mechanistic concept in which carbacetam may act as a multitarget CNS modulator, rather than a "singletarget" benzodiazepinelike ligand. The predicted high-affinity interaction with the benzodiazepine site of the GABA-A receptor (comparable to alprazolam and stronger than diazepam in the present docking set; Table 2) provides a molecular rationale for an anxiolyticlike phenotype, whereas the additional predicted engagement of GABA-B receptors, TSPO, and voltagegated sodium channels suggests plausible complementary pathways that could influence posttraumatic network excitability, stressaxis signaling, and neuroinflammatory tone.

A notable additional signal in the docking profile was the predicted interaction of carbacetam with the Nav1.2 pore region (Table 2). Voltagegated sodium channels (including Nav1.2) are central determinants of neuronal excitability and are increasingly recognized as modulators of downstream injury cascades when ionic homeostasis becomes destabilized. Recent syntheses of the field emphasize that sodium channel subtypes contribute in distinct ways to neuronal firing properties and can be pharmacologically leveraged to dampen pathological hyperexcitability. In experimental TBI, targeting injuryassociated dysregulation of sodium channel expression has been shown to improve structural and functional outcomes, supporting the broader principle that sodium channel modulation can be neuroprotective in the context of neurotrauma. Accordingly, the predicted sodium channel engagement by carbacetam (Table 2) may represent a mechanistically relevant component of its pleiotropic profile rather than a docking "offtarget" artifact [14, 15].

In parallel, the predicted high affinity of carbacetam for TSPO (Table 2) is mechanistically noteworthy because TSPOlinked pathways are positioned at the interface of mitochondrial function, neurosteroid biology, and neuroinflammation. Contemporary reviews highlight that neurosteroid replacement strategies and/or TSPO targeting have been proposed as means to restore altered neurosteroid signaling under neuroinflammatory conditions, while also acknowledging contextdependence and methodological complexity in translating neurosteroidome findings. Importantly for traumatic brain injury, neuroactive steroid receptor modulators (including agents enhancing GABA-A receptor signaling via neurosteroid mechanisms) are actively discussed as pleiotropic candidates capable of addressing multiple posttraumatic pathological processes (edema, inflammation, delayed cell death, neurovascular disruption), a conceptual framework aligned with the present study's rationale for testing a multimechanism compound after closedhead TBI [16, 17].

Taken together, the docking and ADMET results support a testable, integrated hypothesis: carbacetam's

Table 4. Neurological deficit score after closedhead TBI (mean ± SEM)

Group	Day 1	Day 3	Day 7
Intact reference	3.6 ± 2.3	–	–
Vehicle (comparison)	21.3 ± 1.2	21.3 ± 2.1	12.1 ± 1.2
Carbacetam	19.9 ± 1.3	16.4 ± 1.3	8.6 ± 0.9
DCBH	19.5 ± 1.6	18.8 ± 1.6	10.3 ± 1.1

Source: compiled by the authors of this study

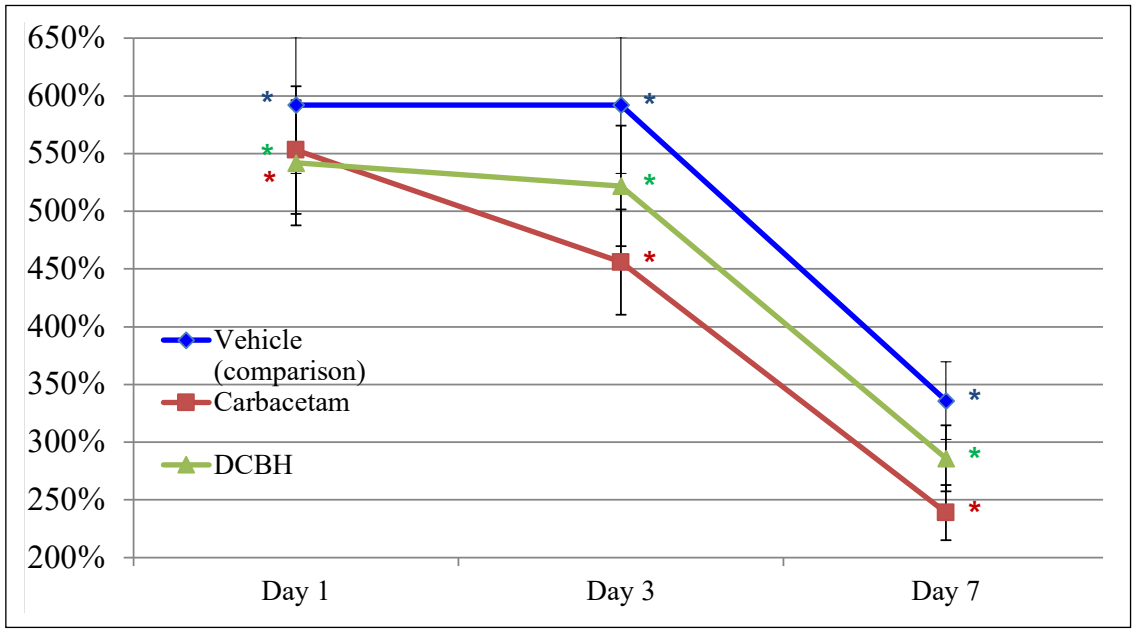

Fig. 1. Neurological deficit dynamics after closed-head TBI. Neurological deficit is presented as percentage of intact reference animals (intact = 100%). Data are shown for Vehicle (comparison), Carbacetam, and DCBH groups at the indicated post-injury time points. Values are mean ± SEM. Absolute neurological deficit scores (points) are provided in Table 4. *p < 0.05 vs intact reference, based on planned contrasts within a mixedeffects model (fixed effects: group, time, and group × time; random effect: animal), with multiplicityadjusted post hoc testing as specified in the Statistical analysis subsection (twosided).
Source: Authors' own work

therapeutic phenotype may arise from synergistic modulation across several CNS targets, in which GABA-A receptor modulation provides an anxiolytic core mechanism, while concurrent engagement of GABA-B receptors, TSPOlinked neurosteroid/neuroinflammatory pathways, and sodium channeldependent excitability may contribute to neuroprotection and cognitive support. This multinode concept is consistent with the broader need for pleiotropic interventions in TBI, where singlepathway neuroprotection has repeatedly underperformed clinically, and it provides a mechanistic scaffold for interpreting the *in vivo* endocrine and behavioral findings presented below.

MORTALITY

Cumulative mortality over the observation period differed significantly across the three injured treatment arms (n = 50 per group at allocation; Table 1). Mortality was highest in the Vehicle group (50%), compared with 16% in the Carbacetam group and 36% in the DCBH group (overall contingency analysis, p = 0.002). Pairwise comparisons indicated a robust reduction in mortality with carbacetam *versus* vehicle (3.1fold lower mortality, p = 0.001), whereas DCBH *versus* vehicle showed a smaller reduction (1.4fold) that did not reach statistical significance (p = 0.096).

NEUROLOGICAL DEFICIT

MOTOR AND REFLEX DOMAINS

On Day 1 after closedhead TBI, all injured groups exhibited a marked increase in neurological deficit relative to intact rats (3.6 ± 2.3 points). In the Vehicle group, the deficit reached 21.3 ± 1.2 points, corresponding to a 5.9fold elevation (i.e., 592% of intact, p < 0.05 vs intact). The Carbacetam group demonstrated a deficit of 19.9 ± 1.3 points (5.5fold; 553% of intact, p < 0.05 vs intact), and the DCBH group showed 19.5 ± 1.6 points (5.4fold; 542% of intact, p < 0.05 vs intact). At this early

Table 5. Behavioral outcomes across groups over time (mean ± SEM)

Outcome	Group	Day 1	Day 3	Day 7	Day 14	Day 30
Open field: squares crossed (count)	Sham	15.7 ± 1.5	15.8 ± 1.6	14.7 ± 1.4	13.7 ± 1.0	12.6 ± 1.3
	Vehicle	6.8 ± 0.5[a]	6.9 ± 0.4[a]	6.7 ± 0.7[a]	5.6 ± 0.5[a]	9.4 ± 1.0[a]
	Carbacetam	6.6 ± 0.4[a]	7.1 ± 0.6[a]	7.2 ± 0.4[a]	5.8 ± 0.4[a]	10.4 ± 0.7
	DCBH	6.5 ± 0.4[a]	6.7 ± 0.4[a]	6.7 ± 0.5[a]	6.6 ± 0.5[a]	9.4 ± 0.8
Open field: holes explored (count)	Sham	13.1 ± 1.2	13.1 ± 1.2	11.4 ± 1.3	9.4 ± 1.0[d]	10.3 ± 0.7[d]
	Vehicle	2.7 ± 0.2[a]	2.9 ± 0.2[a]	3.9 ± 0.3[a]	4.9 ± 0.5[a]	6.6 ± 0.4[a]
	Carbacetam	3.1 ± 0.2[a]	3.4 ± 0.2[a]	6.3 ± 0.4[ab]	6.4 ± 0.5[ab]	8.8 ± 0.6[b]
	DCBH	2.9 ± 0.2[a]	3.0 ± 0.2[a]	4.0 ± 0.2[ac]	4.9 ± 0.4[ac]	6.7 ± 0.5[ac]
Light–dark transition latency (s)	Sham	108 ± 10	108 ± 10	85 ± 11	67 ± 8[d]	55 ± 8[d]
	Vehicle	203 ± 11[a]	188 ± 15[a]	138 ± 12[a]	86 ± 6	70 ± 5
	Carbacetam	180 ± 10[a]	166 ± 10[a]	116 ± 7[a]	84 ± 5	63 ± 4
	DCBH	190 ± 9[a]	176 ± 10[a]	121 ± 7[a]	76 ± 4	59 ± 4
Eightarm radial maze: successful entries (count)	Sham	6.7 ± 0.7	7.1 ± 0.6	7.5 ± 0.6	7.5 ± 0.5	7.6 ± 0.7
	Vehicle	3.2 ± 0.2[a]	4.1 ± 0.3[a]	4.4 ± 0.4[a]	5.7 ± 0.4[a]	6.2 ± 0.7[a]
	Carbacetam	3.4 ± 0.1[a]	4.4 ± 0.2[a]	4.9 ± 0.3[a]	6.8 ± 0.4	7.4 ± 0.6
	DCBH	3.3 ± 0.2[a]	4.1 ± 0.3[a]	4.5 ± 0.3[a]	5.9 ± 0.5[a]	6.7 ± 0.7

Note: Sham animals underwent anesthesia and all experimental handling procedures without impact. Light-dark transition latency corresponds to the latency to enter the dark compartment (burrowreflex paradigm). Successful entries in the eightarm radial maze represent the number of correct entries into baited arms without repetition (index of working memory performance/task acquisition). Values are mean ± SEM. Superscripts indicate statistically significant differences (twosided $p < 0.05$) according to the statistical approach specified in the Statistical analysis subsection (group × time model with appropriate post hoc comparisons/multiplicity control). Superscripts (multiple may apply):
[a] $p < 0.05$ vs Sham at the same time point;
[b] $p < 0.05$ vs Vehicle at the same time point;
[c] $p < 0.05$ vs Carbacetam at the same time point;
[d] $p < 0.05$ vs Day 1 within the same group (habituation in Sham).
Source: compiled by the authors of this study

time point, neurological deficit did not differ significantly among the three injured groups.

By Day 3, neurological deficit in the Vehicle group remained essentially unchanged (21.3 ± 2.1 points), i.e., 100% of the Day1 value (still 592% of intact, $p < 0.05$ vs intact), indicating minimal spontaneous improvement during the first 72 hours. In contrast, the Carbacetam group declined to 16.4 ± 1.3 points, corresponding to 82% of the Day1 value (an 18% reduction vs Day 1) and 456% of intact ($p < 0.05$ vs intact). Compared with vehicle at the same time point, the deficit under carbacetam was 23% lower (reported as 77% of the vehicle value, $p < 0.05$). Clinically, animals in the carbacetam group appeared more stable in consciousness and adaptive behavior, maintained posture, and showed only minimal ataxia. In the DCBH group on Day 3, neurological deficit was 18.8 ± 1.6 points, representing 96% of the Day1 value (a 4% reduction vs Day 1) and 522% of intact ($p < 0.05$ vs intact). At this time point, the DCBH group did not differ significantly from vehicle or carbacetam.

On Day 7, neurological deficit decreased in all injured groups, reflecting partial recovery over the first posttraumatic week. In the Vehicle group, deficit fell to 12.1 ± 1.2 points, i.e., 57% of the Day1 value (a 43% reduction vs Day 1) and 336% of intact ($p < 0.05$ vs intact). In the Carbacetam group, the deficit declined further to 8.6 ± 0.9 points, corresponding to 52% of the Day3 value (a 48% reduction vs Day 3), 43% of the Day1 value (a 57% reduction vs Day 1), and 239% of intact ($p < 0.05$ vs intact). Relative to vehicle, the Day7 deficit under carbacetam was 29% lower (71% of the vehicle value, $p < 0.05$). In the DCBH group, deficit reached 10.3 ± 1.1 points, i.e., 55% of the Day3 value (a 45% reduction vs Day 3), 53% of the Day1 value (a 47% reduction vs Day 1), and 286% of intact ($p < 0.05$ vs intact). Despite a numerically lower score than vehicle (approximately 85% of the vehicle value), this difference was not statistically significant.

Collectively, closedhead TBI induced a 5.4-5.9fold increase in neurological deficit within 24 hours, followed by regression over the first week; however, without pharmacological correction the deficit remained 3.4fold

above intact at Day 7. Both active interventions promoted recovery, but the trajectory differed: carbacetam produced an earlier and more pronounced reduction (already evident by Day 3 and reaching a 57% reduction from Day 1 by Day 7), whereas DCBH showed a slower improvement pattern and did not yield a statistically significant advantage over vehicle in the neurological deficit score at the measured time points.

HIGHER-ORDER BEHAVIORAL AND COGNITIVE PERFORMANCE

(OPEN FIELD, LIGHT-DARK TRANSITION, EIGHTARM RADIAL MAZE)

To probe posttraumatic disturbances of higher-order behavioral and cognitive function – a clinically salient domain of TBI outcomes [5] – we used an integrated battery comprising the openfield test (locomotor activity and exploratory drive), the light-dark transition latency ("burrow reflex" paradigm; emotional reactivity/anxiety-like avoidance), and the eightarm radial maze (correct, nonrepeated entries as an index of task acquisition/working memory performance). Because repeated testing itself can modify behavior over time, we first evaluated the Sham group across the 30day observation period. In sham animals, the number of squares crossed showed a nonsignificant downward trend, reaching ~80% of Day1 values by Day 30. In contrast, hole exploration decreased significantly, to 72% of Day1 values on Day 14 and 79% on Day 30 ($p < 0.05$ for both), consistent with habituation to the testing environment. A similar habituation pattern was evident for the light-dark transition: latency to enter the dark compartment decreased to 62% of Day1 values by Day 14 and to ~51% by Day 30 ($p<0.05$ for both).

In the Vehicle group, behavioral performance was markedly impaired already on Day 1 after TBI compared with timematched sham values ($p < 0.05$ for all reported differences). Locomotor activity (squares crossed) fell to 43% of sham, exploratory activity (holes explored) to 21%, and radialmaze successful entries to 48%. In parallel, light-dark transition latency increased to ~188% of sham (an ~88% prolongation), indicating posttraumatic suppression of adaptive exploratory behavior together with increased avoidance/emotional reactivity. Across the 30day followup, partial recovery occurred but remained incomplete. By Day 30, squares crossed increased to 9.4 ± 1.0, i.e., ~138% of the Vehicle Day1 value, yet still only ~75% of the sham Day30 level ($p<0.05$). Holes explored rose to 6.6 ± 0.4 (~244% of Vehicle Day1), but remained ~64% of sham ($p<0.05$). Radialmaze performance improved to 6.2 ± 0.7 (~194% of Vehicle Day1), but remained ~82% of sham ($p<0.05$). The only parameter that approached normalization without treatment was light-dark latency, decreasing to 70 ± 5 s (~34% of Vehicle Day1; ~66% reduction) and reaching ~127% of sham Day30, which did not differ significantly ($p > 0.05$). Overall, these data indicate that spontaneous recovery after closedhead TBI was insufficient to restore exploratory behavior and workingmemory performance to control levels by one month, consistent with the recognized persistence of cognitivebehavioral sequelae after TBI [5].

In the Carbacetam group, Day1 impairment was comparable to the Vehicle group, supporting baseline comparability of injury severity: squares crossed were ~42% of sham, holes explored ~24%, successful entries ~51%, and latency increased to ~167% of sham. Under carbacetam treatment, recovery became more pronounced over time. By Day 30, locomotor activity reached 10.4 ± 0.7, corresponding to ~158% of Day1 and ~83% of the sham Day30 value (difference not significant, $p > 0.05$). Exploratory activity reached 8.8 ± 0.6, i.e., ~284% of Day1 and ~85% of sham Day30 ($p > 0.05$). Light–dark latency declined to 63 ± 4 s (~35% of Day1) and was ~115% of sham Day30 ($p > 0.05$). Radialmaze performance increased to 7.4 ± 0.6 (~218% of Day1) and approximated sham values (~97% of sham Day30; $p > 0.05$). A particularly early treatment signal was observed for exploratory activity: by Day 7, holes explored under carbacetam (6.3 ± 0.4) were ~162% of the Vehicle value at the same time point (i.e., ~62% higher), indicating earlier restoration of exploratory drive.

In the DCBH group, Day1 performance was also comparable to Vehicle and Carbacetam, again supporting standardization of injury induction. By Day 30, locomotor activity, light-dark transition latency, and radialmaze performance showed substantial improvement and approached sham levels overall (Table 5). However, holes explored remained reduced, reaching 6.7 ± 0.5, i.e., ~231% of DCBH Day1, yet only ~65% of the sham Day30 level ($p < 0.05$), indicating a persistent deficit in exploratory behavior under DCBH. Between the two active interventions, most behavioral endpoints did not differ significantly at any time point. The key exception was again exploratory activity (holes explored): starting at Day 7, DCBH values were consistently lower than under carbacetam ($p < 0.05$). Expressed as deltas, holes explored in the DCBH group were ~63% of carbacetam on Day 7, and ~77% and ~76% of carbacetam on Days 14 and 30, respectively – i.e., ~37% lower at Day 7 and ~23-24% lower at Days 14-30 (Table 5). This pattern supports a more robust restoration of exploratory activity with carbacetam, with the divergence emerging by the end of the first posttraumatic week.

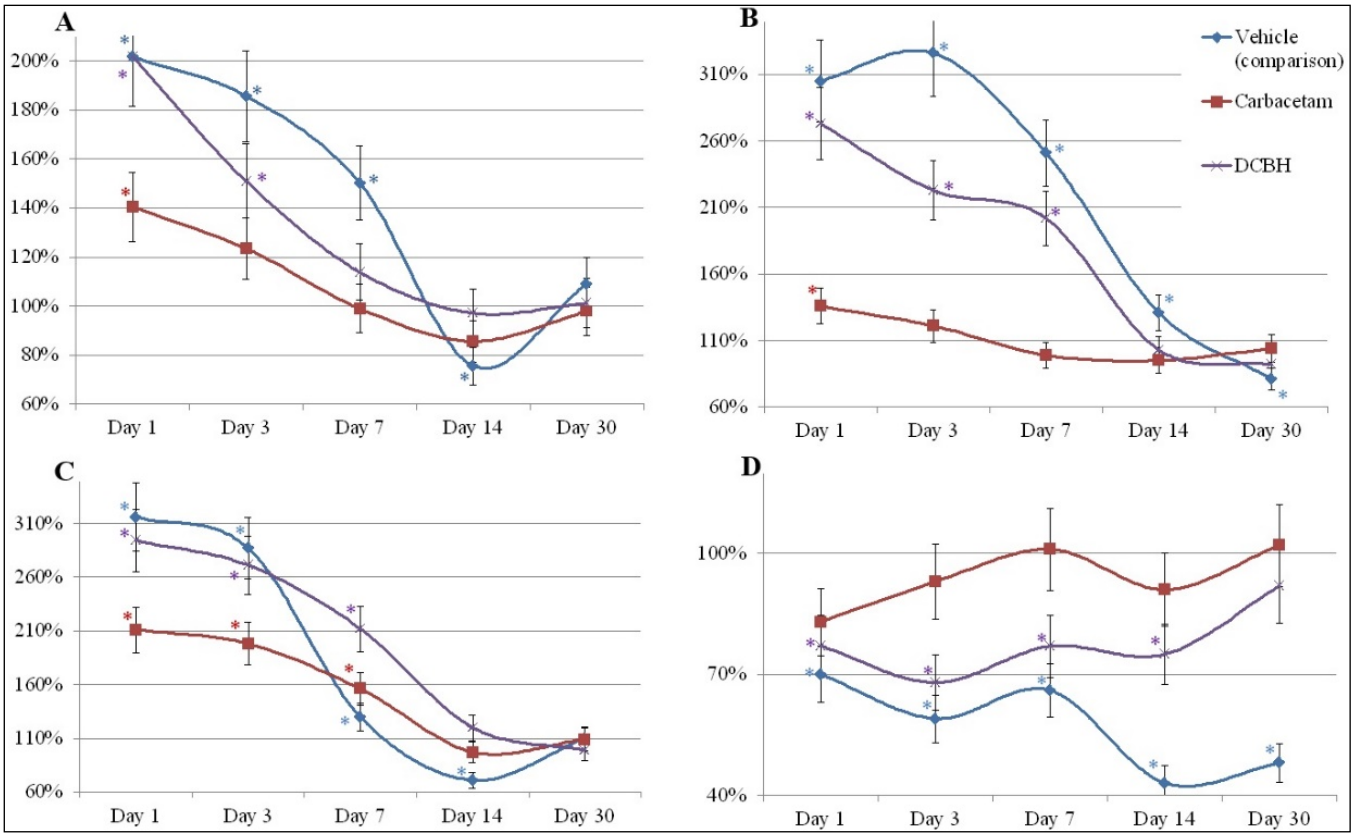

Fig. 2. Neuroendocrine response to closed-head TBI and modulation by carbacetam and DCBH. Plasma hormone levels are shown as percentage of intact reference animals (intact = 100%). Data are shown for Vehicle (comparison), Carbacetam, and DCBH groups at the indicated post-injury time points using terminal sampling (n = 5 per group per time point). Values are mean ± SEM. Absolute values are provided in the text. A – Adrenocorticotropic hormone (ACTH); B – Corticosterone; C – Vasopressin; D – Growth hormone (GH). *p < 0.05 vs intact reference (oneway ANOVA at each time point with Dunnett's post hoc test vs intact; pairwise treatment comparisons performed as described in Statistical analysis subsection).
Source: Authors' own work

NEUROENDOCRINE DYNAMICS

VEHICLE (COMPARISON) GROUP

Closedhead TBI triggered a pronounced acute stress-neuroendocrine response in vehicletreated animals, followed by a delayed suppression ("depletionlike") phase of central neurohormonal output and a later decline of the adrenal component (Fig. 2).

At 24 hours postinjury, the Vehicle group demonstrated a sharp rise in ACTH from 41.8 ± 2.9 pmol/L (intact) to 84.7 ± 8.6 pmol/L, i.e., 203% of intact (+102%, p < 0.05 vs intact). This was accompanied by a disproportionate increase in corticosterone from 379 ± 21 nmol/L to 1156 ± 42 nmol/L, i.e., 305% of intact (+205%, p < 0.05 vs intact). In axis terms, this pattern is consistent with strong feedforward activation along the hypothalamic-pituitary-adrenal (HPA) pathway, with a larger peripheral glucocorticoid output relative to the rise in ACTH. Vasopressin (AVP) also surged from 21.0 ± 1.9 pmol/L to 66.3 ± 4.7 pmol/L, i.e., 316% of intact (+216%, p < 0.05 vs intact), indicating early hypersecretion of posterior pituitary/hypothalamic neurosecretory output. In contrast to the catabolic axes, growth hormone (GH) decreased from 1.45 ± 0.11 μg/L to 1.01 ± 0.11 μg/L, i.e., 70% of intact (~30% decrease, p < 0.05 vs intact), suggesting early suppression of the somatotrophic/anabolic axis after injury.

By Day 3, ACTH began to decline but remained elevated at 77.6 ± 6.1 pmol/L, i.e., 186% of intact (+86%, p < 0.05 vs intact). In contrast, corticosterone continued to rise, reaching 1235 ± 48 nmol/L, i.e., 326% of intact (+226%, p < 0.05 vs intact), consistent with a sustained peripheral glucocorticoid drive and/or emerging decoupling of adrenal output from central ACTH signaling. AVP decreased slightly relative to Day 1 but remained markedly elevated at 60.2 ± 5.8 pmol/L, i.e., 287% of intact (+187%, p < 0.05 vs intact). GH suppression progressed: GH fell to 0.85 ± 0.04 μg/L, i.e., 59% of intact (~41% decrease, p < 0.05 vs intact). Physiologically, this constellation supports an ongoing catabolic stress response with persistent neurosecretory activation and a parallel suppression of anabolic/plasticitysupporting signaling.

At Day 7, ACTH declined to 62.9 ± 5.3 pmol/L, i.e., 151% of intact (+50%, p < 0.05 vs intact), whereas corti-

costerone remained high at 952 ± 40 nmol/L, i.e., 251% of intact (+151%, $p < 0.05$ vs intact). The persistence of hypercorticosteronemia despite a smaller ACTH elevation is compatible with impaired negative feedback within the HPA axis and/or altered glucocorticoid binding/clearance kinetics after injury (interpretation at the level of mechanism). AVP continued to normalize, falling to 27.2 ± 1.8 pmol/L, i.e., 130% of intact (+30%, $p < 0.05$ vs intact). GH showed partial recovery compared with Day 3 but remained 34% lower than intact ($p < 0.05$ vs intact), indicating that restoration of the somatotrophic axis lagged behind normalization of AVP.

At Day 14, ACTH dropped below intact values to 31.3 ± 2.9 pmol/L, i.e., 75% of intact (-25%, $p < 0.05$ vs intact). AVP similarly fell to 14.9 ± 0.8 pmol/L, i.e., 71% of intact (-29%, $p < 0.05$ vs intact). This biphasic course – early hypersecretion followed by a delayed suppression – supports the concept of a posttraumatic transition from acute hyperreactivity to central downregulation/exhaustion of neurosecretory output. Despite this central decline, corticosterone remained elevated at 497 ± 36 nmol/L, i.e., 131% of intact (+31%, $p < 0.05$ vs intact), indicating a sustained hypercorticosteronemic state across the first two postinjury weeks. GH reached its lowest observed level at 0.62 ± 0.04 µg/L, i.e., 43% of intact (-57%, $p < 0.05$ vs intact), aligning temporally with the central suppression phase and supporting prolonged impairment of anabolic and reparative signaling.

By Day 30, ACTH recovered to 45.6 ± 4.7 pmol/L and AVP to 23.1 ± 1.1 pmol/L, with no significant differences vs intact ($p > 0.05$), indicating restoration of central secretory output at the group level. In contrast, corticadrenergic output appeared reduced: corticosterone fell to 307 ± 22 nmol/L, i.e., 81% of intact (-19%, $p < 0.05$ vs intact), consistent with a later decline in the peripheral (adrenal) component after a prolonged period of hyperactivation. GH remained substantially suppressed at 0.70 ± 0.03 µg/L, i.e., 48% of intact (~52% decrease, $p < 0.05$ vs intact), indicating a prolonged somatotrophic deficit extending through the 30day posttraumatic period.

Conclusively, in vehicletreated animals, closedhead TBI produced a biphasic neuroendocrine trajectory: early hyperactivation of ACTH-corticosterone and AVP secretion with concurrent GH suppression (Days 1-3); a delayed suppression of ACTH and AVP below intact values by Day 14, while corticosterone remained elevated; by Day 30, central ACTH/AVP secretion normalized, but corticosterone declined below intact, alongside persistent GH suppression. This pattern is consistent with contemporary concepts of posttraumatic neuroendocrine dysfunction spanning both acute and subacute/chronic phases, where hypothalamic-pituitary axis perturbations (including somatotrophic and posterior pituitary components) can evolve over time and plausibly modulate recovery trajectories [18, 19].

MODULATION OF POSTTRAUMATIC NEUROENDOCRINE DYNAMICS BY CARBACETAM

Compared with the Vehicle group (described above), carbacetam produced an early and sustained "normalizing" shift of the posttraumatic neuroendocrine response, affecting both the central component of the HPA axis (ACTH), the peripheral adrenal response (corticosterone), and hypothalamic vasopressin (AVP) secretion, while largely preserving the somatotropic axis (GH).

At 24 h postTBI, ACTH in the Carbacetam group increased to 58.6 ± 6.5 pmol/L, i.e., 140% of intact (+40% vs intact, $p < 0.05$). Importantly, this early rise was 31% lower than the corresponding Vehicle value (58.6 vs 84.7 pmol/L; $p < 0.05$), indicating that the very first administration of carbacetam materially attenuated the acute central HPA response. A parallel pattern was observed for corticosterone: corticosterone increased to 515 ± 32 nmol/L, i.e., 136% of intact (+36% vs intact, $p < 0.05$), but was ~55% lower than Vehicle at the same time point (515 vs 1156 nmol/L; $p < 0.05$). Thus, both the central drive (ACTH) and peripheral output (corticosterone) were substantially less hyperreactive under carbacetam. AVP also increased, but the magnitude of hypersecretion was attenuated: AVP rose to 44.3 ± 3.9 pmol/L, i.e., 211% of intact (+111% vs intact, $p < 0.05$), yet remained 33% lower than Vehicle (44.3 vs 66.3 pmol/L; $p < 0.05$). This indicates moderated activation of hypothalamic neurosecretory processes (relative to Vehicle) already during the acute postinjury phase. For GH, the carbacetam group showed no statistically significant deviation from intact values and did not differ significantly from Vehicle on Day 1 (as per your comparisons), suggesting that early somatotropic suppression was not a prominent feature under carbacetam at this time point.

By Day 3, ACTH declined to 51.6 ± 4.6 pmol/L, corresponding to 123% of intact (+23% vs intact, not significant) and remained 34% lower than Vehicle (51.6 vs 77.6 pmol/L; $p < 0.05$). Corticosterone decreased to 458 ± 51 nmol/L, i.e., 121% of intact (not significant), and was ~63% lower than Vehicle (458 vs 1235 nmol/L; $p < 0.05$). AVP was 41.6 ± 4.4 pmol/L, i.e., 198% of intact (+98% vs intact; $p < 0.05$) and 31% lower than Vehicle (41.6 vs 60.2 pmol/L; $p<0.05$). A key divergence from Vehicle concerned the somatotropic axis: GH in the carbacetam group was 1.35 ± 0.06 µg/L, not significantly different from intact, but 59% higher than Vehicle at the

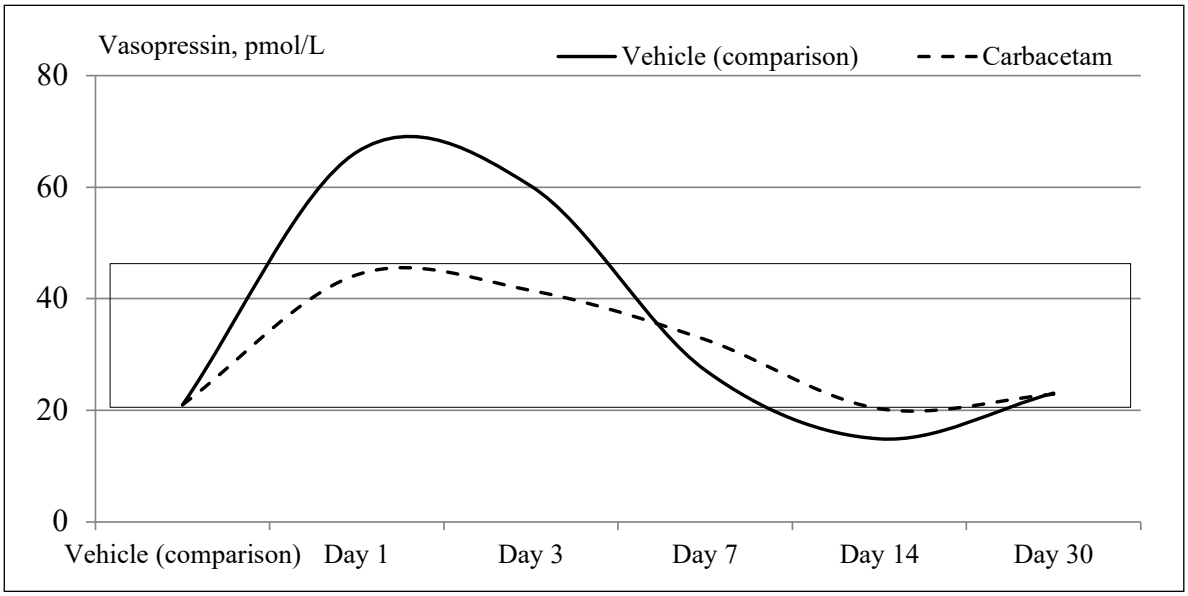

Fig. 3. Carbacetam attenuates early vasopressin hypersecretion and prevents delayed neurosecretory depletion after TBI. Vasopressin dynamics are presented as absolute concentrations (pmol/L) in the Vehicle and Carbacetam groups at post-injury Days 1, 3, 7, 14, and 30 (terminal sampling; n = 5 per group per time point). The boxed/outlined interval highlights the phase interpreted as attenuation of early posttraumatic hyperactivation and prevention of delayed depletion of vasopressin neurosecretion. Data are mean ± SEM. Group differences at each time point were assessed using oneway ANOVA (or Kruskal-Wallis where applicable) with multiplicity adjustment as described in Statistical analysis subsection.
Source: Authors' own work

same time point ($p < 0.05$), indicating preservation of GH secretion during the early subacute phase.

At Day 7, ACTH under carbacetam reached 41.3 ± 3.4 pmol/L, essentially normalized to intact (no significant difference), and remained 34% lower than Vehicle ($p<0.05$). Corticosterone was 374 ± 18 nmol/L, also not different from intact, and ~61% lower than Vehicle ($p < 0.05$). AVP was 32.7 ± 3.4 pmol/L, remaining 56% above intact ($p < 0.05$); however, it did not differ significantly from Vehicle at this time point (despite a numerically higher mean). In parallel, GH was 1.47 ± 0.07 µg/L, again not different from intact and ~53% higher than Vehicle ($p < 0.05$), reinforcing that carbacetam prevented the prolonged suppression of GH observed in Vehicle animals.

At Day 14, the contrast with Vehicle became particularly mechanistically informative. In the Vehicle group, ACTH and AVP fell below intact (a depletionlike phase), whereas under carbacetam both hormones remained within (or close to) the intact range. Specifically, ACTH under carbacetam was 35.8 ± 3.1 pmol/L (no significant difference vs intact and vs Vehicle). Corticosterone was 361 ± 18 nmol/L, not different from intact but 27% lower than Vehicle ($p < 0.05$), consistent with prevention of sustained posttraumatic hypercorticosteronemia. Critically, AVP was 20.3 ± 1.8 pmol/L, not different from intact, but 36% higher than Vehicle ($p < 0.05$), indicating prevention of the delayed AVP decline that characterized the Vehicle group at two weeks (Fig. 3).

GH at Day 14 was 1.32 ± 0.12 µg/L, again not significantly different from intact, but 113% higher than Vehicle ($p < 0.05$), showing that carbacetam prevented the pronounced GH nadir observed in untreated animals during the subacute period.

At Day 30, ACTH under carbacetam was 40.9 ± 3.8 pmol/L, with no significant differences vs intact or Vehicle. Corticosterone was 394 ± 19 nmol/L, not different from intact but 28% higher than Vehicle ($p < 0.05$), consistent with prevention of the late decline in adrenal output that emerged in the Vehicle group. AVP was 22.8 ± 1.9 pmol/L, not different from intact or Vehicle. GH remained preserved: 1.48 ± 0.09 µg/L, again not different from intact and 111% higher than Vehicle ($p<0.05$).

Taken together, across the 30day period, carbacetam acted as a modulator of posttraumatic neuroendocrine stress systems, with two clinically relevant features: immediate attenuation of primary hyperactivation (Day1 reductions vs Vehicle for ACTH, corticosterone, and AVP: -31%, -55%, and -33%, respectively), and prevention of delayed depletion of central neurosecretory output (notably AVP at Day 14), together with sustained preservation of GH across the entire followup (Day 14 and Day 30 GH exceeding Vehicle by +113% and +111%, respectively). These patterns align with contemporary concepts that neuroendocrine dysfunction after TBI is dynamic and phasedependent, spanning acute hyperreactivity and delayed pituitary/hypothalamic dys-

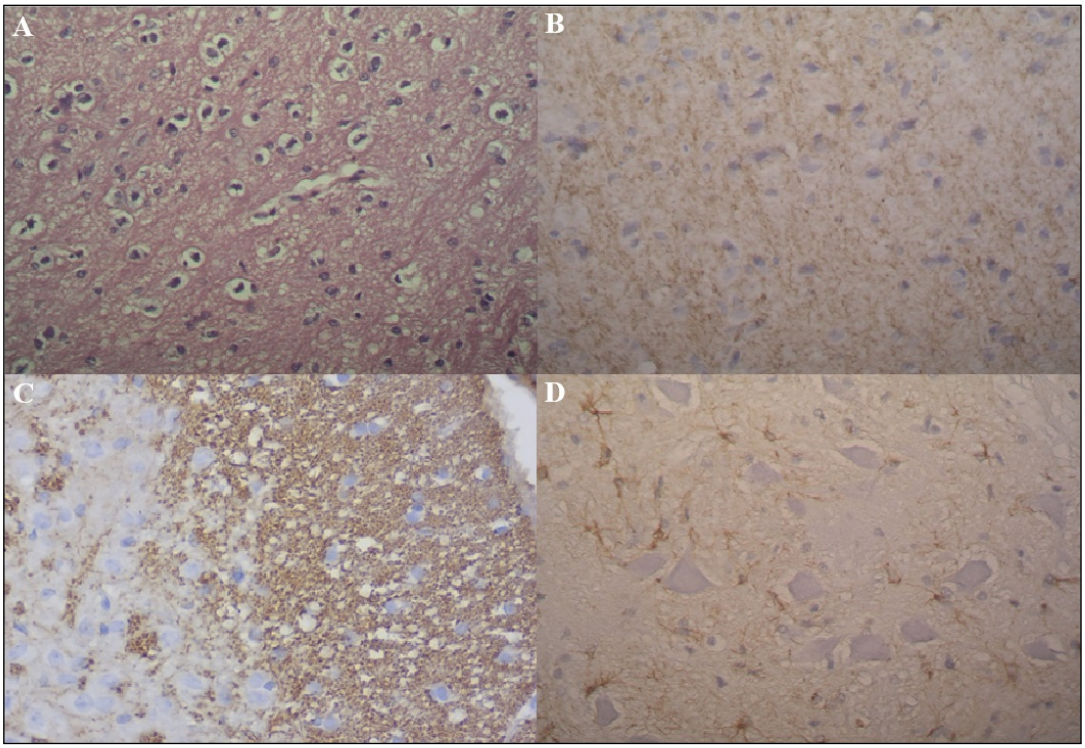

Fig. 4. Representative hypothalamic morphology and immunohistochemistry on Day 7 after closed-head TBI. Representative micrographs obtained on post-injury Day 7 (n=5 per group for tissue analyses). A – Paraventricular nucleus (PVN), H&E staining, carbacetam-treated animal; B – PVN, neuron-specific enolase (NSE) immunohistochemistry, vehicle group; C – PVN, NSE immunohistochemistry, carbacetam group; D – Supraoptic nucleus (SON), glial fibrillary acidic protein (GFAP) immunohistochemistry, vehicle group. Immunoreactivity was visualized using HRP/DAB with hematoxylin counterstain; ×400 magnification.
Source: Authors' own work

regulation. Carbacetam shifted this trajectory toward a more adaptive profile, limiting both the early surge and the later exhaustionlike phase [19].

NEUROENDOCRINE RESPONSE UNDER DCBH

(COMPARATIVE PATTERN)

The endocrine profile under DCBH differed qualitatively from carbacetam: DCBH did not suppress the primary hyperactivation of the central HPA component and AVP secretion during the acute phase, but effectively prevented the delayed depletion at approximately two weeks postinjury. In addition, relative to Vehicle, the reduction of GH secretion was less pronounced under DCBH. These groupwise trajectories are summarized in Fig. 2 (A-D).

HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY

On postinjury Day 7, histological assessment of the neuroendocrine regions most directly implicated in the observed hormonal dynamics – namely the paraventricular nucleus (PVN) and supraoptic nucleus (SON) – together with the hippocampal CA3 field, showed a heterogeneous pattern of posttraumatic tissue injury in the vehicle (comparison) group (Fig. 4B, D). A subset of neurons within these regions displayed morphological signs consistent with cellular stress/injury, including distorted neuronal contours, hyperchromatic (dark) nuclei, and heterochromatin condensation. In parallel, immunohistochemistry supported the presence of a marked glial response: GFAP and S100 immunoreactivity (glial markers commonly used to capture astroglial activation and disturbance of glial-neuronal homeostasis) appeared increased in the hypothalamic neurosecretory nuclei and hippocampus, consistent with reactive astrogliosis and disrupted neuron-glia coupling after TBI. Such astrocytic responses are now understood as contextdependent (potentially both adaptive and maladaptive) components of secondary injury and repair cascades after brain trauma. NSE immunoreactivity within hypothalamic nuclei appeared comparatively weak in this posttraumatic setting, whereas hippocampal NSE staining remained more evident – suggesting regionspecific differences in metabolic compromise across structures involved in neuroendocrine regulation versus cognitive circuitry [20, 21].

Against carbacetam treatment, neurons in the PVN/SON and hippocampal CA3 more frequently exhibited a morphology closer to an "intactlike" profile – round/oval nuclei, higher euchromatin content, and fewer overt dys-

trophic features – consistent with a relative preservation of neuronal functional capacity (Fig. 4A). In the PVN, NSE immunoreactivity in carbacetamtreated animals appeared more pronounced than in the vehicle group (Fig. 4C vs B), which is compatible with better preservation of neuronal metabolic activity under treatment. Qualitatively, serial sections also suggested lower GFAP and S100 immunoreactivity within hypothalamic neurosecretory nuclei under carbacetam versus vehicle, indicating attenuation of the astroglial stress response in the very regions that coordinate HPAaxis signaling and vasopressin neurosecretion. In contrast, in the hippocampus, GFAP/S100 expression remained elevated despite treatment – an observation that can be interpreted in a mechanistically plausible way within modern frameworks: sustained astrocyte activation in hippocampal networks may represent a prorecovery glial program supporting synaptic/metabolic homeostasis and compensation rather than a simple marker of ongoing degeneration. Importantly, this regionspecific pattern provides a coherent morphological correlate to the endocrine phenotype described above – namely, a normalization/modulation of hypothalamic neurosecretion under carbacetam, while hippocampal glial remodeling may persist as part of longerterm plasticity after TBI [20].

CONCLUSIONS

1. Closedhead traumatic brain injury (TBI) induced a marked increase in neurological deficit on Day 1 (5.4-5.9fold vs intact; $p < 0.05$), followed by partial spontaneous regression; however, without treatment the deficit remained significantly elevated on Day 7 (3.4fold vs baseline/intact; $p < 0.05$). The early deficit was driven predominantly by impairment within the reflex domain (37.6% of the total deficit structure). Both carbacetam and deproteinized calf blood hemoderivative (DCBH) were associated with a reduction of neurological deficit, with a larger effect for carbacetam: by Day 7 the deficit decreased by 57% under carbacetam, 47% under DCBH, and 43% in the vehicle group ($p < 0.05$ for the reported withingroup changes).
2. On Day 1 afterTBI, behavioral indices of higherorder CNS function were significantly impaired ($p < 0.05$ vs sham): squares crossed decreased to 43%, holes explored to 21%, and successful entries in the eightarm maze to 48% of sham; light-dark transition latency (burrowreflex paradigm) increased by 88% ($p < 0.05$). During follow-up, these measures partially recovered but, without pharmacological correction, did not fully normalize to sham levels by Day 30 (with persistent deficits in exploratory/locomotor and maze indices, whereas latency approached sham values). Carbacetam restored orienting-locomotor performance to nearcontrol levels (83% for squares crossed and 85% for holes explored vs sham; $p > 0.05$) and normalized the eightarm maze and light–dark latency to controlrange values (97% and 115%, respectively; $p > 0.05$). DCBH also improved behavioral outcomes, but did not normalize holes explored; this parameter remained higher with carbacetam than with DCBH by 24-37% ($p < 0.05$), indicating a more robust recovery of exploratory activity under carbacetam.
3. Following TBI, the hypothalamic-pituitary-adrenal (HPA) axis and vasopressin secretion showed an acute hyperreactive response followed by secondary depletion at approximately two weeks postinjury. A roughly twofold rise in ACTH was accompanied by an approximately threefold rise in corticosterone, consistent with the development and maintenance of a posttraumatic hypercorticosteronemic state. Across the entire observation period, growth hormone (GH) secretion was progressively suppressed, reaching 48% of baseline by Day 30 ($p < 0.05$). Carbacetam exerted an early modulatory effect (evident after the first administration), preventing primary hyperactivation and subsequent depletion of central neuroendocrine outputs, attenuating the development of secondary posttraumatic hypercorticosteronemia, and preventing sustained suppression of GH secretion. DCBH did not attenuate the initial hyperactivation of central HPA signaling and vasopressin secretion, but effectively prevented their secondary depletion; GH remained reduced, but less markedly than in the vehicle group.
4. Immunohistochemical assessment using GFAP, S100, and NSE supported the presence of posttraumatic degenerative/glialreactive changes: GFAP and S100 immunoreactivity was increased (consistent with disturbed neuron–glia interactions), whereas NSE immunoreactivity was reduced in the injury setting. Carbacetam was associated with a decrease in GFAP and S100 and an increase in NSE, indicating attenuation of degenerative/glialstress features and better preservation of neuronal metabolic activity. In hippocampal CA3, carbacetam exerted a protective effect predominantly on the neuronal component of the tissue.
5. Cumulative mortality after TBI was 50% in the vehicle group, compared with 16% under carbacetam and 36% under DCBH (overall $p=0.002$). Carbacetam reduced mortality 3.1fold versus vehicle ($p=0.001$), whereas DCBH reduced mortality 1.4fold ($p=0.096$). These findings are consistent with the interpretation that carbacetam – and to a lesser extent DCBH – promoted a shift of neurohumoral regulatory responses toward a more adaptive pattern (restoration of central and peripheral endocrine components), which was accompanied by improved survival, neurological recovery, and behavioral outcome after closedhead TBI.

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CONFLICT OF INTERESTS

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 29.03.2026
ACCEPTED: 27.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Effect of a group-based intervention programme on self-efficacy in type 2 diabetes mellitus patients

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ABSTRACT

Aim: The aim of this study is to investigate the effectiveness of a group-based intervention of patients with T2DM on self-efficacy. The main objective of the study was to explore the appropriateness and feasibility of diabetes intervention as a motivational strategy to enhance self-care of patients with T2DM and its possible integration in primary care from health professionals.

Materials and Methods: A quasi -experimental study was conducted with a pre- and post-test design in an experimental group and a control group, in Larissa Greece, at the patients with T2DM during their regular visits in Primary Care Units.

Results: The study samples were comprised 2 groups from forty one patients each. None of the patients in either group had received intervention programme previously. The study was conducted throughout in 4 sessions during the period of 3 months. Self-efficacy scores in intervention group differed statistically significantly in all items before and after the experimental procedure, ($p<0.05$). Intervention group achieved significantly higher scores compared with the control group in certain items (Diet, Medical therapy, Medication-feet check, and Physical activity $p=0.001$, $p=0.001$, $p=0.001$ and $p=0.007$, respectively).

Conclusions: Our study confirms the positive effect of a group-based intervention programme of patients with T2DM on self-efficacy. Future research should assess the viability of group-based intervention to be extended to more adults with T2DM in the community.

KEYWORDS: community, diabetes mellitus, diabetes self-efficacy, group intervention program

Wiad Lek. 2026;79(7):1467-1474. doi: 10.36740/WLek/225979 DOI

INTRODUCTION

Type 2 diabetes mellitus (T2DM) accounting for approximately 90% diabetes cases and is a chronic progressive disease with high prevalence, posing significant challenge to healthcare systems worldwide [1]. T2DM in Greece follows the prevalence patterns of other developed countries, and the estimated prevalence of type 2 diabetes in the population is 11,9% %, based on cases [2]. Since the demands and the integration of T2DM is mainly accomplished by patients, these lead to reduced well-being and discouraged [3]. Previous studies have found that persons with diabetes often lacked sufficient knowledge about their condition and reported poor self-care skills [4]. This would mean a high likelihood of diabetes complications leading to a reduction in quality of life, disability, or even death [5]. Self-care refers to the ability to promote and maintain health, prevent disease, and cope with illness, either independently or with the support of health care providers [6]. In addition to structuring the primary care provided, inclusion of a patient education program including instruction in self-management skills show to be valuable [7]. Self-efficacy has been found to have a direct relation with self-care in T2DM therefore developing effective and efficient strategies to promote self-efficacy is important in order to prevent serious complications[8]. Many diabetes intervention studies have explored how challenging it is for diabetic people to maintain active self-efficacy [8]. There is a need to assess whether community-based interventions with a collaborative approach would also be effective in improving self-care among adults with T2DM in Greece, a region with the high prevalence for diabetes [9]. However, increasing patients' knowledge about their condition could potentially be the first step to enhancing diabetes self-care. Although there is some evidence that a program of structured primary care, such a group-based intervention programme is effective in improving self-efficacy on patients with T2DM, the findings are inconclusive and further investigation is necessary.

AIM

The aim of this study is to investigate the effectiveness of a group-based intervention of patients with T2DM on self-efficacy. The main objective of the study was to explore the appropriateness and feasibility of diabetes intervention as a motivational strategy to enhance self-care of patients with T2DM and its possible integration in primary care from health professionals. In T2DM, self-efficacy has been found to have a direct relation with self-care, in a way that this construct owns the predictability power of self-care behavior. Several tools have been developed and used for evaluating self-efficacy of diabetic patients, the most widely used being the Diabetes Management Self-Efficacy Scale (DMSES) [10].

MATERIALS AND METHODS

STUDY DESIGN

A quasi -experimental study was conducted with a pre- and post-test design in an experimental group and a control group, in Larissa Greece, in diabetic patients visiting public primary care units.

POPULATION AND SAMPLE

The study population consisted of a convenience sample of eighty two patients with T2DM. The study samples comprised forty one patients each group and created the Intervention (n=41) and Control (n=41) Group . Groups are randomly created alternaty in order visiting the primary care unit. The study was conducted throughout in 4 sessions during the period of 3 months and conducted by collecting data from the outpatient Primary Care Units, within its territory from November 2024 to January 2025. None of the patient in either group had participated in interventional program previously. Significance level ($\alpha = 0.05$), large level of significance (Cohen's $d = .70$), and test power (90%). Therefore, the subjects selected for this study met the required number.

INTERVENTION

The integration of the intervention programme began with the planning and development stage of the sessions, before the actual intervention programme and the design of every session took place. The Intervention Group participants, instead of receiving standard care, attended a structure 12 hours educational programme group in 4 sessions for 2 weeks. The intervention conducted in small groups of 10-12 people under the guidance of family doctor. Initially, the first session was held with the patients in intervention group, where the objectives of the practice and the characteristics of the intervention programme were discussed. All participants were administered the questionnaires before the study and the same questionnaires were filled in at the end of intervention, and 3 months after, always with informed consent in detail about all the procedures and sessions of the study. Participation in the study was voluntary. Participants were informed that the questionnaires were anonymous and that they could stop participating in the research at any time. The clinical training took place at the outpatient primary health units of the public 5th Regional Health Authority of Thessaly and Sterea in Greece (IRB approval No:44729/23.05.2023).

THE GROUP-INTERVENTION PROGRAM

The Intervention Group participants, instead of receiving standard care, attended a structure 12 hours educational programme group in 4 sessions for 2 weeks. The health education lead by family doctor, community nurse, nutritionists and exercise included information on lifestyle modification, dietary strategies and exercise. Each session was designed for multidisciplinary education and collaborative learning, to teach knowledge and practical skills in diabetes self-care. The emphasis was on empowering individuals to make choices and lifestyle changes. The content and delivery format were designed using the group-based self-empowerment intervention.

A STRUCTURE 12 HOURS EDUCATIONAL PROGRAMME

On the first day the first session, the basic concepts of the intervention programme were introduced. The group-intervention was based on the Consensus Report of the American Diabetes Association [9]. On the first session participants have better understanding on overview of diabetes and perfume regular self-monitoring of blood glucose (BG). On the second session patients with T2DM identify common factors that influence their BG, list causes and common signs and symptoms of high and low BG and discuss ways to prevent and manage diabetes emergencies. At the third session discuss general principles of meal planning, identifying common food groups and preparation which can affect glycemic level and propose ways for dietary adjustment. On fourth session discuss benefits and safety precaution for exercise and physical activity, recap the key points for T2DM and self-care and summarize the intervention programme.

Table 1. Cronbach's alpha for GR-DMSES

	Number of items	Cronbach's alpha
GR-DMSES	20	0.93
Diet (items 4, 5, 9, 10, 13, 14, 15, 16, 17)	9	0.92
Medical therapy (items 1, 2, 3, 6, 18)	5	0.76
Medication and feet check (items 7, 19, 20)	3	0.70
Physical activity (items 8, 11,12)	3	0.79

*GR-DMSES Greek version of the Diabetes Management Self-Efficacy Scale
Source: compiled by the authors of this study

Table 2. Cronbach's alpha for GR-DMSES during the intervention programme

Diabetes Management Self-Efficacy Scale	Pre Intervention	0.95
	Post Intervention	0.92
	3 months after Intervention	0.95
Diet	Pre Intervention	0.94
	Post Intervention	0.94
	3 months after Intervention	0.95
Medical Therapy	Pre Intervention	0.81
	Post Intervention	0.82
	3 months after Intervention	0.84
Medication and feet check	Pre Intervention	0.63
	Post Intervention	0.65
	3 months after Intervention	0.66
Physical therapy	Pre Intervention	0.88
	Post Intervention	0.91
	3 months after Intervention	0.90

Source: compiled by the authors of this study

Participants in the Intervention Group were divided into 3 groups (12-15 people per group) attended four sessions and each session lasting approximately 180 minutes. The purpose of the process evaluation was to explore the strengths of the intervention program and to obtain suggestions from the participants for future improvements.

ASSESSMENT TOOLS

All patients of both groups (intervention and control) were asked to fill in questionnaires both at the beginning and at the end of the study, and 3 months after which, in addition to demographic characteristics, included the validated Greek version of Diabetes Management Self-Efficacy Scale (GR-DMSES). The GR-DMSES contains four items Diet, Medical therapy, Medication-feet check and Physical activity. Responses for all questions usually ranged from 1 to 5, Likert scale (5 = "Certainly YES", 4 = "Probably YES", 3 = "Possibly YES, Possibly NO" 2= "Probably NO" 1= "Certainly NO") with a higher summed scores are indicative of higher levels of self-efficacy in that domain. Based on the available literature this tool has been found to be a reliable and valid self-report measure of diabetes self-efficacy [10].

RECORD SHEET OF INDIVIDUAL CHARACTERISTICS

It included questions about demographic, social and educational characteristics, such as gender, age, marital status, the chronicity of the disease, and adherence to treatment based on self-report, concerning the frequency of skipping the treatment and its possible interruption.

Table 3. Demographical profiles and baseline data of study subjects

Characteristics	Group				P-value
	Control		Intervention		
	N	%	N	%	
Sex					0.83[a]
Female	21	51.2	22	53.7	
Male	20	48.8	19	46.3	
Age	68.8	10.3	71.6	9.5	0.20[b]
Marital status					0.57[a]
Married	35	85.4	33	80.5	
Single	0	0	1	2.4	
Widowed	6	14.6	7	17.1	
Place of residence					0.001[a]
Town	12	29.3	1	2.4	
Village	29	70.7	40	97.6	
Live with family					0.02[a]
No	2	4.9	9	22.0	
Yes	39	95.1	32	78.0	
Education background					0.08[c]
No formal education	2	4.9	3	7.3	
Primary level	24	58.5	28	68.3	
Secondary	2	4.9	5	12.2	
Postsecondary/junior college	8	19.5	3	7.3	
University and above	5	12.2	2	4.9	
Current working status					0.16[a]
Private workers	5	12.2	1	2.4	
Public workers	6	14.6	2	4.9	
Self- employment	2	4.9	1	2.4	
Not working (Homemaker)	8	19.5	7	17.1	
Not working (Retired)	18	43.9	29	70.7	
Technicians/Farmers	2	4.9	1	2.4	
Monthly Household (€)					0.46[c]
<600	18	43.9	19	46.3	
600-1000	14	34.1	17	41.5	
1001-1500	9	22.0	5	12.2	
Insurance Status					0.99[a]
No	1	2.4	0	0	
Yes	40	97.6	41	100	
Body Mass Index (kg/m^2)	30.4	8.2	29.4	4.8	0.49[b]
Waist circumference (cm)	107.4	16.8	104.3	13.8	0.37[b]
Smoking					0.10[a]
No	30	73.2	36	87.8	
Yes	11	26.8	5	12.2	
Daily number of cigarettes	4.9	9.6	1.9	5.9	0.09[d]

[a] chi-square test
[b] independent samples t-test
[c] chi-square trend test
[d] Mann-Whitney test
Source: compiled by the authors of this study

Table 4. Subscales Diet Medical Care Medication Exercise of DMSES

Independent variables	Coefficient b	95% interval of confidence for b	P-value
Item Diet			
Intervention	-3.56	-8.88 to 1.76	0.19
Time	1.65	0.65 to 2.67	0.002
Interaction of intervention and time	3.87	2.12 to 5.61	<0.001
Item Medical therapy			
Intervention	-1.28	-3.89 to 1.34	0.34
Time	0.82	0.14 to 1.49	0.02
Interaction of intervention and time	1.49	0.61 to 2.37	0.001
Item Medication and feet check			
Intervention	-1.47	-3.06 to 0.13	0.07
Time	0.50	0.10 to 0.90	0.01
Interaction of intervention and time	0.98	0.40 to 1.56	0.001
Item Physical activity			
Intervention	-1.69	-3.89 to 0.52	0.13
Time	0.82	0.29 to 1.33	0.002
Interaction of intervention and time	1.05	0.28 to 1.82	0.007

Source: compiled by the authors of this study

STATISTICAL ANALYSIS

We present categorical variables as numbers and percentages. Also, we use mean, standard deviation (SD), median, and interquartile range to present continuous variables. We used the Kolmogorov-Smirnov test and Q-Q plots to examine the distribution of continuous variables. Bivariate analysis included four statistical tests: (a) chi-square to compare two categorical variables, (b) chi-square trend test to compare one categorical with one ordinal variable, (c) independent samples t-test to examine mean differences between two groups when the continuous variable followed normal distribution, (d) Mann-Whitney test to examine median differences between two groups when the continuous variable did not follow normal distribution. To examine the impact of independent variables on the dependent variables, the Generalized Estimating Equations model was employed. This model is particularly suited for clusters of observations that vary over time, making it appropriate for longitudinal data, as it remains robust regardless of the distribution of the variables. The application of the Generalized Estimating Equations model allowed for the control of confounding effects. In this context, the b coefficients of the multivariable models, along with their corresponding 95% confidence intervals and p-values, are presented. P-values less than 0.05 were considered statistically significant. We used the IBM SPSS 28.0 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp) for the analysis.

The GR-DMSES had an overall coefficient alpha of 0.93 (Table 1) The corrected item–total correlations indicated good correlations (different from zero) for all the items. Cronbach's alpha coefficients, for each of the subscales identified during the intervention programme, before intervention (pre-intervention), after the intervention (post-intervention) and after 3 months of the intervention (Table 2).

RESULTS

Each group comprised 41 patients (22 women in control group -54% and 21 women in intervention group-51%). Most participants were married, were retired and had primary level education. The 41 control participants had a mean age of 68.8 years old and 41 intervention participants had a mean age of 71,6 years old. The only variables in which the two groups differed were place of residence and mode of accommodation. More specifically patients living in the city from the control group was 29.3% and patients living in the city from the intervention group it was 2.4% (p=0.001). Patients living with others from the control group were 95.1% and from the intervention group patients were 78% (p=0.02). There was no other statistically significant difference regarding baseline demographic characteristics between the two groups (Table 3).

The GR-DMSES scores in intervention group differed statistically significantly in all subscales before and after the experimental procedure, in contrast with the

control group, since patients in the intervention group improved their scores in all four subscales p<0.05).

Interventional group had a statistically significant higher score on the subscale diet, medical treatment, medication and exercise of Diabetes Management Self-Efficacy Scale compared to control group Table 4.

Regarding the intervention group, gender did not have an effect on explanation and inference. There was no effect of demographic variables on GR-DMSES subscale scores.

DISCUSSION

One reason for the poor outcomes in patients with T2DM is the lack of participation in the treatment of the disease and the low levels of self-care [11]. This participation is important factor in the treatment of T2DM that demands motivation, knowledge, and improve the self-efficacy [12]. The primary outcome variables showed a response to the intervention programme when compared with baseline data and included significantly increased self-efficacy of patients with T2DM [13]. The results of this study show that group interviewing can be an effective method of working with T2DM management, producing improvements in self-care.

According to the findings of the present study, group-based intervention programme on diabetic patients did have an effect on self-efficacy subscales (diet ,medical treatment , medication and exercise) [14]. The importance of group-based intervention programme at all levels of self-efficacy on patients with T2DM has been recognized and a large literature has been generated on the subject. It was concluded that motivational interviewing was a highly acceptable intervention for increasing self-efficacy to change their behaviors [15,16].

This finding was similar to the other study where an intervention based on the integrated health management model including components such as skills training in health management among adults with T2DM [17]. In addition the results was similar to Lynch and colleagues finding, where the lifestyle intervention, a culturally tailored program focusing on diet and physical activity, resulted in an increased nutrition knowledge and diet quality among adults with T2DM. Findings from the lifestyle intervention and the process evaluation results indicated the importance of teaching knowledge and skills on how to eat healthily was essential in facilitating self-care in general diet control among adults with T2DM [18]. In addition, higher levels of knowledge about diabetes have been found to predict higher levels of adherence to self-care behaviors among individuals with T2DM, because they would have a better understanding and be more motivated to perform self-care to manage their condition [19-21].

This study has several limitations.. The study population consisted of a sample of patients residing in the area of the rural medical center. Neither the small training duration nor the sample allow generalizing of our results. The results, which were based on the respondents' self-reported data regarding their self-management , their exercise and diet behavior, may have been subject to recall bias.

CONCLUSIONS

This study has demonstrated that a group-based intervention program designed to teach knowledge and practical skills in diabetes self-care. Goal setting and motivational interviewing could improve the self-efficacy, exercise and general diet among adults with T2DM. Therefore, diabetes care and education community partners, public health professionals, health promotion program planners, and policy makers who have an interest in improving diabetes self-care should work together to extend such programs to more adults with T2DM in the community. Applying the group-based intervention program to diabetic patients presents many advantages. We strongly recommend the incorporation of group-based intervention and motivational practices that systematically promote self-efficacy of patients with T2DM. This research provided evidence for future clinical practices in diabetes care.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 12.03.2026
ACCEPTED: 30.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Clinical-and-neurological symptoms and dynamics of certain biomarkers in the acute and remote periods of combat traumatic brain injury

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ABSTRACT

Aim: To study biochemical markers of brain tissue damage, the levels of which could be used to assess the degree of damage to the nervous tissue and predict the course and outcome of the disease.

Materials and Methods: There were 38 patients in the acute period and 33 in the remote period of MBT were examined. The patients' complaints, levels of brain-specific proteins S-100β, BDNF and inflammatory biomarkers such as interleukins IL-8 and IL-10 were studied in detail.

Results: The study of complaints showed their similarity in both the acute and remote periods, apart from an increase in the number of patients with meteorological sensitivity. An increase in S-100β levels and a decrease in BDNF levels indicate a slowdown in the rate of recovery processes in this category of patients and a deterioration in brain plasticity processes. An increase in IL-8 and IL-10 levels indicates further progression of the disease.

Conclusions: The study of these biomarkers contributes to the prognosis and course of the consequences of mine-blast trauma and the choice of therapeutic agents in treatment.

KEYWORDS: mine-blast trauma, brain-specific interleukin proteins

Wiad Lek. 2026;79(7):1475-1479. doi: 10.36740/WLek/225980 DOI

INTRODUCTION

Mine-blast trauma (MBT) is the main cause of disorders in the central nervous system (CNS) and contributes to further structural, functional and neurochemical disorders in the brain. Traumatic damage to the CNS ranks second in frequency after bullet and shrapnel wounds to the torso and limbs in the structure of combat injuries. In MBT, in addition to the direct impact of the high-pressure zone on the brain, the corresponding receptors of the torso and auditory apparatus are affected by high temperatures and extremely loud sounds. As a result, an extremely powerful flow of afferent impulses is formed in the CNS, leading to the destruction of the entire structural and functional organisation of the brain as the highest integrative control centre of the entire body. Extreme inhibition of brain structure activity causes desynchronisation and imbalance in their activity, i.e. dysregulatory pathology, which impairs the functioning of the brain's functional systems [1].

At the same time, autoimmune processes, a significant increase in antibody production, and an increase in humoral immunity indicators play a significant role in the pathogenesis of MBT.

Post-traumatic changes are a combination of compensatory-adaptive and established pathological processes, which can subsequently develop independently and determine the patient's condition and the course of the disease.

It should be noted that damage to brain tissue in MBT leads to metabolic activation of glial neurons, as well as to energy deficiency and membrane depolarization. Ultimately, the chain of pathological reactions leads to cytotoxic edema and osmotic lysis of cells and, on the other hand, to the initiation of secondary biochemical reactions, among which apoptosis and inflammation play a major role [5].

The diagnosis of mild MBT poses certain difficulties due to the barely noticeable subjective nature of the symptoms. Traditional neuroimaging methods often do not reveal abnormalities in this pathology, which has led to growing interest in various biomarkers that can be objective indicators of brain injury.

These include the S-100β protein, which is found mainly in astrocytes, and the BDNF protein, which is found in the hippocampus, basal parts of the forebrain, as well as fibroblasts, astrocytes, and various types of neurons, and belongs to the neurotrophins, which stimulate and support the development of neurons. Its deficiency reduces the plasticity of neurons and disrupts cognitive functions [2, 4, 6, 7].

The cytokine imbalance that occurs in the acute period of traumatic brain injury persists and maintains a chronic inflammatory process and contributes to the progression of brain damage.

One of such cytokines is interleukins 8 and 10 (IL-8, IL-10). It has been found that IL-8 is released into the cerebrospinal fluid after a brain injury, and its role in the pathophysiology of traumatic brain injury (TBI) may be linked to blood brain barrier dysfunction.

The role of IL-10 in the pathophysiology of TBI is controversial in the literature, although its increase in 6 months period after TBI may indicate long-term chronic inflammation [3].

Damage to the nervous system, as a result of MBT further leads to trophic dysregulation, which is one of the universal components of pathogenesis. Therefore, the study of biochemical markers of brain tissue damage, the levels of which could be used to assess the degree of damage to the nervous tissue and predict the course and outcome of the disease, is protein S100β, a specific protein, the level of which could be used to assess the degree of damage to the nervous substance and predict the course and outcome of the disease, is protein S100β, a specific protein synthesized mainly in astroglial cells (more than 85-90% of their total content in the nervous system). The content of S100β protein in blood serum ranges from 0.005 to 0.105 μg/l [7].

AIM

To study biochemical markers of brain tissue damage, the levels of which could be used to assess the degree of damage to the nervous tissue and predict the course and outcome of the disease.

MATERIALS AND METHODS

There were 38 patients in the acute period and 33 patients in the remote period of mild mine-blast traumatic brain injury who were hospitalised at the neurosurgical clinic of the State Institution 'P.V. Voloshin Institute of Neurology, Psychiatry and Narcology of the National Academy of Medical Sciences of Ukraine' (Kharkiv, Ukraine) during the period of 2022–2025. The control group consisted of men between the ages of 27 and 36 with no history of traumatic brain injury, neuroinfections, somatic pathology, or hereditary diseases.

The study included patients who met the following criteria: a) age between 25 and 37; b) no history of somatic or neurological disorders; c) only a mild traumatic brain injury (with no alcohol poisoning, chest injuries, tuberculosis at the time of injury, and other somatic and mental disorders). These criteria allowed us to exclude factors that could significantly affect the course of the disease. The complaints and neurological status of these patients were studied in detail.

To study the degree of brain damage based on the level of biochemical markers that could be used to assess the degree of brain damage and predict the further course of the disease, we studied the indicators of such biomarkers as S-100β protein, BDNF, and interleukins IL-8 and IL-10.

The level of S-100β protein was studied in blood serum using electrochemiluminescence analysis on an Elecsys 1010 automatic analyzer (Germany), and BDNF was studied using the ELISA immunoassay method with the Human BDNF ELISA Kit in accordance with the manufacturer's instructions. The content of pro-inflammatory cytokines – interleukins (IL-8, IL-10) in blood plasma was determined by immune-enzymatic method using standard kits from "Calbiotech" (Germany) and "Diaclone" (France). Statistical analysis of the results was performed using standard statistical methods with the "Statistika-6.0" and "Microsoft Excel-2010" statistical software packages. The reliability of the results was assessed using Student's t-test. A condition of $p < 0.05$ was considered statistically significant.

ETHICS

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

RESULTS

The duration of mine-blast cerebral trauma at the time of admission to the hospital ranged from 3 to 7 days. These patients were referred for hospitalization with a diagnosis of acute mine-blast trauma, which was diagnosed by neurologists at a military hospital. At the time of injury, 33 of the 38 patients examined lost consciousness (for 5 to 20 minutes). These patients were re-examined and treated in 10–12 months after the injury.

The main complaints in the acute period were constant headache, dizziness, a hearing impairment, memory loss of current events, 'sleep-wake' cycle

Table 1. Main complaints in the acute and long-term periods of mine-blast trauma upon admission to the hospital

Complaints	the acute period MBT (n=38)	the remote period MBT (n=33)	p
headache	36	28	< 0.05
dizziness	32	27	< 0.05
nausea	19	11	
hearing impairment	30	26	< 0.05
memory loss	31	32	< 0.05
sleep disorders	35	26	< 0.05
fluctuations in BP	13	18	
Meteorological sensitivity	3	17	
irritability	34	32	< 0.05

Source: compiled by the authors of this study

Table 2. Indicators of brain-specific proteins in the acute and remote periods in patients with mine-blast trauma

Brain-specific proteins	control indicators	the acute period (n=38)	the remote period (n=33)
S-100β	54.1 ± 1.88ng/l	128.3 ± 6 ng/l	97.2 ± 6.4 ng/l
BDNF	459 ± 21.4 ng/l	388 ± 10.2 ng/l	231± 24.6ng/l

Source: compiled by the authors of this study

Table 3. Data on biomarkers of neuro-inflammation interleukin 8 and interleukin 10 in patients in the acute and remote periods of mine-blast trauma

control values IL-8, IL-10 (0-10 pg/mg)		the acute period of MBT (n=38)		the remote period of MBT (n=33)	
IL - 8	IL - 10	IL - 8	IL - 10	IL - 8	IL - 10
0 - 10		2	-	-	-
11 - 20		7	9	5	4
21- 30		24	16	12	27
31 - 36		5	13	16	2
Total		38	38	33	33

Source: compiled by the authors of this study

disorders, and rapid fatigue. In the remote period, the main complaints were headache, nausea, hearing impairment, noise in the head, meteorological sensitivity, fluctuations in blood pressure (BP), memory loss, daytime sleepiness, irritability, sleep disorders and daytime sleepiness (Table 1).

As can be seen from Table 1, headaches were observed in the acute period of MBT in almost all examined patients (in 36 out of 38 men, $p < 0.05$). A similar picture was observed in patients with the long-term consequences of MBT (in 28 out of 33 men, $p < 0.05$). Slightly fewer patients complained of nausea (19 in the acute period and 11 in the long-term period), while the number of patients with meteorological sensitivity increased (5 in the acute period and 17 in the long-term period). In the rest of the complaints, everything was practically the same in both the acute and remote periods of mine-blast trauma.

A study of the neuroglial protein S-100β showed (Table 2) that it increased almost threefold in the acute period compared to the control group and decreased slightly in the remote period, which may indicate the onset of secondary auto-destructive biochemical processes and the onset of traumatic brain disease. The levels of the brain-derived neurotrophic factor BDNF in the acute period were reduced compared to the control figures, and an even greater decrease was observed in the remote periods of MBT, which indicates a slowdown in the rate of recovery processes in this category of patients and deterioration in brain plasticity processes.

The study of biomarkers of inflammation of such as interleukins 8 and 10 showed that there is an increase in these indicators in the acute and long-term effects of mine-blast trauma (Table 3).

As we can see from Table 3, the highest neuro-inflammation indicators in the acute period of MBT were within the range of 21-30 pg/mg for both IL-8 and IL-10 (in 24 and 16 patients, respectively). In the long-term period of MBT, high levels were within the range of 31-36 (IL-8) in 16 patients and within the range of 21-30pg/mg (IL-10) in 27 patients.

DISCUSSION

At the moment of receiving a mine-blast injury, a series of mechanisms are triggered at both the cellular and molecular levels, leading to a disorder of physiological processes, which subsequently negatively affects the neural system and brain function. Pro-inflammatory factors, such as interleukins and tumour necrosis factor, are activated in response to damage and can exacerbate the negative effects of inflammation, contributing to further tissue damage.

Brain-specific proteins are key components in the pathogenesis and further recovery after cerebral trauma. Studying them helps to understand the mechanisms of trauma, develop therapeutic approaches, and assess the prognosis after trauma.

At the same time, MBT leads to the release of biomolecules into the extracellular space and further into the blood. It is the release of proteins and metabolites into the blood that is a molecular marker of the severity of injury progression (S-100β, BDNF, and others) [6].

The main complaints we received from patients with MBT, both in the acute and remote periods, indicate the presence and persistence in most of them of complaints such as headache, dizziness, hearing and memory impairment, sleep disorders, increased irritability and meteorological sensitivity (which were not observed in patients before the injury). All this indicates that pathological processes continue and cause persistent disorders in the central nervous system.

The increase in S-100β neuroglial protein levels compared to control figures after 9–12 months can be explained by the onset of secondary auto-destructive biochemical processes, indicating the onset of traumatic brain disease and requiring specialized treatment at this stage.

A decrease in the levels of the brain-derived neurotrophic factor BDNF, both in the acute and even more in the long-term period of MBT, is one of the reasons for the slowdown in the rate of reparative processes and brain plasticity that occur in these patients.

Data on the levels of neuro-inflammation biomarkers interleukin 8 and interleukin 10 in patients in the acute and long-term periods of mine-blast trauma showed an increase in levels from 21 to 36pg/mg. The study of these biomarkers is crucial for improving our understanding of the pathophysiology of combat traumatic brain injury and, accordingly, for the diagnosis or prediction of recovery and early outcomes of MBT [7].

CONCLUSIONS

1. In patients in both the acute and remote periods of MBT, the same set of complaints persists, indicating a disorder of the body's compensatory and adaptive mechanisms.
2. An increase in the levels of S-100β protein indicates the onset of secondary auto-destructive biochemical processes, which indicates the onset of traumatic brain injury.
3. A decrease in the levels of the brain-derived neurotrophic factor BDNF is one of the reasons for the slowdown in the rate of reparative processes in individuals who have suffered MBT, since BDNF plays an adaptive role in the formation of brain plasticity.
4. A significant increase in IL-8 and IL-10 levels indicates that chronic inflammation, which is activated after cerebral trauma, leads to neuro-inflammation. These biomarkers can be used not only for diagnosis or prognosis of recovery, but also for long-term outcomes of MBT.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 04.03.2026
ACCEPTED: 29.06.2026

CREATIVE COMMONS 4.0

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ORIGINAL ARTICLE

CONTENTS

Determinants of glycemic control in children and adolescents with type 1 diabetes (T1D) in three care centers of the changing diabetes in children (CDIC) program in the northern regions of Cameroon

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ABSTRACT

Aim: Type 1 diabetes mellitus (T1DM) is the most common endocrine disorder affecting children and adolescents worldwide. Glycemic control is the ultimate goal of diabetes management, and poor glycemic control is associated with serious short- and long-term complications. Only a few studies have addressed this problem in Cameroon. This study aimed to identify the determinants of glycemic control of T1DM in children and adolescents living in the three northern regions of Cameroon.

Materials and Methods: This was a descriptive and analytical cross-sectional study conducted on 100 children and adolescents with type 1 diabetes followed up in the three T1DM management centers of the CDiC program in the northern regions of Cameroon, from November 2016 to March 2017. Glycemic control was assessed on the basis of glycosylated hemoglobin (HbA1c) levels, according to the current ADA Standards of Care in Diabetes—2025 and ISPAD Clinical Practice Consensus Guidelines 2022, both of which recommend a universal target of HbA1c ≤7.0% (≤53 mmol/mol) for all children and adolescents with type 1 diabetes, regardless of age group. Data were entered into CDC Epi-info™ version 7.2 software, then exported to IBM SPSS Statistics™ version 20.0 for analysis. Descriptive and inferential statistics were used. Bivariate analysis was followed by binary logistic regression to calculate adjusted odds ratios (AORs) for determinants of glycemic control, and a p-value <0.05 was considered statistically significant.

Results: The 100 participants were predominantly male (73%), with a mean age of 16.59 ± 2.54 years. The median HbA1c value was 10.08% [8.7–11.63], reflecting very poor overall glycemic control. Applying the current unified ADA/ISPAD target of ≤7.0% (53 mmol/mol), only 19% of participants achieved their glycemic targets — a proportion markedly lower than figures reported in high-income countries such as the United States (approximately 21–25% in the T1D Exchange Registry). In bivariate analysis, variables significantly associated with glycemic control included: being followed in the Adamaoua region (OR=0.21 [0.07–0.62]; p=0.003), living within 20 km of the diabetes clinic (OR=4.4 [0.94–20.84]; p=0.04), and duration of diabetes less than 2 years (OR=2.90 [1.06–8.30]; p=0.03). In multivariate logistic regression, belonging to the Adamaoua region, a treatment regimen of more than 2 injections per day, living less than 20 km from the management clinic, fewer than three episodes of severe hypoglycemia in the last 6 months, duration of diabetes less than 2 years, and participation in more than three therapeutic education sessions were associated with achieving glycemic targets.

Conclusions: The majority of children and adolescents with T1DM in the northern regions of Cameroon had poor glycemic control, with only 19% achieving the current unified target of HbA1c ≤7.0% (≤53 mmol/mol). Belonging to the Adamaoua region, intensive insulin therapy (>2 injections/day), proximity to the care center (<20 km), fewer severe hypoglycemic episodes, shorter disease duration (<2 years), and regular therapeutic education were identified as factors associated with good glycemic control. Although global innovations such as hybrid closed-loop insulin delivery systems and teplizumab represent transformative advances in T1D management, these technologies remain inaccessible in the study setting. Intensification of therapeutic education, creation of satellite CDiC centers, and progressive adoption of affordable monitoring technologies are urgently needed.

KEYWORDS: type 1 diabetes, glycemic control, children and adolescents, northern regions of Cameroon, HbA1c

Wiad Lek. 2026;79(7):1480-1492. doi: 10.36740/WLek/222499 DOI

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia related to a deficit in insulin secretion and/or action [1]. Type 1 diabetes (T1DM) results from a mostly autoimmune destruction of the beta cells of the pancreas [2]. According to the International Diabetes Federation (IDF) Diabetes Atlas, 10th edition (2021), there are now approximately 8.4 million people living with type 1 diabetes worldwide, with over 150,000 new cases diagnosed annually in individuals under 20 years of age [5]. The vast majority (over 90%) reside in low- and middle-income countries [6]. In sub-Saharan Africa, a systematic review by Katte et al. (2023) reported a pooled incidence of 2–10 per 100,000 per year across the region [20]. In Cameroon, approximately 500 to 800 children are estimated to live with T1DM, a figure likely to be an underestimate given the limited diagnostic infrastructure and high rates of misdiagnosis [21]. Including patients over 18 years of age, the total figure may reach 800 to 900 patients [5].

Unlike type 2 diabetes, T1DM results from an autoimmune process and is not lifestyle-related; until recently, there was no known way to prevent or delay its onset. However, a landmark clinical trial demonstrated that teplizumab, an anti-CD3 monoclonal antibody, significantly delayed the onset of clinical T1DM by a median of approximately 3 years in high-risk antibody-positive relatives [29]. Teplizumab (Tzield™) received FDA approval in November 2022, representing the first disease-modifying therapy for T1DM. Nevertheless, such innovations remain inaccessible in sub-Saharan Africa. People with T1DM cannot survive without daily doses of insulin and must adhere to a precise treatment regimen. This is not always easy, and explains the occurrence of serious complications and early mortality, estimated at approximately 10% annually among diabetic children in Cameroon [6]. This is partly attributable to parents' and guardians' lack of awareness of specialized clinics offering free treatment, poor diagnosis by healthcare staff, and patients' non-adherence to treatment [6].

In 2010, a project called Changing Diabetes in Children (CDiC) was implemented to train healthcare professionals, educate patients and their parents, and provide free insulin, with the aim of reducing diabetes-related mortality in children [7].

Numerous factors have been identified as determinants of glycemic control in children and adolescents with T1DM [8, 9]. Multiple studies have examined sociodemographic aspects (age, gender, ethnicity, socioeconomic status, family structure), therapeutic factors (insulin dose, treatment regimen, insulin delivery technology), and behavioral factors (dietary adherence, physical activity, glycemic self-monitoring). In high-income countries, the adoption of advanced diabetes technologies — including continuous glucose monitoring (CGM) systems, insulin pumps (continuous subcutaneous insulin infusion, CSII), and hybrid closed-loop systems such as Medtronic SmartGuard™ and Tandem Control-IQ™ - has dramatically improved glycemic outcomes, with time-in-range exceeding 70% achieved in clinical trials [28]. However, these technologies remain largely unavailable in sub-Saharan Africa, including Cameroon, creating a critical equity gap in diabetes care [19].

In 2013, Djonou et al. identified, in Cameroon, that the duration of diabetes, high insulin doses, and the presence of severe hypoglycemia were associated with poor glycemic control [10]. In the northern regions of Cameroon, characterized by a low level of qualified personnel, a precarious socioeconomic context, and a climate of insecurity, optimal management of T1DM patients remains limited, despite the free supply of insulin.

AIM

To further improve the care of children and adolescents in this part of the country, we set out to identify the factors associated with glycemic control of type 1 diabetes in children and adolescents in the northern regions of Cameroon.

MATERIALS AND METHODS

TYPE, SITE AND STUDY PERIOD

This was a descriptive and analytical cross-sectional study conducted in the regional hospitals of Ngaoundéré, Garoua and Maroua. These hospitals are home to centers for the management of diabetes in children and adolescents, as part of the Changing Diabetes in Children (CDiC) program. This global program, developed by Novo Nordisk, aims to facilitate access to type 1 diabetes treatment for children in low-income countries. Free services encompass both inpatient and outpatient treatment, covering emergencies, outpatient consultations, and regular medical follow-up. Children and adolescents with T1DM benefit from regular pediatric follow-up, including assessments by healthcare professionals, medication refills, and diabetes education sessions. This study was conducted over a period spanning November 2016 to March 2017.

STUDY POPULATION

SOURCE POPULATION

All children and adolescents living with type 1 diabetes in the northern regions of Cameroon.

TARGET POPULATION

All children and adolescents treated at the Changing Diabetes in Children (CDiC) type 1 diabetes centers in the regional hospitals of Ngaoundéré, Garoua and Maroua.

INCLUSION CRITERIA

Patients aged between 6 and 19 years at the time of interview were included. In addition, they were required to have type 1 diabetes and to have been enrolled in the CDiC program for at least 6 months. Children and adolescents were required to give their assent to participate in the study, and parental or guardian written authorization was obtained.

EXCLUSION CRITERIA

Participants with only one HbA1c test in the last six months were excluded. Also excluded were those who withdrew informed consent during the study, failed to complete the interview, or had secondary diabetes (chronic pancreatitis), monogenic diabetes (lipoatrophic), MODY, or neonatal diabetes.

STUDY VARIABLES

The dependent variable was the level of glycemic control. Independent variables were divided into two categories. Socio-demographic factors included age, gender, educational level, parental marital status, displacement status, cohabitation with parents, cultural background, place of residence, and relationship of key caregivers to the child or adolescent. Diabetes-related factors included diabetes history, medical history, insulin treatment modalities, glycemic self-monitoring, geographic accessibility to care, and therapeutic education.

ETHICAL APPROVAL

This study was reviewed and approved by the Institutional Ethics Committee of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I (Reference Number: 293/UY1/FMSB/VDRC/CSD). Written informed consent was obtained from all participants prior to inclusion. For minors, parental or guardian written authorization was obtained, and child assent was secured. Study procedures adhered to the ethical principles outlined in the Declaration of Helsinki (Fortaleza revision, 2013).

OPERATIONAL DEFINITIONS

Glycemic control was assessed using HbA1c, in accordance with the ADA Standards of Care in Diabetes - 2025 and the ISPAD Clinical Practice Consensus Guidelines 2022, both of which recommend a universal HbA1c target of ≤7.0% (≤53 mmol/mol) for all children and adolescents with T1DM, regardless of age group [9, 14]. This replaces the former age-stratified ADA thresholds (HbA1c <8% for ages 6–12 years and <7.5% for ages 13–19 years) used at the time of data collection (2016–2017). For historical transparency, the former thresholds are noted where appropriate in the Results; however, all discussion and conclusions are aligned with the current unified target. Participants were accordingly classified as: (a) achieving glycemic control (HbA1c ≤7.0%) or (b) not achieving glycemic control (HbA1c >7.0%).

Intensive insulin therapy is defined as a regimen comprising 3 or more insulin injections per day, or the use of an insulin pump (continuous subcutaneous insulin infusion, CSII). Conventional insulin therapy is defined as 2 injections per day. In the present study, no participant used an insulin pump due to unavailability in the study setting.

Severe hypoglycemia is defined by the presence of at least one of the following: alteration of the child's state of consciousness or coma, or need for parenteral treatment (glucagon or IV glucose). Geographical accessibility refers to the distance between the patient's place of residence and the nearest CDiC care center. Therapeutic education encompasses all measures designed to help patients better manage their lives with diabetes.

DATA PROCESSING AND ANALYSIS

Data were entered into CDC Epi-info™ version 7.2, then exported to IBM SPSS Statistics™ version 20.0 for analysis. Excel was used for graphical representations. Headcounts and percentages were calculated for qualitative variables; means, standard deviations, maxima and minima for quantitative variables. Variables were tested for normality using the Shapiro-Wilk test. Association between quantitative variables was assessed by Pearson's correlation coefficient. The Pearson Chi-square test was used for categorical variables; odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for dichotomous variables. The threshold of statistical significance was set at $p < 0.05$. Candidate variables with a p-value ≤ 0.25 in bivariate analysis were entered into a binary logistic regression model to calculate adjusted odds ratios (AORs) and identify significant independent predictors of poor glycemic control.

RESULTS

DESCRIPTIVE STUDY

SOCIO-DEMOGRAPHIC CHARACTERISTICS

During our study, we included 100 patients out of 103 in the three northern regions of Cameroon (three were excluded for age >19 years). Among participants, 51% were from the Far North region, 27% from the North, and 22% from Adamaoua. Male participants represented 73% (sex ratio 3:1). The mean age was 16.59 ± 2.54 years, and the most represented age group was [15–20]. The main occupation was education (61%), with 28% in junior secondary school. Seventy-six percent attended school. There were 3% foreign

Table 1. Distribution of participants according to socio-demographic data

Variables	Percentage [%]
Regions	
Far North	51
North	27
Adamaoua	22
Gender	
Male	73
Female	27
Age (in years)	
[15-20]	73
[10-15]	24
[5-10]	03
Average age	16.57 ±2.54
Nationality	
Cameroonian	97
Other	03
Internal refugees	
Yes	06
No	94
Participant's occupation	
Pupil/Student	61
Learning a trade	11
Worker	15
Unoccupied	13
Participant's school level	
No schooling	24
Primary	21
Secondary (6e-3e)	28
Secondary (2nd-Tle)	25
University	02
Parents' marital status	
Married	95
Common-law union	01
Separated or divorced	03
Widow(er)	01
Cultural area	
Sudan-Sahel	98
Grass Field	02

Source: Authors' own work

nationals and 6% internally displaced persons. Socio-demographic characteristics are summarized in Table 1.

Regarding education level, lower secondary: 28%, upper secondary: 25%, primary: 21%, tertiary: 2%. Additionally, 24% had no formal Western education (Fig. 1)

GEOGRAPHIC ACCESSIBILITY AND THERAPEUTIC EDUCATION

Sixty-one percent of participants lived less than 5 km from their care center. However, 30% lived more than 20 km away, with an average distance of 101.34 ± 61.66 km separating them from the CDiC center. More than half attended at least one therapeutic education session per month. All participants were able to test their blood glucose correctly and independently (Table 2).

INSULIN TREATMENT MODALITIES

Nearly 89% of participants received three insulin doses per day. The mean daily dose was 0.80 ± 0.20 IU/kg/day. The main insulin regimen was Actrapid-Mixtard (95%). Only 5% were strictly compliant with their prescribed treatment (Table 3).

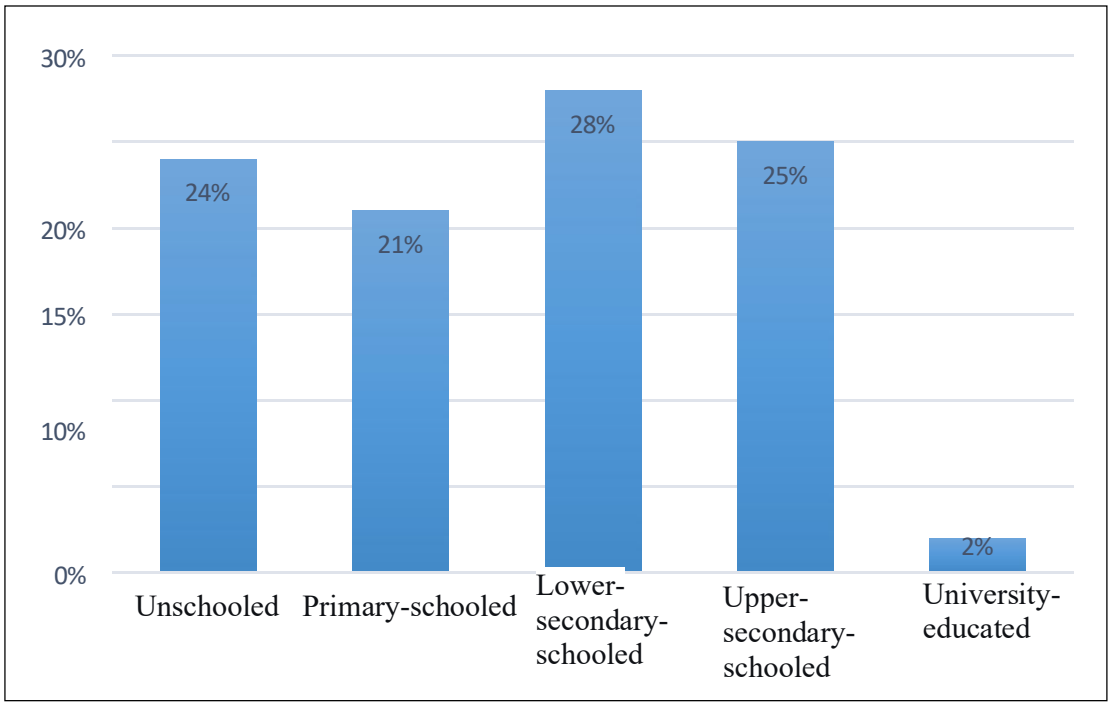

Fig. 1. Participants' level of education
Source: Authors' own work

Table 2. Distance to care center, therapeutic education attendance, and blood glucose testing ability

Variables	Percentage [%]
Distance to care center	
Less than 5 km	61
Between 5 km and 10 km	03
Between 10 km and 20 km	06
Over 20 km	30
Assistance with therapeutic education in the last six months	
Once	03
Twice	15
Three times	20
Four times	01
Five times	03
Six times	58
Ability to test blood sugar correctly	100

Source: Authors' own work

SELF-MONITORING OF BLOOD GLUCOSE

Ninety-eight percent of participants possessed a glucometer and urine test strips. The daily frequency of glycemic self-monitoring was three times per day in 93% of cases (Table 4).

ACUTE COMPLICATIONS

The most frequent acute complication was severe hypoglycemia (93% of cases), followed by infection (7%). No episode of diabetic ketoacidosis was recorded (Table 5).

GLYCEMIC BALANCE AND PARACLINICAL ASSESSMENTS

Only 19% of participants were considered well-controlled (Fig. 2). Mean fasting blood glucose was 192.86 ± 65 mg/dL, and mean glycated hemoglobin was 10.08 ± 2.34% (Table 6). Applying the current unified ADA/ISPAD target of HbA1c ≤7.0% (≤53 mmol/mol), the proportion achieving glycemic control was 19% - far below the target and substantially lower than figures reported in high-income countries (approximately 21–25% in the T1D Exchange Registry, USA [30]).

Table 3. Insulin treatment modalities

Variables	Percentage [%]
Average number of injections per day	
1 injection/day	0
2 injections/day	11
3 injections/day	89
Average daily insulin dose as a function of weight (IU/Kg/day)	0.80 ± 0.20
Insulin therapy regimen	
Actrapid-Insulatard	5
Actrapid-Mixtard	95
Strict compliance with insulin therapy	5

Source: Authors' own work

Table 4. Self-monitoring of blood glucose

Variables	Percentage [%]
Possession of a glucometer	98
Possession of urine dipsticks	98
Daily frequency of glycemic control	
Twice a day	07
Three times a day	93
Therapeutic regimen	
Two injections a day	11
Three injections a day	89

Source: Authors' own work

Table 5. Acute diabetic complications in participants

Variables	Percentage [%]
Ketoacidosis	0
Severe hypoglycemia	93
Infections	07

Source: Authors' own work

Table 6. Paraclinical tests

Variables	
Fasting blood glucose [mg/dl]	192.86 ± 65
Glycated hemoglobin [%]	10.08 ± 2.34

Source: Authors' own work

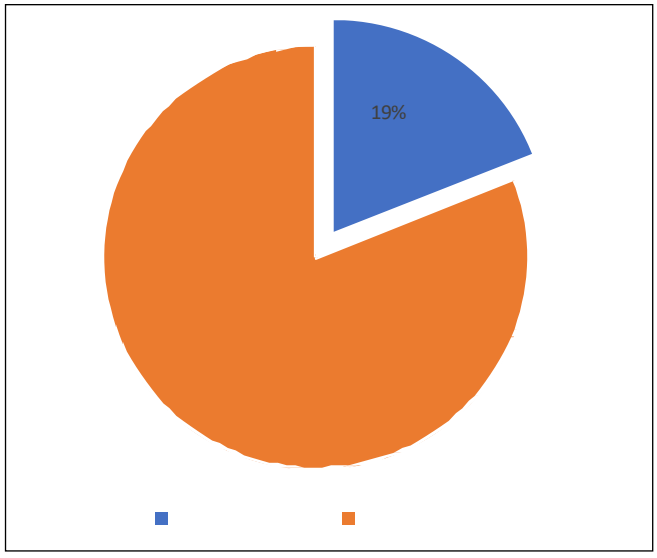

Fig. 2. Participants' glycemic balance
Source: Authors' own work

ANALYTICAL STUDY

SOCIO-DEMOGRAPHIC FACTORS AND GLYCEMIC CONTROL

A statistically significant association was found between glycemic control and being followed in the Adamaoua region (p=0.003; OR=0.21 [0.07–0.62]). No statistically significant association was found between glycemic control and age (p=0.35), gender (p=0.09), occupation (p>0.50), level of education, or family structure (Table 7).

GEOGRAPHIC ACCESSIBILITY AND GLYCEMIC CONTROL

Among the 30% of patients living more than 20 km from their CDiC center, only 2% had a controlled HbA1c. Ninety percent of controlled patients lived less than 20 km from their center (Table 8). In univariate analysis, a significant association was found between distance and glycemic control (p=0.04; OR=4.4 [0.96–20.84]) (Table 9).

Table 7. Association between sociodemographic characteristics and glycemic target attainment

Variables	Glycemic balance		
	Chi²	p	Odd ratio [95% CI]
Age			
Between [6 - 12] years	0.85	0.35	0.44 [0.07 – 2.61]
From [13 - 19] years old	0.85	0.35	2.2 [0.38 – 13.38]
Female gender	2.71	0.09	0.42 [0.148 – 1.199]
Occupation of participants			
Pupils/students	0.04	0.83	0.89 [0.31 – 2.51]
Apprenticeship	0.005	0.94	1.06 [0.21 – 5.37]
Unoccupied	0.12	0.72	1.33 [0.27 – 6.59]
Worker	0.01	0.91	0.92 [0.23 – 3.67]
Participant's level of education			
No	0.06	0.79	0.85 [0.27 – 2.69]
1er secondary school	0.14	0.69	0.80 [0.27 – 2.38]
2nd secondary cycle	1.04	0.30	1.98 [0.52 – 7.49]
Primary	0.00	0.99	0.99 [0.29 – 3.38]
University	1.27	0.25	0.22 [0.01 – 3.76]
Participant's region			
Adamaoua	8.79	0.003	0.21 [0.07 – 0.62]
Far North	3.54	0.06	2.70 [0.94 – 7.83]
North	0.42	0.52	1.50 [0.45 – 4.96]
Family structure			
Living with your mother	1.5	0.21	3.05[0.47– 9.73]
Living with your father	0.85	0.35	2.26 [0.38 – 13.38]

Source: Authors' own work

Table 8. Access to care and glycemic control

Variables	HbA1c normal or not		
	Normal HbA1c	High HbA1c	Total
Distance less than 20 km	17	53	70
Distance over 20 km	2	28	30
Total	19	81	100

Source: Authors' own work

Table 9. Relationship between geographical accessibility to care and glycemic control

	Glycemic balance		
	Chi²	*p-value*	Odd ratio [95% CI]
Distance between home and PEC center (less than 20 km)	4.23	0.04	44 [0.96 – 20.84]

Source: Authors' own work

THERAPEUTIC EDUCATION AND GLYCEMIC CONTROL

No statistically significant association was found between glycemic control and participation in therapeutic education sessions (p=0.59). However, participation in at least one session per month was associated with a 1.3-fold increased likelihood of achieving glycemic targets (OR=1.3 [0.48–3.57]). Possession of a glucometer multiplied by approximately 5 the likelihood of reaching glycemic targets (OR=4.44 [0.26–74.45]) (Table 10).

CURRENT DIABETES MANAGEMENT AND GLYCEMIC CONTROL

A statistically significant association was found between glycemic control and diabetes duration of less than 2

Table 10. Glycemic control and therapeutic education

Variables	Glycemic balance		
	Chi²	*p-value*	Odd ratio [95% CI]
Participation in therapeuticeducation / month	0.27	0.59	1.3 [0.48 – 3.57]
Glucometer ownership	1.27	0.25	4.44 [0.26 – 74.45]
Possession of urine dipsticks	0.47	0.48	1.24 [1.12 – 1.38]

Source: Authors' own work

Table 11. Current diabetes management

Variables	Glycemic balance		
	Chi²	*p-value*	Odd ratio [95% CI]
Insulin			
Therapeutic regimen	0.003	0.95	0.94 [0.09 – 8.88]
Number of injections/day	2.89	0.09	1.27 [1.14 – 1.41]
Duration of diabetes			
[≤ 2 years]	4.59	0.03	2.90 [1.06 – 8.30]
[3- 4 years]	2.33	0.12	0.32 [0.67 – 1.47]
[> 4 years]	0.49	0.49	0.69 [0.24 – 1.94]

Source: Authors' own work

Table 12. Association between glycemic control and the number of severe hypoglycemias

	Glycemic balance		
	Chi²	*p-value*	Odd ratio [95% CI]
Number of severe hypoglycemias in the last 6 months	0.40	0.52	1.19 [0.23 – 16.7]

Source: Authors' own work

years (p=0.03; OR=2.90 [1.06–8.30]). A multiple daily injection regimen (>2 injections/day) was associated with a 1.27-fold increased likelihood of achieving glycemic targets (OR=1.27 [1.14–1.41]) (Table 11).

SEVERE HYPOGLYCEMIA AND GLYCEMIC CONTROL

Approximately 95% of controlled participants had presented with at least 3 episodes of severe hypoglycemia in the last six months. The number of severe hypoglycemic episodes was associated with a 1.19-fold increased risk of poor glycemic control (OR=1.19 [0.23–16.79]; p=0.52) (Table 12).

DISCUSSION

In order to identify the factors associated with glycemic control of type 1 diabetes in children and adolescents in the northern regions of Cameroon, we conducted a study across all CDiC centers in the Far North, North, and Adamaoua regions. Our sample comprised the entire population of T1DM patients in these centers, which enabled us to obtain results reflecting the characteristics of this population. After recruiting 100 patients, we found that the majority were adolescent males enrolled in the first cycle of secondary school, receiving an appropriate insulin dose (0.80±0.20 IU/kg/day) divided into three daily doses, and conducting adequate treatment self-monitoring. The most frequent acute complication was severe hypoglycemia. The mean HbA1c was 10.08±2.34%. The main factors associated with glycemic control were: (1) belonging to the Adamaoua region (OR=0.21 [0.07–0.62]; p=0.003); (2) living less than 20 km from the treatment center (OR=4.4 [0.96–20.84]; p=0.04); (3) having diabetes for less than 2 years (OR=2.90 [1.06–8.30]; p=0.03); and (4) a treatment regimen of more than 2 injections per day (OR=1.27 [1.14–1.41]). These objectives were fully achieved, although the study had limitations. Insecurity in certain regions meant that some patients could not be seen in person. The sample size was also small; a larger sample could have revealed additional significant correlations. Nevertheless, our cross-sectional design has made available original data from the northern regions of Cameroon, a population that is largely under-studied.

SOCIO-DEMOGRAPHIC FACTORS AND GLYCEMIC CONTROL

A large proportion of our study population did not achieve their glycemic targets (81%), with a mean HbA1c of 10.08±2.34%, far exceeding the current unified target of

≤7.0% (≤53 mmol/mol) now recommended for all children and adolescents with T1DM, regardless of age group, by both the ADA Standards of Care 2025 and the ISPAD Clinical Practice Consensus Guidelines 2022 [9, 14]. Under this unified benchmark, only 19% of our participants achieved good glycemic control. This proportion is alarmingly low, and is comparable to — or lower than — figures reported in other low-resource settings. Data from the T1D Exchange Registry (USA) show that approximately 21–25% of youth with T1D achieve HbA1c ≤7.0% [30, 31]. Noorani et al. in Tanzania reported a mean HbA1c of 11.05±2.1% [50], consistent with our results and reflective of shared structural challenges in sub-Saharan Africa. By contrast, Craig et al. in Australia and Petitti et al. in the USA found mean HbA1c values of 8.2% and 8.18% respectively [34], underscoring the profound inequality in global glycemic outcomes. Previous studies have consistently found adolescence to be associated with poor glycemic control [48], partly due to higher insulin elimination and lower insulin sensitivity compared with younger children, as well as psychosocial factors associated with puberty.

In our study, patients followed up in the Adamaoua region demonstrated significantly better glycemic control (p=0.003) than those in the North and Far North regions. This may be explained by a lower patient-to-provider ratio at the Adamaoua CDiC center, greater relational continuity between the center coordinator and patients, and a more stable humanitarian situation compared with the Far North. These observations are consistent with evidence that patient-centered care and lower case loads improve metabolic outcomes [11]. We also note that the older the patient, the 2.2-fold greater the risk of being poorly controlled (OR=2.2 [0.38–13.38]), reflecting diminishing parental supervision with advancing age. Female sex appeared to be better associated with glycemic control (OR=0.42 [0.148–1.199]), in contrast to findings from Germany where female sex was associated with poorer control [51]. Higher educational level and cohabitation with both parents were also associated with improved control, consistent with Argentinian data showing that single-parent family structure and lower maternal education are predictors of poor glycemic control [29].

GEOGRAPHIC ACCESSIBILITY AND GLYCEMIC CONTROL

We found a statistically significant association between the distance separating the patient's place of residence and the CDiC center, and glycemic control (p=0.04; OR=4.4 [0.95% CI: 0.96–20.84]). Living more than 20 km from the CDiC center increased the risk of poor glycemic control by approximately 4.5-fold. This finding is consistent with broader evidence on geographic barriers in chronic disease management. The Atun et al. Lancet Diabetes & Endocrinology commission identified geographic barriers as among the most critical determinants of care quality and diabetes outcomes in sub-Saharan Africa [19]. In our context - where public transport is costly, roads are often unpaved, and humanitarian insecurity limits mobility in the Far North - the burden of distance is amplified. These findings provide empirical justification for the creation of additional satellite CDiC centers or mobile care units in the northern regions of Cameroon.

THERAPEUTIC EDUCATION AND GLYCEMIC CONTROL

While no statistically significant association was found between participation in therapeutic education and glycemic control in bivariate analysis (p=0.59), a clinically meaningful trend was observed: participation in at least one session per month was associated with a 1.3-fold increased likelihood of achieving glycemic targets (OR=1.3 [0.48–3.57]). This finding is consistent with the growing body of evidence supporting structured diabetes self-management education (DSME) as a cornerstone of T1DM care. The ISPAD 2022 Clinical Practice Consensus Guidelines on diabetes education emphasize that structured, ongoing, multidisciplinary education is associated with improved HbA1c, reduced acute complications, and better quality of life in youth with T1DM [12]. The non-significant finding in our study may reflect the limited number of sessions attended and the structural barriers - including low literacy, language diversity, and cultural factors — that reduce the effectiveness of standard educational approaches in the northern regions of Cameroon.

Regarding insulin regimen, participants with more than two injections per day were more likely to achieve their glycemic targets, consistent with ISPAD 2022 recommendations for intensive insulin therapy as the standard of care in T1DM [13]. This result aligns with Ziegler et al., who found that the number of daily insulin injections was proportional to HbA1c improvement [18]. Possession of a glucometer was associated with a 5-fold increase in the likelihood of achieving glycemic targets (OR=4.44 [0.26–74.45]), underscoring the fundamental role of self-monitoring. The lack of a significant association between self-monitoring frequency and glycemic control may reflect the fact that none of our participants achieved the ADA recommendation of at least 4 blood glucose tests per day.

A statistically significant association was found between diabetes duration of less than 2 years and better glycemic control (p=0.03; OR=2.90 [1.06–8.30]). This is consistent with the known phenomenon of residual beta-cell function ("honeymoon period") in the early years after T1DM onset, which facilitates glycemic control. As diabetes duration increases, total insulin deficiency becomes complete, making glycemic management more challenging and reinforcing the importance of early intensive education and follow-up.

Hypoglycemia remains a major limiting factor in improving glycemic control. The fear of hypoglycemia often leads

patients to tolerate higher blood glucose levels [25]. In our cohort, the number of severe hypoglycemic episodes was associated with a 1.19-fold increased risk of poor glycemic control (OR=1.19 [0.23–16.79]; p=0.52), consistent with the finding of Svoren et al. [11]. This could reflect both poorer adherence following a hypoglycemic episode and the effects of excessive correction of hypoglycemia, leading to rebound hyperglycemia.

GLOBAL ADVANCES IN T1D MANAGEMENT AND THEIR RELEVANCE TO OUR SETTING

Over the past decade, the management of T1DM has been transformed by a series of technological innovations that have substantially improved glycemic outcomes in high-income settings. The widespread adoption of continuous glucose monitoring (CGM) systems - particularly real-time devices such as the Dexcom G7 and Abbott FreeStyle Libre 3 - has enabled patients to monitor interstitial glucose continuously, dramatically improving time-in-range (TIR) and reducing hypoglycemic episodes [26]. The integration of CGM with insulin pump therapy (CSII) through hybrid closed-loop systems represents the most significant advancement in T1D management. Systems such as the Medtronic MiniMed 780G with SmartGuard™ technology and the Tandem Control-IQ™ use machine-learning algorithms to continuously adjust basal insulin delivery in response to CGM data, achieving TIR exceeding 70% and significantly reducing HbA1c levels in randomized controlled trials [28]. The ISPAD 2022 guidelines actively recommend these systems for children and adolescents where available [27].

In parallel, the approval of teplizumab (Tzield™), an anti-CD3 monoclonal antibody that delays the onset of clinical T1DM by a median of 32 months in high-risk relatives, has opened a new era of disease modification and secondary prevention [29]. These advances are not currently accessible in Cameroon or sub-Saharan Africa more broadly, due to prohibitive costs, lack of infrastructure, limited healthcare professional training, and absence of supply chains for consumables [19]. The mean HbA1c of 10.08% observed in our cohort stands in stark contrast to the sub-7.0% targets achievable with these technologies in well-resourced settings, and reflects the profound inequity in global diabetes care.

These observations carry concrete implications for advocacy and policy. The continued exclusion of children in low-income countries from evidence-based technological advancements perpetuates the cycle of poor glycemic control, accelerated complications, and premature mortality. Intermediate solutions - such as time-limited, subsidized access to flash glucose monitoring - have been piloted in some African settings and may represent a feasible, cost-effective first step toward improving glycemic monitoring in the northern regions of Cameroon.

CONCLUSIONS

Great strides have been made in the management of T1DM in children and adolescents in Cameroon, notably in improving access to insulin through the CDiC program. The aim of this study was to identify factors associated with glycemic control in a population of children and adolescents with type 1 diabetes living in the northern regions of Cameroon, who have free access to care and medication.

At the conclusion of this work, we state the following: the glycemic control of children and adolescents with T1DM in the northern regions of Cameroon is not optimal, despite free access to care: only 19% achieved the current unified HbA1c target of ≤7.0% (≤53 mmol/mol) as recommended by the ADA Standards of Care 2025 and ISPAD 2022 guidelines [9, 14]. This proportion is alarmingly low and calls for urgent, multifaceted interventions.

Factors associated with good glycemic control included: (1) belonging to the Adamaoua region; (2) a treatment regimen of more than 2 injections per day; (3) living less than 20 km from the care clinic; (4) fewer than three episodes of severe hypoglycemia in the last 6 months; (5) diabetes duration of less than 2 years; and (6) participation in more than three therapeutic education sessions per 6 months.

RECOMMENDATIONS

Based on these findings, we recommend the following priority interventions:

(1) Intensification of structured therapeutic education programs, including culturally adapted content and peer-support diabetes camps;

(2) Creation of additional CDiC satellite centers or mobile care units to reduce geographic barriers;

(3) Strengthening of continuous supply chains for insulin and glucometers;

(4) Advocacy for progressive adoption of affordable glucose monitoring technologies (e.g., flash glucose monitoring) through national health financing mechanisms;

(5) Development of a regional epidemiological registry to generate longitudinal data and monitor outcomes.

In light of global innovations - including hybrid closed-loop insulin delivery systems (SmartGuard™, Control-IQ™) and teplizumab - long-term strategic investments must be made to progressively reduce the technology gap between high-income countries and resource-limited settings such as the northern regions of Cameroon.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 26.04.2026
ACCEPTED: 02.07.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

The analysis of cervicovaginal microbiota and immune mediators in patients with adenomyosis

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ABSTRACT

Aim: To determine and characterise changes associated with microbial, inflammatory and immune factors in patients with adenomyosis.

Materials and Methods: 115 female patients were enrolled in the study, including 85 patients with adenomyosis and 30 healthy individuals. Vaginal cleanliness was assessed by collecting vaginal samples and plating on solid bacterial agar. Urogenital infections were determined by the PCR analysis of the cervicovaginal samples. The immunological characterisation was performed using enzyme immunoassay to analyse cervicovaginal lavage for β-defensin-2 and a defined set of cytokines. Antibody titers were quantified in patients' serum using nephelometry.

Results: Patients with adenomyosis displayed disrupted cervicovaginal microflora that was characterised by elevated levels of pro-inflammatory cytokines and antimicrobial peptide β-defensin-2. Serum analysis of patients with adenomyosis revealed higher concentration of IgG, IgM and IgA antibodies compared to healthy control groups.

Conclusions: Analysis of inflammatory and immunological parameters in patients with adenomyosis revealed distinct features of the pathophysiology of adenomyosis. Specifically, patients with adenomyosis had disrupted cervicovaginal microflora with higher prevalence of anaerobic species. Furthermore, patients with adenomyosis had elevated levels of pro-inflammatory cytokines and interleukins in cervicovaginal lavage, and higher levels of IgG, IgM and IgA antibody serum titres. The analysis presented in the paper represents an important step towards determining precise diagnostic parameters and effective therapies to treat adenomyosis.

KEYWORDS: antimicrobial peptides, adenomyosis, cervicovaginal microbiome

Wiad Lek. 2026;79(7):1493-1499. doi: 10.36740/WLek/218754 DOI

INTRODUCTION

Adenomyosis is a chronic disease of the female reproductive system that affects up to 60% of women of reproductive age worldwide [1]. Adenomyosis is characterised by benign invasion of the endometrium into the myometrium [2]. Disruption of myometrium leads to pathology that affects female reproductive health and includes multiple clinical manifestations including excessive uterine bleeding, dysmenorrhea, chronic pelvic pain, and reduced fertility [3, 4]. Despite well-defined clinical characteristics of adenomyosis, molecular and cellular mechanisms that underlie the disease origin and progression remain poorly understood. Determining the molecular basis of the development of adenomyosis will help design better diagnostic tools and treatment regimens for women.

A number of factors have been proposed to contribute to the development of adenomyosis including angiogenic abnormalities [5], hormonal imbalance [6], and genetic factors [7]. More recently, a number of studies have revealed the importance of the immunological and pathogenic components in a related endometrial condition endometriosis [8, 9]. A local immune dysregulation as well as changes in the microbiome in the genital tract have been shown to play a role in the disease development [10]. Infectious agents including bacteria, viruses and fungi can serve as a trigger to initiate a dysbiosis in the genital tract leading to chronic inflammation and immune dysregulation [11, 12]. The inability of the immune system to resolve inflammation and clear the infection agents results in tissue damage and pathogenesis. Understanding the immunological networks of endometrial-myometrial pathology will be invaluable in defining the biological markers of the disease.

There are multiple lines of defence against the harmful factors that lead to the changes in the female reproductive flora and contribute to the disease progression. The first line of defence includes cytokines and antimicrobial peptides (AMPs) released by immune

cells in response to microbial agent, inflammation and physical damage [13]. There are approximately 39 antimicrobial peptides in cervicovaginal fluid [14] with β-defensins family, cathelicidin and SLPIs being the most dominant AMPs [15]. Multiple reports have demonstrated the importance of AMPs in the protection of vaginal microbiome [16, 17]. However, the role of AMPs in adenomyosis remains poorly understood. Release of AMPs, such as β-defensins, serves as a chemoattractant for immune cells. Following AMPs release, monocytes and dendritic cells migrate to the site of infection and can phagocytose and present pathogen associated molecular patters for T cell recognition. Upon activation, T cells trigger the next line of defence known as the adaptive immunity that involves activation of B cells and production of different antibodies including IgG, IgM and IgA that act at mucosal barriers as well and tissues and blood [18].

In this work, we defined the components of innate and adaptive immunity in the cervicovaginal microenvironment in patients with adenomyosis. We show that both microflora and immune composition is altered when adenomyosis is present, implicating these changes in disease progression.

AIM

To determine and characterize changes associated with microbial, inflammatory and immune factors in patients with adenomyosis.

MATERIALS AND METHODS

PATIENTS INVOLVED IN THE STUDY

115 female patients aged 18-45 years old, who underwent treatment at the clinical bases of the Department of Obstetrics and Gynecology No. 1 of the KhNMU, took part in the study. The patients were assigned into groups as follows. The first clinical group (study group) included 85 (73.9% of the total number of patients enrolled in the study) patients with adenomyosis. The second clinical group (control group) consisted of 30 (26.1% of the total number of patients enrolled in the study) healthy fertile women. All patients underwent a routine general clinical examination during the course of the study, in accordance with the current clinical recommendations of the Ministry of Health of Ukraine. The examination was carried out after obtaining the patient's informed consent for the diagnosis and processing of personal data.

Adenomyosis was diagnosed in patients in the study group based on the diagnostic tools and histological methods of examination of tissues obtained during hysteroscopy. Patient exclusion criteria included extragenital pathology, oncology including leiomyomas, inflammation of the genitals, HPV infection. Patients who were exposed to urogenital viral or bacterial infection in the last 6 months and who underwent antibiotic therapy were excluded from the study.

ETHICS STATEMENT

The study was conducted in compliance with the basic bioethical norms and requirements of the Helsinki Declaration of the General Assembly of the World Medical Association on Human Rights and Biomedicine (1977), in accordance with the provisions of the WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics (1983) and the Order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009. The study protocol was approved by the Bioethics Commission of the KhNMU.

[World Medical Association Declaration of Helsinki "Ethical Principles of Medical Research Involving Human Subjects". 2008. URL : https://zakon.rada.gov.ua/laws/show/990_005]

DETECTION OF UROGENITAL INFECTIONS

Vaginal cleanliness was determined by isolating the *Lactobacilli* strains from vaginal samples using standard procedure of culturing bacteria on solid agar and by performing subsequent PCR sequencing.

To determine the presence of anaerobic bacteria, cervicovaginal lavage (CVL) was analyzed for the presence of the indicated microbial agents (Table 1). Bacterial agents were detected by PCR using standard procedure.

IMMUNOLOGICAL CHARACTERISATION

CVL samples were collected and the levels of cytokines were determined by solid-phase enzyme immunoassay method using Stat Enzyme Immunoassay Analyzer Fax ® 303 Plus (Awareness Technology, Inc. USA) according to manufacturer instructions.

ANALYSIS OF ANTIBODIES IN BLOOD

Blood samples were collected during the study and processed immediately post-collection. Serum was separated from whole blood samples by centrifugation at 2,000g for 15 min at 4°C. Separated serum samples were analysed for total immunoglobulins IgG, IgM and IgA using nephelometry.

Table 1. Cervicovaginal cleanliness in the examined patients

Degree of vaginal cleanliness	Study group (n=85)		Control group (n=30)	
	n	%	n	%
I	8	9.4	24	80
II-III	71	83.5	6	20
IV	6	7.1	-	-

% is percentage out of the total number of examined patients per group
n-number of patients
Source: compiled by the authors of this study

Table 2. Concentration of antimicrobial peptide β-defensin-2 and cytokines in the cervicovaginal lavage of the examined patients.

	Study group	Control group
	n=85	n=30
	Antimicrobial peptide (AMP) ng /ml	
β-defensin-2	30.5±3.5*	46.7±3.8
	Cytokines pg /ml	
IL-4	20.0±1.8*	15.3±1.5
IL-8	4.94±0.6*	3.8±0.4
IL-10	8.37±0.2*	7.1±0.9
IL-18	14.0±1.5*	10.9±1.3
TNFα	43.2±4.5*	33.4±3.6
VEGF	228.5±25.1*	173.5±16.9
IFNg	9.54±1.1*	7.9±0.2

Mann-Whitney U test was performed. Mean±SEM is shown where SEM is standard error of the mean. *P<0.05 was considered statistically significant between the control and study groups. n is number of patients per group
Source: compiled by the authors of this study

STATISTICAL ANALYSIS

Statistical analysis was performed using software package "Statistica 10.0 for Windows". For each variation series, we calculated average arithmetic mean (M), average quadratic deviation (σ), average mistake average arithmetic (m). To identify significant differences, Mann-Whitney U test was used. Differences were considered significant at a level of $P < 0.05$.

RESULTS

ANALYSIS OF BACTERIAL COMPOSITIONS IN THE CERVICOVAGINAL SECRETIONS IN PATIENTS WITH ADENOMYOSIS

There were 115 female patients involved in the study and the patients were divided into two groups. Group 1 constituted patients with adenomyosis diagnosis (study group, 85 patients) and group 2 included healthy patients (control group, 30 patients). The patients were 31.2 ± 2.7 years old, with no statistical difference in age between the study or control groups. 30% of the patients in the study group contained cases of gynecological complications and family history of gynaecological conditions compared to 10% in the control group. Average duration of adenomyosis in patients in study group was between 3 to 5 years. The clinical signs of pathology in the study group included pelvic pain (71% of patients), dysmenorrhea (43% patients) and infertility (84% patients).

First, we aimed to determine the degree of vaginal cleanliness among all the patients enrolled in the study. We used previously reported lactobacilli grading method [19] to define four degrees of purity of vaginal flora: Grade I defined as dominant lactobacilli with no other bacteria present, Grade II defined as having lactobacilli and other bacteria present, Grade II defined as few or no lactobacilli with other bacteria present and Grade IV defined as no lactobacilli and no other bacteria present. Vaginal discharge was collected from patients and bacterial flora was examined using PCR (Table 1). Grade I vaginal purity was detected in 24 (80.0%) women of the control group and in 8 (9.4%) patients in the study group. Grade II-III vaginal purity was identified in 6 (20.0%) control group patients and in 71 patients (83.5%) of the study group. Grade IV vaginal purity was present in 6 (7.1%) of cases in patients of the study group and was not detected in any of the patients in

Table 3. Concentration of antibodies in the serum of the examined patients.

	Study group	Control group
	n=85	n=30
IgM (g/l)	1.85 ±0.26*	1.02±0.09
IgG (g/l)	16.6 ±2.5*	12.3 ±1.6
IgA (g/l)	2.33 ±0.34*	1.10 ±0.08

Mann-Whitney U test was performed, Mean±SEM is shown where SEM is standard error of the mean. *P<0.05 was considered statistically significant between the control and study groups. n is number of patients per group
Source: compiled by the authors of this study

the control group. Together, these data indicated that vaginal purity is negatively affected in patients with adenomyosis.

Decrease in vaginal purity suggested that microbial changes may be present in the vaginal environment. Previous work suggested that there is an association between anaerobic microbes and inflammation of endometrium [20, 21] which prompted us to ask whether anaerobic bacteria may play a role in adenomyosis. To address this question, we examined the anaerobic microbial composition of the cervicovaginal flora in the study and control groups. Samples of cervicovaginal lavage were collected and microbial composition was determined by PCR for an indicated set of microbial agents (Fig. 1). Anaerobic microbial agents constituted seven microorganisms that have previously been reported in vaginal microflora [22, 23]. Comparison between the control and study groups revealed that patients with adenomyosis were characterized by the presence of a number of anaerobic microorganisms with the *Streptococcus* spp. *Staphylococcus* spp. and *Eubacterium* spp. being second and third most abundant anaerobe present respectively. A single case of *Candida* spp., and another case of *Mycoplasma hominis* were identified in the study group, suggesting that the presence of these microorganisms is rare yet detectable. Unlike the study group, the vaginal microflora of healthy patients had no presence of any of the anaerobic microorganisms, consistent with the control group exhibiting Grade I degree with vaginal purity (Table 1). Together, these results suggested a possible link between the adenomyosis and the presence of anaerobic species in likely disrupted cervicovaginal microbiota.

Disruption in microflora in the vaginal environment in patients with adenomyosis lead us to examine the local immunological composition. AMPs produced by commensal microbiota are known to be the key mediators of defence against microbial pathogens in female reproductive tract [12]. AMPs are known to fluctuate in response to hormonal changes as well as in certain disease of female reproductive tract [13]. There different types of AMPs in the endometrial fluid [24] and β-defensins are of the most abundant AMPs present. As a representative analysis, we, chose to look at the levels of β-defensin-2 in CVL fluid in the study and control groups. The levels of b-defensins were downregulated in patients with adenomyosis compared to the control group (Table 2). This result supported the link between adenomyosis and dysregulated microflora.

CERVICOVAGINAL INFLAMMATORY CHANGES IN PATIENTS WITH ADENOMYOSIS

We hypothesised that disrupted microbial composition within the vaginal environment may also be accompanied by immunological changes.

We therefore examined the levels of the key immunological regulators including interleukins (IL-4, IL-8, IL-10, IL-18), pro-inflammatory mediators (TNFα, IFNγ) and endothelial growth factor (VEGF) in the CVL in the study and control groups. In CVL, the concentration of IL-8, IL-10 and IL-18 was significantly higher in patients with adenomyosis compared to the control group (Table 2). Since IL-18 stimulates the formation of Th1-type cytokines (IFNγ, TNFα), its increase at the local level may be aimed at limiting inflammatory processes in the reproductive tract. In addition, by secreting Th1-type mediators, IL-18 may contribute to the development of a T-cell immune response. In line with these observations, higher levels of TNFα were detected in patients with adenomyosis compared to the study group (Table 2). IL-4 and IL-10, that are associated with anti-inflammatory responses, were elevated in patients with adenomyosis. The concentration of VEGF was significantly higher in the study group compared to healthy volunteers indicating a possible disruption in the normal angiogenesis that may result in vascular growth and proliferation of ectopic endometrium.

Next, we examined the levels of three subclasses of antibodies IgG, IgA and IgM in the serum of patients of adenomyosis or healthy women. All three subclasses of antibodies were elevated in patients with adenomyosis

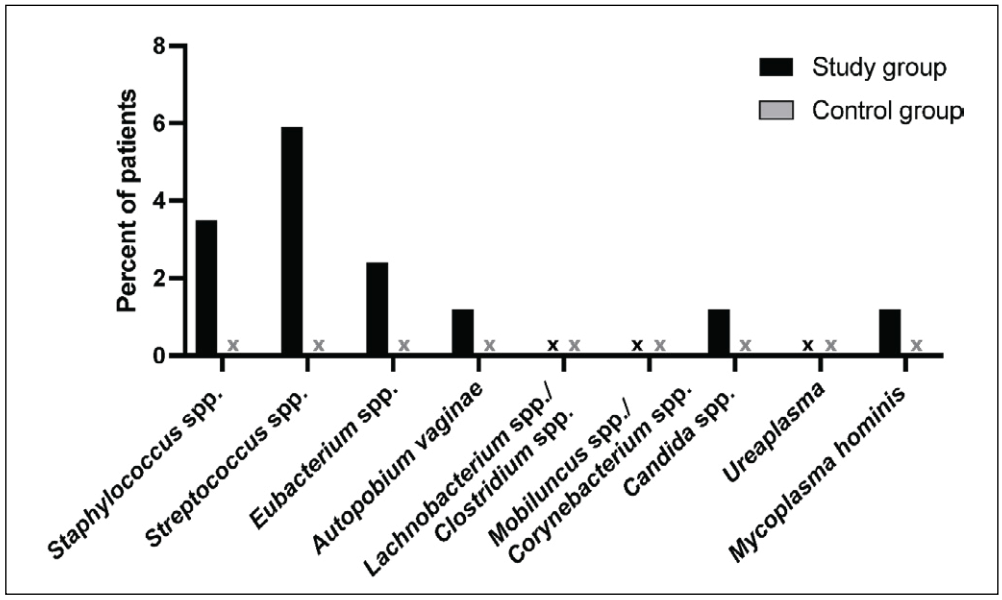

Fig. 1. The presence of anaerobic microbial species in cervicovaginal lavage of patients with and without adenomyosis
The number of patients is presented as a percentage per either study (black) or control (grey) groups. Black bars depict patients in the study group (with adenomyosis, total n=84). No anaerobic bacteria were identified in the control group of patients (n=30) "X" indicates that no patients were identified with a given microbial species.
Authors' own work

compared to the control group, with IgG levels being the most dominant class (Table 3). Collectively these results indicated that the inflammatory microenvironment, defined by elevated levels of pro-inflammatory mediators and increased inflammation-associated immunoglobulins, may be a characteristic of adenomyosis lesions.

DISCUSSION

The results of the study show that adenomyosis may be accompanied by changes in the cervicovaginal microbiome, a drop in b-defensin-2 and changes in the local immunological profile.

Epithelial cells of female reproductive tract are colonized by commensal bacteria that secrete AMPs among others, thereby providing protection against harmful pathogens. Our results showed that patients with adenomyosis displayed disruption in cervicovaginal microbiome that was manifested by the presence of anaerobic microorganisms (Fig. 1, Table 1). Consistent with this observations, other reports indicated that endometriosis-related lesions have distinct microbial compositions [11] and different stages of endometriosis are characterised by specific microbial patters [25]. In our study, we did not look at individual stages of adenomyosis. Furthermore, we only looked at the levels of β-defensin-2, one of many AMPs secreted by commensal bacteria in female genital tract. In future, we would like to determine whether certain stages of adenomyosis present themselves with defined patterns of cervicovaginal microbial composition and expand our analysis to other AMPs in adenomyosis.

The striking difference in pro-inflammatory cytokine profile between the patients with adenomyosis suggests that immune cell composition at the endometrial site differs between the adenomyosis patients and the healthy women. It would be interesting to define immune subsets in the cervicovaginal microenvironment in patients with adenomyosis to pinpoint the dominant immune cell populations that trigger the release of the proinflammatory cytokines identified in the present study. Interestingly, the levels of two anti-inflammatory cytokines IL-4 and IL-10 were similarly elevated in patients with adenomyosis (Table 2), indicating that even these elevated levels of anti-inflammatory cytokines are insufficient to suppress inflammation at the lesion site. Future work will focus on expanding the analysis of cytokine profiles to wider clinical groups to determine whether the cytokines can be used as prognosis markers for the development of adenomyosis [26].

Identifying the association between the elevated antibody levels and the presence of adenomyosis (Table 3) raises an interesting possibility that adenomyosis may have the autoimmune component its pathophysiology, similar to other endometriosis lesions [27]. The elevated levels of the antibodies in the serum of adenomyosis patients suggests that adaptive immunity mediated by T and B cells is activated in these patients.

Follow up studies are necessary to determine whether these antibodies are acting at the site of adenomyotic lesion, thereby exacerbating the disease further. Another outstanding question remains - what are the antigens that are recognised by these antibodies in patients with adenomyosis? Provided the elevated levels of antibodies in patients with adenomyosis recognize self-antigens at the adenomyosis lesion, it would be interesting to investigate whether the mechanism of immunosuppression might be effective at certain stages of adenomyosis development.

Adenomyosis remains to be a topical global problem affecting health of millions of women worldwide and the current treatment does not address prevention or pathophysiological aspects of the disease. The study presented here will contribute to a better understanding of the molecular mechanisms that govern adenomyosis at the cellular level and help advance the development of much needed diagnostic and prevention strategies.

CONCLUSIONS

The pathophysiology of adenomyosis remains understudied largely due to the complexity of the diagnosis and limited understanding of the etiology of the condition. The study presented here revealed several important aspects of the inflammation and immune dysregulation that accompany adenomyosis lesions. The results showed that patients with adenomyosis exhibit disrupted microbial composition within the cervicovaginal environment characterised by high prevalence of anaerobic species. Cervicovaginal lavage collected from patients with adenomyosis was characterized by elevated levels of interleukins (IL-4, IL-8, IL-10, IL-18), pro-inflammatory mediators (TNFα, IFNγ) and endothelial growth factor (VEGF). Furthermore, the serum concentrations of IgG, IgM and IgA antibodies were elevated in patients with adenomyosis compared to healthy individuals. Our study provides an important foundation to further dissect the immunological composition of adenomyosis and ultimately help design better diagnostic and therapeutic tools to treat this debilitating condition.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 07.11.2025
ACCEPTED: 02.03.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Is happiness the strongest predictor of sleep quality? A study on physical activity, stress, and health in Uzbekistan

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ABSTRACT

Aim: To identify key predictors of sleep quality among adults in Uzbekistan, with a focus on health-related factors such as physical activity, psychological well-being (happiness, stress, loneliness), and self-rated health status. The research addresses a critical and growing public health issue in transitional societies, where sleep disturbances remain underexplored despite their widespread impact on overall health and well-being

Materials and Methods: A cross-sectional survey was conducted online in June 2024 among 450 adults aged 18 and older from various regions of Uzbekistan. Sleep quality was assessed using a 5-point Likert scale. Independent variables included physical activity, happiness, stress, loneliness, and health status. Ordinal logistic regression was applied, controlling for socio-demographic characteristics

Results: Happiness ($OR = 1.812$, $p < 0.001$) and physical activity ($OR = 1.293$, $p = 0.045$) were significantly associated with better sleep quality, while stress ($OR = 0.833$, $p = 0.026$) and poor health ($OR = 0.526$, $p < 0.001$) predicted lower sleep quality. Subgroup analysis confirmed that age and gender modulate these associations, with happiness remaining the strongest and most consistent predictor.

Conclusions: These findings highlight the pivotal roles of emotional well-being, lifestyle, and health in shaping sleep quality. Public health strategies should incorporate mental wellness, physical activity, and stress management to improve population-level sleep outcomes in Uzbekistan and similar socio-cultural contexts.

KEYWORDS: sleep, exercise, health status, public health, cross sectional study, Uzbekistan

Wiad Lek. 2026;79(7):1500-1510. doi: 10.36740/WLek/222247 DOI

INTRODUCTION

In today's fast-paced world, sleep deprivation has emerged as a significant public health concern, adversely affecting global well-being. According to ResMed's 2024 Global Sleep Survey (N=36,000), nearly 40% of individuals' experience fewer than three nights of quality sleep per week, leading to excessive daytime sleepiness in 50% of respondents and morning negativity in 40% [1]. These alarming statistics underscore the urgent need to prioritize sleep as a fundamental pillar of health, alongside diet and exercise. Addressing sleep quality is essential for improving overall well-being and mitigating the adverse consequences of the global sleep crisis.

Sleep quality is a crucial determinant of physical and emotional well-being, influenced by biological, psychological, environmental, and cultural factors. Kline [2] defines sleep quality as "one's satisfaction with the sleep experience, integrating aspects of sleep initiation, sleep maintenance, sleep quantity, and refreshment upon awakening". High-quality sleep is essential for cognitive functioning, emotional regulation, and overall health [3]. Conversely, poor sleep quality is associated with impaired cognitive performance, mood disturbances, and an increased risk of chronic illnesses [4]. Understanding the determinants of sleep quality is essential for developing effective public health policies and promoting sleep health advocacy.

While global sleep disturbances have been widely studied, there is limited research on how cultural and socio-economic factors influence sleep quality in transitioning societies such as Uzbekistan. Given the rapid urbanization and changing lifestyles in the region, it is critical to assess the interplay of physical activity, psychological factors (happiness, stress, and loneliness), and health status in shaping self-reported sleep quality. This study aims to bridge this gap by examining these

relationships within Uzbekistan's unique socio-cultural framework. The findings will contribute to the growing body of literature on sleep health determinants, offering insights for public health interventions and policy recommendations tailored to transitional economies and collectivist societies.

Importantly, sleep health is increasingly recognized as a critical component of public health and is directly aligned with Sustainable Development Goal 3 (SDG 3): Good Health and Well-being, which advocates for comprehensive strategies to ensure healthy lives and promote well-being for all at all ages. In this context, improving sleep quality through targeted interventions- particularly those addressing mental well-being and lifestyle behaviors - becomes essential for advancing health equity and resilience in rapidly transforming social environments such as Uzbekistan.

Sleep is a fundamental biological necessity, yet its patterns and quality are significantly influenced by cultural norms, social behaviors, and environmental factors. While biological mechanisms regulate circadian rhythms, cultural traditions dictate when, where, and how individuals sleep, leading to notable variations across societies [5]. In collectivist cultures such as Uzbekistan, strong family and community ties influence sleep arrangements and quality, whereas individualistic societies prioritize personal autonomy in sleep schedules [6]

Uzbekistan presents a unique intersection of traditional and modern sleep behaviors. The collectivist nature of Uzbek society, characterized by extended family structures and community-based living, may act as a protective factor against sleep-related loneliness, mirroring findings in other collectivist cultures (6). Co-sleeping practices, extended family support, and shared social obligations may enhance perceived sleep quality despite external stressors. However, rapid urbanization, increased exposure to digital screens, economic stressors, and shifting work patterns may introduce sleep disturbances similar to those observed in high-income nations [7].

Additionally, sleep patterns in Uzbekistan may differ from those in Western nations due to cultural expectations. For instance, individuals in East Asian countries often experience shorter sleep durations due to academic and work pressures, while Mediterranean and Latin American societies incorporate biphasic sleep (siestas) into daily life [8;9]. These cultural influences suggest that sleep quality in Uzbekistan is shaped by both traditional and modern lifestyle factors, affecting how physical activity, happiness, stress, loneliness, and health status contribute to overall sleep health.

Among health-related variables, physical activity has been widely recognized as a significant predictor of sleep quality. Defined as any bodily movement produced by skeletal muscles that results in energy expenditure, physical activity encompasses occupational, domestic, and recreational activities [10]. As a modifiable behavior, physical activity serves as an effective, non-pharmacological intervention for enhancing sleep quality [11].

Furthermore, structured physical activity programs contribute to improved overall health

[12] and help prevent conditions such as obesity, which is associated with obstructive sleep apnea [13]. Additionally, regular exercise is linked to reductions in symptoms of depression and anxiety - two psychological factors that frequently disrupt sleep - thereby fostering improved mood and better sleep outcomes [14;15]

Psychological and psychophysiological factors, such as happiness, loneliness and stress, play a crucial role in shaping sleep quality. Happiness has been found to regulate neurobiological pathways, including dopamine and serotonin, which are critical for emotional stability and sleep-wake cycles [16].

Lajunen [17] identified a strong correlation between happiness and sleep pattern across 52 countries, linking these variables to broader societal factors such as governance quality, education, and obesity prevalence. Similarly, Otsuka et al. [18] demonstrated that sleep disturbances significantly lower perceived happiness, highlighting the bidirectional nature of this relationship.

In contrast, stress negatively affects sleep by triggering physiological responses that disrupt sleep initiation and maintenance (19). Daily stressors - such as work pressures, financial concerns, and social stress—can lead to hyperarousal of the central nervous system, prolonging sleep latency and reducing slow-wave sleep. This phenomenon has been observed across various occupational groups, irrespective of gender or job position [20].

Moreover, loneliness and sleep quality are closely interrelated. Defined as perceived social isolation, loneliness is increasingly recognized as a key determinant of sleep disturbances. A systematic review identified a significant correlation between loneliness and self-reported sleep disturbances, though sleep duration remained unaffected [21].

Loneliness has been linked to poor subjective sleep quality, extended sleep latency, increased nocturnal awakenings, and reduced daytime functioning [22] It also indirectly impacts sleep quality by heightening pre-sleep arousal and contributing to morning tiredness [23]. However, in collectivist societies like Uzbekistan, strong social support networks may buffer against the negative effects of loneliness on sleep, making its influence less pronounced than in individualistic cultures [24].

Health status is another significant predictor of sleep quality. Individuals with better overall health experience higher-quality sleep, whereas those with chronic health conditions, such as cardiovascular diseases and metabolic disorders, often report poor sleep [25]. Additionally, mental health conditions such as anxiety and depression are strongly linked to sleep disturbances. Poor mental health disrupts sleep initiation and maintenance, and sleep disturbances, in turn, exacerbate mental health conditions, creating a bidirectional relationship [26].

As for socio-demographic factors, including age, gender, marital status, and employment, further influence sleep quality [27;28]. For instance, younger adults and females often report poorer sleep quality due to life stage-specific stressors, hormonal differences, and obesity [29].

Additionally, employment status and socio-economic position play crucial roles, as low-income individuals are more likely to experience sleep disturbances due to financial stress or unstable living conditions [30]. Environmental factors, such as urban noise and light pollution, further interact with these variables, worsening sleep quality [31].

AIM

To identify key predictors of sleep quality among adults in Uzbekistan, with a focus on health-related factors such as physical activity, psychological well-being (happiness, stress, loneliness), and self-rated health status. The research addresses a critical and growing public health issue in transitional societies, where sleep disturbances remain underexplored despite their widespread impact on overall health and well-being

RESEARCH QUESTIONS (RQ):

RQ1: What is the contribution of physical activity, psychological factors (happiness, stress, loneliness), and health status to self-reported sleep quality?

RQ2: Among these predictors, which factor explains the greatest variance in sleep quality?

Hypotheses (H). This study examines the relationship between sleep quality and multiple predictors, including physical activity, psychological factors (happiness, stress, loneliness), and health status. Prior research has established links between these variables, but their relative contribution in transitioning societies such as Uzbekistan remains underexplored. Based on previous findings, the following hypotheses were formulated:

H1: Physical activity positively predicts better sleep quality,

H2: Happiness is positively associated with better sleep quality,

H3: Higher stress negatively predicts sleep quality,

H4: Better self-reported health positively predicts sleep quality,

H5: Higher levels of loneliness negatively predict sleep quality

Among these predictors, physical activity is expected to be one of the strongest determinants of sleep quality (Kline, 2014)

MATERIALS AND METHODS

PARTICIPANTS

This study collected data through an anonymous online survey in June 2023, using convenience sampling via Telegram and WhatsApp. Participants were required to be Uzbek citizens, at least 18 years old, free of evident health or mental impairments, and willing to provide informed consent voluntarily. The final sample included 450 adults after excluding incomplete surveys. The sample had a mean age of 27.79 years (SD = 9.81), with 40.7% identifying as female. Regarding education, participants reported a mean education level of 3.38 (SD = 1.25), corresponding to professional or higher education. Additionally, 40.4% of respondents were married, 77.8% resided in urban areas, and 75.3% were employed.

All participants were informed about the study's purpose and assured of confidentiality. In line with the Declaration of Helsinki, the study ensured voluntary participation, informed consent, and the protection of participants' rights and data privacy.

VARIABLES AND MODEL

The primary outcome variable in this study is sleep quality, assessed using a 5-point Likert scale ranging from 1 ("Very Poor") to 5 ("Excellent"). The mean sleep quality score among participants was 2.35 (SD = 0.99).

To examine the predictors of sleep quality, the following key independent variables were included:

Sleep Duration: Measured on a 3-point scale (1 = "Never/Rarely," 2 = "Sometimes," 3 = "Almost Every Day"), capturing the frequency of sleeping at least seven hours per night.

Physical Activity: Assessed on a 3-point scale (1 = "Never/Rarely," 2 = "Sometimes," 3 = "Almost Every Day"), representing the frequency of engagement in regular bodily movement.

Happiness: Evaluated using a 3-point self-reported scale (1 = "Not Very Happy," 2 = "Fairly Happy," 3 = "Very

Table 1. Descriptive Statistics

Variable	Descriptive	Mean/Percentage	St. Dev.
Age	Age expressed in years.	27.79	9.811
Gender	Percentage of respondents who are female.	40.7%	N/A
Marital status	Percentage of respondents who are married.	40.4%	N/A
Education	Respondent's highest education level achieved: 1. Middle school; 2. Graduated from high school; 3. Professional education (community college); 4. Bachelor's degree (currently enrolled); 5. Bachelor's degree graduated; 6. Post-graduate degree.	3.382	1.252
Children	Percentage of respondents who have children.	38.9%	N/A
Residence	Percentage of respondents living in urban areas.	77.8%	N/A
Employment	Percentage of respondents who are employed.	75.3%	N/A
Labor sector	Percentage of respondents in each labor sector: 1. Public sector; 2. Private sector; 3. Other.	N/A	N/A
Income	Total monthly income level: 1. Less than 2mill. UZS*; 2. 2-4mill. UZS; 3. 4-6mill. UZS; 4. 6 mill. UAS and above.	1.722	1.506
SMU	Time spent on social media daily: 1. Less than 1 hour; 2. 1-2 hours; 3. 2-3 hours; 4. 3-5 hours; 5. More than 5 hours.	2.542	1.218
Sleep duration	Frequency of sleeping at least 7 hours: 1. Never/rarely; 2. Sometimes; 3. Almost every day.	1.376	0.673
Sleep quality	Self-reported sleep quality: 1. Very poor; 2. Poor; 3. Fair; 4. Good; 5. Excellent.	2.347	0.985
Physical activity	Frequency of physical activity: 1. Never/rarely; 2. Sometimes; 3. Almost every day.	1.336	0.691
Happiness	Self-reported happiness: 1. Not very happy; 2. Fairly happy; 3. Very happy.	1.100	0.623
Stress	Self-reported stress: 1. Not stressful or irrelevant; 2. A little stressful; 3. Moderately stressful; 4. Quite stressful; 5. Very stressful.	2.753	1.208
Health	Percentage of respondents with good self-reported health (fair or good).	44.7%	N/A
Sedentary behavior	Sitting time daily: 1. Less than 1 hour; 2. 1-2 hours; 3. 3-4 hours; 4. 4-6 hours; 5. 6-7 hours; 6. More than 7 hours.	2.698	1.947
Loneliness	Self-reported loneliness: 1. Not lonely; 2. Lonely but don't suffer; 3. Suffer a little from loneliness; 4. Suffer greatly from loneliness.	2.502	0.782
BMI	Body Mass Index (BMI) was calculated using an online calculator based on self-reported weight (in kilograms) and height (in centimeters) provided by respondents. BMI categorizes individuals as follows: 1. Underweight: BMI < 18.5 2. Normal weight: BMI 18.5–24.9 3. Overweight: BMI 25–29.9 4. Obesity: BMI ≥ 30	1.413	0.753

* UZS refers to the Uzbekistani sum, the national currency of Uzbekistan.
At the time of data collection, the exchange rate was approximately 1 USD = 12,000 UZS
Source: compiled by the authors of this study

Happy"), reflecting participants' subjective well-being as a determinant of emotional regulation.

Stress: Measured on a 5-point scale (1 = "Not Stressful or Irrelevant," 5 = "Very Stressful"), indicating perceived stress intensity.

Health Status: Self-reported health categorized as "good" (fair or good) or "poor", reflecting participants' perception of their overall physical and mental well-being.

Loneliness: Assessed using a 4-point scale to capture both the presence and severity of loneliness (1 = "Not lonely," 2 = "Lonely but don't suffer," 3 = "Suffer a little from loneliness," 4 = "Suffer greatly from loneliness").

Body Mass Index (BMI): Calculated from self-reported weight (kg) and height (cm), with BMI values categorized as underweight (<18.5), normal weight (18.524.9), overweight (25–29.9), and obese (≥30).

Additionally, socio-demographic variables-including age, gender, marital status, number of children, education level, employment status, income, and place of residence-were included as control variables. Behavioral variables such as social media use and sedentary behavior were also incorporated to account for potential confounding effects on sleep quality.

Table 2. Ordinal Logistic Regression Model: Predictors of Sleep Quality (N = 450)

Variable	Coefficient	Odds Ratio	Std. Error	z-value	p-value	95% Confidence Interval
Physical activity	0.2572	1.293	0.1284	2.00	0.045	[0.0056, 0.5088]
Happiness	0.5943	1.812	0.1553	3.83	0.000	[0.2899, 0.8987]
Stress	-0.1822	0.833	0.0817	-2.23	0.026	[-0.3424, -0.0220]
Health	-0.6430	0.526	0.1775	-3.62	0.000	[-0.9910, -0.2951]

Source: compiled by the authors of this study

STATISTICAL ANALYSIS

The study employed ordinal logistic regression to identify predictors of sleep quality. Odds ratios were calculated to provide an intuitive interpretation of the relationship between predictors and the likelihood of improved sleep quality.

Subgroup analyses were conducted to explore variations in predictors of sleep quality based on:

1. Gender (male and female)
2. Age groups (18–29, 30–49, and 50+ years)
3. Physical activity levels (non-exercising, moderately active, and highly active)

Descriptive statistics (means, standard deviations, and proportions) were reported for all variables. Group differences were evaluated using p-values to identify statistically significant associations (Table 1).

ETHICS

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

AI STATEMENT

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

RESULTS

MAIN RESULTS

KEY PREDICTORS OF SLEEP QUALITY: FULL REGRESSION MODEL

To identify the most significant predictors of sleep quality, Ordinal logistic regression model was estimated, initially including all independent variables. Following the analysis, only those variables that reached statistical significance were retained and reported in Table 2-specifically, physical activity, happiness, stress, and health status. Non-significant variables were excluded from the final presentation of results.

Among the predictors analyzed, happiness emerged as the strongest positive influence on sleep quality. The regression results showed that higher levels of happiness significantly increased the likelihood of reporting better sleep (coefficient = 0.5943, $p < .001$), with an odds ratio of 1.812—indicating an 81.2% greater chance of improved sleep quality. Physical activity also had a positive association (coefficient = 0.2572, p = .045), with each unit increase linked to a 29.3% increase in the odds of better sleep (odds ratio = 1.293). In contrast, stress showed a significant negative relationship (coefficient = -0.1822, p = .026), reducing the odds of good sleep by 16.7% (odds ratio = 0.833). Health status had the largest negative effect (coefficient = -0.6430, $p < .001$), with poor health decreasing the likelihood of quality sleep by 47.4% (odds ratio = 0.526). Together, these findings highlight the central role of emotional well-being, lifestyle, and health in shaping sleep quality (Table 2).

Ordinal Logistic Regression Model: Predictors of Sleep Quality (N = 450)

A visual representation of these findings is provided in Fig. 1, illustrating the relative impact of key predictors on sleep quality. This figure enhances interpretability by demonstrating the magnitude and direction of each predictor's effect.

EXPLORATORY ANALYSIS: GENDER DIFFERENCES

Since gender differences were not a primary hypothesis, a post hoc exploratory analysis was conducted to examine potential variations between male (N = 267) and female (N = 183) participants. The results, summarized in Table 3, indicate notable differences in predictor significance based on gender. For females, happiness (coefficient = 1.1095, p < 0.001) and health (coefficient = -1.0034, p < 0.001) significantly influenced sleep quality, whereas physical activity (p = .155) and stress (p = .289) were not significant predictors. For males, happiness was a marginal predictor (p = .077), while stress (p = .057) and health (p=.087) were negative determinants.

ADDITIONAL ANALYSIS: SLEEP QUALITY ACROSS AGE GROUPS

To further explore age-related trends, ordinal logistic regression analysis was performed across different age groups (18–29, 30–49, and 50+ years). The results are presented in Table 4.

Table 3. Gender-specific predictors of sleep quality

Variable	FEMALE (N=183)					MALE (N=267)				
	Coefficient	Std. Error	z-value	p-value	95% CI	Coefficient	Std. Error	z-value	p-value	95% CI
Physical activity	0.2579	0.1812	1.42	.155	[-0.0972, 0.6129]	0.2629	0.1905	1.38	.168	[-0.1105,0.6362]
Happiness	1.1095	0.2680	4.14	.000	[0.5842, 1.6347]	0.3409	0.1929	1.77	.077	[-0.0371, 0.7189]
Stress	-0.1513	0.1426	-1.06	.289	[-0.4307, 0.1282]	-0.1915	0.1004	-1.91	.057	[-0.3883, 0.0054]
Health	-1.0034	0.2804	-3.58	.000	[-1.5530, -0.4538]	-0.4088	0.2390	-1.71	.087	[-0.8773, 0.0597]

Source: compiled by the authors of this study

Table 4. Ordered logistic regression for sleep quality by age group

Age Group	Variable	Coefficient	Std. Error	z-value	p-value	95% Confidence Interval
18–29 (N=289)	Physical activity	0.2383	0.1790	1.33	.183	[-0.1124, 0.5891]
	Happiness	0.5762	0.1951	2.95	.003	[0.1939, 0.9586]
	Stress	-0.1498	0.1060	-1.41	.158	[-0.3575, 0.0580]
	Health	-0.7499	0.2306	-3.25	.001	[-1.2020, -0.2979]
30–49 (N=145)	Physical activity	0.3689	0.1988	1.86	.064	[-0.0208, 0.7585]
	Happiness	0.5479	0.2601	2.11	.035	[0.0381, 1.0577]
	Stress	-0.2619	0.1349	-1.94	.052	[-0.5263, 0.0026]
	Health	-0.3928	0.3018	-1.30	.193	[-0.9842, 0.1986]
50+ (N=16)	Physical activity	-43.0755	7773.733	-0.01	.996	[-15279.31, 15193.16]
	Happiness	76.5068	11296.22	0.01	.995	[-22063.68, 22216.70]
	Stress	2.4107	1.3842	1.74	.082	[-0.3022, 5.1236]
	Health	19.2010	4095.124	0.00	.996	[-8007.10, 8045.50]

Source: compiled by the authors of this study

Findings indicate that happiness remained a consistent predictor across all age groups, while stress and health status effects varied. Among older adults (50+ years), the results were statistically unstable due to a smaller sample size (N=16).

SLEEP QUALITY BY PHYSICAL ACTIVITY LEVELS

An additional analysis explored how physical activity levels affected sleep quality by categorizing participants into non-exercising, moderately active, and highly active groups. The results, displayed in Table 5, indicate that moderately active individuals exhibited the highest sleep quality, while those engaging in high-intensity physical activity did not show additional benefits.

MEAN SLEEP QUALITY BY GENDER, AGE GROUP, AND PHYSICAL ACTIVITY

The mean sleep quality scores and standard deviations for different demographic groups are presented in Table 6.

The mean sleep quality scores for males (2.356, SD = 0.987) and females (2.333, SD = 0.985) were similar, with no statistically significant difference observed (p = 0.8125).

Age Group: Sleep quality scores varied slightly among the three age groups, with younger adults (18–29 years) reporting the highest mean sleep quality (2.377, SD = 0.986), followed by middle-aged adults (30–49 years) (2.317, SD = 0.991) and older adults (50+ years) (2.063, SD = 0.929). However, these differences were not statistically significant (p = .4205).

*Physical Activity Level***:** Significant differences were observed in sleep quality based on physical activity levels (p = .0003). Non-exercising individuals reported the lowest mean sleep quality (1.860, SD = 1.156), while moderately active (2.432, SD = 0.998) and highly active participants (2.404, SD = 0.885) reported notably higher sleep quality.

Fig. 2 demonstrates a clear trend where moderate levels of physical activity correspond to the highest sleep quality scores, whereas non-exercising individuals report the poorest sleep. However, the lack of further improvement in highly active individuals suggests a potential threshold effect, where excessive exercise may not provide additional benefits for sleep.

Table 5. Ordered logistic regression for sleep quality by physical activity levels

Physical Activity Level	Variable	Coefficient	Std. Error	z-value	p-value	95% Confidence Interval
Non-Exercising (N = 57)	Happiness	0.924	0.440	2.10	.036	[0.061, 1.786]
	Stress	-0.004	0.238	-0.02	.985	[-0.470, 0.461]
	Health	-0.377	0.516	-0.73	.465	[-1.387, 0.634]
Moderately Active (N = 185)	Happiness	0.635	0.253	2.51	.012	[0.138, 1.131]
	Stress	-0.250	0.129	-1.94	.052	[-0.502, 0.003]
	Health	-0.644	0.320	-2.01	.044	[-1.271, -0.017]
Highly Active (N = 208)	Happiness	0.435	0.226	1.92	.054	[-0.008, 0.878]
	Stress	-0.160	0.120	-1.33	.185	[-0.396, 0.076]
	Health	-0.626	0.253	-2.48	.013	[-1.121, -0.131]

Source: compiled by the authors of this study

Table 6. Mean sleep quality by gender, age group, and physical activity level

Category	Group	Mean	Std. Dev.	Observations (N)	p-value
Gender	Male	2.356	0.987	267	.8125
	Female	2.333	0.985	183	
Age Group	18–29 Years	2.377	0.986	289	.4205
	30–49 Years	2.317	0.991	145	
	50+ Years	2.063	0.929	16	
Physical Activity	Non-Exercising	1.860	1.156	57	.0003
	Moderately Active	2.432	0.998	185	
	Highly Active	2.404	0.885	208	

Source: compiled by the authors of this study

DISCUSSION

This study examined the key predictors of sleep quality among adults in Uzbekistan, focusing on the impact of physical activity, psychological factors (happiness, stress, loneliness), and health status. The findings largely confirmed our hypotheses, providing strong empirical support for the role of happiness, stress, and health in shaping sleep quality, while the impact of physical activity exhibited nuanced patterns. These results have significant public health implications, highlighting the need for tailored interventions aimed at improving sleep quality through psychosocial and behavioral modifications.

The hypothesis that physical activity positively predicts better sleep quality (H1) was supported, with higher engagement in physical activity associated with improved sleep outcomes. This finding aligns with previous studies demonstrating that regular physical activity enhances sleep patterns [32]. However, subgroup analysis revealed a non-linear relationship: moderately active individuals experienced the greatest sleep benefits, while highly active individuals did not show a proportional improvement. This aligns with prior research suggesting that excessive physical exertion may lead to physiological stress, delaying sleep onset and reducing overall sleep efficiency [11]. These results emphasize the importance of promoting moderate-intensity physical activity - such as walking, yoga, and recreational sports - as an effective sleep-enhancing strategy in public health interventions [33].

Among all predictors, happiness emerged as the strongest determinant of sleep quality (H2), with individuals reporting higher happiness levels exhibiting significantly greater sleep quality (OR = 1.812, $p < 0.001$). These findings suggest that positive affect, emotional well-being, and life satisfaction play a crucial role in sleep regulation.

On a biological level, happiness is associated with higher levels of serotonin and dopamine, which regulate mood and contribute to sleep-wake balance [17]. Serotonin, a precursor to melatonin, is crucial for initiating and maintaining restful sleep, while dopamine helps modulate arousal and relaxation cycles. Additionally, happiness reduces stress-induced cortisol secretion, which can disrupt sleep quality by increasing nighttime arousal.

Overall, strong social ties and interpersonal relationships in collectivist cultures may provide emotional stability, reduce loneliness, and buffer against the adverse effects of stress, thereby enhancing sleep quality. This is further supported by national well-being indicators, as countries ranking high in the World Happiness Report-such as Finland and Denmark—consistently report superior sleep quality [34]. Uzbekistan's 47th place in

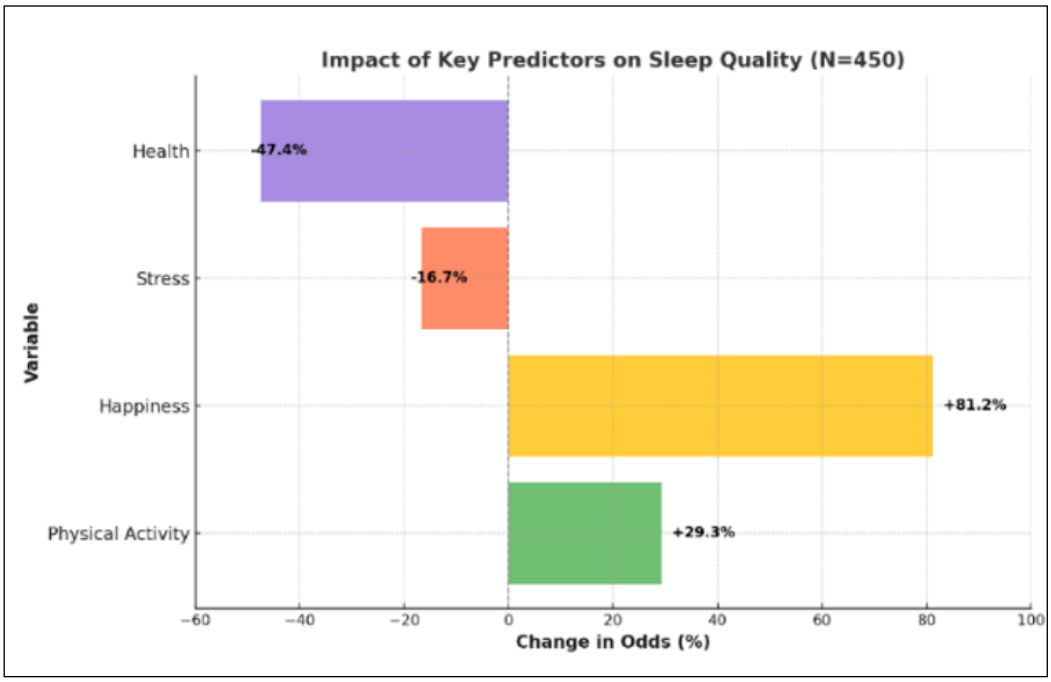

Fig. 1. Impact of key predictors on sleep quality
Authors' own work

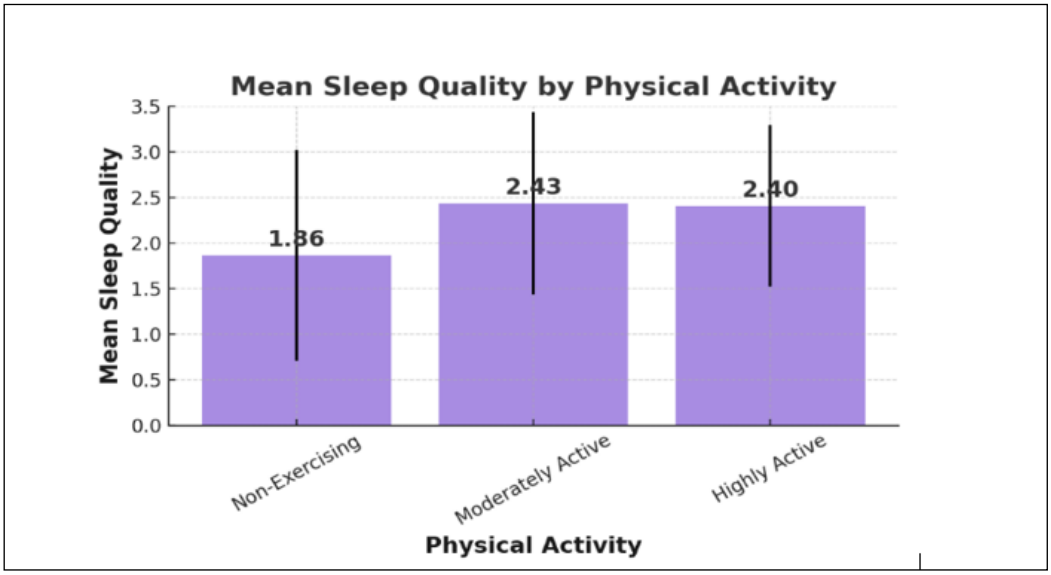

Fig. 2. Mean sleep quality by physical activity
Authors' own work

the 2024 ranking, leading the Central Asian region, suggests that socio-cultural factors may mitigate the negative effects of stress and loneliness on sleep.

The hypothesis that higher stress negatively predicts sleep quality (H3) was also confirmed. Stress has long been established as a key disruptor of sleep, leading to difficulties in sleep initiation, fragmented sleep cycles, and insomnia [35].

Daily stressors—such as work pressures, financial concerns, and social stress—trigger hyperarousal of the central nervous system, prolonging sleep latency and reducing slow-wave (deep) sleep. Previous studies indicate that stress-related sleep disturbances may also exacerbate mental health conditions, creating a vicious cycle of poor sleep and heightened stress [36]. Given these findings, public health strategies should prioritize stress management interventions, such as cognitive behavioral therapy (CBT), relaxation techniques, and mindfulness-based approaches, to improve both sleep quality and psychological resilience.

Consistent with our hypothesis (H4), poorer self-reported health was strongly associated with lower sleep quality. This finding reinforces the bidirectional relationship between physical health and sleep, where chronic conditions contribute to sleep disturbances, and insufficient sleep exacerbates health problems.

Contrary to expectations, higher levels of loneliness did not significantly predict sleep quality (H5). This finding contrasts with previous studies which have consistently shown that loneliness is a strong predictor of sleep disturbances due to pre-sleep cognitive rumination, increased nighttime awakenings, and heightened physiological

arousal [23]. A possible explanation is that Uzbekistan's collectivist culture may mitigate the adverse effects of loneliness, as individuals are more likely to receive social and emotional support from extended family and community networks. Future research should explore whether social cohesion mediates the relationship between loneliness and sleep disturbances.

LIMITATIONS AND FUTURE DIRECTIONS

While this study provides valuable insights into the determinants of sleep quality in Uzbekistan, several limitations should be noted. First, the use of self-reported measures introduces potential bias, as participants may overestimate or underestimate their sleep quality and psychological states. Future studies should incorporate objective sleep assessments, such as polysomnography or actigraphy, to enhance measurement accuracy.

Second, the cross-sectional design limits causal inference. While significant associations were found, longitudinal studies are necessary to determine whether changes in physical activity, happiness, stress, or health status directly lead to changes in sleep quality over time.

Third, this study primarily relied on a convenience sample, recruited via online platforms, which may not fully represent the broader Uzbek population. Future research should aim for more diverse and representative sampling, including participants from various socioeconomic backgrounds, rural areas, and different occupational groups.

Lastly, the study did not assess the role of dietary habits, overall screen exposure, and chronotype—factors that have been shown to influence sleep quality in other populations. Integrating these variables in future studies would provide a more comprehensive understanding of sleep determinants in Uzbekistan.

CONCLUSIONS

This study highlights the complex interplay between happiness, physical activity, stress, and health in shaping sleep quality. Happiness emerged as the strongest predictor, likely due to its role in dopamine and serotonin regulation, emotional resilience, and stress buffering. Moderate physical activity contributed to better sleep outcomes, while excessive exercise showed diminishing returns due to physiological overactivation. Stress remained a key disruptor of sleep, reinforcing the need for stress management interventions.

Future research should employ longitudinal designs to establish causality and develop intervention-based approaches tailored to different demographic groups. Public health policies should prioritize holistic strategies incorporating psychosocial, behavioral, and physiological components to enhance sleep outcomes in Uzbekistan and similar socio-cultural contexts. These findings underscore the importance of integrating mental well-being programs, physical activity guidelines, and stress reduction strategies into national health initiatives to improve population-level sleep quality and overall health as well.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 17.04.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Correlation between interleukin-17 levels and oral manifestations in patients with oral squamous cell carcinoma

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ABSTRACT

Aim: To assess the level of salivary IL-17 in Oral Squamous Cell Carcinoma patients compared to healthy individuals and their association with molecular etiology and oral manifestations.

Materials and Methods: A case-control study was carried out in 56 patients with OSCC and 46 healthy matched subjects. The demographics, tumor molecular profiles (TP53 mutations and EGFR overexpression), and oral examination findings were systematically recorded. ELISA was used to detect the concentration of IL-17 in saliva.

Results: Salivary IL-17 levels were significantly elevated in patients with Oral Squamous Cell Carcinoma (21.34 ± 4.6 pg/mL) compared to the control group (19.72 ± 4.2 pg/mL, $p = 0.040$). IL-17 was higher in TILs of those patients; tumors with overexpression of EGFR exhibited higher levels than those with TP53 mutations. The most common oral manifestations observed in this study included mucositis and xerostomia. The increase in salivary IL-17 was also found to be independently associated with mucosal alterations and changes in pigmentation.

Conclusions: Salivary IL-17 levels were elevated in patients with Oral Squamous Cell Carcinoma, with this biomarker being correlated in such cases with tumors driven by EGFR and with specific oral manifestations. Thus, this biomarker has the potential to be a non-invasive tool for disease detection and follow-up.

KEYWORDS: oral squamous cell carcinoma, salivary IL-17, biomarker, TP53 mutation, EGFR overexpression, oral manifestations

Wiad Lek. 2026;79(7):1511-1517. doi: 10.36740/WLek/222509 DOI

INTRODUCTION

Oral squamous cell carcinoma is the most common among oral cancers accounting for more than 90% of global cases [1]. It is an important public health issue, particularly in some areas where specific risk factors of tobacco intake, alcohol consumption and betel nut chewing are highly prevalent [2]. Although improvements have been achieved regarding improved therapeutic regimens and refined diagnostic tools, OSCC (Oral Squamous Cell Carcinoma) still has a poor prognosis [3]. The survival rate for 5-year-olds ranges mostly from 50 to 60%. Most of the deaths related to this disease can be ascribed to late clinical presentation and its complex biology [4]. In the past years, immune responses in OSCC have been increasingly observed. Of all cytokines, interleukin-17 (IL-17) has emerged as one of the most important pro-inflammatory cytokines that modulates tumor growth, angiogenesis and metastasis [5]. Th17 cells secrete interleukin-17 and have dichotomized roles in both tumor promotion and suppression depending on the specific tumor microenvironment [6]. Elevated expression of IL-17 is seen in a range of tumors but can be particularly pronounced in head and neck cancers. This makes hypotheses about its potential role in disease progression and prognosis [7]. Ulceration, induration, pain and dysphagia are extremely important presenting symptoms in clinical examination of a patient diagnosed with OSCC by the patient themselves [8]. Although they are of diagnostic value, they likely represent patient's immunological and background inflammatory status [9]. Analysis of the association between IL-17 levels and oral clinical features might shed light on cancer biology and lead to new targeted therapies [10]. The present scientific environment highlights precision medicine and the incorporation of immunological indicators for early detection, surveillance, and customized therapy in OSCC [11]. Since IL-17 plays two roles in cancer biology, relating its serum concentrations to particular oral symptoms might uncover clinically significant relationships that would be useful for risk assessment and intervention planning [12]. Anyhow, the association of circulating

levels of IL-17 with oral clinical features is yet to be adequately investigated, and the available evidence remains scarce and inconsistent. To our knowledge, only a few studies have directly addressed whether high circulating levels of IL-17 can be a clinically relevant biomarker connecting tumor biology with the clinical manifestation of oral symptoms in OSCC.

AIM

The researchers seek to systematically examine the correlation between serum levels of interleukin-17 (IL-17) and clinical symptoms of the oral cavity in patients with OSCC and to obtain new information on immunopathological mechanisms that shape the natural history of disease and symptom development. The current study, therefore, aims at integrating systemic cytokine profiling with comprehensive oral clinical assessment to determine if the use of the signature IL-17 to predict OSCC risk, prognosis, and treatment response can be utilized as a non-invasive biomarker of precision medicine in oral oncology.

MATERIALS AND METHODS

PATIENTS AND DATA COLLECTION

This descriptive cross-sectional study took place at the Middle Euphrates Oncology Center in Al-Najaf, Iraq, from December 2024 through June 2025. The study included 56 patients with OSCC and 46 healthy matched subjects. Inclusion criteria for patients required a histopathologically confirmed diagnosis of OSCC and an age of 18 years or older. Participants were enrolled either during routine oncology outpatient visits or while hospitalized for the management of OSCC. Exclusion criteria encompassed patients with chronic systemic conditions such as uncontrolled diabetes mellitus, autoimmune disorders, or cardiovascular diseases. Additionally, individuals with a history of other malignancies, those experiencing ongoing acute infections, or inflammatory conditions not related to OSCC were also excluded. Data regarding demographics and clinical characteristics, including age, gender, tumor location, and disease stage, were collected from hospital medical records.

SALIVA COLLECTION AND PROCESSING

Unstimulated whole saliva was collected from each patient in the morning between 9 and 11 AM to minimize diurnal variation. Participants were advised not to eat, drink (excluding water) or perform oral hygiene activities for at least 1 h before sampling. Each subject was in a comfortable seated position and asked to slightly lean the head downward allowing saliva to pool within their mouth for expectoration into a sterile 15 mL polypropylene collection tube during a period of 5 min. The samples were immediately put on ice and centrifuged at 3000 rpm for 10 min to remove debris. The resulting supernatants were divided into aliquoted and stored frozen at –80 °C up to analysis.

ORAL MANIFESTATION ASSESSMENT

All patients underwent the same oral examination, performed by the same experienced specialist in oral medicine. These assessments were performed with adequate illumination and disposable mouth mirrors and tongue blades. Three major categories of oral findings were reviewed: mucosal changes, salivary gland disease, and mucosal pigmentation alterations. Mucosal changes included differences in mucositis, lichenoid reactions, ulceration and other discernable epithelial lesions. Salivary gland dysfunction was evaluated using patient-reported symptoms (e.g., dry mouth and swallowing discomfort) in conjunction with findings during clinical examination such as decreased salivary pooling or signs of inflammation of the salivary glands. Alterations in mucosal pigmentation were recorded for pigmented or depigmented areas in terms of their location and extent. All findings were documented on standardized clinical data sheets and wherever possible corroborated with photographic documentation for visual aid.

DETECTION OF TP53 MUTATIONS AND EGFR OVEREXPRESSION

Saliva samples were collected from each patient in the morning between 9:00 and 11:00 AM to avoid diurnal fluctuation. Participants were asked not to eat, drink (other than water), or brush their teeth for at least one hour before saliva collection. Depressurized whole saliva was collected in sterile tubes and centrifuged at 3000 × g for 10 minutes, and the supernatants were stored at −80°C until analysis. Genomic DNA was extracted from the pellet of salivary gland cells for analysis of TP53 mutations using a commercial kit. PCR was performed to generate exons 5-8 of the TP53 gene, followed by sequence analysis using the Sanger sequencing technique. The generated sequence data were aligned with the reference TP53 sequence (GenBank accession no. NM_000546) for mutation detection, using point-substitution mutations, insertions or deletions in accordance with IARC guidelines. For EGFR

(Epidermal Growth Factor Receptor) over-expression, the levels of protein in saliva were quantified with the use a commercially available ELISA kit (Diagnostic Systems Laboratories), specific for human epidermal growth factor receptor determined by ELISA. All samples were performed in duplicate and the OD was measured at 450 nm using a microplate reader. Overexpression was assessed based on cut-off values provided by the manufacturer of the ELISA kit, derived from reference ranges established using samples from healthy controls.

MEASUREMENT OF SALIVARY IL-17 LEVELS

Salivary IL-17 concentrations were determined using a high-sensitivity sandwich ELISA kit (specific for human IL-17A) according to the manufacturer's instructions. Prior to analysis, frozen saliva supernatants were thawed at room temperature and centrifuged at 10,000 rpm for 5 minutes to remove residual particulates. Standards and samples were added in duplicate to 96-well microplates pre-coated with monoclonal anti-IL-17 antibodies. After incubation and washing steps, a biotin-conjugated detection antibody was added, followed by streptavidin–HRP solution. The colorimetric reaction was developed using TMB substrate and stopped with 2 N sulfuric acid. OD values were measured at 450 nm using a microplate reader, and IL-17 concentrations were calculated from a standard curve generated using known concentrations of recombinant IL-17A. The detection limit of the assay was 0.5 pg/mL, and intra-assay and inter-assay coefficients of variation were both below 10%.

ETHICAL CONSIDERATION

Approval for conduct of this study was obtained from the Institutional Review Board of the Middle Euphrates Oncology Center and the College of Medicine, University of Kufa (No. 143# in 2025). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Before performing collection of samples, it was mandatory for all patients participating in the study to furnish written consent for their involvement

STATISTICAL ANALYSIS

The SPSS software version 25.0 (SPSS, Chicago) was used for all the appropriate statistical analyses. The normality test which was used to test who want to continuous data for Shapiro Wilk test. All normally distributed data are acted as mean ± standard deviation. Independent t test was used to find out the statistical difference in the respiratory function markers between patients' and control. ANOVA test was used to find out the statistical difference in the respiratory function markers among patients' subgroups classified according to IBs subtypes. A p-value less than 0.05 was considered to indicate a statistically significant difference.

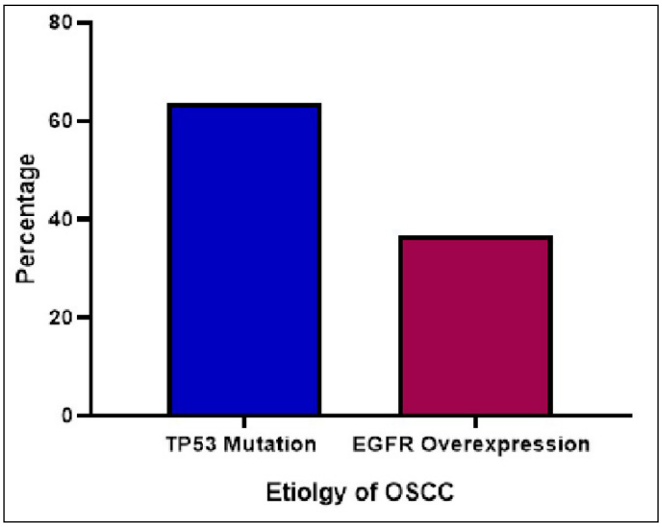

Fig. 1. The proportion of OSCC patients showing TP53 mutations and EGFR overexpression based on salivary molecular analysis. TP53 = tumor protein p53; EGFR = epidermal growth factor receptor. Values are expressed as percentages of the total study population
Source: Own materials

RESULTS

The finding of the demographic data indicated that there was no statistically significant difference in the distribution of age, gender, and residence between oral squamous cell carcinoma patients and the control group ($p > 0.05$ for all). This proves that both groups were comparable, hence minimal demographic confounding effects on further analyses (Table 1).

Regarding molecular etiology of OSCC, TP53 mutations were seen in 64.3% of cases and EGFR overexpressed in 35.7%, making the TP53 mutation the predominant molecular subtype figure 1.

Comparison of salivary IL-17 levels in OSCC patients and healthy controls showed a statistically significant elevation (p value 0.040) in the patient group. The mean IL-17 concentration in OSCC patients was 21.34 ± 4.6 pg/mL, which more than that in the control group which was 19.72 ± 4.2 pg/mL (Table 2).

Differences in salivary IL-17 levels between OSCC patients and control participants

On the other hand, EGFR overexpression presents with much higher salivary IL-17 levels in comparison to those with TP53 mutation p=0.036. This denotes a

Table 1. Demographic characteristics for patients and control groups

Demographic characteristics		Patients (No. = 56)		Control (No. = 46)		Chi square	P value (sig.)
		Freq.	%	Freq.	%		
Age/Years	23–32	18	32.1	15	32.6	0.002	0.998
	33–42	20	35.7	16	34.8		
	≥ 43	18	32.1	15	32.6		
Gender	Male	28	50	23	50	0	1
	Female	28	50	23	50		
Residence	Urban	30	53.6	25	54.3	0.005	0.943
	Rural	26	46.4	21	45.7		

Source: Compiled by the authors of this study

Table 2. Differences in salivary IL-17 levels between OSCC patients and control participants

Groups	No.	Salivary IL-17 (pg/ml) Mean ± SD	T Test (P Value)
Patient	56	21.34 ± 4.56	0.040
Control	46	19.72 ± 4.18	

Source: Compiled by the authors of this study

Table 3. Differences in salivary IL-17 levels between patients' subgroups classified according to molecular etiology

Groups	No.	Salivary IL-17 (pg/ml) Mean ± SD	T test (P Value)
TP53 Mutations	36	58.4 ± 12.6	2.15 (p = 0.036)
EGFR Overexpression	20	66.8 ± 14.1	

Source: Compiled by the authors of this study

Table 4. Oral manifestation for patients participated in the study

Indicators		Patients (No. = 56)	
		Freq.	[%]
Mucosal Change	Oral mucositis	24	42.9
	Lichenoid reactions	10	17.9
	Aphthous-like ulcers	6	10.7
	No Change	16	28.5
Salivary Gland Dysfunction	Xerostomia	22	39.3
	Sialadenitis	8	14.3
	Normal	26	46.4
Mucosal Pigmentation Changes	Hyperpigmentation	12	21.4
	Depigmentation	7	12.5
	No Change	37	66.1

Source: Compiled by the authors of this study

Table 5. Logistic regression analysis for the association between salivary IL-17 levels and oral manifestations in OSCC patients

Oral manifestation	β (coefficient)	SE	Wald χ^2	OR (95% CI)	p-value
Mucosal Change	0.215	0.085	6.38	1.24 (1.05–1.47)	0.012
Salivary Gland Dysfunction	0.178	0.091	3.85	1.19 (0.99–1.42)	0.050
Mucosal Pigmentation Changes	0.241	0.088	7.50	1.27 (1.07–1.51)	0.006

Source: Compiled by the authors of this study

possible connection between the biology of tumors driven by EGFR and an increased inflammatory response (Table 3).

The findings of the oral manifestation indicated that mucositis turned out to be the most frequent mucosal alteration among OSCC patients-42.9%, followed by

lichenoid reaction at 17.9% and aphthous-like ulcers at 10.7%. 28.5% show no any kind of mucosal alterations, describing xerostomia as their primary salivary gland dysfunction accounting for 39.3% with fewer cases of sialadenitis -14.3%. Almost half the patients, or rather 46.4%, have normal salivary function. Hyperpigmentation describes the outcome in 21.4% of patients; depigmentation results in 12.5%. About two-thirds or rather a full 66.1% have no pigmentation changes (Table 4).

Logistic regression analysis revealed that higher levels of salivary IL-17 were significantly related to mucosal change (OR = 1.24, 95% CI: 1.05-1.47, p = 0.012) and mucosal pigmentation change (OR = 1.27, 95% CI: 1.07-1.51, p=0.006) in patients with OSCC. Salivary gland dysfunction showed a borderline association with IL-17 levels (p=0.050). These results could indicate that increased level of this cytokine may be involved in the pathogenesis of some oral lesions in OSCC (Table 5).

DISCUSSION

Our study shows significantly increased salivary IL-17 in OSCC patients (21.34 ± 4.6 pg/mL) when compared to healthy controls (19.72 ± 4.2 pg/mL; p=0.022). This further strengthens the role of IL-17 in inflammation related to OSCC, and hence, potentially as a non-invasive biomarker of the disease for its detection and monitoring [13-14]. Meta-analyses and systematic reviews conducted on salivary cytokines in OSCC verified raised expression levels of IL-6, IL8, TNF-, and recently available data also add IL17 to emphasize salivary cytokine panels as promising tools in diagnostics [15-19]. On the molecular level, IL-17 signals various interacting pathways regarding the pathogenesis of OSCC. It does so by inducing the expression of IL-6, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs) through JAK–STAT3 and NF-κB signaling pathways that would result in the proliferation, angiogenesis, invasion, and survival of malignant keratinocytes [7]. In addition to this, IL-17 facilitates the recruitment of myeloid-derived suppressor cells (MDSCs) and cancer-associated fibroblasts which support epithelial–mesenchymal transition (EMT) immune escape as well as a tumor-promoting microenvironment. The immunohistochemical study gives evidence of more IL-17+ cells both within tumor nests and stroma in OSCC tissues when compared to those in nonmalignant controls [20]. IL-17 levels were considerably greater in EGFR-overexpressing tumors than in TP53-mutant ones p=0.035, indicating a possible cross-talk between oncogenic pathways and inflammatory pathways. This fulfills the first leg of the hypothesis that EGFR on activation increases pro-inflammatory cytokines making more available mediators through which IL-17 can promote tumor progression while TP53 mutation channels carcinogenesis through different, less inflammatory pathways [21]. Clinically, the predominance of mucositis 42.9%, lichenoid reaction 17.9%, aphthous-like ulcers 10.7% and xerostomia 39.3% among dysfunctions underscore inflammatory and immunological pathways in the pathogenesis of OSCC. Higher IL-17 levels with mucosal changes (OR = 1.24; p=0.012) and pigmentation alterations (OR = 1.27; p=0.006) from a logistic regression analysis support that IL-17 is directly involved in epithelial integrity and vascular permeability as well as melanocyte activation most probably through upregulation of INFs and melanogenic mediators by IL-17 [22]. The borderline association with salivary gland dysfunction p=0.050 indicates possible involvement of IL-17 in glandular inflammation which needs further studies for clarification. Inhibitors of the IL-17 pathway are already in therapeutic use for autoimmune conditions; therefore, they may have adjunctive therapeutic potential in the management of tumor-promoting inflammation and mucosal symptoms in OSCC [23]. Preclinical data support that inhibition of IL-17 can further sensitize PD-1 inhibition and reduce progression of oral tumors [24-26]. However, preclinical considerations must include potential risks based on the highly complex and context-dependent functions of IL-17 in mucosal immunity related to tissue repair and host defense [27]. Limitations involved the cross-sectional design, which limited causal inference and potential confounders such as oral hygiene, microbiome shifts, and other concurrent medications. Future longitudinal and multi-marker studies-in which salivary cytokine panels are integrated with molecular tumor profiles [e.g., EGFR, TP53]-are mandated in the interest of further algorithmic refinements in diagnostics and IL-17–targeted interventions in OSCC.

CONCLUSIONS

Salivary levels of IL-17 are increased in patients with OSCC. Salivary IL-17 can be considered a feasible, non-invasive biomarker for disease presence and follow-up. It comes out higher in tumors with overexpression of EGFR; thereby, it denotes possible relationships between tumor biology and inflammatory activity. Mucosal and pigmentation changes relate it to a possible role in some oral lesion developments.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 12.12.2025
ACCEPTED: 26.05.2026

CREATIVE COMMONS 4.0

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ORIGINAL ARTICLE

CONTENTS

Sensitivity and reactivity of the vestibular analyzer in children

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ABSTRACT

Aim: To study the sensitivity and reactivity of the vestibular analyzer in healthy children aged 3 to 14 years. To identify the characteristics of examining children aged 3 to 14 years.

Materials and Methods: Nystagmic thresholds of children between three and seven years were found to be in the area of 8 to 24 radians per second. With age, this amplitude increased to 3 to 30 radians per second, and therefore it was possible to select, for clinical purposes, an optimal angular velocity of 30 radians per second. The reactivity of the vestibular analyzer increased with age, and also, in children over seven, with the increasing speed of rotation.

Results: Children below five were shown to have the least sensitive and reactive vestibular analyzer, while those over seven demonstrated the highest sensitivity and reactivity, which could be related to the development of the vestibular analyzer and the central nervous system.

Conclusions: Nystagmus thresholds in children under 7 years of age are within the range of 8–24 rad/s, with thresholds increasing from 3 to 30 rad/s with age, allowing a minimum angular velocity of 30 rad/s to be adopted for clinical examination. The sensitivity and reactivity of the vestibular analyzer increase with age. The lowest sensitivity and reactivity of the vestibular analyzer are observed in children younger than 5 years old, and the highest in children older than 7 years old, which coincides with the development of the vestibular analyzer and the central nervous system.

KEYWORDS: nystagmus, vestibular analyzer, sensitivity and reactivity, children of 3 to 14 years

Wiad Lek. 2026;79(7):1518-1521. doi: 10.36740/WLek/218748 DOI

INTRODUCTION

It is known that the balance organ, which is formed by the time a child is born, differs significantly from its function in adults. The area of vestibular assessment in children has become popular in audiology and otorhinolaryngology clinics in recent years. If there is a slightest suspicion of vestibular involvement, due to any suspicious symptomatology, the child should be referred for appropriate neurotological evaluation, in an attempt to establish a correct diagnosis and then initiate a more appropriate treatment, if necessary [1-3]. The information available in modern literature on the state of the vestibular analyzer in children is fragmentary, incomplete, and often contradictory. The results obtained are almost always compared with those in adults, although such a comparison is not adequate, so there is a real need to study the sensitivity and reactivity of the vestibular analyzer in children, which is especially important for the further development of rational therapy for various pathological conditions.

AIM

To study the sensitivity and reactivity of the vestibular analyzer in healthy children aged 3 to 14 years. To identify the characteristics of examining children aged 3 to 14 years.

MATERIALS AND METHODS

We studied the sensitivity and reactivity of the vestibular analyzer in healthy children aged 3 to 14 years. In the course of our work, we found that examining children has a number of peculiarities. For example, it is not always possible to obtain a detailed vestibular history, even with the help of parents, so when examining children, it is necessary to focus on objective data. We often encounter a child's lack of understanding and unwillingness to comply with the conditions of the examination, as well as fatigue, so the examination must be conducted in the form of a game, without overloading the child with a large number of techniques.

The vestibular analyzer in children was examined in the vestibulometric laboratory using an electronic automatic device of our own design with dosed angular acceleration. The angular velocity was arbitrarily set from 0 to 100 radians per second (rad/s) with an accuracy of 0.1 rad/s. First, a vestibulometric examination chart was filled out, paying attention to special medical history, vestibular disorders, treatment, past illnesses that could affect vestibular function, concomitant diseases, and the condition of the ENT organs. Then we proceeded to vestibulometry. We examined statokinetic function, using the Fucuda 'writing' test from the age of 5, studying walking in a straight line with eyes open and closed, and flank walking to the right and left.

Spontaneous and positional nystagmus [3-4] were examined in terms of direction and duration while sitting with eyes closed, with the head tilted to the right and left shoulders, forward, backward, lying on the back, right and left sides, and in the Rose position. Electronystagmographic (ENG) recording of spontaneous and positional nystagmus and a rotational test were performed. The sensitivity threshold of the vestibular analyzer was determined for each patient beforehand. The sensitivity threshold was taken as the lowest angular velocity at which at least 3 nystagmus jerks appeared after the stop stimulus. Since the upper limit of the sensitivity threshold is approximately 30 rad/s [5], and the angular velocities are 70 and 90 rad/s, which are often used in clinics to study the functional state of the organ of balance, we settled on these operating modes in order to establish the sensitivity standards of the vestibular analyzer in children. The results obtained will allow us to compare them with data recorded for various pathological conditions in subsequent work. Setting and extinguishing the speed on the installation took 0.3 s, and the time of uniform rotation was 20-50 seconds, i.e., at each speed, 20 rotations to the right and left were performed, and further recording of post-rotational nystagmus was recorded by a four-channel electroencephalograph. When interpreting the ENG data, attention was paid to the duration of the nystagmus response, direction, amplitude, frequency, and slow phase velocity (SPV). Preliminary calibration of the oscillations was performed at a signal voltage of 100 m and calibration of head movements at an angle of 20 radians, with the gain set so that the nystagmus amplitude was 10 mm.

AI STATEMENT

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

ETHICS

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

RESULTS

We studied 120 healthy children in four age groups: 3-5, 5-7, 7-10, and 10-14 years old. Each group was examined three times at three speed settings. It was found that the thresholds for post-rotational nystagmus were within the range of 8-24 rad/s, which is consistent with the data of Vadzyuk et al., 2023 [11] and can be considered the norm for children under 7 years of age. In the younger (3-5 years) and middle (5-7 years) age groups, the threshold decreased to 3 rad/s, which indicates an increase in the sensitivity of the vestibular analyzer in children, which was actually close to that in adults (3-30 rad/s). Spontaneous and positional nystagmus is not detected in children with normal analyzer function. When examining children aged 3–5 years, we did not find significant differences in the parameters of post-rotational nystagmus when changing the angular velocity ($P = 0.5$; see table). Given that the sensitivity of the vestibular analyzer does not change with a change in angular velocity, we can assume a direct relationship between the former and the anatomical and physiological imperfections of both the analyzer and the child's central nervous system.

In the group of children aged 5-7 years, at angular velocities of 30 and 70 rad/s, no significant differences in the studied indicators were recorded (Table 1). At an angular velocity of 90 rad/s, the ENG parameters increased (Table 1), which is probably due to the increased sensitivity of the vestibular analyzer at this age. The same data were obtained in a study of children aged 7-10 years (Table 1). Thus, with an increase in the age of the subjects and the load on the vestibular analyzer, its sensitivity increases.

In children aged 10-14 years, with an increase in angular velocity of rotation, there is also an increase in ENG parameters (Table 1). The data obtained are similar to the previous ones.

Thus, when studying children at low angular velocities (30 rad/s), a decrease in the reactivity of the vestibular analyzer (see table) is observed with increasing age, which is probably due to the inhibitory influence of the central nervous system on the analyzer. With an increase in angular velocity (70 and 90 rad/s), the reactivity of the vestibular analyzer increases. When comparing the data obtained, it can be noted that with an increase in the load on the vestibular analyzer at the age of up to 5 years, its sensitivity does not change. At the age of 5-7 years, there is even a slight decrease in all ENG parameters, and after the age of 7, an increase in the sensitivity of the vestibular analyzer is again noted. The reactivity of the vestibular analyzer also increases (with suprathreshold stimuli) with age.

Table 1. ENG parameters in children during rotational stimulation

Age, years	Rotation to the right					Rotation to the left				
	Reaction duration, s (sec)	Fre-quen-cy, Hz	SMF rad/s	m ±	P	Dura-tion, s (sec)	Fre-quen-cy, Hz	SPV rad/s	m ±	P±
Rotation speed 30 radians per second										
3-5	27.2	1.2	12.2	0.5	0.05	28.5	1.2	12.2	0.5	0.05
5-7	22.0	1.15	13.5	0.1	0.05	21.5	1.3	13.5	0.1	0.05
7-10	28.2	0.6	12.9	0.05	0.01	27.9	0.8	13.1	0.05	0.01
10-14	20.6	1.1	9.9	0.1	0.01	24.2	1.1	10.5	0.3	0.01
Rotation speed 70 radians per second										
3-5	27.2	1.2	11.9	0.5	0.001	28.5	1.2	12.2	0.5	0.01
5-7	22.0	1.2	13.5	0.1	0.001	21.5	1.3	13.5	0.1	0.01
7-10	32.0	1.2	15.3	0.15	0.01	30.0	1.2	15.9	0.15	0.01
10-14	31.8	1.4	16.1	0.2	0.02	31.2	1.5	15.8	0.1	0.02
Rotation speed 90 radians per second										
3-5	27.2	1.0	11.2	0.5	0.5	28.0	1.1	12.7	0.5	0.5
5-7	39.2	1.6	28.5	0.15	0.01	41.1	1.7	28.9	0.17	0.01
7-10	32.5	1.3	16.4	0.2	0.001	33.5	1.3	17.2	0.3	0.001
10-14	31.6	1.6	20.6	0.1	0.001	31.2	1.5	23.3	0.1	0.001

Source: compiled by the authors of this study

DISCUSSION

This study successfully achieved its scientific goal of establishing the parameters of experimental rotatory nystagmus and compiling a graph of normal values for this nystagmus. The data obtained by the study confirm the experimental results determined in the study of the prevalence of vestibular and balance disorders in children and adolescents by renowned researchers Jong Dae Lee, Chang-Hee Kim, Seok Min Hong, Sung Huhn Kim, Myung-Whan Suh, Min-Beom Kim, Dae Bo Shim, Hosuk Chu, No Hee Lee, Minbum Kim, Sung Kwang Hong, and Jae-Hyun Seo [6].

In a study of vestibular function in children with neuropsychiatric disorders Ruth Van Hecke, Frederik J.A. Deconinck, Emmely Van Acker, Maya Danneels, Ingeborg Dhooge, Hilde Van Waelvelde, Jan R. Wiersema, Leen Maes, the integrity of the peripheral vestibular organ in children diagnosed with NDD was studied[7]. Our study of the sensitivity and reactivity of the vestibular analyzer in healthy children opens up opportunities for comparative studies in the future.

The vestibular system, which includes the semicircular canals and otolith organs, is one of the first sensory systems to develop and plays an important role in maintaining balance and spatial orientation [8-10]. Therefore, the conclusion that nystagmus thresholds in children under 7 years of age are in the range of 8-24 rad/s, and that thresholds increase from 3 to 30 rad/s with age, allowing a minimum angular velocity of 30 rad/s to be used for clinical examination, is important to take into account in other related studies.

The data obtained indicate that, along with the incomplete development of the vestibular analyzer, its connections, and the central nervous system, the inhibitory effect on the analyzer is very significant. With increasing age, excitatory processes become more intense, which leads to increased sensitivity and reactivity of the vestibular analyzer.

CONCLUSIONS

Nystagmus thresholds in children under 7 years of age are within the range of 8–24 rad/s, with thresholds increasing from 3 to 30 rad/s with age, allowing a minimum angular velocity of 30 rad/s to be adopted for clinical examination.

The sensitivity and reactivity of the vestibular analyzer increase with age.

The lowest sensitivity and reactivity of the vestibular analyzer are observed in children younger than 5 years old, and the highest in children older than 7 years old, which coincides with the development of the vestibular analyzer and the central nervous system.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 26.09.2025
ACCEPTED: 02.03.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Correlation of serum tumor markers (CEA and CA15-3) with clinicopathological features in breast cancer patients in Iraq

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ABSTRACT

Aim: This research is aimed at looking into the relationship between the levels of the markers and the clinicopathological features of breast cancer patients in Iraq.

Materials and Methods: The study population consisted of 150 breast cancer patients in a cross-sectional correlation study. Demographic, clinical, and pathological data were collected including molecular subtypes, tumor staging and grading. The serum levels of CEA and CA15-3 were determined and the results correlated with patient characteristics. The statistical methods employed were ANOVA, t-tests, and Pearson correlation.

Results: Out of the total patients, 98.5% were females, 52.9% were over 50 years of age and 79.6% were married. The Luminal types were the most frequent (The Luminal types were the most frequent (Luminal A: 45.3%, Luminal B: 36.5%) while the triple-negative (10.1%) and HER2-positive (8.1%) subtypes were the least common. There was an increase in serum levels of CEA and CA15-3 in advanced tumor stage and high grade cancer, a significant difference in CA15-3 levels between the age groups (p=0.010). The marker levels varied according to the molecular subtypes with Luminal A demonstrating a greater CEA and Luminal B showing a higher CA15-3.

Conclusions: The levels of serum CEA and CA15-3 are indicative of the breast cancer patients' tumor stage, grade, and molecular subtype. The regular monitoring of these markers may provide better understanding of the disease progress and help in the formulation of patient-specific management plans.

KEYWORDS: breast cancer, CEA, CA15-3, molecular subtypes, tumor markers

Wiad Lek. 2026;79(7):1522-1528. doi: 10.36740/WLek/221159 DOI

INTRODUCTION

Breast cancer still holds the number one spot as the most common cancer in women worldwide and it is still one of the major contributors to the cancer-related deaths [1-3]. The management of the disease is highly dependent on early detection and monitoring of its progression [2, 10]. Serum tumor markers like carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) are frequently used to evaluate disease activity, the effectiveness of treatments, and risk of recurrence [6, 9]. The presence of these tumor markers indicates that cancer cells are either releasing or the body is producing them in response to the tumor. Among them, CA15-3 derived from the MUC1 gene is found quite often in breast cancer and that too primarily in advanced stages, while CEA is considered to be a nonspecific marker linked to many adenocarcinomas [4, 6]. Therefore, higher blood levels of these markers are associated with larger tumors, presence of cancer in lymph nodes, and spread of disease to distant sites [2, 7]. Molecular subtyping has turned out to be an effective method for breast cancer classification. Luminal A, Luminal B, HER2-rich, and triple-negative are the main subtypes. These subtypes have different prognoses, responses to treatments, and biomarker profiles [11, 12]. Some studies indicate that the levels of serum tumor markers might differ based on the subtype, thus suggesting their potential role in clinical decision-making [14, 22]. Limited studies in Iraq have assessed the association between the levels of serum CEA and CA15-3 and the characteristics of breast cancer. The CEA/CA15-3 serum profile stands for one of the main clinical variables determining the prognosis of breast cancer. Moreover, the monitoring of these markers can lead to the development of personalized therapy based on the patient's individual profile [5-8, 16-19]. Internationally, CEA and CA15-3 have a clinically proven utility in breast cancer management; however, they are not routinely and systematically monitored in Iraq. Apart from being cost-effective, the monitoring of these tumor markers which are very much capable of providing valuable information in real-time disease

surveillance and treatment response assessment has been hindered by the resource limitations, the absence of universally accepted guidelines, and the fluctuation of laboratory facilities. The aforementioned situation underscores the necessity for clinical trials aimed at producing the evidence-based recommendations necessary for integrating serum tumor marker monitoring into the routine clinical workflow for breast cancer patients in Iraq. In addition to the already mentioned, in the case of the diagnostics of the disease, the markers' testing is a cheap and non-invasive procedure, which supports the results obtained by imaging or histopathology in the early detection and prognostication of the disease [15, 17]. CA15-3 has been connected with the recurrence and spread of cancer tumors, while CEA is a marker for aggressive phenotypes of tumors [18, 20]. Using both markers together might make the medical decision-making process better, especially in places with limited resources [21]. Previous studies done in Iraq have revealed a variety of tumor subtypes and different levels of marker expression. This situation suggests that there is a need for an extensive correlation analysis via different demographic as well as clinicopathological factors [3, 5, 19]. Correlation studies are thus necessary not only for the development of guidelines and therapeutic strategies in accordance with the local population but also for the validation of the generalization from other populations.

AIM

The present investigation is designed as a correlation analysis aimed at the evaluation of relationships between the levels of serum markers and the factors of age, tumor grade, stage, and molecular subtypes. The understanding gained could streamline the monitoring, follow-up, and planning of treatment for breast cancer patients in Iraq.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

A cross-sectional correlation study was held in oncological institutions across Iraq by involving 150 breast cancer patients. Histopathology-confirmed breast carcinoma with serum biomarker data was the foremost inclusion criterion. Patients with other malignancies were not included. Gender Distribution and Analysis: The study group included 148 females (98.5%) and only 2 males (1.5%). Male patients were totally eliminated from those statistical analyses of tumor markers and clinicopathological correlations due to not only the significant biological and clinical differences in breast cancer between genders but also the already small sample size of male patients which could not yield a meaningful subgroup analysis. Even so, all the results and comparisons, including statistical ones, still refers to the only female group (n=148) the results come from. Treatment Information: The subjects of the research were all partaking in the standard treatment procedures which were in accordance with the Iraqi federal oncology guidelines. The therapies were different based on the molecular subtype and the stage of disease:

- Luminal A/B: Endocrine therapy (Tamoxifen or Aromatase inhibitors) ± chemotherapy (regimens based on anthracycline or taxane)
- HER2-positive: Targeted therapy (Trastuzumab when available) + chemotherapy
- Triple-negative: Chemotherapy (regimens based on anthracycline and/or taxane)

Surgical options (mastectomy or lumpectomy) were directly determined by the tumor's stage and whether it was operable. Moreover, radiation therapy was granted post-surgically whenever it was clinically necessary.

DATA COLLECTION

Demographic and clinical data such as age, sex, marital status, education, and occupation, living area, and tumor stage, grade, and molecular type were extracted from medical records.

SERUM MARKER MEASUREMENT

Measurement of serum markers Serum levels of CEA (μg/L) and CA15-3 (U/mL) were determined through standard procedures following manufacturer's guidelines with the implementation of accurate quality control measures.

Timing of the measurement of markers: blood specimens for analysis of tumor markers were drawn at the following time intervals: pre-treatment baseline: 45% of patients (n=66) - markers assessed at the time of initial diagnosis before any therapeutic intervention; during active treatment: 38% of patients (n=56) - markers assessed during chemotherapy cycles (usually between cycles or during mid-treatment evaluations); post-treatment follow-up: 17% of patients (n=26) - markers assessed during monitoring after completion of primary treatment.

STATISTICAL ANALYSIS

This study used a test of independence to evaluate the association between serum tumor markers and

Table 1. Demographic and epidemiological features of breast cancer patients

Feature	Frequency or Mean ± SE [%]
Gender	Male: 1.5; Female: 98.5
Age (years)	51.35 ± 1.06
Age groups	<50: 47.1; >50: 52.9
Diagnosis period	<5 years: 88.3; >5 years: 14.7
Marital status	Married: 79.6; Single: 4.4; Widow: 11.8; Divorced: 4.4
Education	Illiterate: 41.3; Primary: 25.2; Intermediate: 8.8; Secondary: 10.3; Diploma: 5.9; Higher diploma: 2.9; Bachelor: 5.9
Occupation	Not employed: 93.6; Employee: 6.5; Retired: 1.5
Residence	Rural: 44.3; Urban: 54.9

Source: Compiled by the authors of this study

Table 2. Cancer stage distribution among molecular subtypes

Cancer Stage	Luminal A [%]	Luminal B [%]	Triple-negative [%]	HER2 [%]	p-value
T2	55.6	54.5	33.3	0.0	0.05
T3	33.3	36.4	66.7	0.0	
T4	11.1	9.1	0.0	100.0	
Total	100.0	100.0	100.0	100.0	

Source: Compiled by the authors of this study

Table 3. Tumor grade distribution among molecular subtypes

Cancer grade	Luminal A [%]	Luminal B [%]	Triple-negative [%]	HER2 [%]	p-value
G1	0.0	14.3	0.0	25.0	0.429
G2	54.5	71.4	50.0	50.0	
G3	27.3	14.3	50.0	25.0	
G4	18.2	0.0	0.0	0.0	
Total	100.0	100.0	100.0	100.0	

Source: Compiled by the authors of this study

clinicopathological features. Based on international oncology guidelines, the following reference (cut-off) values were used:

- CA15-3 > 25 U/mL = Elevated
- CEA > 5 µg/L = Elevated

These cut-offs were incorporated into all statistical comparisons.

- Continuous variables (CEA and CA15-3) were analyzed using:
- ANOVA (comparison across ≥3 groups)
- Independent t-test (comparison across 2 groups)
- Categorical variables (elevated vs. non-elevated markers) were analyzed using:
- Chi-square test of independence
- Fisher's exact test when expected frequencies were <5
- Significance threshold: $p < 0.05$

Table 1 shows demographic and epidemiological features in the studied group of breast cancer patients. Most patients were female, married, and from urban areas.

Most patients were female, married, and from urban areas.

Table 2 shows cancer stage distribution among molecular subtypes. The result of the p-value (0.05) was from a Chi-square independence test, which showed that there was a significant statistical association between the different molecular subtypes and the TNM tumor stage at diagnosis.

HER2-positive tumors predominantly present at T4 stage, whereas triple-negative tumors were mainly at T3.

There was a significant difference in the distribution of cancer stages according to molecular subtypes ($p = 0.05$). The majority of HER2-positive tumors were diagnosed at T4 stage (100%), while the triple-negative were mainly at T3 (66.7%), and the Luminal subtypes had the highest frequency at T2 stage (Table3).

Table 3: Luminal subtypes mostly exhibited G2 grade, while triple-negative showed higher G3 frequency.

Luminal subtypes predominantly exhibited G2 grade, with Luminal B showing the highest proportion (72.2%), followed by Luminal A (55.2%). Triple-negative tumors

Table 4. Serum CEA and CA15-3 levels by cancer stage

Cancer stage	CEA [µg/L] Mean ± SE	CA15-3 [U/mL] Mean ± SE	p-value (CEA)	p-value (CA15-3)
T2	0.83 ± 0.33	16.26 ± 4.06	0.041	0.032
T3	2.05 ± 1.55	203.34 ± 171.09		
T4	3.50 ± 1.10	56.69 ± 35.55		

Source: Compiled by the authors of this study

Table 5. Serum CEA and CA15-3 levels by tumor grade

Tumor grade	CEA [µg/L] Mean ± SE	CA15-3 [U/mL] Mean ± SE	p-value (CEA)	p-value (CA15-3)
G1	0.50 ± 0.10	18.70 ± 5.12	0.021	0.044
G2	13.09 ± 11.27	99.39 ± 48.81		
G3	47.15 ± 44.32	51.32 ± 27.83		
G4	28.00 ± 14.50	45.00 ± 20.30		

Source: Compiled by the authors of this study

Table 6. Serum CEA and CA15-3 levels by molecular subtype

Subtype	CEA [µg/L] Mean ± SE	CA15-3 [U/mL] Mean ± SE	p-value (CEA)	p-value (CA15-3)
Luminal A	74.59 ± 6.43	78.51 ± 7.61	0.008	0.011
Luminal B	13.41 ± 11.20	131.85 ± 8.22		
Triple-negative	0.50	25.69		
HER2	2.05	21.84 ± 3.52		

Source: Compiled by the authors of this study

Table 7. Relationship of age with serum markers

Age group [years]	CEA [µg/L] mean ± SE	CA15-3 [U/mL] mean ± SE	p-value
<50	55.08 ± 43.69	144.56 ± 53.94	0.160
>50	6.41 ± 4.30	27.01 ± 5.25	0.010

*$p < 0.05$ considered statistically significant
Source: Compiled by the authors of this study

Table 8. Relationship of diagnosis period with serum markers

Diagnosis Period [years]	CEA [µg/L] Mean ± SE	CA15-3 [U/mL] Mean ± SE	p-value (CEA)	p-value (CA15-3)
<5	25.63 ± 17.39	78.40 ± 24.76	0.327	0.292
>5	1.16	10.37 ± 3.67		

Source: Compiled by the authors of this study

demonstrated a higher frequency of G3 grade (46.7%) compared to other subtypes. Grade 4 tumors were observed exclusively in the Luminal A subtype (17.9%). No statistically significant difference was found in grade distribution across molecular subtypes ($p = 0.429$) (Table 4).

Table 4: A statistically significant difference was found between cancer stages for both CEA and CA15-3, indicating that tumor markers increase proportionally with tumor advancement ($p < 0.05$ for both markers).

A statistically significant difference was found between cancer stages for both CEA and CA15-3, indicating that tumor markers increase proportionally with tumor advancement ($p < 0.05$ for both markers) (Table5).

Table 5: CEA levels showed statistically significant elevation with increasing tumor grade ($p = 0.021$). CA15-3 values differed significantly across grades ($p = 0.044$), with the highest levels in G2 tumors.

CEA levels showed statistically significant elevation with increasing tumor grade ($p = 0.021$). CA15-3 values differed significantly across grades ($p = 0.044$), with the highest levels in G2 tumors. Significant differences were detected between molecular subtypes for both CEA ($p = 0.008$) and CA15-3 ($p = 0.011$). Luminal A subtype had the highest CEA, while Luminal B subtype had the highest CA15-3 (Table 6).

Table 6: Significant differences were detected between molecular subtypes for both CEA ($p = 0.008$) and

CA15-3 (p = 0.011). Luminal A subtype had the highest CEA, while Luminal B subtype had the highest CA15-3.

At a statistically significant level (p=0.010), patients who were less than 50 years of age (<50 years) had very high CA15-3 levels as compared to the older patients (>50 years), on the other hand, the CEA levels did not show any age-related differences as such (p=0.160). This could imply different patterns of CA15-3 secretion or different tumor biology with respect to age (Table 7).

Table 7: Younger patients exhibited higher CA15-3 levels, with significant differences.

No statistically significant differences were found for either marker according to diagnosis duration (p > 0.05), although levels were higher among recently diagnosed patients (Table 8).

Table 8: No statistically significant differences were found for either marker according to diagnosis duration (p > 0.05), although levels were higher among recently diagnosed patients.

DISCUSSION

In this report, it is shown that the serum tumor markers CEA and CA 15-3 are closely related to the clinical and pathological characteristics of breast cancer patients. The demographic study indicated that the majority of the participants were women older than 50 which conforms to the worldwide epidemiological trends [1-3]. The analysis of tumor markers revealed the presence of higher levels of CEA and CA15-3 in patients with advanced tumor stages and grades thus corroborating with the previous studies that these markers indicate the burden and aggressiveness of the tumor [9, 6, 4, 2]. CA15-3 levels were particularly high among younger patients probably indicating age-related differences in tumor biology or immune response [5, 8, 13, 15]. The molecular subtypes distribution revealed Luminal A as the most prevalent one and the other subtypes as Luminal B, triple-negative, and HER2-enriched. The levels of the markers varied according to the subtype, with Luminal A being the highest CEA producer and Luminal B being the biggest CA15-3 producer, which agrees with earlier studies [12-14, 21-22]. Correlation analysis showed that CEA and CA15-3 could be used as non-invasive markers for the evaluation of tumor characteristics and the prediction of prognosis. Yet, the marker levels were not consistently high in all stages and subtypes, making it even more necessary to take both biomarkers and imaging into account [7, 15, 17, 20]. The current investigation points to the clinical value of serum tumor markers monitoring in parallel with molecular subtyping for the purpose of tailoring treatment strategies more precisely. The combination of CEA and CA15-3 might be a good tool for revealing aggressive tumor cases earlier, monitoring their recurrence, and being a decisive factor in therapy [10-11, 18-19]. This study demonstrated significant associations between serum tumor markers (CEA and CA15-3) and several clinicopathological characteristics of breast cancer patients. The incorporation of reference cut-off values (CEA >5 µg/L, CA15-3 >25 U/mL) allowed for the application of tests of independence, strengthening the statistical interpretation of disease correlations. The significant differences observed across tumor stages indicate that both markers increase proportionally with disease progression, consistent with global findings [6, 9, 12]. Tumor grade also showed statistically significant elevation of markers, particularly CEA (p=0.021) and CA15-3 (p=0.044), suggesting aggressive histopathological behavior reflected through serum biomarkers. Molecular subtypes displayed robust marker variability. Luminal A showed the highest CEA levels, while Luminal B had highest CA15-3, with both relationships being statistically significant (p < 0.05). These findings align with previous molecular biology studies identifying subtype-specific secretory behavior [12, 14, 22]. Such results bring the attention to the necessity of community-based studies since the Iraqi patients had different marker profiles according to their age, stage, and molecular subtype [3, 5, 16, 21].

CONCLUSIONS

The serum tumor marker CEA and CA15-3 are significant indicators for keeping track of the breast cancer process and they are associated with the tumor stage, grade, and molecular subtype. Serum tumor markers CEA and CA15-3 demonstrate significant associations with tumor stage, grade, and molecular subtype in breast cancer patients, serving as valuable indicators for disease monitoring and prognostication. However, routine and systematic measurement of these markers is not currently implemented in standard clinical practice in Iraq due to limited resources, lack of standardized protocols, and inconsistent laboratory availability. The integration of regular CEA and CA15-3 monitoring into routine clinical assessments is strongly recommended, as it could substantially improve early detection of disease progression, optimize treatment planning, and enhance prognostic accuracy for breast cancer patients in Iraq. Establishing evidence-based guidelines for the systematic use of these cost-effective biomarkers should be a priority for improving breast cancer care in resource-limited settings.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 12.12.2025
ACCEPTED: 27.04.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Healthcare-associated infections after maxillofacial and oral surgery procedures in patients with combat-related head and neck injuries in Ukraine: Results of a multicenter study (2022-2025)

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ABSTRACT

Aim: To estimate the prevalence and risk-factor of HAI after maxillofacial and oral surgery procedures in patients with combat-related head and neck injuries in Ukraine, and determine antimicrobial resistance of the responsible pathogens.

Materials and Methods: A multicenter prospective observational cohort study was conducted from June 2022 to December 2025. Definitions of HAIs were adapted from the CDC/NHSN. Antibiotic susceptibility test of bacteria was determined according to the EUCAST.

Results: Among 2,631 patients, 1,075 (40.9%) HAIs were observed. The most frequently reported HAIs were surgical site infections (54%), bone and joint infection (14.2%), pneumonia (12.2%), urinary tract infections (8.6%), bloodstream infections (6.6%), Eye, ear, nose, throat, or mouth infection (4.4%). Risk factor for HAIs were length of stay in ICU, type of injury, intubation, vascular and urinary catheters. The most pathogens isolated from HAIs were *Escherichia coli* (19.2%), *Pseudomonas aeruginosa* (7.6%), *Klebsiella pneumonia* (7.3%), *Enterococcus faecalis* (6.9%), *Acinetobacter baumannii* (6.6%), *Staphylococcus aureus* (5.5%), *Serratia marcescens* (5.5%). Meticillin resistance was reported in 36.1% of S. aureus, and 15.9% of enterococci were resistant to vancomycin. Resistance to third-generation cephalosporins was reported in 64.8% *K. pneumoniae* and in 43.6% *E. coli*.

Conclusions: This study found a high prevalence of HAIs in patients with combat-related head and neck injuries who has undergone maxillofacial or/and oral surgery procedures, caused by MDROs, varying depending on the bacterial species, and antimicrobial group.

KEYWORDS: military conflict, combat-related head and neck injuries, maxillofacial and oral surgery, healthcare-associated infections, risk factors, multidrug-resistant organisms, Ukraine

Wiad Lek. 2026;79(7):1529-1539. doi: 10.36740/WLek/225982 DOI

INTRODUCTION

The protracted military conflict in Eastern areas of Ukraine, beginning in February 2022, has strained the medical capacity of Ukraine's health system during a period of mass casualty and severe injury among both civilians and military combatants [1]. Combat-related in-

juries in Ukraine are characterized by a high prevalence of complex polytrauma driven by artillery, drones, and mines [2]. These often include bone and soft tissue injuries in head and neck, frequently resulting in multiple, simultaneous injuries rather than isolated trauma. [3].

Infectious complications following surgical treatment for patients with combat -related injuries and wounds are serious problem for health-care system of Ukraine. These infections reduce the effectiveness of treatment and increase the duration of hospitalization and the cost of treatment [3]. Despite of advances have been made in infection control practices and availability of antimicrobial prophylaxis; HAIs remain a substantial cause of morbidity [4]. According to published studies, pooled healthcare-associated infection (HAI) rates after maxillofacial and oral surgery procedures ranged from 0% to 62% depending on surgery type [5].

HAIs are one of the most common adverse events following surgical maxillofacial and oral procedures in patients with combat injuries and wounds. According to the literature, HAIs following surgical treatment (maxillofacial and oral procedures) of patients with combat injuries and wounds are high, with a significant 30.2% incidence in head and neck trauma cases [6]. These infections are commonly linked to mine-explosive (25.1%) and gunshot wounds (10.7%), with complications including cellulitis, abscesses, and deep space infections, requiring prompt antimicrobial intervention [6]. Despite the availability of new methods for diagnosing and treating patients, as well as the use of new technologies for infection control, the number of HAIs in patients with combat-related injuries and wounds is not decreasing.

The World Health Organization estimates that HAI caused by multi-drug-resistant organisms (MDROs) is the most frequently occurring adverse event for most country in world [7]. Many countries, including Ukraine have developed and implemented national strategies for the control and prevention of HAIs [8]. However, how this has affected the prevalence of HAIs in patients with combat-related injuries and wounds and the increasing emergence and spread of MDROs is unknown.

In Ukraine the incidence and prevalence of HAIs and antimicrobial resistance of the responsible pathogens in patients with combat-related injuries has rarely been published. A previous study has focused on the prevalence of HAIs among civilian populations and antimicrobial resistance of the responsible pathogens in Ukraine [4, 9]. These studies have found that contamination of environmental surfaces in hospital rooms serves as a reservoir for transmission of MDROs to patients, leading to colonization or/and infection [8, 10-12]. However, these results do not provide sufficient information about to the spread of the incidence of HAIs caused by MDROs in with combat injuries and wounds after maxillofacial and oral surgical procedures, which requires further study. Also, in literature, there is no study estimating the incidence rates of HAIs in patients with combat related head and neck injuries, as well as there is limited information on the AMR of the responsible pathogens and potential risk factors for HAIs associated with maxillofacial and oral surgery procedures.

AIM

The aim of this study was to estimate the prevalence and risk factor of HAI after maxillofacial and oral surgery procedures in patients with combat-related head and neck injuries in Ukraine, and determine antimicrobial resistance (AMR) of the responsible pathogens.

MATERIALS AND METHODS

DESIGN, SETTING AND PATIENTS

A multicenter prospective observational cohort study based on HAIs surveillance data was conducted from 28st June, 2022 to 31st December 2025. This study was performed in eight civilian hospitals which are located in six regions (Kharkiv, Dnipro, Kherson, Zaporizhzhia, Odessa, and Kyiv) of Ukraine. The median number of beds in each participating hospital was 650 beds, and the number of beds in intensive care units (ICUs) ranged from 10 to 12. Overall bed occupancy was 100% in each department of surgery and the ICUs. All participating hospitals are similar in terms of medical equipment, personnel and clinical microbiology laboratory with the capacity to process cultures. These hospitals had at least one full-time hospital epidemiologist or infection control professional and a data manager.

The study included patients with combat related head and neck injuries, aged 24-56 years who has undergone maxillofacial or/and oral surgery procedures and who stayed for more than 48 h in the surgical departments and ICUs, and survived at least 7 days after surgery. A total of 2,631 patients fulfilled the requirements of inclusion criteria were included in the study. Of which 2,584 (98.2%) were military combatants and 47 (1.8%) were civilians that has combat trauma or injured in military conflict area. Patients who passed away within 48 hours after the operation, maxillofacial or/and oral surgery procedures performed for wound infection complication and patients that had injuries and wounds in head and neck outside of the active war theaters were excluded.

DEFINITIONS

Specific HAI site definitions were adapted from the Centers for Disease Control and Prevention's (CDC's) 2008 National Healthcare Safety Network (NHSN) case definitions [13]. In this study an HAI cases was defined as an infection arising >48 h. after maxillofacial or/ and oral surgery and other medical procedures in hospital, and not present or incubating on admission, unless the patient had been discharged from hospital within a defined period. An incident HAI was defined by microbiologically confirmed CDC/NHSN HAI epidemiological case definitions. Institution of antibiotics by a physician without microbiological confirmation was not considered to be sufficient for diagnosis of an HAI because of widespread use of antimicrobial drugs for infection prevention in surgery and ICUs of hospitals in Ukraine [8]. Serologic and antigen test results were not included in case definitions. An HAI cases was deemed to be ICU-acquired where it occurred on or after the third calendar day in the ICU. Multi-drug resistance was defined in accordance with current published interim standard definitions [14]. Cases were described as community acquired if MDROs were isolated from patients on either of the first two days of hospital admission.

ETHICS

The study protocol complies with the ethical principles of the Helsinki Declaration and approval of the Ethics Committee of the Ukrainian Association of Infection Control and Antimicrobial Resistance (Protocol No. 3 dated 17.03.2022). Participants' data were anonymized prior to the analysis. According to the Act of Ukraine, surveys and evaluations that are intended to ensure that diagnosis and treatment produce the intended results do not require patient consent.

DATA COLLECTION

Surveillance data on all HAI cases, both in patients and their causative pathogens, were collected on a form specifically designed by the investigators using medical records comprising charts, daily flow sheets, and laboratory (microbiology) results. The collected data included demographics, Injury type/ethiology, time from the moment of injury to the first specialized medical help, surgical interventions on the previous evacuation stages, presence of a tracheostomy, trauma-associated complications, concomitant damage to other organs and systems, presence of concomitant somatic pathologies, type of concomitant somatic pathologies, harmful habits, location of soft tissue wounds of the head, burns, facial fractures, type of fractures (mandible body, mandible symphysis, mandible ramus, Le For I, Le For II, Le For III, zygoma, naso-orbito-ethmoid complex, orbit, nose, frontal sinus), dimensions of the soft tissue defect, dimensions of the bone defect, presence and the character of the foreign body, character of the dento-alveolar trauma, concomitant head and neck injuries, masticatory deficiency, visual functions, vascular and urinary catheter, endotracheal intubation, type of HAI, date of infection onset, clinical signs, administered antibiotics, isolated pathogens with antibiogram results, and outcome on discharge from the hospitals. The risk factors were evaluated from the time of admission until the onset of HAI.

MICROBIOLOGICAL METHODS

All sample isolates from HAI cases were sent to the local microbiology laboratory. Microbial isolates were identified and antimicrobial susceptibilities were determined using an automated microbiology system (Vitek-2 Compact; bioMe´rieux, Marcy l'Etoile, France). Antibiotic susceptibility testing of bacteria was determined according to the protocol of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://eucast.org). Multi-drug resistance (MDR) for *Acinetobacter* spp. and *Pseudomonas aeruginosa* was defined in accordance with published definitions, which were used in NHSN Antimicrobial Resistance (AMR) report [15]. MDR for *Klebsiella pneumoniae* and *Escherichia coli* was defined as being non-susceptible to at least one drug in three antimicrobial agents/groups [16].

STATISTICAL ANALYSIS

HAIs after maxillofacial and oral surgery procedures in patients with combat related head and neck injuries were analyzed as a binary exposure variable (no HAI, any HAI). Statistical data analysis was performed using Microsoft Excel for Windows (Microsoft Corp, Redmond, WA). Results are expressed as median (range), mean ± standard deviation for continuous variables, and the number with the corresponding percentage for qualitative variables. A descriptive analysis was performed by calculating the number and percentage for each value. Pearson's chi-square test was performed to check the matching performance between the case and comparison groups and compare the differences between groups for categorical variables. Multivariate analysis was carried out using stepwise logistic regressions to assess the predictive factors of the HAIs after maxillofacial and oral surgery procedures. In this study, statistical significance was defined as $P < 0.05$.

AI STATEMENT

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

RESULTS

PREVALENCE OF HAIS

During the study period (2022-2025), 1,075 of 2631 patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures were found to have HAIs. The prevalence of this patients with at least one HAI was 40.9% [95% confidence interval (CI) 39.9-41.9]. In this study ninety-seven percent of patients who has undergone maxillofacial or/and oral surgery procedures with an HAI were receiving at least one antimicrobial on the day of the survey. The most frequent types of HAI in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures were surgical site infections (SSIs) (54%), bone and joint infection (osteomyelitis) (14.2%), pneumonia (PNEU) (12.2%), Urinary tract infections (UTIs) (8.6%), bloodstream infections (BSIs) (6.6%), and eye, ear, nose, throat, or mouth (EENT) infection (4,4%) (Table 1).

CHARACTERISTICS OF COMBAT-RELATED HEAD AND NECK INJURIES

Among patients with HAI a mild combat-related head and neck injuries was diagnosed in 4.7% (114/2631) of the wounded, severe – in 71.8% (1746/2631), extremely severe – in 23.5% (571/2631). The cause of combat-related head and neck injuries in 85.6% (2133/2631) was mine-explosive injury (MIE), in 10.1% (217/2631) - gunshot wounds (GSW), in 4.3% (81/2631) - combined injury (MIE+GSW). Isolated head and neck injuries were diagnosed in only 9.2% of observations.

Most often, a combat-related injury to the head and neck was combined with injuries to the limbs (47.3%) and chest (54.8%). In the case of mine-explosive injury blind penetrating wounds of the head and neck (83.2%) significantly prevailed through (16.8%). In the case of gunshot bullet wounds in head and neck injuries, the specific gravity of through wounds (48.2%) was significantly higher than that of shrapnel wounds (18.3%). Facial fractures of the damaged organs of the maxillofacial area and oral (88.6%) significantly prevailed through (11.4%).

In the structure of the facial fractures, the fractures of the mandible were damaged in 48.5%, the mandible body - in 46.0%, the mandible symphysis - in 31.4%, the mandible ramus - in 26.3% of the wounded, the fractures of the midface - in 23, 5% of wounded, Le For I – in 17.1%, Le For II -in 18.3%, Le For III – in 19.6%, zygoma – in 18.8%, naso-orbito-ethmoid complex – in 16.5%, orbit – in 5.9%, nose - in 5,2%, and frontal sinus - in 4.9%.

PATIENT DEMOGRAPHICS AND RISK FACTORS FOR HAIS

Characteristics in patients with combat-related head and neck injuries and risk factors for HAI in patients are shown in Table 2.

The prevalence of HAIs was highest among patients admitted to ICUs with length of stay in ICU ≥5 days. The most common HAI types in the ICU were PNEU, UTIs and BSIs. Unsurprisingly, Surgical site infection, bone and joint infection (osteomyelitis), and Eye, ear, nose, throat, or mouth infection were the predominant HAI

Table 1. Distribution of 1,075 healthcare-associated infections (HAIs) after maxillofacial and oral surgery procedures among 2,631 patients with combat-related head and neck injuries in Ukraine, 2022-2025

Type of HAI	Total	
	n (%)	95% CI
Surgical site infection	581 (54.0)	53.0 - 55.0
Bone and joint infection	153 (14.2)	13.5 - 14.9
Pneumonia	131 (12.2)	11.7 - 12.8
Urinary tract infection	92 (8.6)	8.1 – 9.2
Bloodstream infection	71 (6.6)	6.1 – 7.1
Eye, ear, nose, throat, or mouth (EENT) infection*	47 (1.9)	4.0 – 4.8
Total	1,075 (100.0)	39.9 – 41.9

CI, confidence interval.

* EENT infection including: conjunctivitis (14 cases), mastoid infection (14 cases), eye infection, other than conjunctivitis (3 cases), sinusitis (5 cases), oral cavity infection (3 cases), upper respiratory tract infection (3 cases), pharyngitis (4 cases), laryngitis (2 cases), and epiglottitis (1 cases).

Source: compiled by the authors of this study

Table 2. Characteristics and risk factors for healthcare-associated infections (HAIs) (n=1.075) in patients (2,631) with combat-related head and neck injuries in Ukraine, 2022-2025

Variable	All patients n	HAI				P-value
		Yes		No		
		n	%	n	%	
Age (full years at the time of injury)						0.24
20-25	388	268	69.1	120	30.9	
26-30	311	129	41.5	182	58.5	
31-35	619	201	32.5	418	67.5	
36-40	311	136	43.7	193	56.3	
41-45	310	98	31.6	212	68.4	
46-50	386	112	29.0	274	71.0	
51-55	231	118	51.1	113	48.9	
≥56	75	13	17.3	62	82.7	
Social status						0.31
civilian	47	17	36.2	30	63.8	
military	2.584	1.058	40.9	1.526	59.1	
Harmful habits						0.074
smoking	2.417	718	29.7	1.699	70.3	
alcohol	136	43	31.6	93	68.4	
Type of injury						<0.001
Mine-Explosive Injury (MIE)	2.133	673	31.6	1.460	68.4	
Gunshot wound (GSW)	217	25	11.5	192	88.5	
Combined injury (MIE+GSW)	81	14	17.3	67	82.7	
Location of soft tissue wounds of the head						0.04
oral/perioral auricular	1.873	621	33.2	1.252	66.8	
scalp	437	47	10.8	390	89.2	
periorbital	95	8	8.4	87	91.6	
perinasal	112	9	8.0	103	92.0	
other	47	3	6.4	44	93.6	
Time from the moment of injury to the first specialized medical help (hours)						<0.001
less then 24 h.	1.109	176	15.9	933	84.1	
24-48 h.	798	341	42.7	457	57.3	
>48 h.	724	558	77.1	166	22.9	
Length of stay in ICU >5 (days)	574	447	77.9	127	22.1	<0.001
Intubation within 48 h before onset	242	131	71.2	111	28.8	0.002
Central venous catheter within 48 h before onset	102	66	64.7	36	35.3	0.05
Peripheral vascular catheter within 48 h before onset	9	3	33.3	6	66.7	0.19
Urinary catheter within 7 days before onset	93	86	92.5	7	7.5	0.001

Source: compiled by the authors of this study

type in surgery departments.

In this study patient risk factors for HAIs included 2,631 patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures. All risk factors were significantly associated with the prevalence of HAIs (P<0.001 after adjustment for all factors in the model) in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures. Also, 76.5% risk factor sub-levels included in the model were significantly associated at the P<0.001 level, while five risk factor levels were included for consistency although they were signifi-

Table 3. Distribution of micro-organisms (n=2,794) causing HAIs isolated from patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures in Ukraine, 2022-2025

Microorganism	n	%
Gram-positive cocci	**648**	**23.2**
Staphylococcus aureus	154	5.5
Coagulase-negative staphylococci (CoNS)	128	4.6
Enterococcus faecalis	192	6.9
Enterococcus faecium	93	3.3
Streptococcus pneumoniae	69	2.5
Other Gram-positive cocci	12	0.4
Gram-negative bacilli	**1.990**	**71.2**
Escherichia coli	536	19.2
Enterobacter cloacae	127	4.5
Enterobacter aerogenes	88	3.1
Citrobacter spp.	28	1.1
Klebsiella pneumonia	203	7.3
Klebsiella oxytoca	81	2.9
Proteus vulgaris	37	1.3
Proteus mirabilis	72	2.6
Proteus morganii	23	0.8
Providencia rettgeri (Proteus rettgeri)	42	1.5
Serratia marcescens	153	5.5
Acinetobacter lwoffii	93	3.3
Acinetobacter baumannii	184	6.6
Pseudomonas aeruginosa	211	7.6
Stenotrophomonas maltophilia	112	4.1
Fungi	**156**	**5.6**
Candida spp.	125	4.5
Aspergillus spp.	24	0.9
Other fungi	7	0.3
Total	**2.794**	**100.0**

Source: compiled by the authors of this study

cantly associated at the P<0.05 level (type of fractures, location of soft tissue wounds of the head, and central venous catheter within 48 h before onset). The strongest independent associations for HAIs in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures were observed for length of stay in ICU ≥5 days before HAI onset, mine-explosive injury (MIE), time from the moment of injury to the first specialized medical help and the presence of intubation within >48 h before onset and urinary catheters within 7 days before onset (before the onset of pneumonia and UTIs, respectively).

RESPONSIBLE PATHOGENS OF HAIS AND ANTIMICROBIAL RESISTANCE

In this study 2,794 specimens were isolated from 1,075 patients with HAIs. In the structure of the responsible pathogens of HAIs the Gram-positive cocci were damaged in 24.9%, Gram-negative bacilli - in 78.3%, and fungi - in 5.8%/ Overall, predominated *E. coli* (19.2%), *Pseudomonas aeruginosa* (7.6%), *Klebsiella pneumonia* (7.3%), *Enterococcus faecalis* (6.9%), *Acinetobacter baumannii* (6.6%), *Staphylococcus aureus* (5.5%), *Serratia marcescens* (5.5%), followed by Coagulase-negative staphylococci (CoNS) (4.6%), *Enterobacter cloacae* (4.5%), *Candida* spp. (4,5%), *Stenotrophomonas maltophilia* (4.1%), *Enterococcus faecium* (3.3%), *Acinetobacter lwoffii* (3.3%), *Enterobacter aerogenes* (3.1%), and other pathogens (Table 3).

Gram-positive bacteria were the most common causes of SSIs, bone and joint infection (osteomyelitis), eye, ear, nose, throat, or mouth infection (EENT) and BSIs, and Gram-negative bacteria were the most common causes of respiratory tract infections (pneumonia),

Table 4. Distribution of micro-organisms causing HAIs isolated from patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures, and AMR phenotype, by bacterial species and antimicrobial group/agent, Ukraine, 2022-2025

Bacterial species	Antimicrobial group/agent	AMR, %
Escherichia coli	Aminopenicillin (amoxicillin/ampicillin) resistance	64.7
	Third-generation cephalosporin (cefotaxime/ceftriaxone/ceftazidime) resistance	43.6
	Carbapenem (imipenem/meropenem) resistance	14.7
	Fluoroquinolone (ciprofloxacin/levofloxacin/ofloxacin) resistance	40.1
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	24.1
	Combined resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides	11.2
Klebsiella pneumoniae	Third-generation cephalosporin (cefotaxime/ceftriaxone/ceftazidime) resistance	64.8
	Carbapenem (imipenem/meropenem) resistance	49.8
	Fluoroquinolone (ciprofloxacin/levofloxacin/ofloxacin) resistance	66.5
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	61.7
	Combined resistance to fluoroquinolones, third-generation cephalosporins, and aminoglycosides	55.2
Pseudomonas aeruginosa	Piperacillin-tazobactam resistance	47.3
	Ceftazidime resistance	45.6
	Carbapenem (imipenem/meropenem) resistance	59.8
	Fluoroquinolone (ciprofloxacin/levofloxacin) resistance	53,3
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	51.1
	Combined resistance to ≥3 antimicrobial groups (among piperacillin-tazobactam, ceftazidime, carbapenems, fluoroquinolones, and aminoglycosides)	48.4
Acinetobacter baumannii	Carbapenem (imipenem/meropenem) resistance	65.8
	Fluoroquinolone (ciprofloxacin/levofloxacin) resistance	71.1
	Aminoglycoside (gentamicin/netilmicin/tobramycin) resistance	69.8
	Combined resistance to carbapenems, fluoroquinolones, and aminoglycosidesc	65.3
Staphylococcus aureus	Meticillin-resistant S. aureus (MRSA)	35.1
Streptococcus pneumoniae	Penicillin non-wild-type	6.7
	Macrolide (azithromycin/clarithromycin/erythromycin) resistance	5.9
	Combined penicillin non-wild-type and resistance to macrolidesc	3.7
Enterococcus faecalis	High-level gentamicin resistance	49.1
Enterococcus faecium	Vancomycin resistance	15.9

Source: compiled by the authors of this study

UTIs and SSIs.

Antimicrobial susceptibility testing data were available on the day of the survey for 97.1% of Gram-positive and Gram-negative bacteria as causing HAIs. Distribution of micro-organisms causing HAIs isolated from patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures, and AMR phenotype, by bacterial species and antimicrobial group/agent, Ukraine, 2022-2025 are shown in Table 4.

DISCUSSION

The results presented in this study are based on multicentre prospective surveillance data for HAI in patients with combat-related head and neck injuries in Ukraine and antimicrobial resistance of responsible pathogens these infections. This study expands upon the previous reports [6, 8, 9, 17] and is the first study to publish frequent pathogens and characterization of the phenotypic mechanisms of responsible pathogens of HAIs isolated from patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures in Ukraine.

This survey identified a high prevalence of HAI (40.9%) among patients with combat related head and neck injuries, who has undergone maxillofacial or/and oral surgery procedures. A previous study in Ukraine found that prevalence of HAI rate among patients with com-

bat-related injuries and wounds were from 30.2% [6] to 68.6% [17]. No data in the literature on the prevalence of HAIs among patients with combat injuries and wounds in other countries.

In this study the most frequent types of HAI in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures were SSIs (54%), bone and joint infection (osteomyelitis) (14.2%), PNEU (12.2%), UTIs (8.6%), BSIs (6.6%), and EENT infection (4,4%). The prevalence of HAI among patients with combat-related head and neck injuries varied widely in regions of Ukraine. A previous study in Ukraine found that of the total cases of HAIs in patients with combat-related injuries and wounds, 27.3% were SSI, 25.6% bone and joint (BJ) infections, 15.7% skin and soft tissue infections (SST), 9.7% BSI, 7.9% central nervous system infections (CNS), 5.3% PNEU, 4.1% UTI, 1.7% EENT infections [17]. Our results are consistent with those of previous studies [6, 17].

In this study among patients with HAIs a mild combat-related head and neck injuries was diagnosed in 4.7% of the wounded, severe – in 71.8%, extremely severe – in 23.5%. The cause of combat-related head and neck injuries in 85.6% was mine-explosive injury (MIE), in 10.1% - gunshot wounds (GSW), in 4.3% - combined injury (MIE+GSW). Isolated head and neck injuries among patients with HAIs were diagnosed in only 9.2% of observations. A previous study in Ukraine found that among patients with HAIs a mild combat injury was diagnosed in 15.5% of the wounded, severe – in 72.4%, extremely severe – in 12.1% [17]. Most often, a combat-related injury to the head and neck was combined with injuries to the limbs (47.3%) and chest (54.8%). In the case of mine-explosive injury blind penetrating wounds of the head and neck (83.2%) significantly prevailed through (16.8%). In the case of gunshot bullet wounds in head and neck injuries, the specific gravity of through wounds (48.2%) was significantly higher than that of shrapnel wounds (18.3%). Facial fractures of the damaged organs of the maxillofacial area and oral (88.6%) significantly prevailed through (11.4%).

In this study all risk factors were significantly associated with the prevalence of HAIs in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures. The strongest independent associations for HAIs in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures were observed for length of stay in ICU ≥5 days before HAI onset, mine-explosive injury (MIE), time from the moment of injury to the first specialized medical help and the presence of intubation within >48 h before onset and urinary catheters within 7 days before onset (before the onset of pneumonia and UTIs, respectively). Also, while five risk factor levels were significantly associated at the P<0.03 - 0.05 level (type of fractures, location of soft tissue wounds of the head, and central venous catheter within 48 h before onset).

In the structure of the responsible pathogens of HAIs the Gram-positive cocci were damaged in 24.9%, Gram-negative bacilli - in 78.3%, and fungi - in 5.8%/ Overall, predominated *E. coli*, *P. aeruginosa*, *K. pneumonia*, *E. faecalis*, *A. baumannii*, *S. aureus*, and *S. marcescens*. In this study, Gram-negative bacteria (GNB) were the most common pathogens of HAIs, in agreement with several studies in the USA [18], Europe [19], Mexico [20], Brazil [21], China [22], Singapore [23], and Turkey [24].

Antimicrobial susceptibility testing data were available on the day of the survey for all of micro-organisms reported as causing HAIs in patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures. Of the all isolates antibiotic susceptibility tested, 31.7% were found to be multidrug-resistant organisms (MDROs). In this study meticillin resistance was found in 35.1% of *S. aureus* (MRSA) isolates, and vancomycin resistance was found in 15.9% of enterococci. Non-susceptibility to third-generation cephalosporins was detected in 64.8% of *K. pneumoniae* and in 43.6% *E. coli*. Carbapenem resistance was found in 59.8%, 65.8%, 14.7% and 49.8% of *P. aeruginosa* and *A. baumannii* isolates, *E. coli*, and *K. pneumoniae,* respectively. The high rates of resistance to multiple antibiotics found in this study are of concern. According to the literature, there has been an increase in antibiotic-resistant pathogens of HAIs since the start of the Eastern Ukraine military conflict [17]. This was also observed in conflicts in Afghanistan and Iraq [25, 26], Syria [27], Congo [28], and Gaza [29].

STRENGTHS AND LIMITATION

One strength of the present study was that it was a prospective multicentre observational cohort study, based on surveillance data of HAIs in patients with combat-related injuries. Also, this was the first study to publish frequent pathogens and characterization of the phenotypic mechanisms of responsible pathogens of HAIs isolated from patients with combat-related head and neck injuries after maxillofacial and oral surgery procedures in Ukraine. The limitations of this study include in conduct only a civilian hospital of Ukraine. Therefore, the results may not be representative of military hospitals with different distributions of HAI cases in patients with combat-related head and neck injuries and antimicrobial resistance of responsible pathogens

of HAIs after maxillofacial and oral surgery procedures.

CONCLUSIONS

This study found a high prevalence of HAIs in patients with combat-related head and neck injuries who has undergone maxillofacial or/and oral surgery procedures caused by MDROs, varying depending on the bacterial species, and antimicrobial group. The incidence of HAIs after maxillofacial and oral surgery in these patients varying widely depending on risk factors such as length of stay in ICU ≥5 days before HAI onset, mine-explosive injury, time from the moment of injury to the first specialized medical help and the presence of intubation within >48 h before onset, urinary catheters within 7 days before onset, AMR of the responsible pathogens, location of soft tissue wounds of the head, and central venous catheter within 48 h before onset, and surgical approach. These findings highlight the need for targeted prevention strategies to mitigate these risks and implement advanced infection control measures based on HAI and AMR surveillance data. Additionally, perioperative management strategies, particularly the use of prophylactic antibiotics, play a crucial role in HAIs prevention in patients with combat-related head and neck injuries who has undergone maxillofacial or/and oral surgery procedures.

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The authors would like to thank all the nurses and physicians who contributed to the prevalence surveys, and the infection control teams in all of the participating hospitals for their cooperation and support. The findings and conclusions in this study are those of the authors.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 09.03.2026
ACCEPTED: 30.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Clinical and epidemiological characteristics and risk factors for the development of recurrent respiratory diseases in children

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ABSTRACT

Aim: To investigate the influence of risk factors on the development of recurrent respiratory diseases, to analyze clinical and epidemiological components for the possibility of their prevention.

Materials and Methods: The research was conducted on the basis of the Uzhhorod Children Primary School. Children studied groups (n=118) aged 6–9 years with a diagnosis of "acute recurrent respiratory diseases" were formed.

Results: Childrens inflammatory episodes were most often observed, in particular: bacterial tonsillitis – 86 (72,89%), otitis – 56 (47,46%), pneumonia and sinusitis – 37 (31,36%) (identical indicators). In our study, respiratory infection caused by at least one pathogen was observed in 63,4±4,1% of patients. The proportion of viral infections was 48,34±3,6%, in particular, coinfections were identified in 36,4±2,9% of cases. The distribution of confirmed viruses is as follows: AdV (26,34±1,7%), SARS-CoV-2 (19,5±1,6%), RV (18,2%), RSV (17,8%), influenza A/B (11,4%) and others in minimal structural components (6.8%).

Conclusions: Recurrent respiratory tract infection is a multifactorial disease caused by a complex interaction of environmental and host factors. We suggest that an "additive effect," depending on the number of factors involved, most of which may be underestimated, may be a plausible explanation for the recurrence of respiratory tract infection in a significant proportion of healthy children with unexplained frequent respiratory symptoms.

KEYWORDS: recurrent respiratory diseases, acute respiratory viral infections, COVID-19, clinical and epidemiological characteristics, risk factors, children

Wiad Lek. 2026;79(7):1540-1548. doi: 10.36740/WLek/225983 DOI

INTRODUCTION

There is currently no generally accepted consensus on the definition of recurrent respiratory diseases in children. When counting the number of episodes of respiratory diseases (to determine the recurrent nature), it is important to take into account their severity, duration and localization of the pathological process. Some authors propose the following definition of recurrent respiratory diseases: 8 or more respiratory diseases during the year in children under three years of age, and 6 or more in children over three years of age, with the prerequisite being the absence of any pathological conditions that could cause recurrent diseases [1, 2].

Some authors claim that the following number of mild cases of respiratory infectious diseases can be considered normal: in children under 4 years old – 11 episodes per year; in preschoolers (3–5 years old) – 8; in schoolchildren (6–12 years old) – 4 [3]. Other authors believe that 6–10 self-limiting viral infectious diseases, lasting 1–2 weeks, during the year are the norm [4].

Infectious rhinitis is considered recurrent if more than five episodes are recorded within a year; pharyngitis or tonsillitis – more than three episodes; otitis media – three episodes within six months, or four episodes within a year. Bronchitis and pneumonia are defined as recurrent if more than three episodes occur per year [5].

The most significant etiological agents of severe lower respiratory tract infections are bacteria such as Streptococcus pneumoniae and Haemophilus influenzae, as well as viruses such as SARS-CoV-2, influenza virus, and respiratory syncytial virus (RSV) [6]. A study conducted in Bulgaria during the COVID-19 pandemic showed that Haemophilus influenzae and RSV were the most common co-pathogens in patients testing positive for SARS-CoV-2 [5]. Our previous studies also found that the presence of an additional viral or bacterial co-pathogen is a risk factor for developing severe COVID-19. Respiratory infections are the most common reason for primary care visits among children. An increase in the number of episodes of recurrent infections is observed with the beginning of attending children's groups [6].

Acute respiratory viral infections are a global health problem, as they are the leading cause of morbidity and mortality in children worldwide [7, 8]. Respiratory infections are the most common pathology between the ages of 6 months and 6 years. There are two peaks in the incidence of recurrent respiratory infections in children. The first is observed between the ages of 6 months and one year. The reason is a decrease in the level of immunoglobulins inherited from the mother and the still insufficient production of its own immunoglobulins. The second – with the beginning of attending children's groups [9, 10].

Immunological immaturity is considered one of the main causes of recurrent respiratory infectious diseases in children. The development of the immune system in childhood occurs gradually. In the prenatal period, the immune system must be tolerant to maternal antigens. A baby is born with an immature immune system that gradually matures. Early protection against infections is provided by maternal antibodies, which are transmitted transplacentally and in milk. After breastfeeding ends, children become more susceptible to infections, but at this time the innate and adaptive immune systems are already better developed. Vaccination provides additional protection. As we age, the immune system's defenses increase, and young adults become sick much less frequently [11, 12].

AIM

The aim of the study was to investigate the influence of risk factors on the development of recurrent respiratory diseases, to analyze clinical and epidemiological components for the possibility of their prevention.

MATERIALS AND METHODS

The research was conducted on the basis of the Uzhhorod Primary School "Yalynka" of the Uzhhorod City Council of the Transcarpathian Region and the Municipal Non-Profit Enterprise "Transcarpathian Regional Children's Sanatorium «Malyatko»" of the Transcarpathian Regional Council. Three groups of children diagnosed with «acute recurrent respiratory diseases» were formed: group 1 – treatment with optimized therapy (n=62), group 2 – treatment with basic therapy (n=56). For comparative characteristics of the obtained data, group 3 was created – a control group of children identical in age and sex, without subjective and objective signs of acute recurrent respiratory diseases (n=26). The group of studied children (n=118) was characterized by the following parameters: age – 7,48 ± 0,68 years (min – 6 years; max – 9 years), weight – 28,41 ± 6,65 kg (min – 17,80 kg; max – 49,5 kg), height – 131,42 ± 8,22 cm (min – 114,5 cm; max – 156,5 cm). The diagnosis of recurrent respiratory tract infection is established if at least one of the following criteria is met: six or more episodes of acute respiratory infections per year, one or more cases of upper respiratory tract infection per month from September to April, or three or more episodes of lower respiratory tract infections per year [13, 14, 15]. All studies performed and treatment methods applied were carried out with the voluntary written consent of the patients, in compliance with all requirements for ensuring the anonymity and confidentiality of the results obtained. The methodology of scientific and practical monitoring was carried out in accordance with the criteria of the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the norms of the Council of Europe Convention on Human Rights and Biomedicine, as well as the current norms of Ukrainian legislation and the requirements of the local medical commission of the State Higher Educational Institution «Uzhhorod National University».

RESULTS

Respiratory tract infections (RTIs) are a common reason for specialist consultations and medication prescriptions [1]. RTIs are mostly caused by viruses and are usually self-limiting. It is estimated that a quarter of preschool children experience RTIs recurrences. Social factors such as kindergarten attendance, smoking, family size, sibling contact with kindergarten, and environmental pollution play an important role in the occurrence of RTIs in many children [16].

In the subgroup of children with recurrent RTIs, characterized by severe and frequent symptoms, primary immune defects, local anatomical factors, and genetic abnormalities play a leading role. It has been hypothesized that in children with recurrent RTIs, transient immunosuppression caused by the virus may predispose them to subsequent RTIs, creating a "vicious cycle" [16]. However, this hypothesis has never been proven. Other considerations, such as genetic susceptibility to infection, low levels of certain trace elements, and respiratory microbiota, may explain why some children develop RTIs and others do not. According to our research, an analysis of many factors that may have an impact on the development of pathology was conducted. In particular, among the children from the studied contingent, the consumption of vegetables and fruits in the diet was observed in 103 (87,29%) children, only fruits were consumed by 13 (11,02%) children, and neither fruits nor vegetables were consumed by 2 (1,69%) children. 114 (96,61%) children attended preschool education-

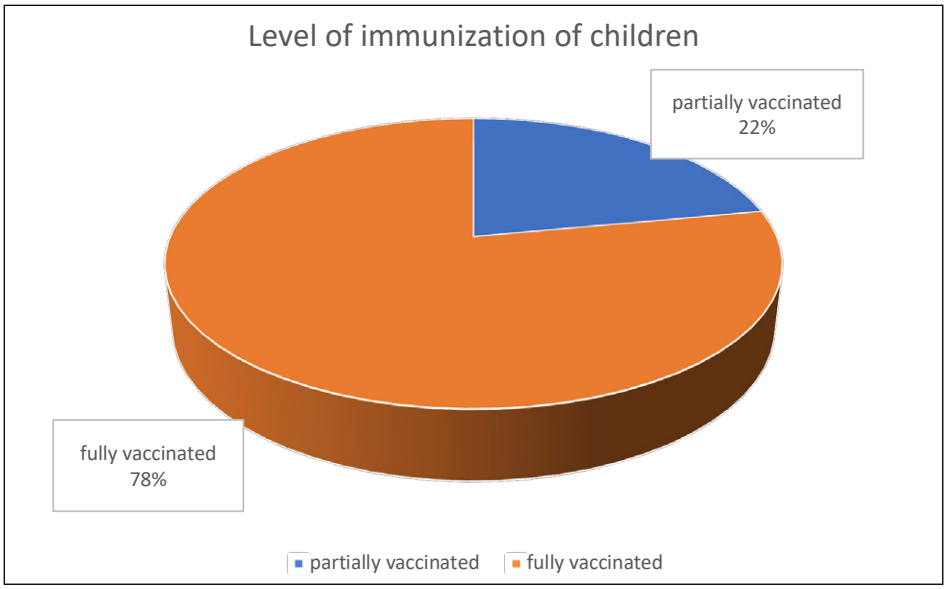

Fig. 1. Level of immunization of children
Picture taken by the authors

al institutions before starting primary school, and 4 (3,39%) children did not attend. In the autumn-winter period, children spent 101,61 ± 49,13 minutes outdoors (min – 30 minutes; max – 180 minutes). Sports activities were noted in 83 (70,34%) children, and the absence of sports activities was noted in 35 (29,66%) children. Iatrogenic factors, including the inappropriate use of antibiotics, nonsteroidal anti-inflammatory drugs, or glucocorticoids, can also contribute to the development of respiratory pathology. According to our data, the use of antibiotics was most often observed after 2 years of age – in 47 (39,83%) children, in the period from 12 to 24 months – in 32 (27,12%) children, from 6 to 12 months – in 14 (11,86%) children, and up to 6 months – in 25 (21,19%) children. Since 2013, the American Academy of Pediatrics (AAP) has encouraged the rational use of antibiotics for uncomplicated pediatric RTIs. However, while overall antibiotic prescribing rates for children have declined, unnecessary, overuse of antibiotics remains a devastating problem worldwide, leading to antibiotic resistance and contributing to rising healthcare costs and increased risk of side effects. An interesting report by Margolis et al. found a twofold increased risk of developing uncomplicated RTI in individuals who had received antibiotics for acne treatment within the previous 12 months. The intrinsic immunomodulatory effect of antibiotics has long been proven. Thus, overuse of antibiotics may impair the body's natural resistance to respiratory infections in several ways: by disrupting the respiratory microbiota and interfering with the innate immune response against viruses and bacteria [1, 17-20].

The study of the functional state of the respiratory tract revealed the following characteristics: chest circumference (inhalation) – 63,89 ± 2,89 cm (min – 53 cm; max – 78 cm); chest circumference (exhalation) – 61,49 ± 5,25 cm (min – 51 cm; max – 76 cm); chest circumference (pause) – 62,61 ± 5,18 cm (min – 52 cm; max – 77 cm); chest circumference (excursion) – 2,41 ± 0,81 cm (min – 2 cm; max – 4 cm).

Neonatal data are important. The following was found. Birth weight was 3357,03 ± 541,96 g (min – 2040 g; max – 4600 g). Duration of breastfeeding was, in particular:

- less than three months – 22 (18,64%) children;
- duration from 3 to 12 months – 36 (30,51%) children;
- duration 12 months and more – 25 (21,19%) children;
- duration up to two years – 33 (27,97%) children;
- artificial feeding – 2 (1,69%) children.

Therefore, the most common duration of breastfeeding was up to the age of three to twelve months (36 (30,51%) children), as well as up to two years (33 (27,97%) children). Despite the fact that among the children in the study group, artificial feeding occurred in only two children (1,69%), in a significant part of the children - 83 (70,34%), breastfeeding was completed by the age of twelve months. The average sleep duration of children was 8,71 ± 0,72 hours (min – 7 hours; max – 10 hours).

The Ruffier index characterizes the functional state of the cardiovascular system. According to our data, the following indicators ware observed:

- september 2021 – 8,15 ± 2,05 (min – 4; max – 15,2);
- september 2022 – 8,13 ± 2,11 (min – 4; max – 16,2);
- september 2023 – 8,23 ± 1,69 (min – 5,6; max – 12,8).

Number of days of illness with acute respiratory viral infections during the academic year:

- 2021–2022 – 8,85 ± 7,46 days;
- 2022–2023 – 20,64 ± 15,79 days;
- 2023–2024 – 11,28 ± 7,46 days.

Did children take vitamin D_3 in the autumn-winter period?
• yes – 48 (40,68%);
• no – 70 (59,32%).

We analyzed the level of immunization of the studied children (Fig. 1):
• fully – 92 (77,97%);
• partially – 26 (22,03%).

Let us consider the structural components of vaccination in children from the study group.
–Vaccination against tuberculosis:
• yes – 112 (94,92%);
• no – 6 (5,08%).
–Vaccination against diphtheria, pertussis, tetanus (vaccination 1):
• yes – 107 (90,68%);
• no – 11 (9,32%).
–Vaccination against diphtheria, pertussis, tetanus (vaccination 2):
• yes – 106 (89,83%);
• no – 12 (10,17%).
–Vaccination against diphtheria, pertussis, tetanus (vaccination 3):
• yes – 98 (83,05%);
• no – 20 (16,95%).
–Vaccination against diphtheria, pertussis, tetanus (revaccination 1):
• yes – 80 (67,79%);
• no – 38 (32,21%).
–Vaccination against diphtheria, tetanus (revaccination 2):
• yes – 34 (28,81%);
• no – 84 (71,19%).
–Vaccination against polio (vaccination 1):
• yes – 116 (98,31%);
• no – 2 (1,69%).
–Vaccination against polio (vaccination 2):
• yes – 113 (95,76%);
• no – 5 (4,24%).
–Vaccination against polio (vaccination 3):
• yes – 110 (93,22%);
• no – 8 (6,78%).
–Vaccination against polio (revaccination 1):
• yes – 109 (92,37%);
• no – 9 (7,63%).
–Vaccination against polio (revaccination 2):
• yes – 95 (80,51%);
• no – 23 (19,49%).
–Vaccination against measles, mumps, rubella (vaccination 1):
• yes – 117 (99,15%);
• no – 1 (0,85%).
–Vaccination against measles, mumps, rubella (vaccination 2):
• yes – 79 (66,95%);
• no – 39 (33,05%).

The vaccination rate in children is shown in Fig. 2.

The highest vaccination rate, according to our data, was for vaccination against measles, mumps, and rubella (vaccination 1) – 99,15%, and the lowest level was observed for vaccination against diphtheria, tetanus (revaccination 2) – 28,81%.

Environmental factors play a key role in the etiopathogenesis of respiratory infections. The most common environmental factors include biota diversity, winter season, overcrowding, nighttime crowding, household size (the smaller the house, the higher the risk), attendance at daycare, and attendance at daycare by siblings aged one to three years.

Of course, acute respiratory infections are also associated with maternal smoking during pregnancy and passive smoking. Exposure to harmful factors in children has been associated with several respiratory diseases, including lower respiratory tract infections, wheezing, and respiratory symptoms in the first year of life. This connection has been proven to be stronger in those whose mothers smoke heavily (more than one pack of cigarettes per day).

Among these environmental factors that increase the risk of wheezing and RTIs, several studies have shown that aerosolized cleaning chemicals (such as bleach) act as irritants, damaging the airway epithelium, maintaining inflammatory processes, and promoting infection by local flora [17, 18, 21-23].

We also examined the components of the respiratory tract disease group. Below are the results of the questionnaire and anamnestic data:
–Has the child had any cases of pneumonia?
• yes – 37 (31,36%)
–1 time in a lifetime – 34
–2 times in a lifetime – 1
–3 times in a lifetime – 2
• no – 81 (68,64%)
–Has the child had any cases of otitis media?
• yes – 56 (47,46%)
–1 time in a lifetime – 36
–2 times in a lifetime – 10
–3 times in a lifetime – 2
–4 times in a lifetime – 6
• no – 62 (52,54%)
–Has the child had any cases of acute bronchitis?
• yes – 37 (31,36%)
–1 time in a lifetime – 21
–2 times in a lifetime – 9
–3 times in a lifetime – 1
–4 times in a lifetime – 0
–5 times in a lifetime – 6

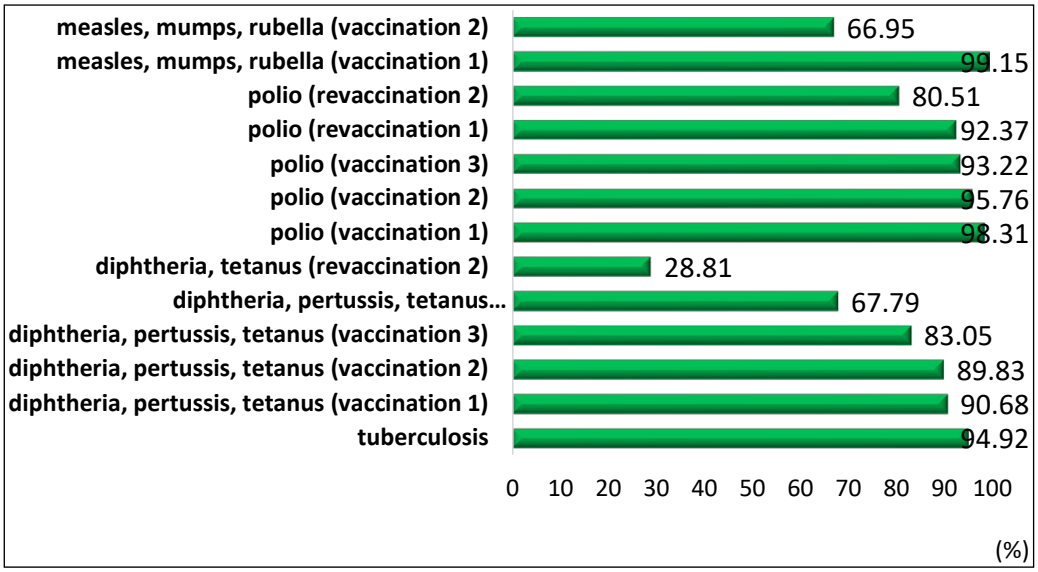

Fig. 2. Vaccination rate
Picture taken by the authors

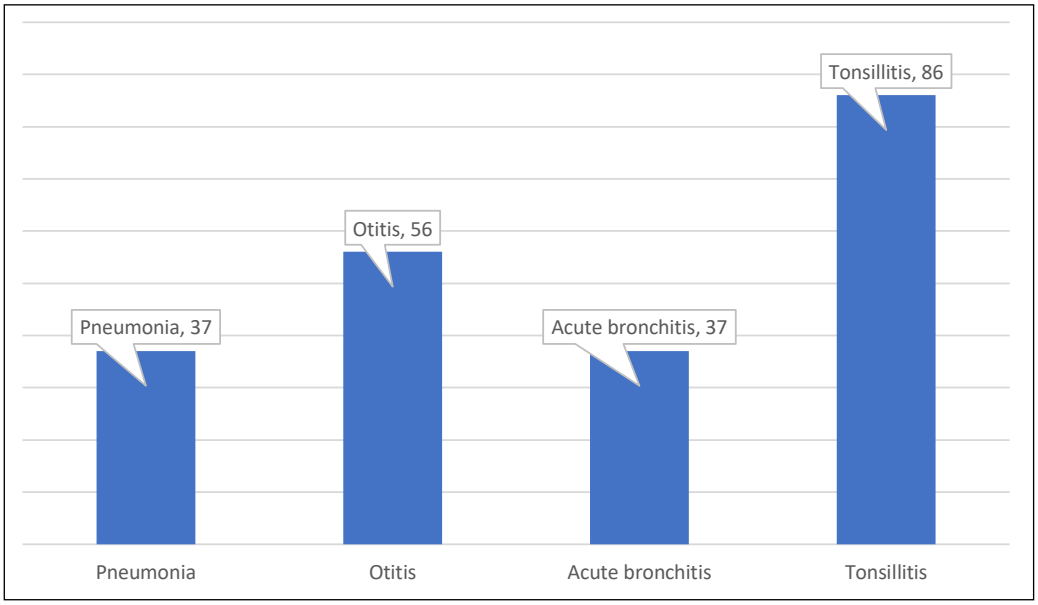

Fig. 3. Cases of recorded diseases during life
Picture taken by the authors

• no – 81 (68,64%)
–Has the child had any cases of tonsillitis?
• yes – 86 (72,89%)
–1 time in a lifetime – 32
–2 times in a lifetime – 30
–3 times in a lifetime – 14
–4 times in a lifetime – 3
–5 times in a lifetime – 7
• no – 32 (27,11%)

We present a graphical representation of the obtained data (Fig. 3).

So, the most frequent cases were acute tonsillitis (86 (72,89%)), otitis media (56 (47,46%)), and pneumonia and acute bronchitis with the same frequency – 37 (31,36%). We considered the data excluding children with a history of pneumonia. In our study, respiratory infection caused by at least one pathogen was observed in 63,4±4,1% of patients. The proportion of viral infections was 48,34±3,6%, in particular, coinfections were identified in 364±2,9% of cases.

The distribution of confirmed viruses is as follows: AdV (26,34±1,7%), SARS-CoV-2 (19,5±1,6%), RV (18,2%), RSV (17,8%), influenza A/B (11,4%) and others in minimal structural components (6,8%). It is important to note that atopy is an important additional risk factor for recurrent RTIs as it likely underlies immune dysfunction. Atopic disorders (allergic rhinitis, atopic dermatitis) are associated with pneumococcal infections in children almost twice as often as in healthy children in the control group. Furthermore, the duration of the disease in patients with atopy is longer than in patients without atopy. A defective innate immune response (e.g., associated with impaired interferon response) leads to more severe asthma exacerbations due to rhinovirus infection. In particular, early onset (up to two years) of severe RTIs correlates with an increased risk of later

Table 1. Results of psychodiagnostics of fluid intelligence (CF2A)

Parameters	IQF1	IQF2	IQF3	IQF4	General IQF
M ± m	101.51 ± 13.87	106.76 ± 11.59	103.71 ± 21.34	79.75 ± 26.23	90.49 ± 9.92

Source: compiled by the authors of this study

development of asthma/atopy, likely due to a Th1/Th2 shift in the cytokine milieu. In addition, atopic dermatitis is associated with increased fragility and permeability of the skin barrier to pathogens. This sensitivity may be explained by the insufficient synthesis of cutaneous antimicrobial peptides, such as cathelicidin (LL-37) and beta-defensin, which are involved in the innate immune response against bacterial and viral pathogens. Children with atopic dermatitis are at increased risk of sore throats, colds, bronchitis, flu, pneumonia, sinus infections, ear infections, chickenpox, and urinary tract infections. In particular, the combination of atopic dermatitis and other atopic conditions correlates with a higher number of infections than those who had only one of these conditions [23].

According to our research, negative impacts of mediated environmental factors were observed:

–Does anyone in the family smoke?
• yes – 44 (37,29%)
• no – 74 (62,71%)
–Is there a pet living at home?
• yes – 67 (59,78%): cat – 16; dog – 51
• no – 51 (40,22%)
–Does the child have any allergic diseases?
• yes – 5 (4,42%)
• no – 113 (95,76%)
–Are there any allergic diseases among relatives?
• yes – 15 (12,71%)
• no – 103 (87,29%)

Starting in the womb and throughout the "first 1000 days of life," harmful environmental factors on the development of the immune and respiratory systems can lead to a reduced ability to fight infections and an increased risk of allergic symptoms later in life. According to the authors, it has been proven that exposure to environmental tobacco smoke during pregnancy was associated with recurrent RTIs in children followed from birth to 4 years of age, as well as with early markers of atopic status (elevated total IgE or atopic dermatitis). Exposure to tobacco smoke has been shown to increase the duration and severity of RTIs symptoms by increasing bacterial adhesion or damage to the mucociliary apparatus of the respiratory tract and loss of local defenses [21].

Scientists have also identified a link between the presence of intracellular atypical pathogens (Mycoplasma pneumoniae and Chlamydia pneumoniae) and the development of recurrent RTIs, especially in preschool-aged patients (<5 years). Presumably, atypical bacteria cause both respiratory disorders and pathology of the nasopharyngeal area [21].

Overweight and obesity are also considered by scientists, along with other independent risk factors (exposure to cigarette smoke, allergies, history of chronic respiratory symptoms) and are significant risk factors for recurrent respiratory infections in children with pathological signs. There is a direct correlation between the frequency of RTI and body mass index (BMI). It has been reported that leptin deficiency in severely obese children may make them more sensitive to pro-inflammatory stimuli and infections [24].

Many studies highlight the immunomodulatory role of vitamin D_3, documenting an inverse relationship between 25(OH)D levels and the incidence of upper respiratory tract infections, with the risk depending on the degree of deficiency. On the other hand, there is some evidence that vitamin D_3 supplements help prevent acute pediatric RTIs. Hair zinc and selenium levels were lower in a cohort of pediatric patients with recurrent wheezing compared with healthy children. A higher incidence of recurrent infections has also been reported in patients with suboptimal antioxidant levels (β-carotene, retinol, α-tocopherol, ascorbic acid, glutathione). In addition, micronutrient supplementation during pregnancy apparently has a potential immunomodulatory effect on the offspring at the epigenetic level from the prenatal period [25].

A psychodiagnostics of fluid (potential) intelligence was also conducted using the "Culturally Free Intelligence Test (CF2A)" by R. B. Cattell and A. K. Cattell [26]. We present the results of four groups of psychodiagnostic subtests (Table 1).

According to the research, the general IQF corresponded to the range of the average level of social creativity of the individual.

The level of intelligence corresponded to the following gradation:

• good norm – 53 (44,92%);
• low – 32 (27,12%);
• borderline zone – 4 (3,38%);
• average – 29 (24,58%).

DISCUSSION

According to the World Health Organization (WHO), the largest number of acute respiratory diseases occurs at

the age of 6–18 months and 5–7 years, which can be explained by the expansion of children's contacts and the characterological features of the children's immune system [27, 13].

Proposals from pediatricians and family doctors regarding the unification of requirements for the frequency and nature of nosological forms that may indicate that a child belongs to the group of «frequently ill children» or recurrent respiratory diseases differ significantly in different countries.

Equally important is the study of the biological role of acute respiratory diseases in the development of a child. According to some researchers, cases of rhinitis, nasopharyngitis, and even bronchitis trigger the body's defenses [27, 28].

According to our data, repeated episodes of acute respiratory infections lasted 3–4 days and tended to recur after 3–4 weeks, during which no clinical symptoms were observed [14].

There was also a predominance of episodes of acute respiratory infections in winter and spring, while in summer episodes of acute respiratory diseases were almost not observed. The general condition of the children between exacerbations was satisfactory, and there were no complaints [27].

Scientific evidence suggests that children with recurrent RTIs (6–8 episodes of RTIs per year) have different health status than those with fewer such episodes. In particular, they are more likely to develop complications requiring hospitalization. The level of inpatient treatment depends on the season and the child's age [15, 29, 30].

CONCLUSIONS

1. According to our study, negative impacts of indirect environmental factors were observed: smoking in the family – in 44 (37,29%), presence of pets at home – in 67 (59,78%), of which cats (in 16 children) and dogs (in 51 children), 22 children (18,64%) were breastfed until three months of age, vitamin D intake was not carried out in 70 children (59,32%), 35 children (29,66%) did not attend sports clubs.
2. According to our research, the vaccination rate of children was not at the proper level. The highest vaccination rate, according to our data, was observed for vaccination against measles, mumps, and rubella (first vaccination) – 99,15%, and the lowest level was for vaccination against diphtheria and tetanus (second booster) – 28,81%.
3. Among the children in the study group, inflammatory episodes were most often observed, in particular: bacterial tonsillitis – 86 (72,89%), otitis – 56 (47,46%), pneumonia and sinusitis – 37 (31,36%) (identical indicators). 71 children (60,17%) had experience with antibiotic therapy under the age of two, 25 children (21,19%) were used antibiotics before the age of six months.
4. Respiratory infection caused by at least one pathogen was observed in 63,4±4,1% of patients. The proportion of viral infections was 48,34±3,6%, in particular, coinfections were identified in 36,4±2,9% of cases. The distribution of confirmed viruses is as follows: AdV (26,34±1,7%), SARS-CoV-2 (19,5±1,6%), RV (18,2%), RSV (17,8%), influenza A/B (11,4%) and others in minimal structural components (6,8%).
5. Recurrent RTI is a multifactorial disease caused by a complex interaction of environmental and host factors. We suggest that an "additive effect," depending on the number of factors involved, most of which may be underestimated, may be a plausible explanation for the recurrence of RRI in a significant proportion of healthy children with unexplained frequent respiratory symptoms.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 10.03.2026
ACCEPTED: 29.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

In vivo induction of different venous thromboembolism by use of DC electrical shock on jugular vein endothelium

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ABSTRACT

Aim: This study aimed to establish a novel rabbit model using direct electrical stimulation to induce internal jugular vein injury and subsequent organ-specific thrombosis.

Materials and Methods: Twenty-one male New Zealand White rabbits underwent jugular vein thrombosis induction *via* direct current (DC) electrical stimulation. D-Dimer assays confirmed thrombus formation. Histopathological analyses of the lungs, heart, and brain assessed thromboembolic changes. Thrombosis induction was tested at varying DC voltages and exposure durations: 6 V for 60 s, 12 V for 120 s, and 24 V for 180 s.

Results: In the lungs (6 V/60 s), thrombi exhibited characteristic lines of Zahn with dense platelet and fibrin deposition, alveolar wall destruction, emphysematous changes, and wedge-shaped hemorrhagic pulmonary infarctions. In the heart (12 V/120 s), thrombus formation was observed in coronary arteries, with interstitial hemorrhage and altered cardiac muscle fibers showing rounded and oval nuclei. In the brain (24 V/180 s), acute neuronal injury was evident, including shrunken eosinophilic "red neurons," cerebral edema, liquefactive necrosis, small-vessel hemorrhage, and astrocyte proliferation. These findings indicate voltage- and duration-dependent induction of thromboembolism with organ-specific distribution.

Conclusions: Direct DC electrical stimulation induces deep vein thrombosis in rabbits in a voltage- and duration-dependent manner. Lower voltage and shorter exposure primarily affect the lungs, moderate settings target the heart, and higher voltage with longer duration affect the brain. This model provides a controlled platform for studying organ-specific thromboembolism and evaluating therapeutic interventions, especially if laboratory tests related to coagulation parameters are applied, they may help in identifying the components of the formed clot. Similarly, using lower or higher electrical currents at different intervals may reveal more precise pathological changes, thus contributing to the application of more specialized pharmaceutical treatments..

KEYWORDS: venous thrombosis, pulmonary infarctions, thrombus formation

Wiad Lek. 2026;79(7):1549-1555. doi: 10.36740/WLek/222507 DOI

INTRODUCTION

Thrombosis means the formation of blood clots within blood arteries and veins, which can block blood flow and lead to serious conditions such as a heart attack, myocardial infarction (MI), stroke, pulmonary embolism (PE), or tissue ischemia, depending on the thrombus site [1]. A common clinical condition is venous thrombosis (VT), with a significant financial burden on the US healthcare system. Despite its spread, VT diagnosis and management remain primitive [2]. Dysfunction in the endothelium has been associated with VT that activates the clotting system, with both thrombus types. White thrombus is rich in platelets and fibrin with few erythrocytes, and red thrombus consists of erythrocytes and less fibrin that is trapped in [3]. Deep vein thrombosis (DVT) and PE are called venous thromboembolism (VTE), which is a very common health problem [4]. Trauma is a powerful trigger factor for VTE, the leading cause of death worldwide [5]. Internal Jugular Vein thrombosis (IJV) is a potentially life-threatening disease due to the development of a localized ischemic stroke thrombus [6]. Animal models of thrombosis are crucial in pathophysiological research to understand the complex mechanisms of hemostasis and thrombogenesis, and to screen anticoagulant drugs. Numerous in vitro and in vivo models are routinely used in thrombosis research [7]. The experimental rabbit was employed as a model because the Coagulation factor (FX) profile in rabbit and human plasma has equivalent binding affinity to enzyme substrate and small–molecule with FXa inhibitors [8]. Electrical currents that are implemented in animals, e.g., electrical stunning during slaughter or electro-cauterization as a surgical tool, or accidentally when animals contact with electrical currents, can cause DVT in organs. The physiological impact is modulated by current, voltage, heat production, and the duration

of the electrical flow. Once introduced into the body, electrical currents travel the most direct path from the contact source to the grounding or exit site [9].

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Twenty-one healthy male New Zealand White rabbits, weighing between 3.0 and 3.5 kg, were utilized in this experimental study, kept in good health under standard laboratory conditions. During quarantine, animals undergo an acclimation period to adapt to the housing environment and routine management practices. Rabbits were maintained on standard feed with unrestricted access to water. All housing, handling, and experimental procedures adhered to the regulations of the Local Ethics Committee for Animal Experimentation (University of Basra, College of Pharmacy), under the affiliation of the National Ethics Committee for Animal Experimentation, Polish Ministry of Science and Higher Education. "In order to ensure adequate adaptation to the laboratory environment, rabbits were transported from the farm to the animal housing facility 14 days prior to the initiation of experiments [10].

LATEX AGGLUTINATION ASSAY

As the latex agglutination assay, D-Dimer latex (ATLAS Medical GmbH, Germany) was used pre- and post-experiment to be sure that platelet aggregation and the clot formation process began. Plasma is prepared by centrifugation of whole blood collected using a sodium citrate anticoagulant tube. Place 20 µL of the reagent within a field on the reaction slide. A 20 µL aliquot of undiluted plasma or control solution was accurately pipetted next to the Latex Reagent. The mixture was homogenized using a stirrer until uniform. The slide was mounted on a mechanical rotator operating at 80–100 rpm, and agglutination was examined under intense illumination exactly at 3 minutes.

SURGICAL PROCEDURE

All experimental rabbits were pretested with D-Dimer test (a protein that breaks down blood clots) before performing any surgical procedures, to be sure that all animals didn't have any coagulation problems. Anesthesia was induced and maintained using ketamine (50 mg/kg initial dose, followed by 50 mg/kg/h, intramuscularly) in combination with xylazine (10 mg/kg initial dose, followed by 10 mg/kg/h, intramuscularly).

After achieving adequate anesthesia, the fur in the cervical region was carefully shaved. The rabbit was subsequently transitioned to isoflurane anesthesia, and vital parameters—including respiratory rate, blood oxygen saturation, and heart rate—were continuously monitored.

An incision was made in the shaved cervical region to expose and carefully isolate the jugular vein from surrounding tissues. Thrombosis was induced by electrical stimulation of the left jugular vein at different times as shown in Table 1, and Fig. 1 with a DC electrical supply (3-24V, 3A,72W) using a stainless-steel probe [11-12].

As shown in Figures 2, and 3 the jugular veins were carefully exposed by dissecting the fascia and exposing and preparing the sternocleidomastoid muscle. To accelerate the thrombus formation, wet the leg that is tied with a negative charge probe. The vein is exposed simply by placing the positive electrode on the external surface of the jugular vein. As a result of the electric current passing through the body, which leads to the induction of trauma in the venous endothelium, it is noticeable as a result of the color change of the vein surface to white. Its intensity depends on the voltage of the current and the period of exposure. Suturing of the incision and treatment with antibiotics were applied to prevent contamination.

HISTOPATHOLOGICAL EXAMINATION

Primary fixation by perfusion of an entire animal with normal saline solution is performed by pumping it via the left ventricle (IV). The fixative arrives at and fills the capillary net, where most of the perfusion occurs by allowing the fixative solution to leave the body, following preservation in 10% formaldehyde, kidney and lung specimens were embedded in paraffin wax. Sections of 3 µm were obtained using a rotary microtome. Histological evaluation was performed after staining the sections with hematoxylin and eosin (H&E).

RESULTS

LUNG

Characteristic alternating layers of erythrocytes and platelets/fibrin, termed the lines of Zahn, are observed during thrombus formation. A thromboembolus (TE) introduced into the pulmonary artery may, over time and with survival of the rabbit, undergo both structural organization and dissolution. The clot formation can occur in a blood vessel near the shock, and if the clot travels through the bloodstream, it may end up getting trapped in the pulmonary artery, leading to a pulmonary embolism. Additionally, the lung is a receptive location for clot formation due to slower blood flow.

Table 1. Mean ± SD values according to agglutination test results for Lung, Heart, and Brain under different voltages and exposure times (P<0.05 considered significant)

Organ	6V 60s	6V 120s	6V 180s	12V 60s	12V 120s	12V 180s	24V 60s	24V 120s	24V 180s
Lung	2.29±0.90*	2.86 ± 0.38	3.00±0.00	3.00±0.00	2.86±0.38	2.86±0.38	2.86±0.38	2.86±0.38	3.00±0.00
Heart	4.00±2.16*	0.43±0.53*	0.57±0.53*	1.14±1.05*	2.57±0.53*	2.29±1.13*	0.29±0.49*	3.00±0.00	3.00±0.00
Brain	2.57±0.79	2.86±0.38	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00*

Source: Compiled by the authors of this study

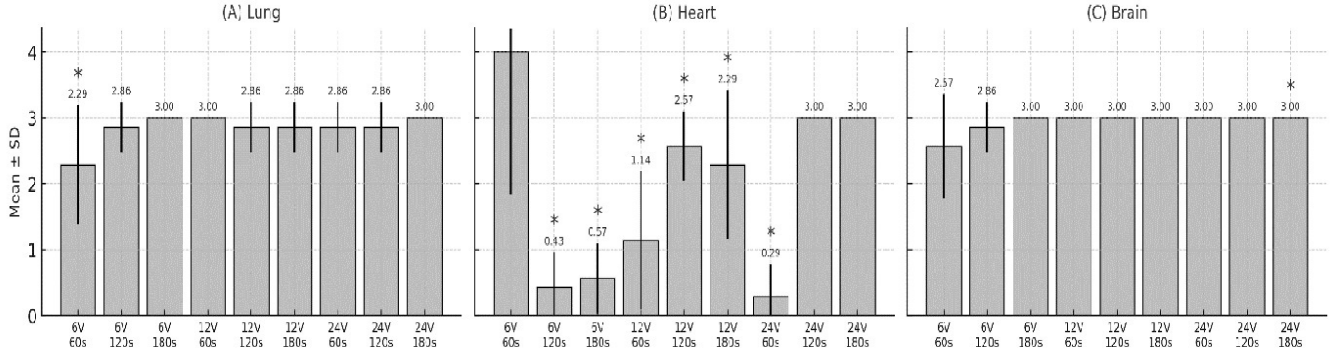

Fig. 1. Effect of voltage and exposure time on organ responses measured by agglutination test. Mean ± SD are shown for lung **(A),** heart **(B)**, and brain **(C).** Significant differences (P < 0.05) are indicated by an asterisk (*). Each bar represents the mean of three independent experiments
Source: Own materials

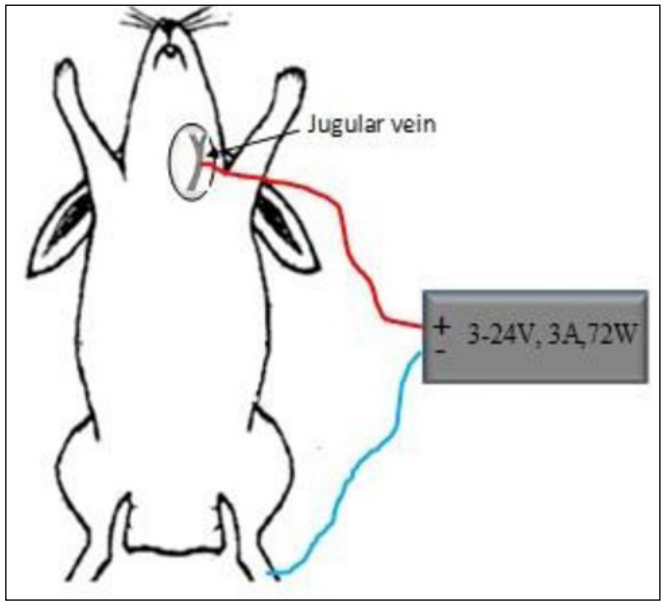

Fig. 2. Rabbit neck incision exposing the jugular vein. An electrode placed on the vein is connected to a DC source to deliver an electrical shock and induce thrombosis
Source: Own materials

As illustrated in Figure 4, the thrombus is adherent to the arterial wall, with characteristic lines of Zahn indicated by the arrow. At high magnification, microscopic examination reveals destruction of alveolar walls accompanied by emphysematous changes, with remaining airspaces markedly dilated. Hemorrhagic pulmonary infarctions are wedge-shaped and sub pleurally based, reflecting the survival of the tissue. These infarcts are hemorrhagic because, despite obstruction of the pulmonary artery - which supplies the majority of blood and oxygen to the lungs - the bronchial arteries from the systemic circulation, providing approximately 1% of pulmonary blood flow, remain patent.

HEART

Cardiac muscle fibers running in different directions with an acidophilic cytoplasm and central pale oval nuclei, with areas of interstitial hemorrhage and rounded or square nuclei, and some rounded nuclei (Fig. 5).

BRAIN

Shrunken, eosinophilic neurons (also known as "red neurons") badge of acute neuronal injury. Cerebral edema and liquefactive necrosis with hemorrhage in small vessels and Astrocyte proliferation were noticed (Fig. 6).

DISCUSSION

The present study demonstrated that electrical current can induce thrombus formation in multiple organs, including the lungs, heart, and brain, with the severity and location of thrombosis being dependent on both voltage intensity and duration of exposure. These findings support previous hypotheses suggesting that electrical injury can directly damage vascular endothelium, thereby promoting thrombogenesis.

Fig. 3. Intraoperative view demonstrating surgical exposure of the rabbit's jugular vein. A stainless-steel electrode is applied directly to the jugular vein and connected to a DC power source to induce endothelial injury and thrombus formation via controlled electrical stimulation
Source: Own materials

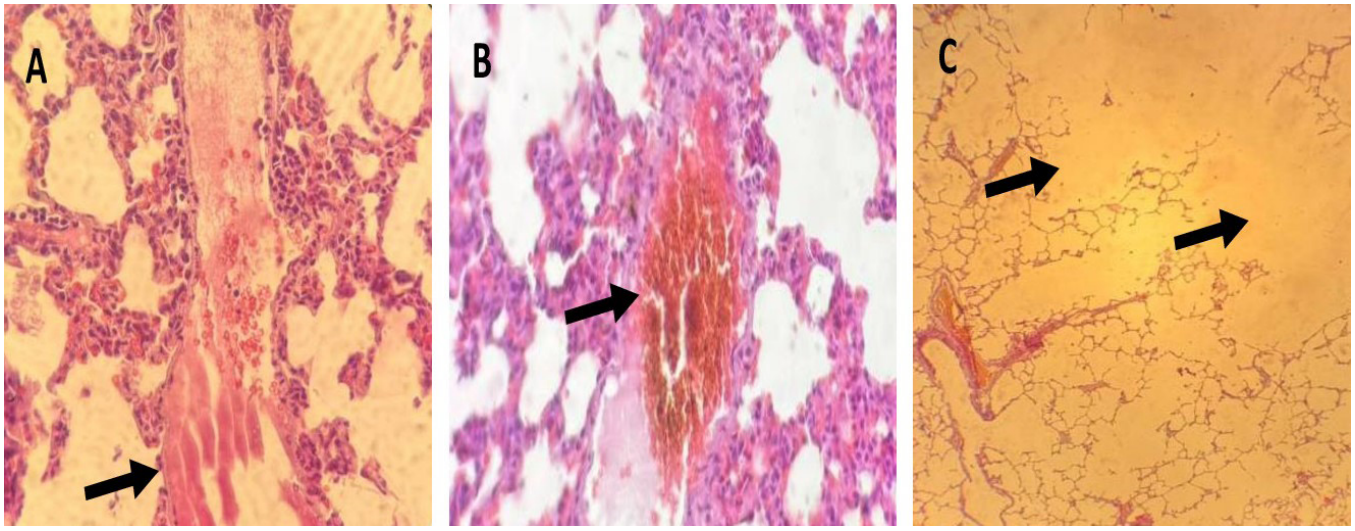

Fig. 4. Lung histology from 6 voltages for 60 sec. **(A):** Show thick white and red line of Zahn with condense of platelets deposition all over thrombus (H&E stain ×40). **(B):** Loss of alveolar walls with emphysema (H&E stain ×20). **(C):** Clear hemorrhagic pulmonary infarction in bronchial artery (H&E stain x 40)
Source: Own materials

In the lungs, significant thrombus formation was observed following relatively low-voltage exposure, accompanied by pulmonary infarction and vascular congestion. Similar mechanisms have been proposed in clinical studies where direct electrical injury to the vascular endothelium increased the risk of deep venous thrombosis (DVT), particularly during the early period following electrical exposure [13]. Furthermore, evidence-based reviews of electrical injuries have suggested that both thermal and electrical damage to endothelial cells contribute to venous and arterial thrombotic events [14]. The observed pulmonary thrombosis in the present study may therefore result from endothelial dysfunction combined with alterations in blood flow and local coagulation activity.

The findings of this study are also consistent with reports indicating that the severity of tissue and vascular injury increases with higher electrical current and voltage, regardless of whether alternating current (AC) or direct current (DC) is involved [15, 16]. Previous investigations have shown that electrical injuries cause heat-induced tissue destruction, vascular endothelial

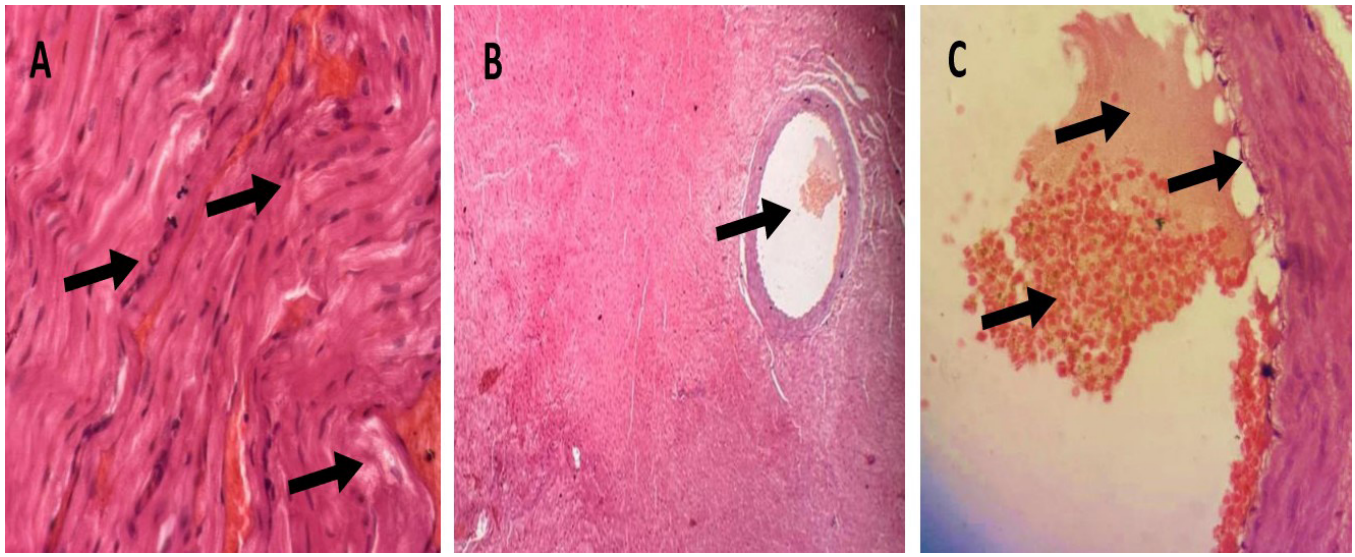

Fig. 5. Heart histology from 12V for 120 sec. **(A)**: Show different direction fibers with rounded and oval nuclei (H&E stain x 40). **(B&C)**: Show thrombus formation in the coronary artery with clear fibrin (H&E stain ×10 A; ×40 B)
Source: Own materials

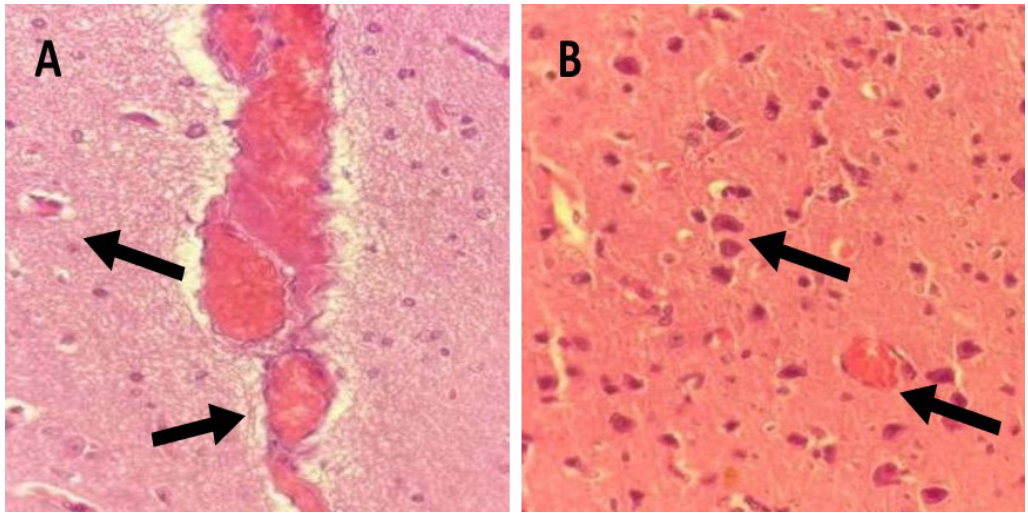

Fig. 6. Brain histology from 24V for 180 sec. (A): Shrunken, eosinophilic neurons with cerebral edema and liquefactive necrosis and Hemorrhage in small vessels with clear Astrocyte proliferation (B) Cerebral cortex demonstrating acute ischemic neuronal injury ("red neurons"), characterized by shrunken eosinophilic neurons with pyknotic nuclei, consistent with early hypoxic–ischemic brain damage (H&E stain ×10 A; ×40)
Source: Own materials

damage, and vessel thrombosis, supporting the pathological findings observed in the current experimental model [17].

In the heart, coronary artery thrombosis and fibrin deposition were observed at intermediate voltage and exposure duration. These findings correlate with several clinical reports describing coronary vascular injury after electrical exposure. Jacob et al. documented a case of coronary artery thrombosis and dissection following a 6,000-volt electrical shock, suggesting that electrical current can directly damage coronary vessels and trigger thrombotic events [18]. Similarly, comprehensive reviews of electrical cardiac injuries have highlighted arrhythmias, myocardial infarction, and coronary thrombosis as important consequences of electrical exposure [19]. Additional case reports described acute myocardial infarction with refractory cardiogenic shock after high-voltage electrocution [20], while delayed ventricular dysfunction and myocardial fibrosis have also been reported in survivors of severe electrical injuries [21]. Moreover, multiple cases of coronary thrombosis requiring surgical intervention following electric shock have been documented, further supporting the relationship between electrical injury and coronary vascular thrombosis [22].

In the brain, prolonged exposure to higher voltages resulted in marked neuronal injury, cerebral edema, hemorrhage, and vascular thrombosis. These findings are in agreement with several reports describing cerebrovascular complications after electrical injury. Huan-Jui et al. reported an acute ischemic stroke following low-voltage electrical exposure and suggested that vasospasm may play a major role in the pathogenesis of cerebral ischemia [23]. Patel et al. described cerebral venous thrombosis after accidental electrocution, emphasizing that thrombotic events may occur even when

the brain is not directly within the electrical pathway [24]. Likewise, cerebral infarctions involving the frontotemporal and parietal regions have been reported following low-voltage electrical injury [25], while acute ischemic stroke after high-voltage electrocution has also been documented [26]. These observations support the current findings and indicate that electrical injury may produce both direct vascular damage and secondary thrombotic complications within the central nervous system.

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CONCLUSIONS

Overall, the present study demonstrates a clear relationship between electrical exposure and thrombus formation across multiple organ systems. The experimental findings suggest organ-specific susceptibility, with pulmonary thrombosis occurring at lower electrical settings, coronary thrombosis developing at intermediate exposure levels, and cerebral vascular injury appearing predominantly after higher voltage and prolonged exposure. These results support existing clinical and experimental evidence indicating that electrical injury can induce thrombosis through endothelial damage, thermal injury, vascular dysfunction, and coagulation activation. Consequently, this rabbit model provides a reproducible platform for investigating the mechanisms of electrical injury-induced thrombosis and may facilitate the development of preventive and therapeutic strategies for thromboembolic complications associated with electrical shock.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 07.01.2026
ACCEPTED: 26.05.2026

CREATIVE COMMONS 4.0

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ORIGINAL ARTICLE

CONTENTS

Features of structural organization of the urinary bladder under conditions of experimentally modeled hyperglycemia

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ABSTRACT

Aim: To establish the features of morphofunctional organization of the rat urinary bladder in modeled hyperglycemia, taking into account the complex of structural-metabolic changes in the organism in the dynamics of its development.

Materials and Methods: The work was performed in compliance with the rules of humane treatment in experiments on 80 one-year-old male Wistar rats. Streptozotocin diabetes was modeled in 50 animals by single intraperitoneal administration of streptozotocin with material collection on days 14, 28, 42, 56, and 70 of the experiment. Histological, electron microscopic, biochemical, massometric methods, measurement of consumed water and daily diuresis, morphometric methods, cluster analysis, and statistical methods were applied.

Results: Urothelial cells form four clusters with histometric and ultrastructural characteristics. Chronological features of streptozotocin diabetes development were confirmed by blood glucose level, glycosylated hemoglobin content, urine glucose concentration, its pH, massometric indicators of body weight and urinary bladder weight, and volumes of consumed liquid and diuresis. Disruption of structural-functional balance between urothelial cell clusters is detected from day 14 until the end of the experiment. On days 14-28 of streptozotocin diabetes development, compensatory hypertrophy of smooth myocytes of the bladder wall with ultrastructural signs of their functional stress was detected, as well as the first signs of diabetic microangiopathy in the bladder wall. From day 56, generalization of sludge syndrome, dystrophic changes in endotheliocytes, and basement membrane edema are observed. Against the background of pronounced microangiopathy, diuresis and polydipsia decrease, desquamation of urothelial cells intensifies, and dehydration of smooth myocytes and urothelial cells occurs, which is pronounced on day 70 of the experiment.

Conclusions: In the dynamics of induced hyperglycemia development, disruption of structural-functional balance between urothelial cell clusters was detected, leading to imbalance between proliferative potentials of basal urothelial cells and integrity of the urothelial cellular barrier, as well as stages of differentiation of intermediate urothelial cells. Diabetic microangiopathy intensifies until day 70 of the experiment. Overall, during this period, diabetic cystopathy remains compensated (percentage of smooth myocyte area is 1.16 times greater than control and percentage of collagen fiber area is 1.23 times less than control).

KEYWORDS: urinary bladder, hyperglycemia, urothelium, diabetic cystopathy, microangiopathy

Wiad Lek. 2026;79(7):1556-1562. doi: 10.36740/WLek/225987 DOI

INTRODUCTION

The prevalence of diabetes mellitus has pandemic characteristics [1,2]. Almost half a billion people in the world live with diabetes mellitus, and it is predicted that their number will increase by 25% by 2030 and by 51% by 2045 [3]. Diabetes mellitus increases the risk of developing urological disorders, while urological pathology can cause decompensation of diabetes course [4]. In the lower urinary tract, complex interaction of neuronal, myogenic, and urothelial dysfunction causes the development of functional disorders, among which disorders of accumulation and emptying of the urinary bladder occupy a leading place [5]. Diabetic cystopathy is one of the specific complications of diabetes mellitus and significantly reduces the quality of life of patients from both social and economic points of view, and complicates the course of diabetes mellitus itself, which indicates the social significance of further research on this scientific topic [6,7].

Works in which the results of morphological and morphometric studies of structural components of the urinary bladder are correlated against the background of streptozotocin diabetes development with biochemical indicators of urine, which is aggressive to the urothelium, are few [8,9]. Diabetic cystopathy is associated primarily with dysfunction of the detrusor muscle of the urinary bladder, which leads to changes in its contractile ability, but research results are

ambiguous [10,11]. Changes in the urinary excretory capacity of the urinary bladder occur with structural reorganization of its muscular coat, but information on this issue is contradictory [12-14]. The connection between urodynamic disorders in diabetes mellitus and microvascular complications has been clinically proven [15,16]. However, these results are not confirmed by morphological changes in vessels and their metric characteristics. Therefore, the study of reorganization of urothelial cells, smooth myocytes, collagen fibers, and blood vessels of the urinary bladder wall during the development of diabetic cystopathy requires comprehensive research using morphological, morphometric, biochemical methods and multidimensional statistics methods.

AIM

To investigate light-optical, morphometric, and ultrastructural reorganization of urothelial cells and their cluster structures, smooth myocytes, and collagen fibers at stages of experimental streptozotocin diabetes course; to trace chronological features of microvessel changes during the development of diabetic microangiopathy in the urinary bladder wall.

MATERIALS AND METHODS

The study was conducted on 80 one-year-old male Wistar rats weighing 214-256 g. The animals were divided into three groups: intact/normal (10 animals); experimental (50 rats in which streptozotocin diabetes was modeled by single intraperitoneal administration of Sigma (USA) streptozotocin in 0.1 M citrate buffer (6 mg/100 g animal body weight)); control (20 animals - only citrate buffer was administered). Material collection for morphological and biochemical studies, determination of rat body weight and their urinary bladder weights were performed in the morning on an empty stomach on days 14, 28, 42, 56, and 70 of the experiment. Euthanasia was performed under thiopental anesthesia. The work performed does not contradict the basic bioethical norms of the Helsinki Declaration adopted by the General Assembly of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedicine, relevant provisions of WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics, and Directive 2010/63/EU of the European Parliament and Council of Europe on the protection of animals used for scientific purposes. Histological sections were stained with hematoxylin and eosin and by Masson's trichrome method. Semi-thin sections were stained with 1% methylene blue solution. Electron micrographs were digitized on an Epson Perfection V550 Photo scanner at 1200 dpi resolution. Blood glucose level (mmol/L) was studied by glucose oxidase method. Glycosylated hemoglobin (HbA1C) content in blood (%) was determined using the "ACCENT-200 HbA1c DIRECT" kit (PZ Cormay S.A., Poland). Urine pH was measured with a portable "Checker 1" pH meter (Hanna Instruments, Italy).

RESULTS

Using Ward's cluster analysis method (Euclidean metric), it was determined that the cluster structure of transitional epithelium cells of the urinary bladder consisted of 4 clusters, with cell percentages equal to 10.96, 39.47, 34.21, and 15.35%, respectively. It was established that normally, the percentages of cells falling into clusters 1 and 4 and clusters 2 and 3 did not differ significantly from each other ($p>0.05$). Cluster 1 is represented by basal urothelial cells that were poorly differentiated, as indicated by their high nuclear-cytoplasmic ratio (NCR) (0.81±0.30). They are the smallest in area (42.2±5.09) μm^2 and nuclear area (18.3±4.79) μm^2. Their features include attachment to the basement membrane of the urothelium and paucity of organelles. We determined that cluster 4 is formed by umbrella cells that had the lowest NCR (0.49±0.18) and were highly differentiated. These are the largest cells by area (115.6±11.18) μm^2 and their nuclear area (36.7±7.84) μm^2. They had plaque and hinge-like regions, junctional complexes, many urothelial vesicles and primary and secondary lysosomes, and a well-developed Golgi complex. We found that clusters 2 and 3 are urothelial cells of the intermediate layer of the transitional epithelium of the urinary bladder. Cluster 3 cells characterized the final stage of intermediate urothelial cell differentiation, while cluster 2 represented the initial stage, which was argued by the following: cluster 3 urothelial cells were located under umbrella cells, while cluster 2 cells were closer to the basement membrane of the urothelium; the NCR of cluster 3 cells was (0.62±0.20), while cluster 2 was (0.79±0.28), indicating a higher degree of differentiation of cluster 3 cells; in terms of cell size (85.4±7.48) μm^2 and their nuclei (31.7±6.58) μm^2, cluster 3 urothelial cells were more mature cells than cluster 2, which were smaller - (62.5±6.21) μm^2 and (26.8±5.05) μm^2, respectively; according to ultrastructural cell structure, cluster 3 cells differed little from umbrella cells, i.e., belonged to highly differentiated cells, while cluster 2 belonged to moderately differentiated cells.

In the experiment, we established that blood glucose level increased until day 42 (by 4.75 times, $p<0.01$),

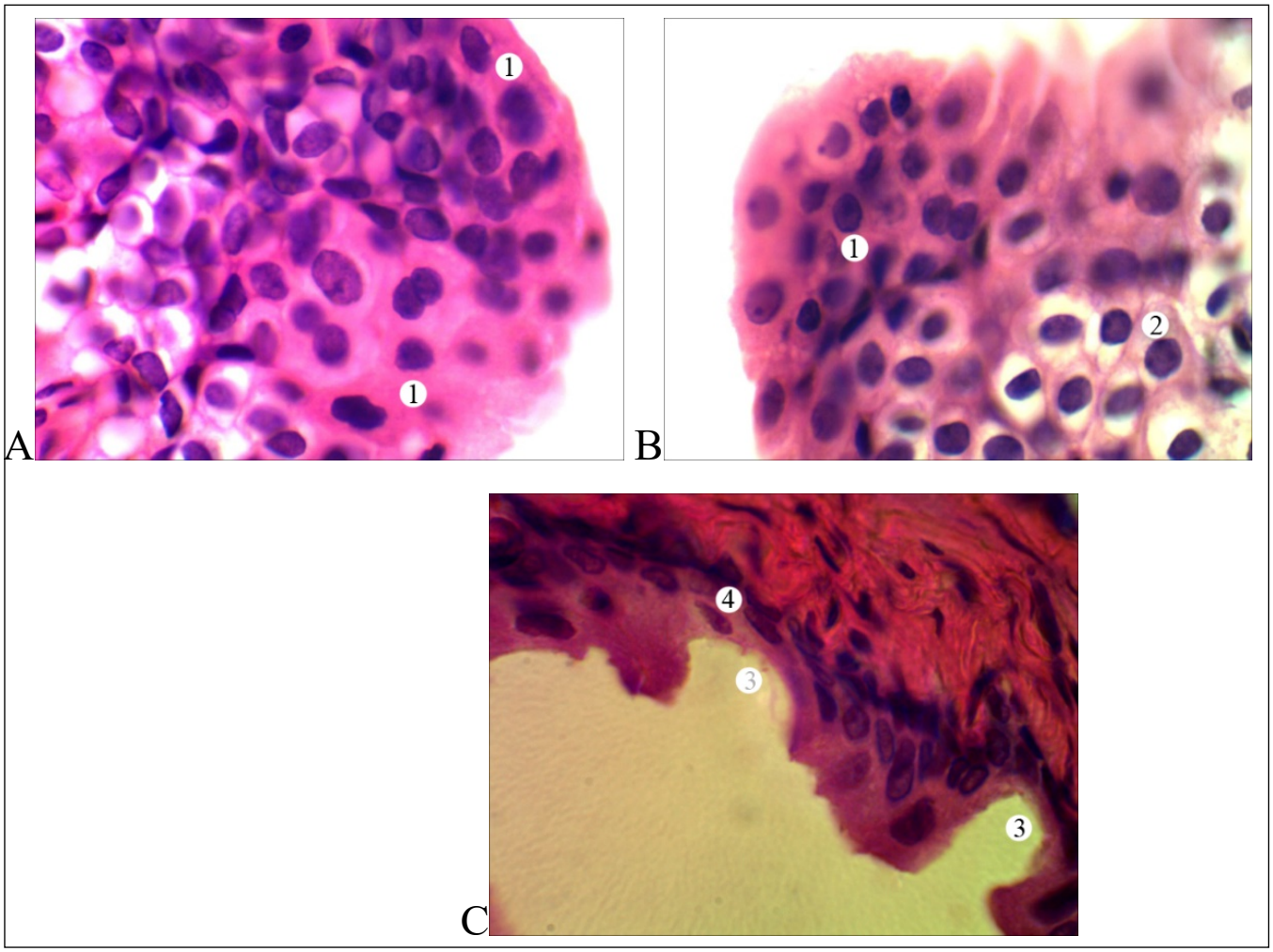

Fig. 1. Urinary bladder wall at different stages of experimentally induce hyperglycemia. A - Day 14 of the experiment: (1) umbrella urothelial cells. B - Day 28 of the experiment: (1) umbrella urothelial cells; (2) vacuolated epithelial cells of the intermediate and basal layers. C - Day 56 of the experiment: (3) loci of desquamation; (4) basal layer of the transitional epithelium. Staining: hematoxylin and eosin. Magnification: (A, B) ×400; (C) ×200.

reaching a level of (24.98±2.16) mmol/L, and did not change until the end of the experiment ($p>0.05$). Urine glucose content increased 21.38-fold ($p<0.01$) by day 42 of the experiment and did not change thereafter ($p>0.05$). HbA1C content in blood increased significantly already on day 14 of the experiment (by 3.48 times, $p<0.01$), and by day 70 became 5.34 times greater than control ($p<0.01$). The most acidic urine reaction was determined on day 56 of the experiment. Body weight of diabetic rats gradually decreased and by day 70 of the experiment became 1.46 times less than control ($p<0.01$). The amount of water consumed by diabetic rats and daily diuresis increased by day 28 of the experiment by 7.61 and 18.54 times respectively ($p<0.01$), and by the end of the experiment decreased somewhat but remained greater than control, by 5.84-4.90 and 13.64-11.10 times respectively ($p<0.01$).

In streptozotocin diabetes, structural-functional balance between cells was disrupted due to an increase in the percentage of cluster 1 cells ($p<0.001$) and a decrease in clusters 3 and 4 ($p<0.01$) with no changes in the proportion of cluster 2 cells ($p>0.05$), which indicated disorganization (imbalance) of systemic mechanisms of urothelial functioning, which occurred from the first terms of the experiment. It was established that the cause of these changes is desquamation of urothelial cells, which led to disruption of morphological stratification of the urothelium, which became one- or two-layered.

We established that by day 28 of the experiment, hypertrophy of smooth myocytes developed, which was morphometrically confirmed by an increase in their area percentage by 1.31 times ($p<0.001$), and ultrastructurally by signs of functional stress (light myocytes with clearly expressed myofilaments that had large nuclei with numerous protrusions and invaginations of the nuclear envelope; in the cytoplasm - a large number of free ribosomes and glycogen inclusions located near the nuclear poles; increased size and content of mitochondria detected near nuclei or in sarcoplasm).

Simultaneously, the percentage of collagen fiber area decreased by 1.92 times ($p<0.001$), which is a compensatory reaction that provided the possibility of bladder wall stretching.

We established that diabetic microangiopathy of the urinary bladder was determined by sludge, the degree and prevalence of which corresponded to HbA1C content. By day 28 of the experiment, arteriole dilation was revealed, on day 42 - spasm, and from day 56 - their secondary expansion. Dystrophic changes in capillary endotheliocytes were determined from day 28 of the experiment, and on day 42 became significant (their cytoplasm thickened, heterochromatin shifted marginally, organelle content decreased, microvilli appeared, cytoplasm thickening and microclasmatosis occurred). From day 56, dystrophic changes in endotheliocytes dynamically increased, which caused plasma permeation of perivascular connective tissue. The thickness of capillary basement membrane of the suburothelial hemomicrocirculatory bed gradually increased and on day 70 thickened by 3.22 times, and from day 56 the basement membrane became lamellar.

Results of comprehensive assessment of urinary bladder reorganization in streptozotocin diabetes showed that on days 14-28 of the experiment, primary increasing hyperglycemia triggered a cascade of changes that caused: minor glucosuria; expansion of arteriole lumen; hemoglobin glycosylation (HbA1C). This caused sludge formation in venules; increasing dystrophic changes in endotheliocytes; disruption of basement membrane structure. Large volume of urine led to significant polydipsia, desquamation of umbrella cells and reorganization of cluster structures of the urothelium, compensatory hypertrophy of smooth myocytes, and an increase in their area percentage. Vacuolar dystrophy developed and urothelial cell sizes increased (Fig. 1A, 1B). Arteriole expansion, polydipsia, and sludge in venules caused the development of interstitial edema.

On days 42-70 of the experiment, persistent high hyperglycemia caused: pronounced glucosuria; narrowing of arteriole lumen; sludge formation in capillaries, and from day 56 - generalization of sludge syndrome; significant dystrophic changes in capillary endotheliocytes and lamellation and thickening of their basement membranes. Diuresis and polydipsia decreased. Plasma permeation increased. Against the background of pronounced microangiopathy, myocyte dehydration occurred with a decrease in their area and an increase in the content of dark involutive types and an increase in collagen fiber area; desquamation of cells of all urothelial layers developed, and in combination with glucosuria - their dehydration and size reduction (Fig. 1C).

DISCUSSION

Taking into account our own results and literature data, it can be stated that normally, the levels of cell proportions in clusters 1 and 4 indicate structural-functional balance between proliferation of basal cells and integrity of the urothelial cellular barrier, and the levels of cell proportions in clusters 2 and 3 indicate balance between initial and final stages of differentiation of intermediate urothelial cells [17]. Basal urothelial cells are cambial committed cells that differentiate into cells of other layers of transitional epithelium [18]. Cluster 4 urothelial cells form the urothelial cellular barrier [19,20]. A number of authors identify an additional type of urothelial cells located under umbrella cells and capable of quickly replacing them in case of damage [21,22] - these were cluster 3 cells.

In the experiment, we established that blood glucose level increased until day 42 (by 4.75 times, $p<0.01$), reaching a level of (24.98±2.16) mmol/L, and did not change until the end of the experiment ($p>0.05$). Authors who used Wistar rats indicate the same dynamics of hyperglycemia development [23]. In contrast, in experiments involving Sprague-Dawley rats, blood glucose levels of 21-28 mmol/L are observed already on days 3-14 from induction of streptozotocin diabetes [24].

The decrease in body weight of experimental animals is associated with protein metabolism disorders. We used one-year-old rats, in which the insignificant natural increase in body weight could not affect the experiment results, based on the fact that in recent years there have been data proving the dependence of body weight decrease in animals with streptozotocin diabetes on sex, rat line, and especially on age[25,26]. It was found that the urinary bladder weight of diabetic rats increased by day 70 by 1.57 times ($p<0.01$).

On days 14-28 of the experiment, the area of urothelial cells ($p<0.001$) and their nuclei ($p<0.01$) of all clusters increased due to the development of vacuolar dystrophy triggered by hyperglycemia, which led to an 18.54-fold increase (day 28) in daily urine volume. The development of vacuolar dystrophy can be explained by the fact that as a result of a multiple increase in diuresis rate, urine against the background of minor glucosuria became of low specific gravity. The movement of such (hypotonic) urine into the depth of the urothelium was facilitated by desquamation of its cells and wedge-shaped ruptures that sometimes reached the basement membrane of the urothelium. Water transport to urothelial cells occurred passively - as a result of diffusion along a concentration gradient. On days 42-70 of the experiment, persistent significant glucosuria against the background of decreased polyuria increased urine specific gravity, which led to

dehydration of urothelial cells, decrease in their area and nuclei ($p<0.01$), and ultrastructural reorganization (ruptures of junctional complexes; osmiophilic cytoplasm matrix; fusion of vacuoles into balloons that compressed the nucleus and organelles; short cisterns of granular endoplasmic reticulum and Golgi complex; small mitochondria with crest destruction and dark matrix; secondary lysosome disintegration; decreased content of urothelial vesicles).

From day 42 of the experiment, the percentage of collagen fiber area increased and smooth myocyte area decreased, but even on day 70, the myocyte area proportion remained greater than control (by 1.16 times, $p<0.001$), indicating their hypertrophy, while collagen fibers were less (by 1.23 times, $p<0.001$), and there was no basis to speak of diffuse fibrosis of the bladder wall. This indicates that diabetic cystopathy remained compensated during our experiment. From day 56, the content of dark myocytes of involutive type with paucity of organelles, subsarcolemmal vesicles, and myofilaments increased. Myocytes with sarcolemma sequestration and individual areas of interstitial substance fibrosis were detected. The obtained results are consonant with data from other researchers [27].

It is known that primary hyperglycemia causes release of vasodilators, and high hyperglycemia causes vasoconstrictors [28,29]. Secondary expansion of microvessels is due to disruption of their sympathetic innervation.

Such dynamics of morpho-functional changes was traced when studying the effect of experimental streptozotocin diabetes on seromucous cells of the submandibular salivary gland. The authors state that the reaction of glandulocytes of seromucous acini to hyperglycemia development is typical and non-specific. Alterative changes have a pronounced dystrophic character in early stages and increase until day 42 of the experiment. On day 70, histological adaptation of seromucous cells was observed, which is confirmed by the development of atrophic changes, decrease in cell area, and normalization of the relative area of their granules [30].

CONCLUSIONS

In the dynamics of experimental streptozotocin diabetic cystopathy development, two periods can be distinguished. The first lasts during days 14-28 and manifests as increasing hyperglycemia and glucosuria, pronounced polyuria and polydipsia, reorganization of cluster structures of urothelial cellular composition, desquamation of umbrella cells and wedge-shaped ruptures of the urothelium, increase in area and vacuolar dystrophy of urothelial cells, compensatory hypertrophy of smooth myocytes of the bladder wall, and development of initial signs of diabetic microangiopathy. The second period covers days 42-70 and is characterized by high stable hyperglycemia and glucosuria, decrease compared to day 28 in polyuria and polydipsia, dehydration of urothelial cells (with corresponding qualitative and morphometric changes) and smooth myocytes (with decrease in their area proportion and prevalence of dark involutive types), occurring against the background of pronounced diabetic microangiopathy.

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This work was conducted within the research program of the Department of Human Anatomy at Ivano-Frankivsk National Medical University, as part of the project "Possibilities of Radiological and Morphological Methods for Diagnosing Diseases Caused by Endothelial Dysfunction of Multifactorial Origin" (0124U004858).

CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 18.03.2026
ACCEPTED: 29.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Comparative characteristics of structural changes in the kidneys of experimental rats 1 and 3 hours after exposure to the *Leiurus macroctenus* scorpion venom

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ABSTRACT

Aim: To compare structural changes in the kidneys of experimental rats 1 and 3 hours after exposure to the Leiurus macroctenus scorpion venom.

Materials and Methods: The venom of scorpions from the Buthidae family, genus Leiurus, species *Leiurus macroctenus*, was administered to rats (0.5 ml of venom solution; 28.8 μg/ml; LD_{50}=0.08 mg/kg). The study utilised 20 white male laboratory rats weighing 200 g (±10 g). The selected rats were divided into two groups: a control group – 10 rats, to which no venom was administered, and biological material was collected 1 and 3 hours after the administration of saline solution experimental group of 10 rats, from which histological material was collected 1 and 3 hours after the administration of the scorpion venom. For microscopic examination, kidney samples were taken from all groups. The specimens were fixed in a 10% formalin solution for 1 day. Histological samples of rat kidneys were stained with haematoxylin and eosin. Histological slides were examined using an SEO SCAN light microscope.

Results: One hour after the administration of *Leiurus macroctenus* scorpion venom to rats, significant hemodynamic disorders and the onset of inflammatory processes in the organ were noted, along with acute renal failure due to severe damage to the glomerular apparatus. Disruptions in the cytoskeleton of podocytes and the tubular apparatus of nephrons, along with manifestations of desquamation, hydropic, and hyaline-droplet dystrophies of the tubular epithelium, were established. The introduction of *Leiurus macroctenus* scorpion venom to rats was accompanied by markedly pronounced dystrophic changes in the structural components of the kidneys three hours after the beginning of the experimental study, in contrast to the earlier time point of the study. The emergence of inflammatory processes in the organ interstitium, characterised by plasmacytic, lymphocytic, and macrophage infiltration, was observed. Vascular and hemodynamic disorders were also detected, manifesting as desquamation of the endothelial lining of the vascular wall and increased permeability. The development of tubular necrosis characterised the renal tubular apparatus.

Conclusions: More pronounced histological changes in the structural organisation of rat kidneys were found 3 hours after exposure to *Leiurus macroctenus* scorpion venom than after 1 hour of study.

KEYWORDS: scorpions, kidneys, nephron, endothelium, rats

Wiad Lek. 2026;79(7):1563-1571. doi: 10.36740/WLek/222245 DOI

INTRODUCTION

According to the WHO, approximately 1.5 million cases of poisoning due to scorpion bites are reported annually worldwide, resulting in 2,000 to 3,000 deaths. Scorpionism is prevalent in the United States, Canada, Europe, and Australia. However, this issue is most widespread in Africa, India, Mexico, Brazil, Iran, Saudi Arabia, and Venezuela, where over 2 billion people face a constant threat. Climate change and the rapid urban development near the typical habitats of scorpions significantly heighten the likelihood of encounters with them. Specifically, in Brazil alone, the number of scorpion bite cases has increased from 64,000 to 124,000 per year since 2012 [1-8].

To date, data exists on approximately 2,000 species of scorpions. Most of these are dangerous to humans, particularly those from the families Buthidae, Scorpionidae, and Hemiscorpionidae. The clinical manifestations of poisoning from scorpion toxins vary significantly, depending on the species and the amount of venom that has entered the victim's body [9-12]. Researchers note that the composition of their venom is highly complex and heterogeneous. The most well-studied components are currently small

peptides, mainly due to their diversity and extensive pharmacological properties. Small peptides are classified into three major superfamilies based on their structure: peptides containing cysteine-stabilised (CS) α/β motifs with disulfide bridges, calcins, and peptides that lack disulfide bridges. However, it has been established that among the toxic components of scorpion venom, enzymes, mixtures of inorganic salts, free amino acids, nucleotides, and lipids are also recognised. [13-19].

According to scientific literature, the toxic components of scorpion venom adversely affect the nervous, cardiovascular, respiratory, and blood coagulation systems. In recent years, their role in the development of lesions in the excretory system has been demonstrated.

A thorough analysis of the scientific literature has shown that to date, studies on the effects of scorpion venom on structural and functional parameters of mammalian kidneys are extremely limited. In addition, the composition of the Leiurus macroctenus scorpion venom is not yet sufficiently known, and therefore the features of its influence on morphological changes in internal organs, including kidneys, have not been established. It is worth noting that the study of microscopic changes in the structure of the kidneys under the influence of scorpion venom at different time intervals is important for understanding the main pathogenetic links in the development of complications of scorpion bites. Thus, our study is timely and relevant.

AIM

The study aims to compare the structural changes in the kidneys of experimental rats one and three hours after exposure to Leiurus macroctenus scorpion venom.

MATERIALS AND METHODS

The venom from scorpions of the Buthidae family, genus Leiurus, species Leiurus macroctenus, was administered intramuscularly to rats (0.5 ml of venom solution previously dissolved in saline; 28.8 μg/ml; LD_{50}=0.08 mg/kg) [20].

The study utilised 20 white male laboratory rats, each weighing 200 g (±10 g), reared in the vivarium of the Educational and Scientific Centre «Institute of Biology and Medicine» at Taras Shevchenko National University of Kyiv (Agreement on Scientific and Practical Cooperation between Taras Shevchenko National University of Kyiv, National Pirogov Memorial Medical University, Vinnytsia, and I. Horbachevsky Ternopil National Medical University, dated 1 February 2021). The rats were provided with a standard diet in an accredited vivarium, in accordance with the «Standard Rules for the Arrangement, Equipment and Maintenance of Experimental Biological Clinics (Vivaria)." The experiments were conducted in accordance with current regulatory documents governing the organisation of work involving experimental animals and adhering to the principles of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» [21]. Furthermore, all interventions involving the animals complied with the Law of Ukraine No. 3447-IV of 21 February 2006, «On the Protection of Animals from Cruelty and Ethical Norms and Rules for Working with Laboratory Animals.»

The rats chosen for the experiment were divided into two groups: a control group consisting of 10 rats, which received no venom, with biological material collected 1 and 3 hours after saline solution administration; and an experimental group of 10 rats, for which histological material was collected 1 and 3 hours after administration of scorpion venom. The rats were euthanised using carbon dioxide inhalation. Their kidneys were isolated at 4 °C immediately following euthanasia.

For microscopic examination, kidney samples were collected from all animal groups. The samples were fixed in a 10% formalin solution for one day. Subsequently, the samples were dehydrated in increasingly concentrated alcohols and embedded in paraffin blocks. Histological samples of rat kidneys were stained with hematoxylin and eosin [22]. Histological slides were examined using an SEO SCAN light microscope and photodocumented with a Vision CCD Camera, equipped with an image output system for histological preparations.

AI STATEMENT

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

RESULTS

Studies of histological samples from the kidney, taken one hour after the introduction of scorpion venom into the experimental animals, revealed the onset of acute renal failure due to significant damage to the glomerular apparatus of the nephrons, the emergence of considerable haemodynamic disturbances, and the onset of inflammatory processes within the organ.

The fibrous capsule is oedematous and easily detached from the organ. The venous vessels dilate, and the arterial vessels ischaemiate. In the

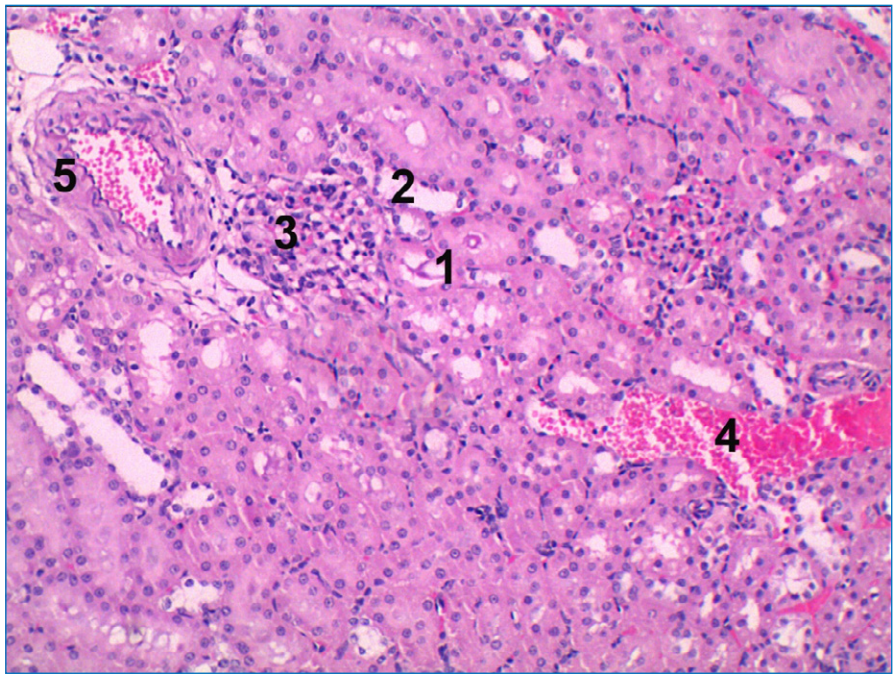

Fig. 1. Microscopic structure of the kidney of white rats 1 hour after the introduction of scorpion venom. 1 - dystrophy of proximal tubules with accumulation of cellular detritus in the lumen, 2 - destruction of distal tubules, 3 - hypertrophied renal corpuscle, 4 - area of haemorrhage, 5 - destructured artery. Staining with hematoxylin and eosin. Magnification. x 200
Authors' own work

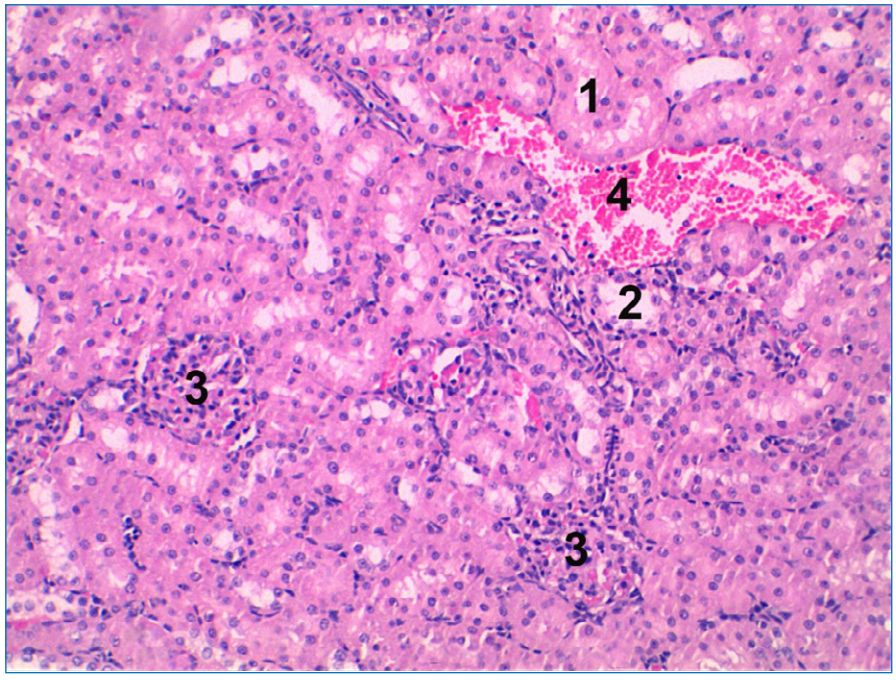

Fig. 2. Microscopic structure of the kidney of white rats 1 hour after the introduction of scorpion venom. 1 - dystrophy of proximal tubules, 2 - destruction of distal tubules, 3 - renal corpuscles with no urinary space, 4 - haemorrhage area. Staining with hematoxylin and eosin. Magnification x 200
Authors' own work

interstitium of the organ surrounding the vessels of the microcirculatory bed, perivascular oedema with infiltration by lymphocytes, macrophages, and histiocytes occurs.

In the cortical layer adjacent to the renal corpuscles, which retain their typical histological structure, hypertrophied renal corpuscles are observed where the lumen of the afferent and efferent arterioles is narrowed. Erythrocyte aggregation is enhanced within their lumens. The walls of the arteries and arterioles are thickened due to hypertrophy and slight leukocyte infiltration of the tunica media, while smooth myocytes, losing their spindle shape, become rounded. Their nuclei are hyperchromic, and the cytoplasm appears brightly oxyphilic. There is mild oedema and lympho-histiocytic infiltration in the subendothelial layer of the tunica intima and tunica adventitia. The endothelial cells of the tunica intima exhibit a cubic shape, and slight oedema can be seen in the cytoplasm. The nuclei are hyperchromic, and their plasmalemma is indistinct, showing significant protrusions into the lumen and invaginations. The basement membrane is continuous, although in some areas, signs of endothelial desquamation are present (Fig. 1).

In the glomeruli, stasis is present. In some areas, their lumens have collapsed due to spasms of the afferent

arteriole. The urinary spaces are sharply dilated. In the walls of the glomerular capillaries, initial signs of focal endothelial dystrophy are evident. The cytoplasm of the endothelial cells is swollen; the nuclei are hypertrophied and hyperchromatic, significantly protruding into the vessel's lumen. The basement membrane has thickened and appears swollen. Due to increased proliferative activity of mesangiocytes, there is an expansion of the mesangium and a rise in the amount of matrix. Mesangial hyperplasia has caused a sharp narrowing of the urinary space of the renal corpuscles, which, in most fields of view, appears slit-like or is not visualised at all (Fig. 2). The cells of the outer layer of Bowman's capsule are sharply flattened, their nuclei are hyperchromatic with invaginations of the karyolemma, and the basement membrane is thickened. The growth of the mesangium disrupts the cytoskeleton of the podocytes in the inner layer of the capsule. There is disorganisation of the cytopodia and cytotrabeculae of podocytes, resulting in the onset of filtration disorders.

In the proximal and distal renal tubules, the lumens are unevenly expanded with thinning of the walls. In the proximal and distal tubules, the epithelium is damaged. The brush border and basal striation are especially noticeable in dystrophic changes. In the foci, the cells almost wholly lose them, and desquamation of the epithelium into the lumen of the tubule is observed. The cells exhibit manifestations of hydropic and hyaline-droplet dystrophies, characterised by the appearance of vacuoles and single acidophilic granules in the weakly oxyphilic cytoplasm, indicating cytoplasmic swelling. The nuclei are hyperchromic and compacted, with indistinct contours. Fibrin accumulations were detected in the lumens of the tubules. In the thin tubules, hyperplasia of epithelial cells is due to the swelling of their cytoplasm. On the cross-section, thin tubules with slit-like lumens. When tubules with moderately dilated lumens are collected, epithelial cells almost completely lose microvilli and basal striation. Their cytoplasm is edematous, with single vacuoles in a weakly oxyphilic cytoplasm. The nuclei are hyperchromic and compacted. Along the course of the vessels of the myocirculatory bed of the interstitium, both in the cortex and medulla, there are locally single haemorrhages (Fig. 2).

Microscopic examination of the kidneys of white rats three hours after the introduction of scorpion venom into the experimental animals revealed a greater degree of dystrophic changes in the organ's structural components compared to the previous experimental group. Local inflammatory infiltrates were observed in the organ against a backdrop of significant vascular changes, coagulopathy, acute renal failure, and tubular necrosis.

Due to the oedema, the fibrous capsule is thickened. The organ shows dilation of the venous vessels and spasm of the arteries. The vessel wall thickens due to hypertrophy of the tunica media, accompanied by perivascular oedema and lymphohistiocytic infiltration. In their lumens, thrombi consisting of erythrocytes and solitary leukocytes are present, without clearly visualising the boundaries between them.

Within the interstitium of the organ surrounding the vessels of the myocirculatory bed, there is notable perivascular oedema and infiltration by lymphocytes, plasma cells, macrophages, tissue basophils, and eosinophils. In the lumens of the vessels, there is stasis, thrombi, and erythrocytes lacking clear boundaries. The capillary wall features an endothelium with disrupted intercellular contacts, and the basement membrane is significantly thinned and discontinuous. Focal areas of endothelial desquamation are present. The nuclei of endothelial cells are hyperchromatic and compacted. They protrude markedly into the lumen—increased vascular-tissue permeability of the interstitium results in pronounced hydration of the ground substance in loose connective tissue. Collagen fibres in areas with significant oedema appear brightly oxyphilic, creating spaces while retaining their fascicular nature, but are defibrotic. Disruptions in blood clotting processes and the integrity of the microcirculatory vessels within the organ lead to the emergence of small, multiple haemorrhages with pronounced erythrocyte hemolysis and substantial lymphohistiocytic infiltration at their periphery.

In comparison to the previous term of the experiment, there is an intensification of destructive-degenerative changes in the glomerular and tubular apparatus of nephrons within the cortical layer, alongside stromal-vascular disorders. The renal corpuscles of the nephrons exhibit varying sizes, predominantly being hypertrophied and deformed. Afferent and efferent arterioles remain in a spasmodic state, with thickened walls resulting from pronounced mucoid swelling, and stasis occurs within their lumens, characterised by significant hemolysis of erythrocytes. Lymphocytes, macrophages-histiocytes, and eosinophils significantly infiltrate the walls of the arterioles. Smooth myocytes of the tunica media appear shortened, displaying hyperchromic nuclei and markedly eosinophilic cytoplasm. The brightly eosinophilic collagen fibres swell in the subendothelial layer of both the tunica intima and tunica adventitia, maintaining their fascicular nature while demonstrating defibrotic characteristics. There is significant dehydration of the ground substance, which shows an increased presence of weakly basophilic glycosaminoglycans.

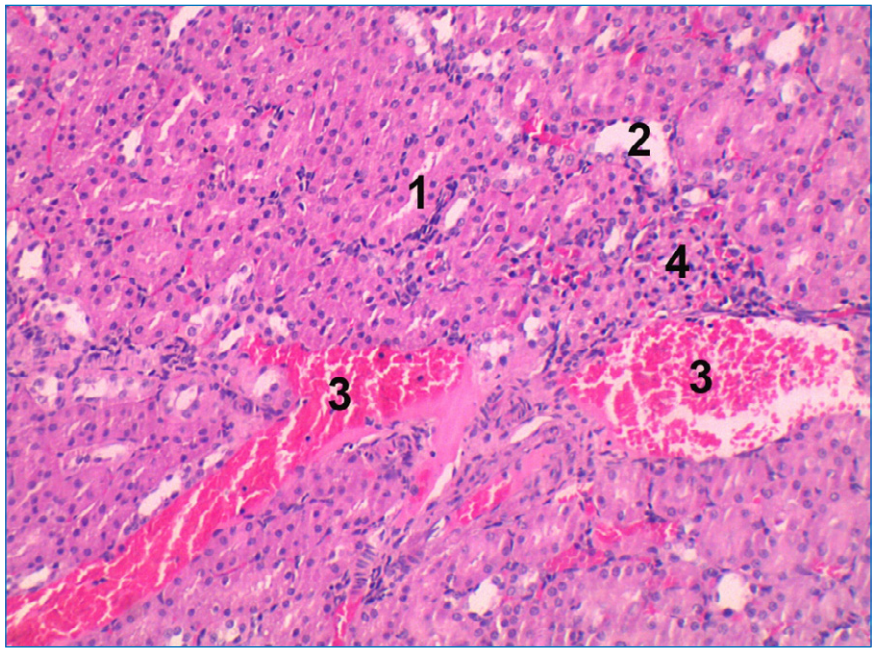

Fig. 3. Microscopic changes in the kidney of white rats 3 hours after the introduction of scorpion venom. 1 - proximal tubules with dystrophically altered epithelial cells, 2 - destruction of the distal tubule, 3 - areas of haemorrhage, 4 - deformed renal corpuscle with absent urinary space. Staining with hematoxylin and eosin. Magnification x 200
Authors' own work

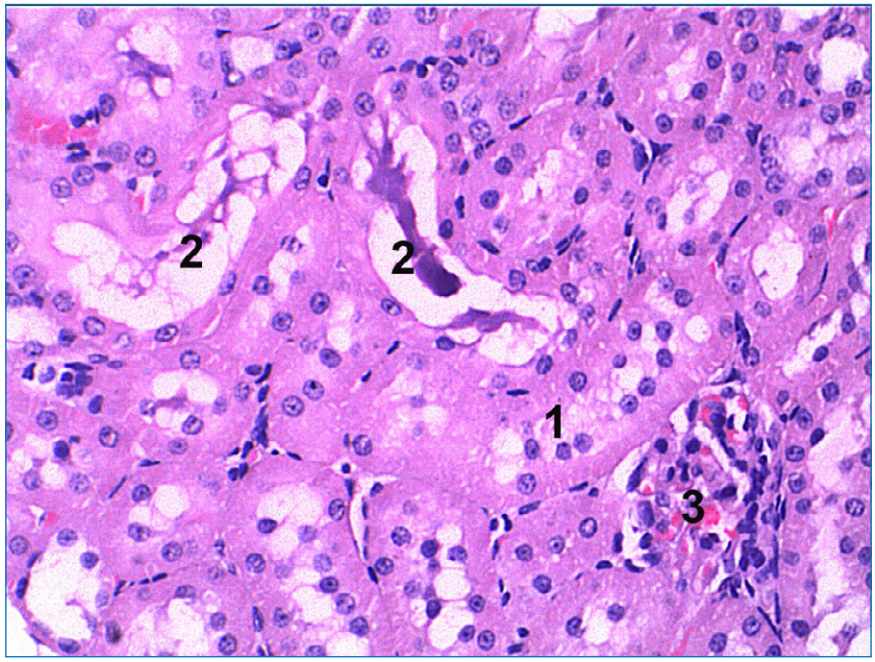

Fig. 4. Microscopic changes in white rats 3 hours after introducing scorpion venom. 1 - proximal tubules, 2 - cellular detritus in the lumen of the tubules, 3 - deformed renal corpuscle. Staining with hematoxylin and eosin. Magnification x 200
Authors' own work

Endotheliocytes display a swollen tunica intima and contain pyknotic, hyperchromic nuclei. The basement membrane is discontinuous and appears thinned, with focal areas of complete endothelial desquamation.

In the wall of glomerular capillaries, destructive degenerative disorders resemble those in the afferent arterioles. The expansion of the urinary spaces is preserved. The cells of the outer layer of the capsule are flattened; the cytoplasm is oxyphilic, and their nuclei are compacted and basophilic, while the basement membrane is thickened. The mesangium increases due to an augmentation in the matrix and active proliferation of mesangiocytes, resulting in significant oedema and destruction of the cytopodia and cytotrabeculae of podocytes. The urinary space was not visualised in such renal corpuscles (Fig. 3).

The proximal and distal renal tubules exhibit dilated lumens. Some tubules are characterised by acute necrotic damage to the epithelial cells and complete desquamation of the epithelium. The epithelium is rounded and loses its complete brush and striated borders; its cytoplasm is brightly oxyphilic. The nuclei are pyknotic, compacted, and intensely basophilic. There are individual cells with nuclei that possess an indistinct karyolemma. This indicates that the nuclei are undergoing lysis and the onset of acute necrosis. In the lumens of such tubules, there is an accumulation of cellular detritus. Simultaneously, a significant number of

tubules display manifestations of hydropic and hyaline-droplet dystrophy of the epithelium, complete loss of brush and basal striation, and desquamation into the tubule lumen. In their lumens, accumulations of protein masses and cellular detritus were observed (Fig. 4).

The above changes were fully confirmed during the morphometric study. Morphometric studies of the cortical substance of the kidneys of white rats after 1 hour of scorpion venom administration established that the average value of the area of the renal corpuscles is (5868,7±289,3) μm^2. In contrast, the average values of the area of the vascular glomerulus and the capsule cavity are (4618,1±221,9) μm^2 and (1250,6±60,5) μm^2, respectively. The average value of the diameter of the proximal renal tubules is (43,98 ±1,96) μm, respectively, but the average area of the tubules is (1518,38 ±73,90) μm^2. The average values of the diameter and area of their lumen are (25,06 ± 1,21) μm and (492,98 ± 22,64) μm^2, respectively. The parameters of the epithelial cells of the proximal nephron changed, consisting of a partial increase in height and width, with values of (14,75 ± 0,63) μm ($p < 0,05$) and (16,28 ± 0,74) μm, respectively. Taking into account the specified changes in the height and width of the cells, their average area is (240,13 ± 11,05) μm^2. The average value of the area of the epithelial cell nucleus is (28,35 ± 1,39) μm^2, and the nuclear-cytoplasmic ratio is 0,118 ± 0,003. The average value of the diameter of the distal tubules is insignificantly equal to (41,43 ± 1,86) μm, but their area has increased and is (1347,41 ± 66,37) μm^2. The average diameter of the lumen of the distal tubules is (22,85 ± 1,08) μm. It was found that the average value of the lumen area of the distal tubules is (409,51 ± 18,78) μm^2. The average height of the epithelial cells of the convoluted distal tubules of the nephron is (8,35 ± 0,37) μm, respectively, but the average value of the cell width decreases and is (12,90 ± 0,63) μm. The average area of the epithelial cells increases due to cytoplasmic swelling and is (107,72 ± 5,28) μm^2. The average value of the area of the epithelial cells' nuclei is (26,07 ± 1,25) μm. Violation of the structure of epithelial cells increased the nuclear-cytoplasmic ratio, which was 0,242 ± 1,25.

In contrast to these data, a significant change in the above parameters is observed in rats 3 hours after the introduction of scorpion venom into the experimental animals: the average value of the renal corpuscle area is (6646,4 ± 313,5) μm^2. The average values of the area of the vascular glomerulus and the capsule cavity are (5273,8 ± 254,1) μm^2 and (1372,6 ± 60,3) μm^2, respectively. The average diameter of the proximal renal tubules has decreased to (42,71 ± 1,94) μm. At the same time, the average area of the tubules is significantly reduced, measuring (1431,95 ± 69,75) μm^2. The average value of the diameter of the tubular lumen is (26,85±1,32) μm, and the average value of the tubular lumen area significantly ($p<0,001$) increases and reaches the value of (565,92±26,05) μm^2. It was established that the average value of the height of epithelial cells is (13,08±0,59) μm, and the value of the average width of cells significantly ($p<0,05$) increases and equals (17,35±0,81) μm. Accordingly, the average value of the area of epithelial cells is (226,94 ± 10,47) μm^2. The average value of the area of the epithelial cell nucleus decreases and is (25,19±1,20) μm^2. An insignificant decrease in the nuclear-cytoplasmic ratio to the value of 0,111±0,001 was established.

The average diameter of the distal tubules decreases to a value of (40,02 ± 1,78) μm, while the average area of the tubules significantly decreases ($p < 0,001$) to (1257,26 ± 61,18) μm^2. The average indicator of the diameter of the lumen of the distal tubules is (23,51 ± 1,11) μm. There is a significant ($p<0,001$) increase in the average value of the lumen area of the distal tubules, which is (433,89 ± 20,39) μm^2. The average value of the height of the epithelial cells of the convoluted distal tubules of the nephron is (9,28 ± 0,41) μm, and their width is (11,35 ± 0,55) μm. In contrast, the average area of the epithelial cells is (105,33 ± 5,23) μm^2. The average value of the area of epithelial cell nuclei decreases to (23,76±1,16) μm^2, and the nuclear-cytoplasmic ratio is 0,226±0,009.

DISCUSSION

Among the currently known pathological processes associated with scorpionism, kidney damage is regarded as one of the most critical complications, often resulting in fatality [23, 24, 25]. Notably, Hemiscorpius lepturus and Androctonus australis are observed to exhibit a pronounced nephrotoxic effect. The toxic compounds in their venom can directly or indirectly impact kidney tissue, leading to conditions such as mesangiolysis, glomerulonephritis, vasculitis, interstitial nephritis, cortical and tubular necrosis, as well as hypoxia and renal infarction [26, 27]. Among these conditions, tubular necrosis is most frequently reported in victims, characterised by reduced reabsorption and increased secretion processes of Na^+, K^+, and Cl^-. Studies have shown that venom inoculation affects Na^+ channels within the kidneys. Concurrently, there is an elevation in the blood levels of catecholamines among the victims. In the cytosol of nephron tubular cells, during scorpion toxin-induced poisoning, the concentration of Ca^{2+} ions rises. The body's protective mechanisms lead to an increase in pro-inflammatory cytokines within the renal parenchyma, including IL, TNF-α, NO, platelet aggregation factor, PG, leukotrienes, kinins, and angiotensin.

Clinical cases of bites accompanied by rhabdomyolysis, haemolysis, and disseminated intravascular coagulation have been documented to date. These processes have contributed to the development of acute renal failure through damage to the glomerular apparatus and have been associated with haemoglobinuria and proteinuria. The action of venom PLA2 can also lead to acute kidney injury. The enzymes in these conditions exhibit haemolytic and cytotoxic effects on the organ. Additionally, it promotes the generation of arachidonic acid and, consequently, the synthesis of eicosanoids. There is also evidence of increased activity of the hypothalamic-pituitary-adrenal system, which stimulates corticosteroid production. Simultaneously, an increase in the levels of acute phase proteins and significant haemodynamic disturbances has been recorded. It is known that the accumulation of a substantial number of haemoproteins accompanies haemolytic phenomena. Under these conditions, heme groups enhance the production of free radicals that accumulate in the renal cortex, exerting a toxic effect. An increase in NADPH oxidase activity facilitates the production of reactive oxygen species (ROS). In this context, apoptosis or necrosis of kidney cells is a frequent consequence [28].

Movahed A. et al. [29] studied the effect of the scorpion Hemiscorpius lepturus venom on the morphology of the kidneys. During the histological examination, they observed an expansion of the lumen and thinning of the walls of the proximal tubules of the nephron, as well as the accumulation of fibrin in the glomerular apparatus. The glomerular capillaries contained clusters of erythrocytes, and the endothelium of their walls was swollen four hours after the introduction of the venom to laboratory animals. In the proximal tubules, flattening of the epithelial cells of their inner membrane, loss of their brush border, and an atypical response of the nuclei of these cells were noted, indicating the development of acute tubular necrosis.

A thorough analysis of the scientific literature has demonstrated the involvement of macrophages in the development of acute kidney injury due to scorpion stings. Consequently, the level of MCP increases in the victim's body, indicating the initiation of an inflammatory process in the organ and signalling the risk of complications, as it directly affects the migration of macrophages and the proliferation and differentiation of leukocytes in the epithelium of the human kidney tubules. MCP-1 stimulates the secretion of IL-6 and the intercellular expression of ICAM-1. Additionally, binding to the chemokine receptor CC2 on the surface of podocytes can reduce the expression of both microRNA and nephrin protein. The latter is involved in forming filtration gaps; therefore, defects in this protein lead to renal failure, disruption of the podocyte cytoskeleton, and disorders of the filtration process [30].

CONCLUSIONS

1. One hour after administering *Leiurus macroctenus* scorpion venom to rats, we observed the development of significant haemodynamic disorders and the initiation of inflammatory processes in the organs, as well as acute renal failure resulting from damage to the glomerular apparatus. Disturbances in the cytoskeleton of podocytes and the tubular apparatus of nephrons, accompanied by desquamation, hydropic changes, and hyaline-droplet dystrophies of the tubular epithelium, were established.
2. The introduction of *Leiurus macroctenus* scorpion venom to rats resulted in significantly pronounced dystrophic changes in the structural components of the kidneys three hours after the commencement of the experimental study, in contrast to the earlier phase of the investigation. Inflammatory processes in the organ interstitium were observed, featuring plasmacytic, lymphocytic, and macrophage infiltration. Vascular and haemodynamic disorders were also detected, as evidenced by the desquamation of the endothelial lining of the vascular wall and increased permeability. The development of tubular necrosis characterised the renal tubular apparatus.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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RECEIVED: 19.03.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Exploring the therapeutic potential of Beta-3 adrenergic agonists in overactive bladder: A comparative analysis of monotherapy *versus* combination therapy strategies

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ABSTRACT

Aim: to compare effects and side-effect profiles between mirabegron monotherapy and 2 combination treatments including mirabegron in patients with OAB.

Materials and Methods: Study is nonrandomized, prospective, and observational; conducted in 22 centers, 400 patients with OAB symptoms. Patients administered 50 mg monotherapy of mirabegron for 8 weeks. Responders received monotherapy (Group 0, n=109) and non-responders allocated to double therapy with mirabegron plus Solifenacin 5mg (Group 1, n=145) or mirabegron plus Flavoxate/Uripas (Group 2, n=146) for an additional eight weeks. Providing bladder volume, postvoid residual and BWT served as primary end points.

Results: Prevalence of urgency, frequency, and nocturia was high in cohort (92.8%, 88.5%, and 76.5%), but that of UUI35 was low at 9%. This indicates that patients are clearly of OAB-dry phenotype. Mixed storage-voiding symptom was most frequent among 53.8%. An unusual baseline comparison population along to chew, - 200% male and severe symptom' GROUPS 2 BWT a benefit of mirabegron\+\-Solifenacin); one could get; other couldn't (-36ml *vs.* -31ml). End-point analysis did not allow exclusion of therapeutic equipoise of REGULAR vs. DROP for final PVR (between-group difference: -1.48 mL, p = 0.065).

Conclusions: Beta-3 agonist as monotherapy is a potent, multimodality first-line therapy with strong influence of severe disease. Each of combinatory treatments show characteristic effect on phenotype-specific increase in bladder capacity. Mirabegron flaoxate could be considered for detrusor hypertrophy and combination of mirabegron Solifenacin would stimulate voiding facilitate. These proof of concept data justify PPTP based on objective biomarkers such as BWT and PVR to select combination therapy in what is another small step in our journey towards precision medicine for OAB.

KEYWORDS: overactive blasser, mirabegron, beta-3 adrenergic agonist, solifenacin, flavoxate, combination therapy, bladder wall thickness, personalized medicine; phenotyping

Wiad Lek. 2026;79(7):1572-1580. doi: 10.36740/WLek/222508 DOI

INTRODUCTION

Introduction Overactive bladder (OAB) is a prevalent and clinically relevant syndrome that encompasses urgency urinary incontinence (UUI) with or without urgency usually with frequency, and nocturia, in the absence of any causal condition or infection of the urinary tract [1]. GB is both a physical and psychological burden to the patients, causing an impairment of (HRQoL), anxiety and depression as well as limitations in social activities and work. According to the clinical and epidemiological data, it has been estimated that OAB occurs in 16-17% of adult population according to age (thereby becoming a significant public health issue in various places around the world [2]. The management of OAB progresses with step therapy by 1 consisting of non-invasive behavioral therapies as the first approaches (bladder training and pelvic floor muscle training). The limited pharmacologic therapies also recommended by the AUA and SUSU include muscarinic or beta-3 agonist medications [3]. Antimuscarinic, like Solifenacin, oxybutynin and tolterodine suppress involuntary detrusor contractions by blocking muscarinic receptors and yet more they may be under-prescribe due to these anticholinergic side effects and hence low treatment continuation rate especially in the elderly [4]. Beta-3AGONISTS OAB Paradigm Shifters Beta-3 agonists are the next game changers in the treatment of OAB. Mirabegron as a beta-3 agonist, mirabegron is the prototype of this class, with a unique mode of action in that it causes detrusor muscle relaxation during storage phase via specific activation of beta-3 adrenoreceptors

and thus increases bladder capacity [5]. It has similar efficacy to Antimuscarinic without an anticholinergic adverse event profile and thus has higher tolerability, with better adherence to treatment. Suspect: B-A Prescribing This 'B' with a good side-effect profile (on rate of dry mouth and constipation) is increasing dramatically in usage, especially in the elderly and C/I Antimuscarinic patients [6]. Despite the efficacy of monotherapy, a good proportion of patients fail to achieve sufficient control of symptoms through the use of a single drug. It is a highly active treatment for these patients. Recent pristine RCT-based data on combination therapy of a beta-3 agonist with an Antimuscarinic from SYNERGY and BESIDE studies are available [7-8]. The 2024 AUA/SUFU guideline on idiopathic overactive bladder significantly moves away from "step-wise" therapies to personalized, patient centered treatment paradigms with shared decision-making [9]. This paradigm shift acknowledges that interventions may not be applicable for all patients based on the phenotype or the urodynamic profile of an individual patient, and thus supports exploring mechanisms which result in OAB in each patient and treating them individually. Equally exciting is movement toward phenotype-specific OAB treatment using biomarkers and objective measures in personalized urology [10]. Prospective data for combination therapy vs. Antimuscarinic monotherapy in the case of OAB is equally strong, but there is an important void between β-3 agonist monotherapy and when we assess various combinations of therapies. At present uptake of a enter into clinical decision making, yet to date there has been no clear evidence as to whether treatment will be commenced with mirabegron monotherapy or addition added on combination therapy as in the case for an invasive good symptom control marginal safe. In addition, EGFR and other biomarkers which have anticancer potentiality in personalized treatment decision for combination are also a question that has not been solved [11]. Methods for consideration of this, proof was sought from evidence-based literature to compare the effectiveness and safety between the mirabegron monotherapy and combination therapy with specific two drugs (mirabegron combined with Solifenacin (an Antimuscarinic) or Flavoxate (a direct muscle relaxant)), in terms of efficacy and safety in patients with OAB. All patients were assessed clinically and by U/S (bladder wall thickness; BWT, post void volume; PVR) both before and after treatment.

AIM

The aim of this study is to compare effects and side-effect profiles between mirabegron monotherapy and 2 combination treatments including mirabegron in patients with OAB.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This prospective, randomized, controlled, open-label, parallel-group clinical trial was conducted at the urology outpatient clinics in Sadr Teaching Hospital, Basra, Iraq. Data collection was planned over a period of 18 months from the start of recruitment through the treatment and follow-up phases ending in final evaluation of patients. The study protocol was reviewed and approved by the Committee of Ethics in Basra Health Directorate and by the Scientific Committee of Basra University. All participants gave written informed consent prior to participation. Data confidentiality and patient privacy were ensured throughout the study.

STUDY POPULATION

Treatment-seeking adult patients (≥18 years) of either sex presenting with symptoms consistent with OAB were eligible for inclusion. OAB was defined according to the International Continence Society (ICS) criteria updated to 2018 as urinary urgency, with or without urgency urinary incontinence, usually accompanied by frequency and nocturia, for at least 3 months, in the absence of urinary tract infection or other obvious pathology.

INCLUSION CRITERIA

1 Adults aged 18 years or older of either sex;
2 Clinical diagnosis of OAB fulfilling the ICS definition;
3 Presence of urinary urgency as the predominant symptom for at least 3 months;
4 Willingness to participate after providing informed consent.

EXCLUSION CRITERIA

1 Active urinary tract infection or hematuria;
2 Neurogenic bladder;
3 History of bladder outlet obstruction or post-void residual urine volume greater than 150 mL;
4 Patients who reported using the study medications within the past 3 months;
5 Pregnancy or breastfeeding;
6 Cognitive impairment that can affect reporting capability of patient's symptoms and other information required to fill the data collection form.

SAMPLE SIZE CALCULATION

Based on previous studies such as the SYNERGY and BESIDE trials, the expected mean difference in reduction of urgency episodes per 24 hours between groups would be 0.5 episodes. Considering a standard deviation of 1.0 and to achieve 80% power at a 5% level of significance, a minimum of 63 patients per arm was required. To compensate for dropout, 15% was added to the estimated sample size. Therefore, 400 patients were considered sufficient to bring the required power and measure the significant differences when present.

TREATMENT PROTOCOL AND RANDOMIZATION

After reaching the planned sample size of 400 patients, all participants received monotherapy with the beta-3 agonist mirabegron at a dose of 50mg once daily for 8 weeks. Patients were classified into three severity grades (mild, moderate, and severe) according to a symptom grading system. Medications were ceased for 1 week (clearance period), and patients were re-evaluated during this washout period. Following the monotherapy phase, 110 patients had complete resolution of symptoms and were classified as monotherapy responders (Group 0, n=109 in final analysis). The remaining 290 non-responders were randomized into two arms of 145 patients each. One arm received mirabegron 50mg plus solifenacin 5mg once daily (Group 1, n=145). The second arm received mirabegron 50mg plus flavoxate (Uripas) (Group 2, n=146). The combination treatment continued for an additional 8 weeks. All medications were taken orally. Patients were instructed to take the assigned medication(s) once daily with no dose modification. Adherence to the treatment protocol was monitored by pill count and patient records.

OUTCOME MEASURES

PRIMARY EFFICACY OUTCOMES

The main efficacy parameters that were assessed included:

1 The relative change from baseline in FAC measured by TAUS,

2 The relative changes of PVR and BWT. All subjects underwent the measurement of PVR and BWT by a routine ultrasound technique.

SECONDARY EFFICACY OUTCOMES

Secondary measures of efficacy included:

1 Mean change from baseline in number of micturition per 24 hr. after 12 weeks;

2 Change from baseline to week 12 in the number of urgency episodes per 24 hr.;

3 Incontinence episodes/24 hr.;

4 Volume voided at a single micturition for all participants who completed scores on the PPBC and patients with a response option ≥2% of days from treated as missing data Summary statistics were used for patient-reported outcome measure based on OAB-q SF responses by response option for each category, each individual question, and four domains and symptom bother total without re cording them back between categories;

5 Patients 'Perception of Bladder Condition.

SAFETY OUTCOMES

Safety was evaluated by:

1 incidence of AEs and SAEs;

2 changes in BP, HR and P VR after voiding; and

3 withdrawal due to tolerability.

DATA COLLECTION AND MONITORING

Patients were evaluated at 4 intervals: baseline, week 4, week 8, and week 12. At each visit, the following information was obtained: symptom diary (3-day diary); OAB-q and PPBC questionnaires; vital signs and adverse event monitoring; urinalysis; and ultrasound measurement of post-void residual and bladder wall thickness.

ULTRASOUND PROTOCOL

Bladder wall thickness was measured using transabdominal ultrasound with a 3.5-5 MHz convex transducer at a standardized bladder volume of 150-250 mL. Measurements were taken at the anterior bladder wall in the midline, perpendicular to the bladder surface. A BWT threshold of 3 mm was considered indicative of detrusor hypertrophy based on established literature. Post-void residual volume was measured within 10 minutes of voiding using standard volumetric calculations.

STATISTICAL ANALYSIS

All statistical analyses were performed using IBM SPSS Statistics (version 26, IBM Corp., Armonk, NY, USA) and GraphPad Prism (version 10, GraphPad Software, San Diego, CA, USA). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables as frequencies and percentages. Normality of distribution was assessed using the Shapiro-Wilk test. For normally distributed continuous variables, comparisons between baseline and follow-up within groups were conducted using paired-samples t-tests, while independent-samples

Table 1. Symptom prevalence and ranking (N=400)

Rank	Symptom	n/N	Prevalence	95% CI
1	Urgency	371/400	92.8%	90.3-95.3%
2	Polyuria/Frequency	354/400	88.5%	85.4-91.6%
3	Nocturia	306/400	76.5%	72.3-80.7%
4	Intermittent stream	257/400	64.3%	59.6-69.0%
5	Dribbling	245/400	61.3%	56.5-66.1%
6	Loin pain	166/400	41.5%	36.7-46.3%
7	Incontinence	36/400	9.0%	6.2-11.8%
8	Hematuria	0/400	0.0%	-

Notes: CI = confidence interval
Source: Compiled by authors of this study

Table 2. Baseline patient characteristics by treatment group

Characteristic	Group 0 (n=109)	Group 1 (n=145)	Group 2 (n=146)	p-value
Age, years (mean ± SD)	38.68 ± 8.96	39.06 ± 7.06	38.18 ± 6.10	NS
Male sex, n (%)	82 (75.2%)	145 (100%)	113 (77.4%)	<0.001
Married, n (%)	72 (66.1%)	116 (80.0%)	128 (87.7%)	<0.001
Symptom duration, months	10.69 ± 10.71	16.75 ± 13.55	10.30 ± 9.17	<0.05
Severity, n (%)				<0.001
Mild	24 (22.0%)	0 (0%)	0 (0%)	
Moderate	44 (40.4%)	0 (0%)	43 (29.5%)	
Severe	41 (37.6%)	145 (100%)	103 (70.5%)	
Ultrasound parameters				
BWT, mm	2.44 ± 0.75	2.49 ± 0.50	3.56 ± 1.74	<0.001
Pre-void volume, mL	244.33 ± 41.28	220.51 ± 28.33	232.21 ± 36.97	0.003
PVR, mL	24.27 ± 10.32	34.28 ± 3.91	32.42 ± 6.50	<0.001

Notes: Group 0 = monotherapy responders; Group 1 = mirabegron + Solifenacin; Group 2 = mirabegron + Flavoxate. BWT = bladder wall thickness; PVR = post-void residual; NS = not significant. Data presented as mean ± SD or n (%)
Source: Compiled by authors of this study

t-tests were employed for between-group comparisons. In cases where assumptions of normality were not met, equivalent non-parametric tests (Wilcoxon signed-rank test or Mann-Whitney U test) were applied. Categorical outcomes were analyzed using the Chi-square test of independence (or Fisher's exact test when cell counts were <5). For repeated measurements across treatment stages (baseline, post-monotherapy, post-combination), repeated-measures ANOVA was used with post hoc Bonferroni correction for multiple comparisons. Where sphericity was violated, the Greenhouse-Geisser correction was applied. Effect sizes were reported as Cohen's dz for within-group paired comparisons and Cohen's d for between-group contrasts, with 95% confidence intervals (CIs) calculated for mean changes. Adjustment for multiple pairwise contrasts was performed using the Holm-Bonferroni correction to control family-wise error. Statistical significance was defined as a two-tailed p-value < 0.05.

RESULTS

PATIENT ENROLLMENT AND DISPOSITION

The final group consisted of 400 patients who were eligible. After the 8 weeks of monotherapy with mirabegron 50mg, patients were classified by response: 109 (27.3%) continued with only one drug (Group 0) and 291 patients (72.7%) switched to combination therapy. Mirabegron plus Solifenacin was administered to one hundred and forty five patients (Group 1) while mirabegron plus Flavoxate/Uripas received by one hundred and forty six of them (Group2).

DURATION OF OVERACTIVE BLADDER SYMPTOMS

Among the sample (n=400), the median symptom duration was 6.0 months (IQR: 2.5-21.0), and the mean and median of symptom presence were 12.74 ± 11.71 and 1-41 months,

Table 3. Within-group changes after β3-agonist monotherapy phase

Group	Outcome	Mean Change [95% CI]	t (df)	p	dz
Group 1 (n=145)	BWT [mm]	-0.38 (-0.71, -0.06)	-2.34 (144)	0.021	-0.20
	Pre-void [mL]	+57.38 (52.17, 62.60)	21.76 (144)	<0.001	+1.81
	PVR [mL]	-5.90 (-6.51, -5.29)	-19.10 (144)	<0.001	-1.59
Group 2 (n=146)	BWT [mm]	-0.15 (-0.30, -0.01)	-2.07 (145)	0.040	-0.18
	Pre-void [mL]	+37.54 (30.78, 44.31)	10.97 (145)	<0.001	+0.91
	PVR [mL]	-2.83 (-3.49, -2.16)	-8.39 (145)	<0.001	-0.70

Notes: BWT = bladder wall thickness; PVR = post-void residual; dz = Cohen's effect size for paired comparisons. Negative changes in BWT and PVR indicate improvement; positive changes in pre-void volume indicate increased bladder capacity

Source: Compiled by authors of this study

respectively. A bias to the right was found because most patients had early access to medical review shortly after their illness onset. Symptoms lasted ≤6 months in 50.2% of the entire cohort (n=201) and >36 months in 5.0% only (n=20).

SYMPTOM PREVALENCE AND PATTERNS

Frequency OAB syndrome symptoms were the most common (Table 1). Urgency (92.8%, n=371) was reported as being the predominant symptom, followed by polyuria/ frequency (88.5, n=354), with nocturia (76.5%, n=306) coming third. The most frequent voiding symptoms were not non-associated with storage, and intermittent stream (64.3%, n = 257) and dribbling (61.3%, n = 245), respectively, were the most common among them. Note that only 9.0% of the patients were on UI (n=36) indicating a prevalence for OAB-dry phenotype. Loin pain describers.

SYMPTOM BURDEN AND CLUSTERING

The mean value for the reported number of symptoms was 4.34 ± 1.24 (median, 5.0). The majority of patients (n=329, 82.3%) reported an intermediate number of symptoms (3–5), with 45 CSF-positive patients experiencing a high symptom burden (≥6; 11.3%). Low burden (≤2 symptoms) was observed in 6.5% of all patients only (n=26). Three sets of clusters of symptoms were discovered: the storage core triad (urgency + nocturia + polyuria), which presented in 69.3% (n=277); voiding stimuli bundle (intermittent stream - dribbling), 53.8% (n=215); and men with pure storage complaints but an absence of any voiding-related complaints, only seen in 15.0% with acute urinary retention symptom presentation (n=60).

BASELINE DEMOGRAPHICS AND CLINICAL CHARACTERISTICS BY TREATMENT GROUP

All groups showed significant differences at baseline (Table 2). Comparison is the crucial lens through which we should be viewing all those other effect findings. All male patients were 100% in group I (mirabegron & Solifenacin, both drugs). All these had very high symptoms. Symptom duration in Group 1 (16.75 ± 13.55 months) was significantly longer compared with both Group 0 (10.69 ± 10.71 months) and Group 2 (10.30 ± 9.17 months). The average age of the groups were compared and found not to be statistically different (Group 0: 38.68 ± 8.96, Group 1: 39.06 ±7.06, Group 2: 38.18 ± 6.10). The rate of incidence in the severe cases was higher 70.5% than that in Group 0 (37.6%), and now we can understand why monotherapy might not be enough. The only meaningful objective difference was observed for BWT: the BWT mean in G2 (3.56 ±1.74 mm) appeared to be significantly different from group O and group I (p<0.001). Baseline pre-void residual volumes differed significantly: Group 0 had the highest values (244.33 ± 41.28 mL), followed by Group 2 (232.21 ± 36.97 mL) and Group 1 (220.51 ± 28.33 mL). Baseline PVR was lowest in Group 0 (24.27 ± 10.32 mL) and higher in Groups 1 (34.28 ± 3.91 mL) and 2 (32.42 ± 6.50 mL).

THERAPEUTIC EFFICACY OF B3-AGONIST MONOTHERAPY

The multimodal efficacy of beta-3 agonist was histometric in OAB pathophysiology (Table 3). Summary of Results (continued) Variable Key: M = Mean; SD = Standard Deviation Outcomes Placebo Details Placebo Adjusted mirabegron Efficacy All 3 treatment groups 25 mg b.d.50 mg q.d.100 mg q.d.P value NE Enrolment NE Yes (P>0.0007 It is clear from Table 1 that statistically significant and clinically meaningful improvements in the primary UDS variables have been found, for each group of interventions). Pre-void residual was elevated in both groups. Effect sizes in all groups were large: Group 1 (dz = +1.81, p<0.0002); Group 2 (dz = +1.51; p<0.005) and; Group 3 (d=+4.82; p<0.000001). 18; p=0.040).

Table 4. Combination therapy outcomes: Final values and total change from baseline

Parameter	Group 1 (Mirabegron + Solifenacin)	Group 2 (Mirabegron + Flavoxate)	p-value†
Bladder Wall Thickness [mm]			
Baseline	2.49 ± 0.50	3.59 ± 1.74	<0.001
Post-combination	1.86 ± 0.39	2.63 ± 1.11	<0.001
Mean change [95% CI]	-0.64 (-0.72, -0.55)	-0.96 (-1.15, -0.77)	0.002
Pre-void Residual Volume [mL]			
Baseline	220.51 ± 28.33	232.58 ± 36.84	0.003
Post-combination	328.69 ± 48.68	332.47 ± 40.72	0.521
Mean change (95% CI)	+108.19 (99.95, 116.42)	+99.89 (93.82, 105.96)	0.110
Post-void Residual Volume [mL]			
Baseline	34.28 ± 3.91	32.43 ± 6.52	0.052
Post-combination	22.86 ± 6.58	24.40 ± 6.94	0.081
Mean change (95% CI)	-11.42 (-12.35, -10.49)	-8.03 (-9.03, -7.03)	<0.001

Notes: Data presented as mean ± SD or mean change (95% CI). †Between-group comparison using independent t-test. Within-group comparisons all p<0.001 by paired t-test

Source: Compiled by authors of this study

COMBINATION THERAPY OUTCOMES

Following escalation to combination therapy, both regimens demonstrated robust efficacy with distinct differential effects (Tables 4 and 5).

FINAL TREATMENT OUTCOMES

Significant baseline differences persisted between the two combination groups. Group 2 started with significantly higher BWT (3.59 ± 1.74 mm *vs.* 2.49 ± 0.50 mm, p<0.001) and pre-void residual volume (232.58 ± 36.84 mL *vs.* 220.51 ± 28.33 mL, p=0.003). Following combination therapy, both groups demonstrated substantial improvement. For BWT, Group 1 achieved a final value of 1.86 ± 0.39 mm compared to Group 2's 2.63 ± 1.11 mm (p<0.001). The total change from baseline was significantly greater in Group 2 (-0.96 mm) compared to Group 1 (-0.64 mm), with a between-group difference of p=0.002. For pre-void residual volume, final values were comparable between groups (328.69 ± 48.68 mL *vs.* 332.47 ± 40.72 mL, p=0.521). Both groups showed large, equivalent increases: Group 1 increased by +108.19 mL and Group 2 by +99.89 mL, with no significant between-group difference (p=0.110). For PVR, final values were similar between groups (22.86 ± 6.58 mL *vs*. 24.40 ± 6.94 mL, p=0.081). However, the reduction was significantly greater in Group 1 (-11.42 mL) than in Group 2 (-8.03 mL), with a between-group difference of p<0.001

ADDITIONAL THERAPEUTIC EFFICACY OF COMBINATION THERAPY ESCALATION

The additional change in urodynamic parameters following escalation from monotherapy to combination therapy revealed distinct group-dependent effects (Table 5). For BWT, Group 1 showed a non-significant additional reduction of -0.25 mm (dz = -0.13, p=0.133). In contrast, Group 2 demonstrated a highly significant additional reduction of -0.76 mm (dz = -0.70, p<0.001). For pre-void residual volume, both groups showed significant additional increases: Group 1 increased by +50.46 mL (dz = +1.32, p<0.001) and Group 2 by +62.55 mL (dz = +1.78, p<0.001). Both groups showed significant additional PVR reductions: Group 1 reduced by -5.55 mL (dz = -1.44, p<0.001) and Group 2 by -5.21 mL (dz = -0.92, p<0.001).

PRIMARY ENDPOINT ANALYSIS: BETWEEN-GROUP COMPARISON

Non inferiority of both combination therapies was not demonstrated in the between-group analysis presented in (Table 6) based on justifiable use using the primary outcome item (post-void residual volume at final visit). The adjusted mean PVR was 1.48 mL higher in group 2 than in group 1 (adjusted MD = -1.48 ml, SE=0.80 and CI 95% = -3.05 to ≈ +0.09). This 'negative' delta value relates to a (small), however, "positive" tendency of better voiding efficiency in Group 1. This differed from control [(t = −2.23, p = 0.040), but this did not reach the conventional threshold of alpha = 0.05 when corrected for multiple comparisons using Holm procedure (t = −1.85, Holm-adjusted p = 0.065)]. Post void residuals (23-24 ml) determined in the two study groups were close to being very low, clinically and obviously below the 50-ml safety limit. 2014 Service At least at the time of this analysis, based on primary PVR reduction the

Table 5. Additional change after escalation to combination therapy

Group	Outcome	Mean Change [95% CI]	t (df)	p	dz
Group 1 (n=145)	BWT [mm]	-0.25 (-0.57, 0.08)	-1.51 (144)	0.133	-0.13
	Pre-void [mL]	+50.46 (44.16, 56.75)	15.85 (144)	<0.001	+1.32
	PVR [mL]	-5.55 (-6.19, -4.92)	-17.32 (144)	<0.001	-1.44
Group 2 (n=146)	BWT [mm]	-0.76 (-0.94, -0.58)	-8.35 (145)	<0.001	-0.70
	Pre-void [mL]	+62.55 (56.79, 68.31)	21.46 (145)	<0.001	+1.78
	PVR [mL]	-5.21 (-6.14, -4.29)	-11.11 (145)	<0.001	-0.92

Notes: Paired t-tests quantify additional benefit after adding combination agent, dz = Cohen's effect size (mean difference / SD of differences)
Source: Compiled by authors of this study

Table 6. Primary endpoint: Pairwise adjusted mean differences at final visit (Holm-corrected)

Contrast	Adjusted Mean Difference (mL)	SE	t	p (Holm)
Group 1 vs. Group 2	-1.48	0.80	-1.85	0.065

Notes: Negative values favor Group 1 (lower = better PVR). Holm adjustment controls family-wise error across pairwise contrasts. At final visit, Group 1 had 1.5 mL lower mean PVR compared with Group 2; this did not reach significance after correction
Source: Compiled by authors of this study

two combination therapies would hence be regarded as equivalent in result.

DISCUSSION

These results have an impact on the treatment hierarchy and offer valuable evidence for stratified management of OAB. Our results demonstrate that while baseline demographics and potential performance indicators such as BWT are important confounders (the subject of the study is simply not only fit to be studied) in an unbalanced study, they also make good predictor(s) for response rate against time-to-escalation. It may be helpful to have more personal information of the medical history, disease symptoms and consultation habit to better understand 400 cases enrolled in our series; therefore its application value could be determined whether these information are consider as important or tenable. The right-skewed symptom-duration distribution (median, 6 mo) suggests that most patients experienced their symptoms and presented to our clinic in relatively short order (e.g., bothersome enough for seeking evaluation fairly soon after becoming symptomatic). The symptom prevalence pattern (92.8% urgency, 88.5% frequency, 76.5% nocturia) is characteristic for the classical OAB syndrome complex. Also, the low rate of UUI (9.0%) and the tendency toward the OAB-dry subtype were remarkable. This further supports the fact that it is urgency and frequency which is motivating patients to care, giving early disease a chance. The presence of widely associated voiding symptoms (53.8% storage-voiding dysfunction overlap) does not favor a dichotomous diagnostic separation. Such a clinical overlap also reflects the practice at which we treat male patients suffering both (BPH) and OAB, when already today it makes sense if somehow that benign prostatic hyperplasia (BPH) and OAB are in bad company in most patients who will need combined treatments for storage and voiding lUTS [12]. The beneficial differences in baseline characteristics between groups are a key consideration in interpreting treatment effectiveness. The significant additional BWT in Group 2 (3.56 ± 1.74 mm compared with 2.44 ± 0.75 mm for Group 0) is anatomical evidence of marked detrusor hypertrophy and forms a pathoanatomical basis for combined therapy in excess of an effort to discontinue treatment alone, while observational are only one method of (WHOERS) who allow such differences in funnel plot symp analyzing clinical data, and differences between groups tometry at baseline to be quantified. Our data indicate that BWT is a candidate for an objective criterion that will enable clinicians to select patients at risk of requiring step-up of combination therapy sooner. This strategy is consistent with the 2024 AUA/SUFU guideline, i.e., we should individualize treatment by taking note of patient features and phenotypes [13-14]. For the β-3 agonist (mirabegron), a multi-modal effect as monotherapy was observed for great improvement of BLC along with substantial decrease in PVR and BWT. This suggests that treatment with mirabegron confers positive structural remodeling in addition to the symptomatic relief. But the difference in their reactions was extreme. The modest decrement in BWT experienced by nonresponders meant that monotherapy "worked" for them to some level but they remained unmet clinically and they were a rational biological foundation for escalating. This model of escalating the intensity of Management in

response to failure aligns well with emerging emphasis on moving away from step therapy and back towards a personalized patient centered approach together via shared-decision making, as underscored by 2024 AUA/SUFU guideline [13-14]. The finding that both combination regimens lead to significant increases in bladder capacity (approximately + 100 mL) indicates that they were equally efficacious at one of the most important OAB end points. This parallels pharmacodynamics synergy of drugs shown in trials such as SYNERGY where combination therapy with mirabegron and Solifenacin were more effective than monotherapy [15-16]. Heterogeneity in secondary end points is informative. The significantly higher BWT reduction with mirabegron/Flavoxate reflects a giant myogenic synergy driving the favorable picture of muscle hypertrophy turn-around. On the other hand, the more prominent reduction in PVR pressure with mirabegron + Solifenacin seems to make patients feel less threatened about retention by making voiding protocol presumably little more organized. The potential for phenotype-specific therapy is therefore an attractive clinical concept in light of this finding. When the bladder wall thickening was severe (BWT >3 mm), mirabegron and Flavoxate can be combined for structural normalization. Combination mirabegron and Solifenacin Combination therapy with mirabegron + Solifenacin may be of value in the patient with high PVR or voiding dysfunction to improve voiding. This specific assumption is consistent with the general premise of 2024 guidelines, which emphasizes the choice of personalized intervention according to patient clinical condition, symptoms and priority.

RELEVANCE

Clinical rational for balance presented with main outcome. It hint that a B3-agonist + MR min combination would have equivalent or greater efficacy to the comparator NMJ-min-combination as far as primary efficacy variables. This is further support for the paradigm shift advocated in the new 2024 AUA/SUFU guideline of step fixed therapy to individualized therapy [14]. The present equipoise provided herein could impact clinical practice for MCOs. Flavoxate combination is an evidence based approach for patients intolerant to anticholinergic AEs (a clinically relevant concern in view of real-world medication persistence and risk profiles 1) [17]. Possibility to perform shared decision-making between clinicians and patients by using combination regimens according to patient-related variables, symptom complex and over-ruling specific intolerances [13]. Several limitations warrant consideration. Observational design at first sight, we noticed that in this analysis there were important differences at baseline. Secondly, this post-hoc stratification of the patient cohort is exposed to selection bias. Third, long-term follow-up is limited by the relatively short follow-up period. Fourth, PROs findings on QoL could not be obtained for this analysis. Fifth, the study was a single-center study and results may not be generalizable. Such limitations would obviously be overcome by subsequent prospective (with stratification), large RCTs, with patients randomized to the intervention arm based on BWT at presentation.

CONCLUSIONS

The β-3 agonists have the clearest evidence base for multi-modality benefit as effective monotherapy and first-line treatment, but less so in patients with high symptom intensity. Both complex regimens are equally effective in promoting a phenotype-selective drive to increase bladder capacity. The MBG/Flavoxate response is better at structural remodeling in unashamed detrusor hypertrophy (BWT high) group, and the MBG/Solifenacin treatment has a higher voiding efficiency. Such findings strengthen a phenotype-model-based personalized therapy with objective biomarkers such as BWT and PVR for combination therapeutics, conditioning toward the ultimate goal of precision medicine in OAB.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 19.01.2026
ACCEPTED: 26.05.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Validation of Ukrainian breast and cervical cancer screening questionnaire

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ABSTRACT

Aim: This study aimed to develop and validate the Ukrainian Breast and Cervical Cancer Screening Questionnaire (UBC-SQ) to assess women's believes, behavior, knowledge and practices toward breast cancer and cervical cancer screening.

Materials and Methods: The validation study was conducted between June 2023 and January 2024 in Uzhhorod, Ukraine, among women aged 21–74, recruited through social centers for internally displaced persons and a family medicine clinic. Participants completed the questionnaire either online or on paper.

Results: The UBC-SQ was adapted from the two prototypes about breast and cervical cancer screening behaviour using translation and back-translation. Content validity was confirmed by expert review (CVR ≥ 0.62, CVI ≥ 0.79). External validity was assessed through item impact scores (IIS ≥ 1.5). Reliability was evaluated using test-retest (Weighted Kappa ≥ 0.3) and internal consistency (Cronbach's alpha ≥ 0.7).

Conclusions: The UBC-SQ is a valid, reliable, and culturally appropriate tool for assessing BC and CC screening believes, behavior, knowledge and practices in Ukrainian women.

KEYWORDS: health promotion, neoplasms, war, health services organization, noncommunicable diseases, global burden of disease

Wiad Lek. 2026;79(7):1581-1590. doi: 10.36740/WLek/222250 DOI

INTRODUCTION

Breast cancer (BC) emerges as the most common diagnosis among women, spanning both developing and developed countries. In 2022, approximately 2.3 million breast cancer cases were who had been diagnosed with breast cancer in the past five years, making it the most prevalent cancer worldwide [1]. Cervical cancer (CC) on the other hand is on the 4th position as the commonly diagnosed cancer in women [2]. Notably, 604,000 episodes of cervical cancer were diagnosed worldwide. Moreover, CC ranks first place in women in 36 low-and middle-income countries [3].

In Ukraine there are no state based screening program have been organized for BC and CC [4]. National screening programs were reported in Ukraine in 2002–2006, [5] but they are no longer mentioned. Now in Ukraine, the primary strategy for screening of BC is annual preventive gynecological examinations to all females aged 18 and above, which include a clinical breast examination with follow-up diagnostic mammography if needed [6]. The opportunistic BC and CC screening carried out across Ukraine varies in quality as well as the initiatives to increase awareness of women and vigilance of doctors [7]. The report of World Health Organization (WHO) showed that the incidence of BC in Ukraine was 43.4% and 19.4% for CC. Mortality rate was 13.3% and 7.0% for BC and CC respectively [1]. National Cancer Registry of Ukraine (NCRU) in 2022 reports that five-year prevalence of BC stands at 25.1% and 6.0% for CC as of 2022. In 2021, only 28.8% of BC episodes were detected during screening [8], with 24.2% for CC [9].

Ongoing war in Ukraine over the past two years forced millions of Ukrainian women to leave their homes [10]. Many of them were forced to find themselves in conditions where prolonged stress, social and economic problems shift the vector of priority from disease prevention and life extension to solving urgent issues [11]. In addition, the change of place of residence, the destruction of health care infrastructure, the outflow of medical professionals, the absence of state screening programs shifts focus of patients and doctors from prevention to management immediate medical needs [12].

Table 1. UBC-SQ first version

Statement	Answers
Q1. Treatment prescribed to breast cancer patients can only prolong the period of suffering.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q2. Treatment for cervical cancer patients can only prolong the period of suffering.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q3. Even if breast cancer is diagnosed at early stages, there is a very small chance that the patient will survive.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q4. Even if cervical cancer is diagnosed at early stages, there is a very small chance that the patient will survive.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q5. If a woman's fate is to get breast or cervical cancer, she will definitely get it.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q6. Screening for breast and cervical cancer should only be done if there are relevant complaints.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q7. A healthy lifestyle (healthy diet and exercise, avoiding smoking, alcohol, etc.) will help me avoid breast and cervical cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q8. I only see a doctor when I feel sick or have symptoms of illness.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q9. Only women with a family history (among close relatives) of the disease are at risk of getting breast cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q10. Only women with a family history (among close relatives) of the disease are at risk of getting cervical cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q11. I am afraid to have a Pap smear because I don't understand what will be done.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*

Table 1. Cont.

Q12. I know that screening for early detection of breast and cervical cancer in our country is free.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q13. A cervical smear (Papanicolaou test) will help me detect cervical dysplasia early	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q14. Mammography is the only scientifically proven method for early detection of breast cancer	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q15. I am worried that having a mammogram will hurt my breasts	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q16. Human papillomavirus can cause cervical cancer	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q17. I regularly perform breast self-examination for early detection of breast cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q18. Having a Pap smear is too embarrassing.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q19.It would be difficult to arrange transportation for getting a mammogram.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*

Source: compiled by the authors of this study

The analysis of a few studies examining BC and CC incidence shows that there is tendency of increasing BC and CC attributed to the impact of armed conflicts on healthcare infrastructure, access to screening, and overall health-seeking behaviors of the population [13].

Research group of family doctors of the international family medicine clinic (Uzhhorod, UA) dedicated to help internally displaced people (IDPs) who fled from the East to the West of Ukraine because of Russian aggression, evaluated the numbers of referrals of IDPs women for BC screening [14]. A total of 2,500 medical records of family doctors were analysed (378 of them were women of 40-74y.o.). During the observation period, only 17 (4.5%) of all IDPs women aged 40-74 who visited the clinic underwent mammography. Among the indicated number, 6 of the women visited a doctor for screening, the rest sought medical help for other gynecological pathology and were referred by a doctor for screening tests [14] .

Since our data suggests that screening rates among IDPs women backgrounds are low and literature search show that there is a lack of evidence which conclusively demonstrates specific knowledge, beliefs, and attitudes that significantly influence women's cancer screening behaviors in Ukraine. Consequently, the development of a reliable assessment tool for evaluating believes,

Table 2. Final version of UBC-SQ

Statement	Answers
Q1. Treatment prescribed to breast cancer patients can only prolong the period of suffering.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q2. Treatment for cervical cancer patients can only prolong the period of suffering.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q3. Even if breast cancer is diagnosed at early stages, there is a very small chance that the patient will survive.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q4. Even if cervical cancer is diagnosed at early stages, there is a very small chance that the patient will survive.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q5. Screening for breast and cervical cancer should only be done if there are relevant complaints.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q6. A healthy lifestyle (healthy diet and exercise, avoiding smoking, alcohol, etc.) will help me avoid breast and cervical cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q7. I only see a doctor when I feel sick or have symptoms of illness.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q8. Only women with a family history (among close relatives) of the disease are at risk of getting breast cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q9. Only women with a family history (among close relatives) of the disease are at risk of getting cervical cancer.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q10. I know that screening for early detection of breast and cervical cancer in our country is free.	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*
Q11. A cervical smear (Papanicolaou test) will help me detect cervical dysplasia early	*- Strongly Agree* *- Agree* *- Undecided* *- Disagree* *- Strongly Disagree*

Table 2. Cont.

Q12. Mammography is the only scientifically proven method for early detection of breast cancer	*- Strongly Agree - Agree - Undecided - Disagree - Strongly Disagree*
Q13. Human papillomavirus can cause cervical cancer	*- Strongly Agree - Agree - Undecided - Disagree - Strongly Disagree*
Q14. I regularly perform breast self-examination for early detection of breast cancer.	*- Strongly Agree - Agree - Undecided - Disagree - Strongly Disagree*
Q15. Having a Pap smear is too embarrassing.	*- Strongly Agree - Agree - Undecided - Disagree - Strongly Disagree*

Source: compiled by the authors of this study

behavior, knowledge and practices related to BC and CC screening is crucial. Such tool would be essential for devising effective interventions aimed at promoting screening practices for both BC and CC.

AIM

This study aimed to develop and validate the Ukrainian Breast and Cervical Cancer Screening Questionnaire (UBC-SQ) to assess women's believes, behavior, knowledge and practices toward breast cancer and cervical cancer screening.

MATERIALS AND METHODS

To achieve the objective, we utilized two validated questionnaires: Breast Cancer Screening Beliefs Questionnaire (BCSBQ) and the Pap Smear Belief Questionnaire (PSBQ) [15,16]. Validation process was conducted between June 2023 and January 2024 in Ukraine. Women were recruited through social centers for IDPs and family medicine clinic in Uzhhorod, Ukraine. The research on the validation of the UBC-SQ received ethical approval from the Ethics Committee of Uzhhorod National University.

All participants invited to the research were informed about the study details, including potential risks and benefits. All participants gave informed consent prior to their involvement in the study. The study included women aged 21-74 who were literate. Participants with a history of BC or CC were excluded. They could then choose to complete the questionnaire either on paper or online. Additional participants were recruited through "snowball" method, where current participants invited others to join the study online. Those who gave agreement to participate completed the questionnaire, available in Ukrainian, which took approximately 10 minutes (Table 1, Table 2).

Participation by a minimum of 40 women out of 100 invited to participate and 10 experts in cancer screening were recruited into the validation process [17,18]. Methodological steps followed in fig.1:

INSTRUMENT

BCSBQ was originally developed and validated by Kwok et al. (2010), as instruments for assessing women believes, knowledge and attitudes regarding BC screening. After that BCSBQ undergone validation process in women in various cultural contexts, including Vietnamese [19], African [20], Indian [21], Arabic [22], and Korean [23] communities. Each adaptation of the BCSBQ has demonstrated reliability and validity, as evidenced by positive validation outcomes. PSBQ assessing women's attitudes and beliefs concerning the Pap test and CC screening. The questionnaire was developed by Ackersone et al to support public health initiatives and gynecological services for women in the USA and showed a role in understanding believes about CC screening and informing interventions aimed at promoting women's health [16].

AI STATEMENT

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

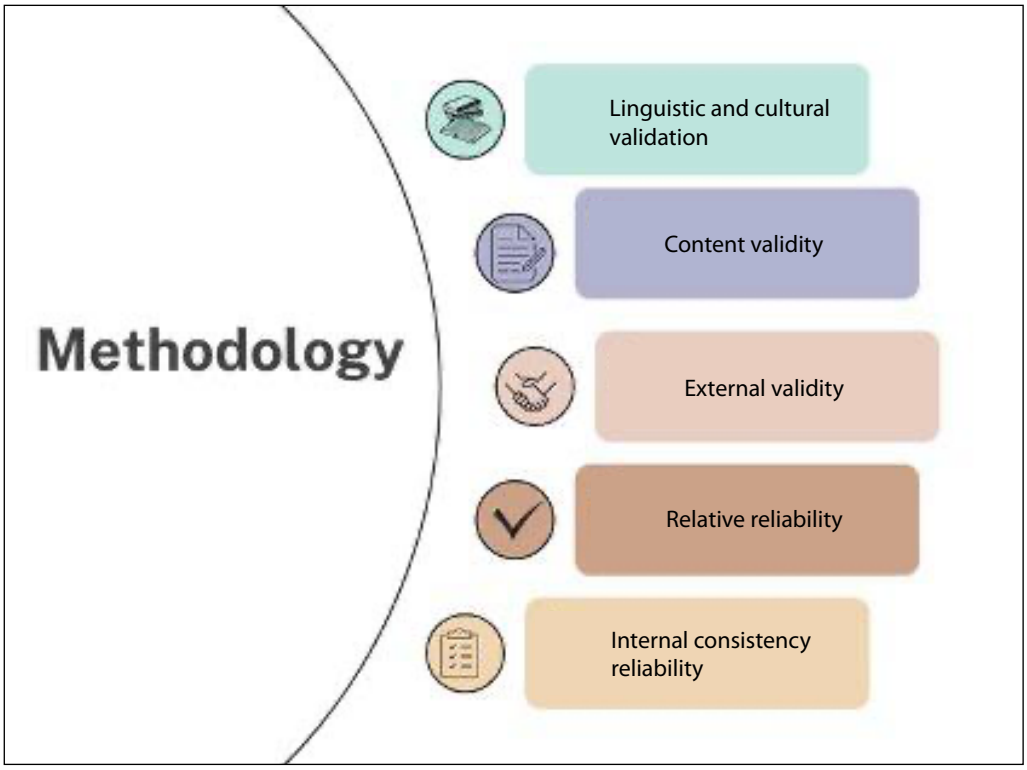

Fig. 1. Methodological steps of validation of UBC-SQ

RESULTS

LINGUISTIC AND CULTURAL VALIDATION

The first stage of validation was conducted, which included linguistic and cultural validation. Both prototype questionnaires were translated into Ukrainian based on the forward-backward translation protocol [24]. Initially, two English speaking doctors - experts independently translated the questionnaires into Ukrainian and then, the authors developed a combined final translation of UBC-SQ based on their translations and the aim of study. After that, two other experts (English speaking general medicine practice specialists) independently back translated the final version of the questionnaire into English [25]. The two translators and researcher combined the results of the translations. They made a final translation of UBC-SQ.

VALIDITY ASSESSMENT

Content validity assessment was conducted in two stages: qualitative and quantitative. Qualitative stage involved surveying 10 independent experts, including PhD in medicine, gynecologist, general practitioners, psychologist, and medical education instructors. They selected questions that corresponded to the set goal and objectives of the study. Experts could recommend changes to the questionnaire items based on linguistic and cultural peculiarities of surveyed population. Selected statements reproduce patients' beliefs, knowledge, attitudes regarding BC and CC screening.

In quantitative stage we calculated Content Validity Ratio (CVR) and Content Validity Index (CVI). Ten experts were asked to evaluate the importance and relevance of the questionnaire statements, providing additions and comments as necessary. For CVR calculation, the experts rated UBC-SQ items as "Essential" (scored 1), "Not essential, but useful" (scored 2), and "Not essential" (scored 3), with a minimum required CVR of 0.62 (Table 3) (26,27). To calculate CVI, the specialists rated the relevance of the items on the following: 1 - "Irrelevant"; 2 - "Somewhat relevant"; 3 - "Acceptably relevant"; 4 - "Completely relevant". CVI of 0.79 were acceptable (table 3) [26,27].

External validity. For this purpose, 10 women aged 21-74 y.o assessed the importance of each questionnaire item based on 5-point Likert scale (where 1 - not important at all, and 5 - very important). Based on the survey, the Item Impact Score (IIS) was assessed. IIS ≥ *1,5* is accepted as relevant for research (table 3).

Table 3 shows CVR values ranges from 0.43 to 1.0. Value 0.62 and more indicates the expert's agreement that the item must be included to questionnaire. CVR value less than 0.45 explains the experts disagree and statements were eliminated from the questionnaire. Based on CVR calculations we excluded from the ques-

Table 3. Content validity and external validity results of Ukrainian Breast and Cervical Screening questionnaire

Statement	Content Validity Ratio (CVR)	Content Validity Index (CVI)	Item Impact Score (IIS)
Q1. Treatment prescribed to breast cancer patients can only prolong the period of suffering	0.63	0.9	1.95
Q2. Treatment for cervical cancer patients can only prolong the period of suffering	0.63	0.9	2.00
Q3. Even if breast cancer is diagnosed at early stages, there is a very small chance that the patient will survive	1	1	2.04
Q4. Even if cervical cancer is diagnosed at early stages, there is a very small chance that the patient will survive	1	1	1.81
Q5. If a woman's fate is to get breast or cervical cancer, she definitely will get it	0.63	0.9	2.2
Q6. Screening for breast and cervical cancer should only be done if there are relevant complaints	1	1	2.08
Q7. A healthy lifestyle (healthy diet and exercise, avoiding smoking, alcohol, etc.) will help me avoid breast and cervical cancer	0.82	1	1.91
Q8. I only see a doctor when I feel sick or have symptoms of illness	0.82	0.9	1.87
Q9. Only women with a family history (among close relatives) of the disease are at risk of getting breast cancer	0.82	0.9	1.95
Q10. Only women with a family history (among close relatives) of the disease are at risk of getting cervical cancer	0.63	1	1.91
Q11. I am afraid to have a Pap smear because I don't understand what will be done	0.43	0.65	
Q12. I know that screening for early detection of breast and cervical cancer in our country is free	0.63	1	1.87
Q13. A cervical smear (Papanicolaou test) will help me detect cervical dysplasia early	1	1	1.77
Q14. Mammography is the only scientifically proven method for early detection of breast cancer	1	1	2.04
Q15. I am worried that having a mammogram will hurt my breasts	0.42	0.62	
Q16. Human papillomavirus can cause cervical cancer	0.82	1	2.1
Q17. I regularly perform breast self-examination for early detection of breast cancer	0.63	1	2.0
Q18. Having a Pap smear is too embarrassing	0.60	0.70	
Q19.It would be difficult to arrange transportation for getting a mammogram	0.80	0.65	1.2

Source: compiled by the authors based on [26, 27]

tionnaire Q11 and Q15 and Q18. In table 1 the level of CVI were <0,79 in Q11, Q15, and Q19 which showed that experts decided that they are not relevant for the aim of study. External validity was assessed by 10 women from target population. They evaluated how relevant and important each statement of the UBC-SQ. The results of calculation are presented in Table 1 in the 3rd column. After of the evaluation of the IIS index Q19 has been esteemed by women as not important (IIS Q19 = *1,2)*.

RELIABILITY

Relative reliability was assessed using the test-retest method, where 30 participants completed the UBC-SQ twice, with a two-week interval. We calculated interrater reliability (IRR) using Weighted Kappa (calculation of more than 0.3 were accepted), reflecting the level of agreement between two rates [28] (table 4).

Based on evaluation we excluded Q5 from the questionnaire as Weighted Kappa was less than 0.3 (p=0.142). Internal consistency which reflected how closely related the questionnaire items were evaluated by Cronbach's alpha. The coefficient ranges from 0 to 1 indicate greater reliability. A minimum acceptable value is generally considered to be 0.7. Low level of Cronbach alpha means there is low degree of homogeneity in the questionnaire [29]. In our study, the Cronbach's alpha coefficient was calculated as 0,75. This result indicates

Table 4. Calculation of IIR of statements of the UBC-SQ

Statements	Weighted Kappa	p-value	Statements	Weighted Kappa	p-value
Q1	.915	<.001	Q9	.948	<.001
Q2	.956	<.001	Q10	1.000	<.001
Q3	.847	<.001	Q12	.902	<.001
Q4	1.000	<.001	Q13	.953	<.001
Q5	.151	.142	Q14	.930	<.001
Q6	.881	<.001	Q16	.953	<.001
Q7	1	<.001	Q17	1.000	<.001
Q8	.443	<.001			

Source: compiled by the authors based on [28]

a satisfactory level of internal consistency, suggesting that the statement in the questionnaire is adequately aligned for evaluation Ukrainian women believes, behavior, knowledge and practices towards BC and CC screening.

DISCUSSION

The aim of this study was to validate the UBC-SQ. Our findings demonstrate that this questionnaire can be effective tool for understanding women believes, behavior, knowledge and practices towards BC and CC screening. The high scores on CVR an CVI suggest that the included items are both relevant and essential for the target population additionally the satisfactory ICR reflected by Cronbach's alpha confirms that the questionnaire is reliable for repeated measures.

One of the major strengths of this study is the methodological approach, including forward-backward translation, expert reviews, and comprehensive validity and reliability assessments.

This thorough process ensures that the questionnaire is appropriate for the Ukrainian context culturally and linguistically. Similar approaches are considered essential in international research, where forward-backward translation, expert committee review, and pretesting are standard steps for cross-cultural adaptation to preserve semantic, conceptual, and functional equivalence of the instrument [24].

However, a limitation of the study is the relatively small sample size used for test-retest reliability and external validity assessments. Our findings are consistent with previous validation studies were conducted in various cultural contexts, including Vietnamese, African, Arabic, Korean and the US communities [16,19-23]. This cross-cultural consistency highlights the robustness of the questionnaires in diverse settings. From the other side in Ukraine there is lack of validated questionnaires for evaluation believes, behavior, knowledge and practices of BC and CC screening. The validated UBC-SQ can become a reliable tool for researches and healthcare practitioners to understand what Ukrainian women believes, behaviour, knowledge and practices towards BC and CC screening. Similar questionnaires has been used in many countries to link knowledge, believes, attitudes with actual screening behaviours in different countries [31-33]. This knowledge can help to design better public health campaigns and education programs to promote early detection of BC and CC in Ukraine. Policymakers can also use UBC-SQ to check how current screening recommendations work and make better decisions to improve women's health. Longitudinal studies focused on more people from different demographics could assess the stability of the UBC-SQ over time. Additionally, we can use this tool to study how education programs affect people's knowledge, beliefs, and attitudes about cancer screening and could give useful ideas for improving public health in Ukraine.

CONCLUSIONS

The validation process confirmed that the Ukrainian breast and cervical cancer screening questionnaire is culturally relevant, valid, and reliable for evaluating believes, behavior, knowledge and practices related to breast and cervical cancer screening.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 05.08.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Changes in the levels of nerve growth factor and middle molecular mass molecules in patients with post-traumatic neuropathies and plexopathies accompanied by chronic neuropathic pain

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ABSTRACT

Aim: To investigate and compare the levels of the beta-subunit of NGF (Beta-NGF) and middle molecular mass molecules (MMM) in the blood of patients with post-traumatic non-firearm and firearm neuropathies and plexopathies accompanied by chronic neuropathic pain.

Materials and Methods: We examined 93 men aged 21-59 years with compression-ischemic (groupI - comparison), post-traumatic non-firearm (groupII) and firearm (groupIII) neuropathies and plexopathies with and without chronic neuropathic pain syndrome. Quantitative determination of Beta-NGF in serum was performed by enzyme-linked immunosorbent assay. The content of MMM was determined by spectrophotometric method (fractions MMM238, MMM254, MMM260, MMM280). The following integral indices were calculated: peptide-nucleotide ratio, distribution coefficient, aromaticity coefficient.

Results: We revealed an increased level of Beta-NGF in the blood serum of patients with post-traumatic firearm and non-firearm neuropathies and plexopathies accompanied by chronic neuropathic pain (Mann-WhitneyU-test,p=0.0077 and Mann-WhitneyU-test,p=0.04608, respectively). The level of the CR280/254 in patients of groupIII with post-traumatic firearm neuropathies and plexopathies at a statistically significant level depends on the presence of chronic neuropathic pain (Mann-WhitneyU-test,p=0.0113). The accumulation of biologically active molecules involved in the activation of compensatory, protective mechanisms in patients with post-traumatic firearm neuropathies and plexopathies accompanied by chronic neuropathic pain was established.

Conclusions: The results confirm the existing data on the pathophysiological role of Beta-NGF and MMM in the development of chronic neuropathic pain and indicate the potential use of NGF as a therapeutic target, while the CR280/254 ratio may serve as a biomarker reflecting the accumulation of biologically active molecules associated with chronic neuropathic pain in patients with post-traumatic neuropathies and plexopathies.

KEYWORDS: Beta-NGF, molecules of medium mass MMM, post-traumatic non-gunshot and gunshot neuropathies, plexopathies, chronic neuropathic pain

Wiad Lek. 2026;79(7):1591-1597. doi: 10.36740/WLek/222252 DOI

INTRODUCTION

Nerve Growth Factor (NGF) is a regulatory protein synthesized by neurons, as well as cells in the neuronal environment, such as fibroblasts, smooth muscle cells, keratinocytes, Schwann cells, mast cells, and macrophages. The nerve growth factor was isolated in 1956 by Rita Levi-Montalcini and Stanley Cohen, who received the Nobel Prize for this discovery in 1986 [1,2].

The main physiological effects of nerve growth factor are support of nerve cell proliferation and regeneration, stimulation of axonal growth and sprouting, and NGF is involved in the regulation of the endocrine system and immune processes. Nerve growth factor is synthesized as pro-NGF, a complex of three subunits (α-, β- and γ-NGF), and undergoes post-translational transformation with the release of the active component, β-NGF. Apart from neurons, nerve growth factor receptors are widely expressed on many immunocompetent, endocrine, and stromal cells in the human body. Two major NGF receptors are known: tropomyosin receptor kinase A (TrkA) and the low-affinity receptor NGFR/p75NTR [3-5].

Nerve Growth Factor (Beta-NGF) is involved in the development of central sensitization, the biological basis of which is reversible changes in the neuronal membrane at the level of synapses of the posterior horns of the spinal cord, accompanied by a persistent decrease in pain threshold, hyperalgesia and allodynia. This effect is especially pronounced against the background of an inflammatory reaction accompanied by activation and chemotaxis of macrophages, mastocytes and other lymphohistiocyte cells to the site of injury. At the same time, Beta-NGF can

have a direct effect on nociceptors through TrkA activation of transmembrane channels, which leads to changes in transmembrane potential and increased excitability of nerve endings. Beta-NGF can indirectly affect nociceptors by activating inflammatory response cells and increasing the synthesis of many pain and inflammatory mediators, such as prostaglandins, leukotrienes, bradykinin, histamine, substance P [6-8].

According to modern research, in the acute period of trauma, endogenous intra- and extracellular molecules specifically formed due to tissue damage are released, which activate immune cells and cause the development of an inflammatory response of excessive intensity with subsequent undesirable consequences for the injured organism. The substances responsible for the development of endogenous intoxication include middle molecular mass molecules - protein toxins that affect various pathophysiological processes, namely, promote hypercoagulation, microcirculatory disorders, functional and structural changes in cell membranes, and hypoxia. On the other hand, low-molecular-weight oligopeptides have a wide range of biological effects and coordinate the performance of biological functions by various organs and tissues, can affect the repair processes in body tissues, and the antioxidant property of medium-molecular peptides has also been proven [9-11].

In patients with post-traumatic neuropathies and plexopathies, an increase in the level of nerve growth factor correlates with the presence of chronic neuropathic pain. This increase is accompanied by an elevated CR 280/254 ratio, indicating the accumulation of biologically active molecules involved in the activation of compensatory and protective mechanisms. Therefore, Beta-NGF and MMM may serve as biomarkers for the development and maintenance of chronic neuropathic pain and represent potential therapeutic targets for its management in patients with post-traumatic neuropathies and plexopathies, which has not been systematically evaluated in peripheral nerve injuries before. By combining quantitative immunological measurements with clinical pain assessment, the study provides a comprehensive approach to understanding the pathophysiology of chronic neuropathic pain in post-traumatic peripheral nerve injuries, contributing new knowledge that can guide future research and therapy.

AIM

The aim of the study is to investigate and compare the levels of the beta subunit of NGF (Beta-NGF) and middle molecular mass molecules (MMM) in the blood of patients with post-traumatic non-firearm and firearm neuropathies and plexopathies accompanied by chronic neuropathic pain.

RESEARCH HYPOTHESIS

We hypothesized that patients with post-traumatic neuropathies and plexopathies accompanied by chronic neuropathic pain would exhibit significantly elevated serum levels of Beta nerve growth factor (β-NGF) and middle molecular mass molecules (MMM) compared with patients without chronic neuropathic pain, and that these alterations would be more pronounced in firearm-related injuries, reflecting greater tissue damage and a more intense and persistent neuroinflammatory response.

MATERIALS AND METHODS

We examined 93 men aged 21 to 59 years with compression (group I - comparison), post-traumatic non-firearm (group II) and firearm (group III) neuropathies and plexopathies with and without chronic neuropathic pain syndrome. Group I included 30 patients with compression neuropathies and plexopathies. Group II included 30 patients with post-traumatic non-firearm neuropathies and plexopathies due to injuries, limb fractures and joint dislocations. Group III included 33 patients with post-traumatic firearm neuropathies and plexopathies due to shrapnel and bullet wounds of the limbs.

The inclusion of Group I (patients with compression neuropathies and plexopathies without chronic neuropathic pain) was necessary to differentiate post-traumatic mechanisms of neurotrophic and metabolic alterations from non-traumatic nerve damage. This group served as a reference model of peripheral nerve pathology without pronounced neuroinflammatory and pain-associated neurotrophic activation, allowing more accurate interpretation of β-NGF and MMM changes specifically related to post-traumatic neuropathies accompanied by chronic neuropathic pain.

Patients with documented chronic viral or bacterial infections, central nervous system disorders, autoimmune, oncological, or hematological conditions were excluded from the study. Patients with compression neuropathies or plexopathies were screened for chronic neuropathic pain using the validated Visual Analog Scale (VAS) and questionnaires PainDETECT, DN4, MPQ and those identified with such pain were also excluded.

To achieve the objectives of the study, we analyzed the following materials and data sources: patients' medical records, including case histories, discharge summaries, clinical examination protocols, and surgical reports; laboratory results, including general and biochemical blood tests; and instrumental examination data, such as radiography (X-ray), computed tomography (CT), ultrasound, and electroneuromyography (ENMG).

Quantitative determination of the beta subunit of human nerve growth factor (β-NGF) in serum was performed by enzyme-linked immunosorbent assay using the Ray Bio Human Beta-NGF ELISA Kit. The study was performed on a STATFAX

3200 enzyme-linked immunosorbent assay analyzer (USA). Non-heparinized peripheral blood (5 ml) was collected from the antecubital vein under sterile conditions. The samples were centrifuged at 3000 rpm at room temperature to obtain serum, which was subsequently frozen at –20 °C or lower and stored for 3 to 6 months.

The content of middle molecular mass molecules (MMM) was determined on a Shimadzu UV-2600 spectrophotometer (Japan) by extinction at wavelengths of 238 nm, 254 nm, 260 nm and 280 nm according to the method of Gabrielyan N.I. The concentration of MMM was expressed in optical units of the optical density of the centrifugate obtained after precipitation of plasma proteins with 10% trichloroacetic acid solution. At a wavelength of 238 nm, the fraction containing biologically active oligopeptides was determined, at a wavelength of 254 nm - the MMM254 fraction, which reflects the level of intermediate products of intensive proteolysis, the nucleotide fraction at a wavelength of 280 nm - the MMM280 fraction, which contains aromatic chromatophores. The following integral indices were calculated: peptide-nucleotide ratio, distribution ratio, and aromaticity ratio.

Assessment of pain severity and characteristics was performed using the Visual Analog Scale (VAS) and neuropathic pain questionnaires, including DN4, PainDETECT, and the McGill Pain Questionnaire (MPQ).

The data obtained were statistically processed using the STATISTICA 6.0 program. Quantitative indicators are presented in the form Me [LQ; UQ], where Me is the median, LQ is the lower quartile, and UQ is the upper quartile. The statistical significance of differences was tested using the Mann-Whitney U-test (MWU). Differences were considered statistically significant at $p<0.05$.

ETHICS

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

AI STATEMENT

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

RESULTS

Fig. 1 shows the quantitative content of nerve growth factor (Beta-NGF) in patients of group II with post-traumatic non-firearm neuropathies and plexopathies with chronic neuropathic pain and without chronic neuropathic pain.

The level of Beta-NGF in patients of group II with post-traumatic non-firearm neuropathies and plexopathies was significantly associated with the presence of chronic neuropathic pain (Mann-Whitney U-test, p=0.04608). Thus, in patients with post-traumatic non-firearm neuropathies and plexopathies in the presence of chronic neuropathic pain, the level of Beta-NGF is 165.8 [69.3;510] pg/ml, which is significantly higher than in patients with post-traumatic non-firearm neuropathies and plexopathies without chronic neuropathic pain, in whom the level of Beta-NGF is 47.9 [25;93] pg/ml.

Fig. 2 shows the quantitative content of nerve growth factor (Beta-NGF) in patients with post-traumatic firearm neuropathies and plexopathies with and without chronic neuropathic pain.

The level of Beta-NGF in patients of group III with post-traumatic firearm neuropathies and plexopathies at a statistically significant level depends on the presence of chronic neuropathic pain (Mann-Whitney U-test, p=0.0077). Thus, in patients with post-traumatic firearm neuropathy and plexopathy in the presence of chronic neuropathic pain, the level of Beta-NGF is 337.1 [39.2; 579.25] pg/ml, which is significantly higher than in patients with post-traumatic firearm neuropathy and plexopathy without chronic neuropathic pain, in whom the level of Beta-NGF is 25.9 [25; 35.3] pg/ml.

Fig. 3 demonstrates the CR level of 280/254 in patients with post-traumatic firearm neuropathy and plexopathy with chronic neuropathic pain and in patients with post-traumatic firearm neuropathy and plexopathy without chronic neuropathic pain.

The level of the distribution coefficient of CR 280/254 in patients of group III with post-traumatic firearm neuropathies and plexopathies at a statistically significant level depends on the presence of chronic neuropathic pain (MWU, p=0.0113). Thus, in patients with post-traumatic firearm neuropathies and plexopathies accompanied by chronic neuropathic pain, the CR 280/254 was found to be significantly higher and equal to 1.17 [1.15; 1.21] than in patients with post-traumatic firearm neuropathies and plexopathies without chronic neuropathic pain, the CR 280/254 was 1.13 [1.12; 1.14]. The data obtained indicate the accumulation of biologically active substances involved in the activation of compensatory and protective mechanisms in patients with post-traumatic firearm neuropathy and plexopathy accompanied by chronic neuropathic pain.

Table 1 presents the levels of β-NGF and the CR 280/254 ratio in patients with compression, post-traumatic non-firearm and firearm neuropathies and plexopathies depending on the presence of chronic neuropathic pain. The inclusion of Group I (compression neuropathies without chronic pain) as a reference allows us to differentiate post-traumatic neurotrophic and neuroinflammatory responses from non-traumatic nerve alterations. In Group II patients with post-traumatic non-firearm neuropathies and plexopathies, the presence of chronic neuropathic pain was associated with a statistically significantly higher β-NGF level compared with patients without pain 165.8 [69.3; 510] vs. 47.9 [25; 93] pg/mL, p = 0.04608. At the same time, for the CR 280/254

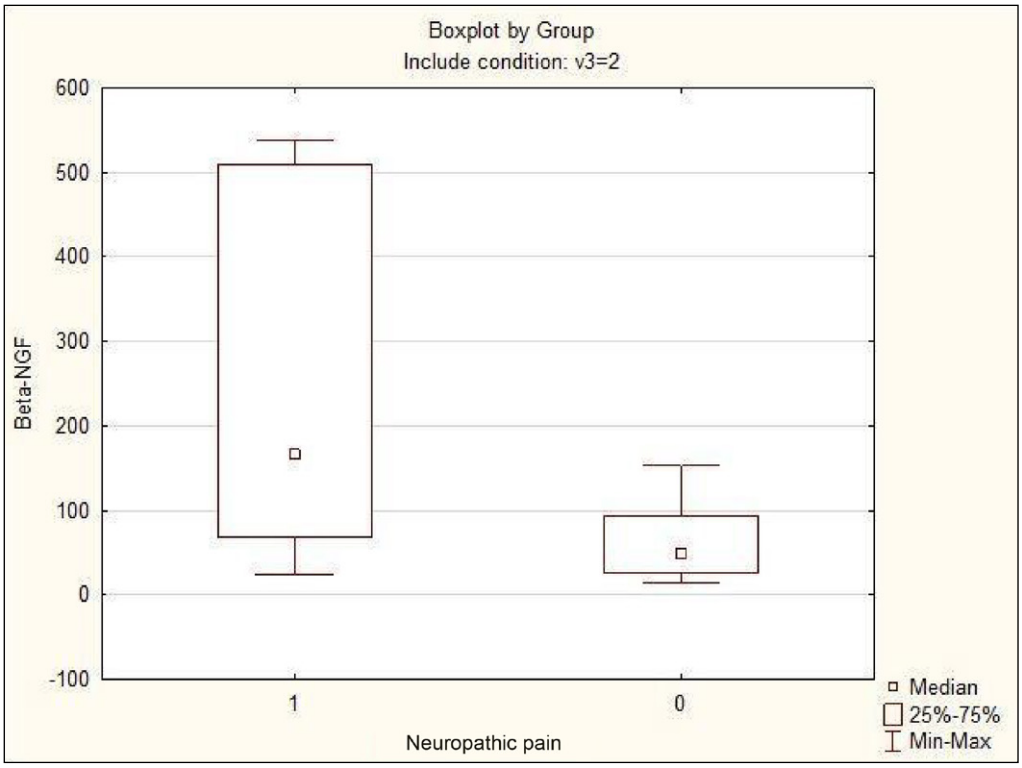

Fig. 1. Quantitative content of nerve growth factor (Beta-NGF) in patients with post-traumatic non-firearm neuropathies and plexopathies with chronic neuropathic pain and without chronic neuropathic pain (pg/ml)
Picture taken by the authors

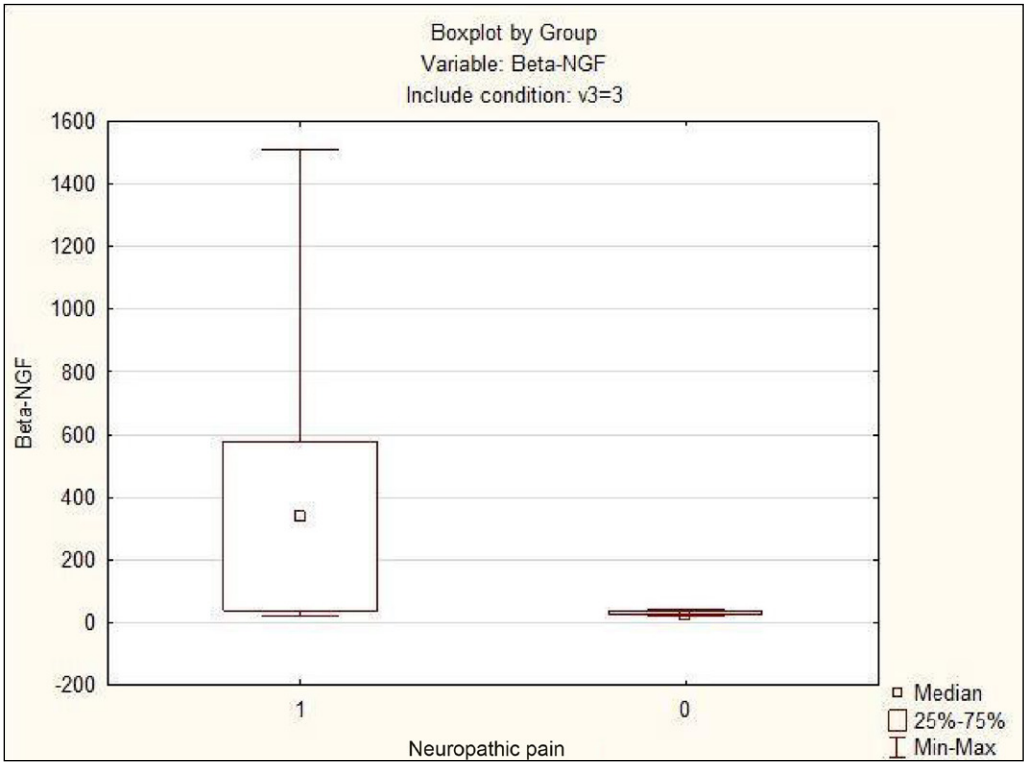

Fig. 2. Quantitative content of nerve growth factor (Beta-NGF) in patients with post-traumatic firearm neuropathies and plexopathies with chronic neuropathic pain and without chronic neuropathic pain (pg/ml)
Picture taken by the authors

ratio in this group, a borderline level of association with chronic neuropathic pain was observed: values were higher in patients with pain 1.213 [1.141; 1.263] than in those without pain 1.137 [1.0795; 1.2025], corresponding to a trend toward differences at p = 0.1006. In Group III patients with post-traumatic firearm neuropathies and plexopathies, both β-NGF levels and the CR 280/254 ratio were statistically significantly higher in the presence of chronic neuropathic pain. Specifically, β-NGF concentrations were markedly higher in patients with pain than in those without pain 337.1 [39.2; 579.25] vs. 25.9 [25; 35.3] pg/mL, p = 0.0077. Similarly, the CR 280/254 ratio was significantly elevated in patients with chronic neuropathic pain 1.17 [1.15; 1.21] compared with pain-free patients 1.13 [1.12; 1.14], p = 0.0113.

Overall, the summarized data indicate that β-NGF and the CR 280/254 ratio demonstrate statistically significant

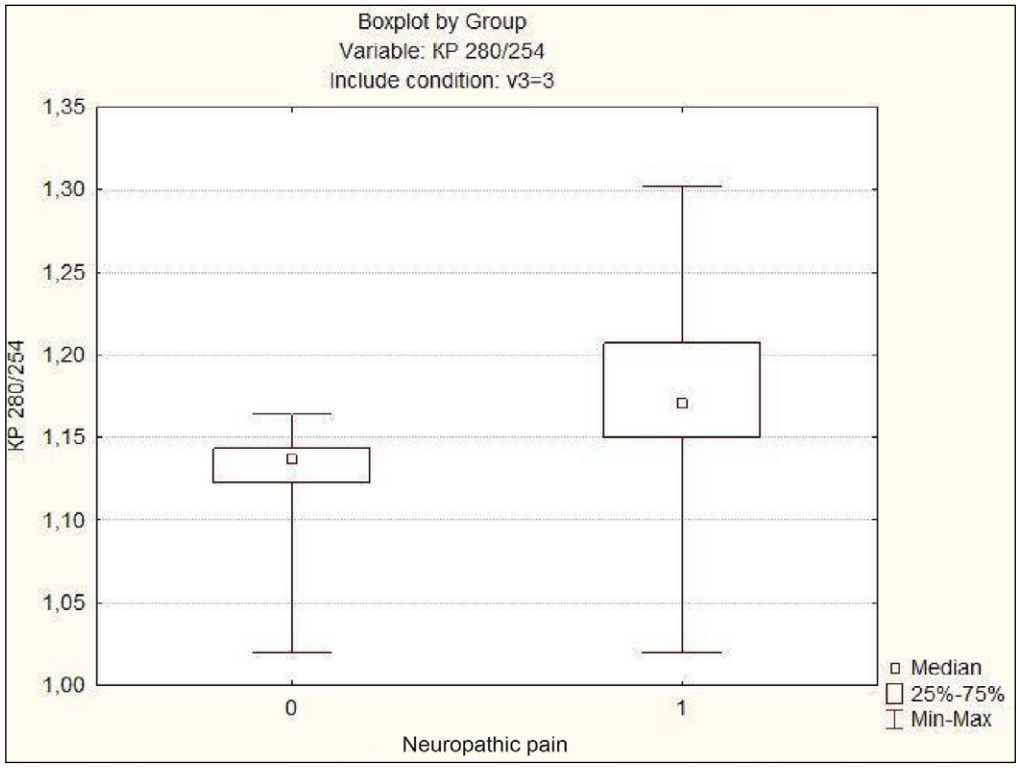

Fig. 3. CR level 280/254 in patients with post-traumatic firearm neuropathy and plexopathy accompanied by chronic neuropathic pain and without chronic neuropathic pain
Picture taken by the authors

Table 1. Content of β-NGF and CR 280/254 in patients with compression, post-traumatic non-firearm and firearm neuropathies and plexopathies depending on the presence of chronic neuropathic pain

Group	Parameter	With chronic neuropathic pain	Without chronic neuropathic pain	p-value
Group I (compression neuropathies and plexopathies)	β-NGF, pg/mL	_	83.6 [42; 335.4]	_
	CR 280/254	_	1.186 [1.137; 1.232]	_
Group II (post-traumatic non-firearm neuropathies and plexopathies)	β-NGF, pg/mL	165.8 [69.3; 510]	47.9 [25; 93]	0.04608
	CR 280/254	1.213 [1.141; 1.263]	1.137 [1.079; 1.202]	0.1006
Group III (post-traumatic firearm neuropathies and plexopathies)	β-NGF, pg/mL	337.1 [39.2; 579.25]	25.9 [25; 35.3]	0.0077
	CR 280/254	1.17 [1.15; 1.21]	1.13 [1.12; 1.14]	0.0113

Source: compiled by the authors of this study

associations at the $p < 0.05$ level in patients with post-traumatic firearm neuropathies and plexopathies, whereas for the CR 280/254 ratio in patients with post-traumatic non-firearm neuropathies and plexopathies, borderline significance at approximately $p \approx 0.1$ was observed, suggesting less robust but potentially clinically meaningful associations. This pattern suggests that the severity and mechanism of nerve injury strongly influence the intensity of neurotrophic and neuroinflammatory activation.

DISCUSSION

The results of the study on changes in the content of nerve growth factor in patients with post-traumatic neuropathies and plexopathies accompanied by chronic neuropathic pain are consistent with the data of other researchers who emphasize the role of NGF in the pathogenesis of chronic neuropathic pain.

According to the study by Schaefer et al, NGF plays an important role in the development of chronic pain syndrome through the activation of signaling pathways, in particular the modulation of sodium channels in neurons, which leads to increased excitability and sensitization to pain [12]. This confirms the correlation between Beta-NGF levels and the presence of chronic neuropathic pain in patients with post-traumatic firearm and non-firearm neuropathies and plexopathies.

Another study conducted by Binshtok et al. showed that NGF activates macrophages and other inflammatory response cells that release biologically active molecules that contribute to nociceptor sensitization [13]. This explains the increase in the levels of middle molecular mass

molecules observed in our study in patients with chronic neuropathic pain, since their accumulation is a marker of the inflammatory response, contributes to homeostasis and the development of endogenous intoxication.

The results are also consistent with the findings of Barker et al. and McMahon et al. who indicate that NGF is a critical regulator of glial cell proliferation and activation that maintains chronic pain by increasing sensitivity to pain stimuli. Studies in animal models have shown that blocking NGF activity leads to a decrease in hyperalgesia and allodynia, which confirms the importance of this factor in maintaining chronic pain in patients [14, 15].

In addition, modern clinical trials confirm the effectiveness of therapy aimed at blocking NGF to reduce chronic pain in patients with nervous system diseases. Thus, a study conducted by Kreiner et al. demonstrated that the use of NGF inhibitors helps to reduce pain scores and improve the functional status of patients with chronic pain [16].

Importantly, most previously published studies have focused on the role of NGF in chronic pain syndromes of inflammatory or degenerative origin, while data regarding its involvement in post-traumatic peripheral nerve injuries, especially in firearm trauma, remain limited. Our study expands existing knowledge by demonstrating that β-NGF elevation is not only associated with the presence of chronic neuropathic pain but also varies depending on the mechanism and severity of nerve injury. The significantly higher β-NGF levels observed in patients with firearm neuropathies and plexopathies compared with non-firearm injuries suggest a more pronounced neurotrophic and neuroinflammatory response, likely reflecting extensive tissue damage, persistent immune activation, and long-term sensitization of nociceptive pathways. Unlike previous studies that evaluated NGF in isolation, our work also assessed middle molecular mass molecules (MMM) and their integral ratios, allowing a more comprehensive characterization of endogenous intoxication and compensatory metabolic processes associated with chronic neuropathic pain. The identified increase in the CR 280/254 ratio in patients with firearm injuries and chronic pain represents a novel finding, indicating the accumulation of biologically active aromatic compounds and peptides that may contribute to sustained neuroinflammation and pain chronification. Thus, the combined assessment of β-NGF and MMM parameters provides new insight into the biochemical and immune mechanisms underlying chronic neuropathic pain following severe peripheral nerve trauma and may serve as a basis for developing targeted neurotrophic or anti-inflammatory therapies. These findings highlight the clinical relevance of β-NGF and CR 280/254 as potential biomarkers for pain severity and for monitoring therapeutic interventions in post-traumatic neuropathies and plexopathies.

CONCLUSIONS

The study revealed elevated levels of Beta-NGF in the serum of patients with post-traumatic firearm and non-firearm neuropathies and plexopathies accompanied by chronic neuropathic pain. Studies of the role of NGF in nerve and plexus injuries indicate the importance of this factor not only in the regulation of neurotrophic activity, but also in the modulation of pain that accompanies most injuries and diseases of the nervous system.

As a result of the study, we found the accumulation of biologically active molecules involved in the activation of compensatory and protective mechanisms in patients with post-traumatic firearm neuropathies and plexopathies accompanied by chronic neuropathic pain.

Further study of the peculiarities of neurotrophic control and changes in the content of biologically active molecules in injuries and diseases of the nervous system accompanied by chronic pain is an urgent and important task of modern medicine. This opens up prospects for the development of new therapies aimed at modulating neurotrophic regulation and correcting pathogenetic mechanisms in patients with chronic neuropathic pain. Thus, the results of our study confirm existing data on the pathophysiological role of NGF and MMM in the development of chronic neuropathic pain and indicate the potential use of NGF as a therapeutic target, while the CR 280/254 ratio may serve as a biomarker reflecting the accumulation of biologically active molecules associated with chronic neuropathic pain in patients with post-traumatic neuropathies and plexopathies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 23.03.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Molecular profiling of apoptotic, inflammatory, and angiogenic markers in prostate cancer: Focus on p53, IL-6, IL-8, Bax/Bcl-2 ratio, and VEGF

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ABSTRACT

Aim: To perform a comprehensive molecular profiling of key apoptotic, inflammatory, and angiogenic markers - p53, IL-6, IL-8, Bax/Bcl-2 ratio, and VEGF -in peripheral blood samples of patients with prostate cancer.

Materials and Methods: A total of 60 male participants were enrolled in the study, comprising 40 patients with histologically confirmed prostate cancer and 20 age-matched healthy controls. The median age in both groups was 68 years (range: 55–78). Patients were stratified according to Gleason score, PSA levels, and tumor stage from the begging of August 2024 to the end May 2025. Blood samples from patients suffering Prostate cancer (PCa). ELISA Human-specific ELISA kits (e.g., from R&D Systems, BioLegend, or Abcam) (from Serum) for IL-6, IL-8, VEGF. As well as Bax/Bcl-2 Ratio. RNA Quality Check: The concentration and integrity of RNA will be evaluated using a NanoDrop agarose gel. cDNA Synthesis: A reverse transcription kit (such as Thermo Fisher's High-Capacity cDNA Reverse Transcription Kit) will be used to create cDNA from 1 µg of RNA. Target Genes p53, Primers, and Probes for Quantitative Real-Time PCR (qRT-PCR).

Results: p53 Expression, qPCR analysis revealed significantly decreased expression of p53 mRNA in prostate cancer patients compared to controls (mean $\Delta Ct = 6.1 \pm 0.8$ vs. 4.3 ± 0.6; $p < 0.001$. Serum IL-6 levels (by ELISA) were elevated in prostate cancer patients (mean = 18.4 ± 6.2 pg/mL) compared to controls (7.1 ± 2.3 pg/mL); $p < 0.001$. Similarly, IL-8 levels were significantly higher in patients (27.5 ± 9.8 pg/mL) than in controls (10.2 ± 3.5 pg/mL); $p < 0.001$. Serum VEGF concentrations were significantly increased in prostate cancer patients (median = 296 pg/mL; IQR = 230–360 pg/mL) compared to controls (median = 122 pg/mL; IQR = 90–160 pg/mL); $p < 0.0001$.

Conclusions: Prostate cancer patients exhibited decreased p53 and Bax/Bcl-2 ratio, indicating impaired apoptosis. IL-6 and IL-8 were significantly elevated and positively correlated, reflecting a pro-inflammatory environment.

KEYWORDS: prostate cancer, p53, Bax/Bcl-2 ratio, IL-6 , IL-8 , VEGF levels

Wiad Lek. 2026;79(7):1598-1605. doi: 10.36740/WLek/220739 DOI

INTRODUCTION

Being a major source of cancer morbidity and mortality globally, prostate cancer (PCa) continues to be a major global health concern. Additionally, a thorough comprehension of the illness is required. An important factor in the development of prostate cancer is inflammation of the prostate brought on by hereditary or environmental causes [1]. Genetic, inflammatory, apoptotic, and angiogenic pathways interact intricately to drive the onset and spread of prostate cancer. Due to their crucial functions in the tumor microenvironment, a number of molecular markers have drawn attention in this study [2]. The five major biomarkers that are the focus of this initiative are p53, the Bax/Bcl-2 ratio, IL-6, IL-8, and VEGF. These biomarkers collectively offer a multifaceted profile and spread of prostate cancer. Several molecular markers have been highlighted in this study because of their critical roles in the tumor microenvironment [3]. This study focuses on five significant biomarkers. of the biological activity of the tumor. The fundamental way that bodily tissues react to damage is through inflammation. When damage or intruders are detected in the tissue, a relatively limited set of reactions takes place that eliminate the damaged cells and structures, eliminate and kill the invaders, and encourage the replacement and repair of cells and matrix. The original cells and tissue function are restored under normal circumstances [4]. The presence of many types of inflammatory cells in the normal gland, as well as in individuals with prostate cancer, suggests that inflammation plays a role in prostate diseases [5]. The term "apoptosis," sometimes known as "programmed cell death," describes the death of cells that happens as a regular and regulated aspect of an organism's development. The name

"apoptosis" was first used in the final decades of the 20th century, despite the fact that this process was first noted in 1842 [6]. Nowadays, apoptosis is thought to be a ubiquitous, genetically encoded process that allows cells to experience highly controlled cell death in response to particular signaling, which has been extensively studied along with its intracellular effectors [7]. Understanding the cause of Benign prostatic hyperplasia (BPH) , the mode of action of the medications already in use, and—most importantly - the creation of novel, more effective compounds may all benefit greatly from an understanding of both inflammation and apoptosis [8]. The tumor suppressor p53 has a major impact on apoptosis, cell cycle arrest, and the DNA damage response. Tumor aggressiveness and treatment resistance are often correlated with p53 mutations or functional inactivation, which are present in many malignancies, especially high-grade prostate cancer. One effect of p53 activation is the transcriptional regulation of apoptotic proteins such as Bax (pro-apoptotic) and Bcl-2 (anti-apoptotic) [9]. As a result, the Bax/Bcl-2 ratio functions as a predictor of a cell's propensity for apoptosis: a high ratio denotes a pro-apoptotic environment, often induced by therapies, whereas a low ratio suggests resistance to apoptosis, promoting tumor survival and progression [10]. Prostate carcinogenesis is known to be characterized by chronic inflammation. The pleiotropic cytokine interleukin-6 (IL-6) activates pathways like JAK/STAT3 and PI3K/Akt to promote tumor growth, survival, and immune evasion. Castration-resistant prostate cancer (CRPC), which is connected to worse outcomes and more aggressive prostate cancer, has been linked to elevated serum IL-6 levels [11].

Another pro-inflammatory cytokine that plays a part in angiogenesis, metastasis, and the recruitment of tumor-associated neutrophils is interleukin-8 (IL-8) [12]. Additionally, IL-8 increases VEGF expression, creating a clear connection between angiogenesis and inflammation in the prostate tumor microenvironment. Tumor angiogenesis is primarily driven by Vascular Endothelial Growth Factor (VEGF), which allows tumors to create a vascular network for the provision of nutrients and oxygen. Increased tumor grade, metastasis, and resistance to androgen deprivation treatment have all been linked to elevated VEGF levels. VEGF is known to be upregulated by both IL-6 and IL-8, resulting in an inflammatory-angiogenic axis that promotes tumor growth [13].

AIM

1. To evaluate p53 expression levels and ascertain its relationship to apoptotic regulation using the Bax/Bcl-2 ratio.
2. To measure the pro-inflammatory cytokines IL-6 and IL-8 in circulation and assess how they relate to the severity and course of the disease.
3. To quantify VEGF levels and look into how they relate to tumor angiogenic activity, IL-6, and IL-8.
4. To investigate how apoptotic, inflammatory, and angiogenic pathways interact by combining molecular information from the chosen markers
5. To assess these markers' potential as non-invasive prognostic or diagnostic markers for prostate cancer.

MATERIALS AND METHODS

STUDY DESIGN

The study was conducted 60 samples from the begging of August 2024 to the end May 2025. Blood samples from patients suffering Prostate cancer (PCa) were obtained from the primary screening program, either as the primary sample or during secondary follow-up of abnormal findings within the program.

SAMPLE COLLECTION

Five ml of blood was withdrawn from all study groups in serum gel tube and centrifuged for 10 minutes at 3000 rpm. The serum was separated and placed in Eppendorf serum tubes for immunological markers stored at -20c until analysis for (p53, IL-6, IL-8, Bax/Bcl-2 Ratio, and VEGF). As well as 2ml of peripheral blood will be collected via venipuncture Blood will be drawn into EDTA tubes for RNA extraction (gene expression analysis).For RNA Extraction and cDNA Synthesis: Blood will be processed within 2 hours using a blood RNA extraction kit RNA will be extracted from whole blood using a specialized kit such as the (PAXgene Blood RNA Kit or Qiagen RNeasy Protect System). RNA Quality Check: NanoDrop agarose gel will be used to assess RNA concentration and integrity. cDNA Synthesis: Using 1 µg of RNA, cDNA will be synthesized with a reverse transcription kit (e.g., High-Capacity cDNA Reverse Transcription Kit, Thermo Fisher). Quantitative Real-Time PCR (qRT-PCR) Target Genes p53 and Bax/Bcl-2 Ratio, Primers and Probes: Custom or commercially available validated primers/probes will be used. PCR Conditions: Standard qRT-PCR protocol with SYBR Green or TaqMan chemistry.

CONVENTIONAL PCR ASSAY

PCR MASTER MIX REACTION PREPARATION

The PCR master mix reaction was prepared following the manufacturer's instructions provided by TransGen Biotech Inc., China. *EasyTaq*® PCR SuperMix is a ready-to-use mixture of *EasyTaq*® DNA Polymerase, dNTPs, and optimized reaction buffer at a concentration of 2×. For DNA amplification, simply add the template, primers, and water to allow the SuperMix solution to react at a concentration of 1×. For 2×*EasyTaq*® PCR

SuperMix (-dye), the PCR products which contain dA overlangs at the 3' end can be directly cloned to *pEASY*®-T vectors. For 2×*EasyTaq*® PCR SuperMix (+dye), the PCR products can be analyzed by electrophoresis directly, and need to be purified to remove dye when applied in cloning.

After preparing the PCR master mix according to the specified components, standard PCR tubes were filled with the mixture. These tubes contained lyophilized Taq DNA Polymerase, dNTPs, and 6 mM MgCl2 at pH 8.7, necessary for Multiplex PCR. The tubes underwent centrifugation in an Existing vortex centrifuge for three minutes to ensure thorough mixing before being transferred to a Multigene PCR thermocycler for amplification.

PCR THERMOCYCLING CONDITIONS

PCR thermocycler conditions were set using a conventional PCR system, detailed in the accompanying Table 1.

STATISTICAL ANALYSIS

All test results and questionnaire data were compiled into a datasheet. Data analysis was conducted using IBM SPSS version 28.0 and Real Statistics Resource Pack for Excel 2016.

RESULTS

MOLECULAR ANALYSIS

PCR AMPLIFICATION OF TUMOR PROTEIN p53 GENE USING SPECIFIC PRIMERS – GEL ELECTROPHORESIS ANALYSIS

Polymerase chain reaction (PCR) was performed to amplify a 421-base-pair fragment of the p53 gene, a key tumor suppressor involved in the regulation of cell cycle progression, DNA repair, and apoptosis. The samples used in this analysis were collected from patients with prostate cancer (Fig. 1). p53-specific primers were used to amplify 42 genomic DNA samples, and agarose gel electrophoresis was used to examine the PCR results. Molecular weight markers (L) for size estimate and a negative control (N) to confirm reaction integrity surround the two sample rows in the gel mapping (lanes 1–32 in the upper panel and lanes 33–42 in the lower panel). In most lanes, a clear band at roughly 421 base pairs was seen, suggesting accurate and targeted amplification of the desired p53 area. High-quality DNA templates and ideal PCR conditions are reflected in the bands' consistency and clarity.

IL-6 LEVELS BY GROUP STUDY

Serum IL-6 levels (by ELISA) were elevated in prostate cancer patients (mean = 25.4 ± 6.2 pg/mL) compared to controls (17.1 ± 2.3 pg/mL); $p < 0.001$ (Fig. 2).

IL-8 LEVELS BY GROUP STUDY

IL-8 levels were significantly elevated in prostate cancer patients (mean = 27.5 ± 9.8 pg/mL) compared to healthy controls (mean = 10.2 ± 3.5 pg/mL), supporting its role as a pro-inflammatory and pro-angiogenic cytokine (Fig. 3).

P53 LEVELS BY GROUP STUDY

P53 was significantly decreased in prostate cancer patients, indicating potential p53 inactivation and impaired apoptotic control. This aligns with observed reductions in Bax/Bcl-2 ratio and increased tumor survival (Fig.4).

BAX/BCL-2 RATIO LEVELS BY GROUP STUDY

The Bax/Bcl-2 ratio was significantly reduced in the prostate cancer group ($p < 0.001$), indicating a shift toward anti-apoptotic signaling in tumor-bearing individuals (Fig. 5).

VEGF LEVELS BY GROUP STUDY

VEGF levels were markedly increased in the prostate cancer group, highlighting enhanced angiogenic signaling. VEGF correlated significantly with IL-6 and IL-8, supporting an inflammation-driven angiogenesis (Fig. 6).

CORRELATION ANALYSIS

Spearman correlation analysis within the prostate cancer group demonstrated strong interrelations among the biomarkers. p53 expression positively correlated with inflammatory and angiogenic markers (IL-6, IL-8, VEGF), while showing a negative correlation with the Bax/Bcl-2 ratio. These findings support the hypothesis that inflammatory and angiogenic signaling pathways may suppress apoptosis and promote tumor progression (Tables 2- 3).

ROC CURVE FOR VEGF

The receiver operating characteristic (ROC) curve for VEGF levels demonstrates a high diagnostic accuracy with an area under the curve (AUC) of 0.91, indicating strong discriminative power between prostate cancer patients and healthy controls (Fig. 7).

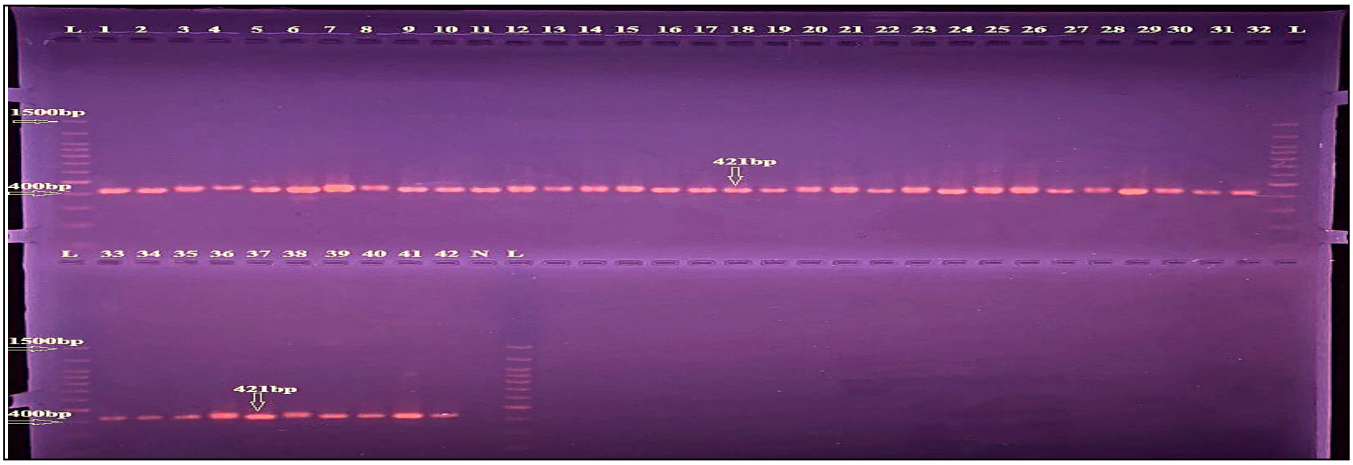

Fig. 1. PCR products generated from the p53 gene employing primers specific to a 421 bp region were electrophoresed on an agarose gel. Lanes 1–42 depict individual DNA samples, with clear amplification detected at the expected product size. The DNA size marker, which ranges from 400 to 1500 bp, is represented by lane L, while lane N is the negative control (no amplification). The outcomes validate the p53 amplification reaction's efficiency and specificity
Source: Own materials

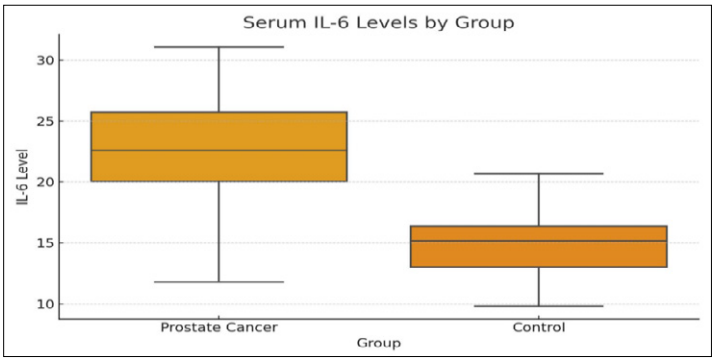

Fig. 2. IL-6 levels by group study
Source: Own materials

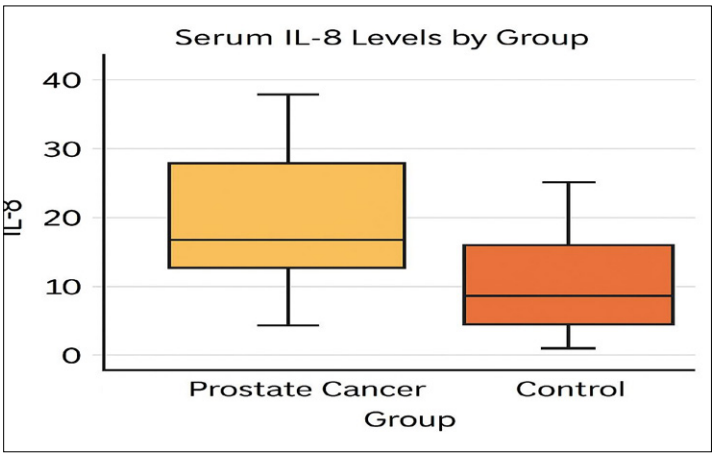

Fig. 3. IL-8 levels by group study
Source: Own materials

DISCUSSION

In peripheral blood samples from individuals with prostate cancer, this study offers thorough molecular profiling of important apoptotic, inflammatory, and angiogenic markers, including p53 [14], the Bax/Bcl-2 ratio, IL-6, IL-8, and VEGF. The results provide credence to the theory that the pathophysiology and progression of prostate cancer are influenced by deregulation of apoptosis, chronic inflammation, and increased angiogenesis [15]. Cancer patients showed a marked decrease in p53 expression and the Bax/Bcl-2 ratio, indicating compromised apoptosis and cellular resistance to programmed cell death [16]. Malignant cells may be able to accumulate mutations and avoid immune clearance due to this anti-apoptotic state. The function of functioning p53 in preserving apoptotic equilibrium is further supported by the negative relationship between p53 and the Bax/Bcl-2 ratio [17].

Prostate cancer is associated with persistent inflammation, as evidenced by elevated serum levels of IL-6

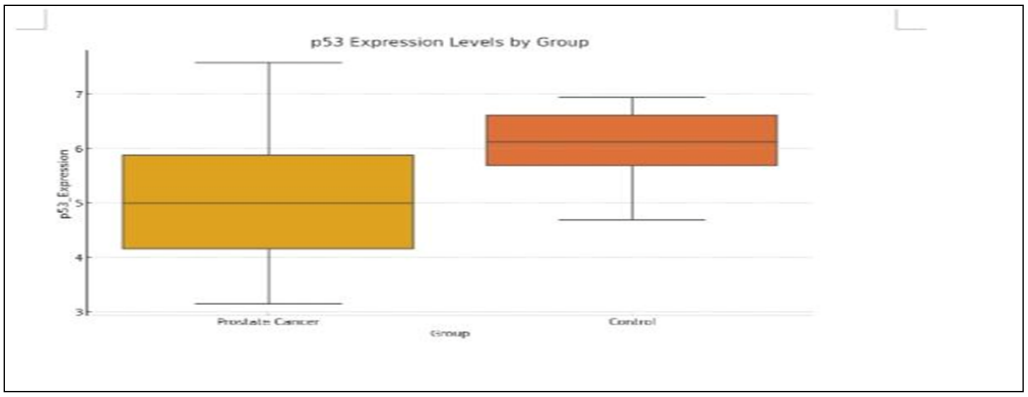

Fig. 4: P53 levels by group study
Source: Own materials

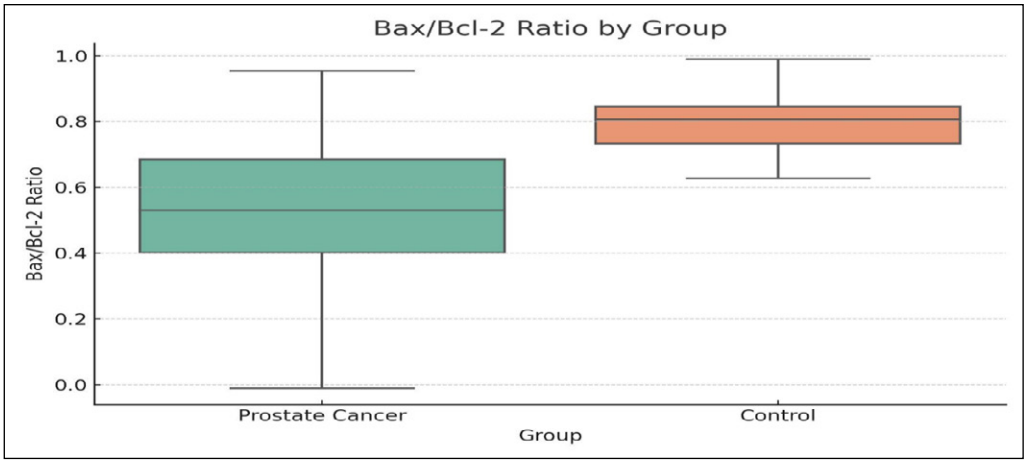

Fig. 5. Bax/Bcl-2 ratio levels by group study
Source: Own materials

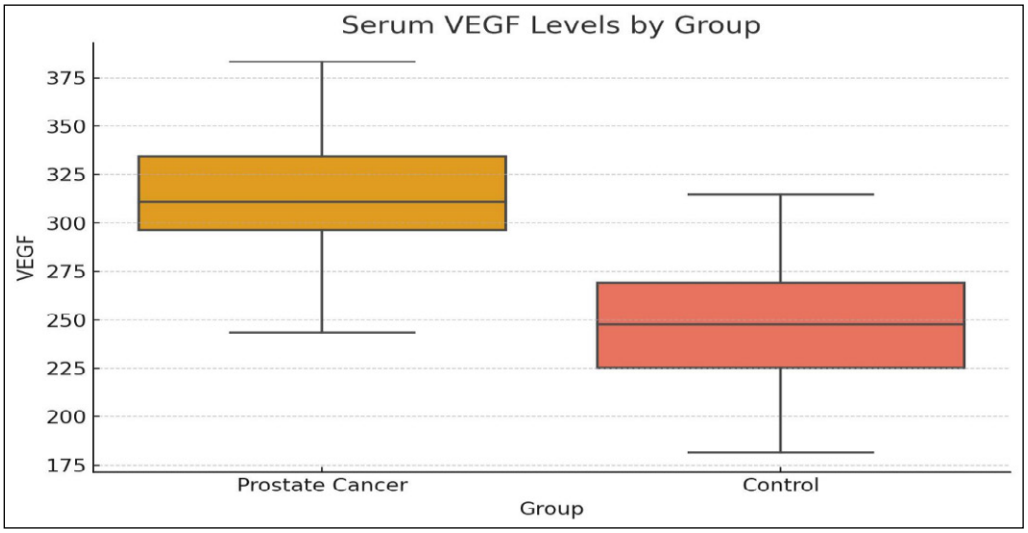

Fig. 6. VEGF levels by group study
Source: Own materials

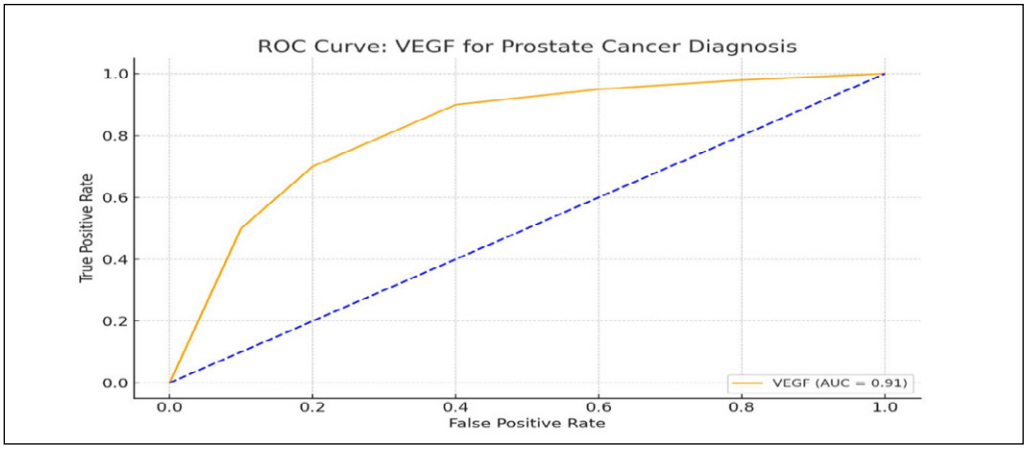

Fig. 7. ROC Curve for VEGF prostate cancer
Source: Own materials

Table 1. PCR amplification program for PCR

Step	Temperature (˚C)	Time	No. of cycles
Initial denaturation	94˚C	3 min	1
Denaturation	94˚C	30 s	35
Annealing	Variables according to (primer's TM)	30 s	
Extension Step	72˚C	1 min	
Final extension	72˚C	5 min	1
Hold temperature	4˚C	∞	-

Source: Compiled by authors of this study

Table 2. Comparison of biomarker levels between groupsv

Marker	Mean (patients)	Mean (controls)	P-value
P53_Expression	5.04	6.08	0.0000
Bax_Bcl2_Ratio	0.65	0.43	0.0001
IL6	12.77	20.52	0.0001
IL8	20.56	27.06	0.0603
VEGF	201.95	304. 18	0.0000

Source: Compiled by authors of this study

Table 3. Comparison of biomarker levels between prostate cancer group

Marker 1	Marker 2	Spearman r	P-value
P53_Expression	Bax_Bcl2_Ratio	-0.60	0.0000
P53_Expression	IL6	0.66	0.0000
P53_Expression	IL8	0.73	0.0000
P53_Expression	VEGF	0.59	0.0001
Bax_Bcl2_Ratio	IL6	-0.58	0.0001
Bax_Bcl2_Ratio	IL8	-0.62	0.0000
Bax_Bcl2_Ratio	VEGF	-0.64	0.0000

Source: Compiled by authors of this study

and IL-8 (18). By activating the STAT3 and NF-κB signaling pathways, both cytokines are known to increase tumor development, angiogenesis, and resistance to treatment. This study's high positive correlation between them and VEGF points to a molecular connection between angiogenesis and inflammation. By promoting neovascularization, aiding metastasis, and allowing food supply, this inflammatory-angiogenic axis may aid in the growth of tumors [19].

Crucially, the correlation analysis showed a consistent biological interaction: IL-6 and IL-8 associated negatively with the Bax/Bcl-2 ratio and positively with VEGF, suggesting that inflammation may inhibit apoptosis in addition to promoting angiogenesis [20]. These connections highlight the intricate molecular crosstalk that occurs in the tumor microenvironment, where several signaling pathways come together to maintain cancer. A therapeutically significant benefit of using non-invasive blood-based biomarkers is that they allow recurrent sample to track the course of a disease or the effectiveness of a treatment [21]. Incorporating apoptotic, inflammatory, and angiogenic indicators should improve the accuracy of prostate cancer diagnosis and help move toward customized medicine [22].

CONCLUSIONS

1 Prostate cancer patients exhibited decreased p53 and Bax/Bcl-2 ratio, indicating impaired apoptosis.
2 IL-6 and IL-8 were significantly elevated and positively correlated, reflecting a pro-inflammatory environment.
3 VEGF levels were markedly increased, with strong associations to both inflammatory markers and tumor burden.
4 Combined profiling of these markers demonstrates diagnostic potential and sheds light on the molecular crosstalk between apoptosis, inflammation, and angiogenesis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 29.01.2026
ACCEPTED: 17.04.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Diagnostic criteria for oligosymptomatic choledocholithiasis in patients with acute calculous cholecystitis

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ABSTRACT

Aim: To identify clinically significant diagnostic criteria for oligosymptomatic choledocholithiasis and determine their diagnostic value in patients with acute calculous cholecystitis (ACC) based on instrumental and laboratory examination data.

Materials and Methods: The study included 129 patients divided into two groups: the study group (I) and the comparison group (II). The study group included patients with ACC and oligosymptomatic choledocholithiasis (n=83), while the comparison group included patients with ACC without OCL (n=46).

Results: A univariate analysis using logistic regression identified nine factors with prognostic value for determining the presence of OCL. A high quality of OCL prediction was observed (AUC=0.99). The optimal decision threshold for diagnosing OCL was 0.5. Sensitivity at the established threshold was 100%, specificity was 88.90%, and accuracy was 96.88% ($p<0.001$). a multivariate regression model based on six factors was developed to enhance the clinical applicability of OCL prediction in ACC. This model also demonstrated a high predictive quality (AUC=0.98) compared to the nine-factor model. The decision threshold for the presence or absence of OCL was 0.6. The sensitivity of this model was 95.65%, specificity was 88.90%, and accuracy was 93.75%.

Conclusions: The diameter of the common bile duct, the activity of ALT, AST, alkaline phosphatase, total bilirubin levels, as well as the expression levels of miR-122 and miR-21 in serum and bile, serve as reliable diagnostic criteria associated with the presence of oligosymptomatic choledocholithiasis.

KEYWORDS: acute calculous cholecystitis, oligosymptomatic choledocholithiasis, miR-122, miR-21

Wiad Lek. 2026;79(7):1606-1614. doi: 10.36740/WLek/218750 DOI

INTRODUCTION

Choledocholithiasis is a common complication in surgical practice observed in patients with gallstone disease. The clinical presentation of gallstones in the common bile duct (CBD) can vary widely, ranging from asymptomatic incidental findings to severe complications such as obstructive jaundice, acute cholangitis, and pancreatitis [1, 2].

The presence of CBD stones in patients with acute calculous cholecystitis (ACC) requires comprehensive surgical treatment to prevent further complications. According to meta-analysis data, the incidence of choledocholithiasis in ACC patients is 13.7% (95% confidence interval 11.8–15.9) [3]. However, in the absence of overt clinical signs of choledocholithiasis, surgical interventions in such patients often do not include a CBD revision [4, 5].

The diagnosis of choledocholithiasis is based on clinical assessment, blood biochemistry, and imaging techniques [6, 7]. Traditionally, indicators of cholestasis in blood biochemistry (direct bilirubin, alkaline phosphatase, gamma-glutamyl transferase) have significant diagnostic value in choledocholithiasis [8]. However, changes in biochemical blood markers, as well as clinical symptoms, indicate only bile flow disturbances rather than their cause. Moreover, ACC itself, regardless of choledocholithiasis, may be associated with elevated cholestasis markers, reducing the diagnostic value of liver function tests [9].

Transabdominal ultrasonography (TAUS) allows for the detection of gallstones, changes in wall thickness and CBD diameter, and inflammatory changes in acute cholecystitis. However, the accuracy of TAUS in detecting choledocholithiasis is only 50-70% since gallstones may lack acoustic shadows or be located in the distal CBD, obscured by bowel gas and duodenal contents [9]. Additionally, the accuracy of the examination largely depends on the specialist's experience and the adequacy of patient preparation, which is not always feasible in acute surgical conditions [4].

Endoscopic retrograde cholangiopancreatography (ERCP) is one of the most informative diagnostic tech-

niques for choledocholithiasis; however, it is an invasive procedure with potential complications and is therefore not recommended for diagnostic purposes alone [10].

The use of other highly informative imaging techniques, such as magnetic resonance cholangiopancreatography (MRCP), various types of computed tomography (CT), and endoscopic ultrasonography (EUS), for all patients with gallstone disease is problematic in emergency surgery settings and financially unjustifiable, especially in the presence of ACC [3, 4].

Recently, the role of small RNA molecules (microRNAs) in liver disease diagnostics has been actively studied. MicroRNA expression is tissue-specific, and changes in the concentration levels of various molecules may accompany pathological processes in corresponding organs and tissues [11, 12]. MiR-122 is a widely distributed liver-specific microRNA [13, 14], constituting approximately 52% of the total hepatic microRNA in humans, with minimal expression in other cell types. Increasing evidence suggests the crucial role of miR-21 in liver diseases [15], particularly its association with the pathogenesis of cholestatic liver injury [11, 13]. Therefore, studying microRNA involvement in the etiology, pathogenesis, and diagnosis of hepatobiliary disorders, including choledocholithiasis, is becoming increasingly relevant. MicroRNA levels are stable and easily detectable in blood plasma, making them superior biomarkers for diagnosing, predicting, and assessing liver diseases compared to traditional markers [14, 16].

Thus, considering the growing importance of diagnosing oligosymptomatic choledocholithiasis (OCL), its diagnostic techniques require further improvement with detailed justification. In emergency settings, determining an adequate minimal scope of examinations is crucial to avoid treatment delays and prevent complications. This prompted us to explore the feasibility of using a combination of biochemical markers and microRNA expression levels for the diagnosis of OCL in ACC patients by developing a regression-based mathematical model.

AIM

To identify clinically significant indicators of oligosymptomatic choledocholithiasis and determine their diagnostic value in patients with acute calculous cholecystitis based on instrumental and laboratory examination data.

MATERIALS AND METHODS

The study included 129 patients who underwent treatment at the Department of Surgery No. 1 of Lviv National Medical University from 2016-2023. Patients were divided into two groups: the study group (I) and the comparison group (II). The study group included patients with ACC and OCL (n=83), and the comparison group included ACC patients without OCL (n=46). The first group consisted of 61 females (73.49%) and 22 males (26.51%), with a mean age of 56±14.4 years. Patients in group II were matched by age and gender. The gender distribution was reciprocal. An additional control group (III) included conditionally healthy individuals (volunteers, n=20) of corresponding age and gender. All patients underwent TAUS of the liver, gallbladder, and bile ducts, as well as blood biochemistry and the detection of miR-122 and miR-21 expression levels in blood serum and bile.

The criteria for inclusion in group I were confirmed by the diagnosis of ACC, lack of a clear clinical picture of choledocholithiasis, the absence of direct signs of choledocholithiasis during TAUS (absence of echogenic mass with acoustic shadow in the lumen of the common bile duct), diameter of common bile duct in TAUS was less than 10 mm; no jaundice (total bilirubin less than 34.5 μmol/L). The exclusion criteria for patients in group I were as follows: no confirmation of ACC diagnosis; the presence of jaundice; direct signs of choledocholithiasis according to TAUS; diameter of the common bile duct in TAUS was more than 10 mm; total bilirubin exceeding 34.5 μmol/L.

In the study group (83 patients) and the comparison group (46 patients), quantitative determination of miR-122 and miR-21 was performed using real-time polymerase chain reaction (PCR) in blood serum and bile. In the control group (20 volunteers), it was conducted only in serum.

A two-stage study was used according to the manufacturer's protocol to study the expression level of miR-122 and miR-21 in serum and bile. Initially, RNA was extracted from serum and bile using a Trizol RNA-prep kit (Isogen) to isolate total RNA. The method is based on the use of Trizol reagent.

Reverse transcription was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), specific loop primers to reach the maturation of each miRNA and 10 ng of total RNA. Real-time quantitative PCR was performed using TaqMan MicroRNA Assays (Applied Biosystems, USA): hsa-miR-122, hsa-miR-21, and U6 snRNA as endogenous control. Temperature mode: initial denaturation 95°C – 10 minutes; 45 cycles of 95°C – 15 seconds and 60°C – 60 seconds. The miRNA level was calculated by the formula ($2\Delta Ct \times 100$), normalized to U6 snRNA and represented in relative units (RU). Amplification was performed with 7500 Fast Real-time PCR (Applied Biosystems, USA). The

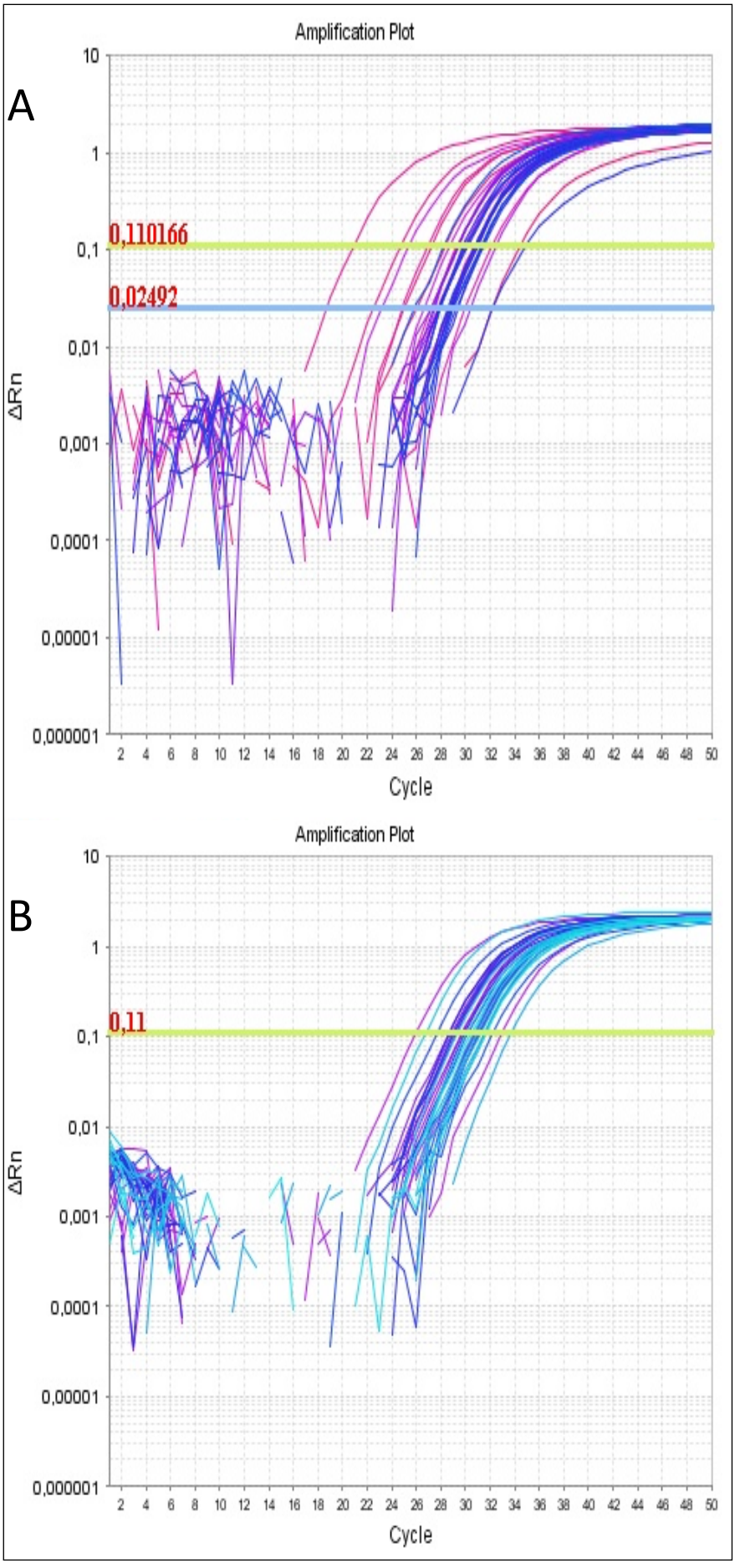

Fig. 1. Graph of the increase in fluorescence intensity during real-time PCR. A – miR-122, B – miR-21
Picture taken by the authors

Table 1. Prognostic Risk Factors for OCL Diagnosis using Logistic Regression (General Model)

No.	Indicator	Abbreviation	Regression Coefficient (β_i)
1	Constant (β_0)		-128.410
2	ALP (alkaline phosphatase)	V1	1.103
3	Common bile duct diameter	V2	-73.040
4	Total bilirubin	V3	13.380
5	AST (aspartate aminotransferase)	V4	772.000
6	ALT (alanine aminotransferase)	V5	58.530
7	miR-21 in serum	V6	-3.389
8	miR-122 in serum	V7	0.816
9	miR-21 in bile	V8	-0.539
10	miR-122 in bile	V9	15.050

Source: compiled by the authors of this study

Table 2. Results of logistic regression for risk factors of oligosymptomatic choledocholithiasis (simplified model)

No.	Indicator	Abbreviation	Regression Coefficient (β_i)
1	Constant (β_0)		-17.440
2	ALP	V1	0.020
3	Common bile duct diameter	V2	0.745
4	Total bilirubin	V3	0.230
5	AST	V4	10.804
6	ALT	V5	1.506
7	miR-122 in serum	V6	0.018

Source: compiled by the authors of this study

data obtained were analyzed using 7500 Fast Real-time PCR software and displayed using a graph (Fig. 1).

STATISTICAL CALCULATIONS

Statistical calculations were performed using RStudio software v.1.1.442 and R Commander v.2.4-4. Logistic regression was used to create a mathematical model for the prediction of OCL depending on the microRNA expression level in serum and bile. ROC analysis (The Receiver Operating Characteristic) was used to evaluate the quality of the model. As a result, in addition to the graph of the ROC curve, we analyzed the area under the curve (AUC), and the sensitivity, specificity, and accuracy of the model. Subsequently, the selection of the optimal indicator of the threshold value of decision-making (DM) using the graphs of sensitivity, specificity, and accuracy, as well as analyzing the following calculated DM: DM with equal values of sensitivity and specificity, DM at the maximum values of sensitivity and specificity, DM at the maximum value of Cohen's kappa coefficient, DM at the maximum value of the proportion of available and missing correctly defined cases, optimal DM for a given ROC curve. After determining the optimal DM value, the final values of sensitivity, specificity, and accuracy of the model were calculated.

ETHICS

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

AI STATEMENT

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

FRAMEWORK

The study was approved by the Ethics Committee of the Danylo Halytsky Lviv National Medical University, Ukraine (decision number 6 on 28.09.2020). The article was carried out within the framework of the research theme of the Department of Surgery No. 1 of the Danylo Halytsky Lviv National Medical University state registration number 011511000048.

RESULTS

The results of TAUS and laboratory tests were used to determine the criteria for diagnosing OCL in patients with ACC. A univariate analysis using logistic regression identified 9 of the 51 indicators that have prognostic value for diagnosing OCL: common bile duct diameter

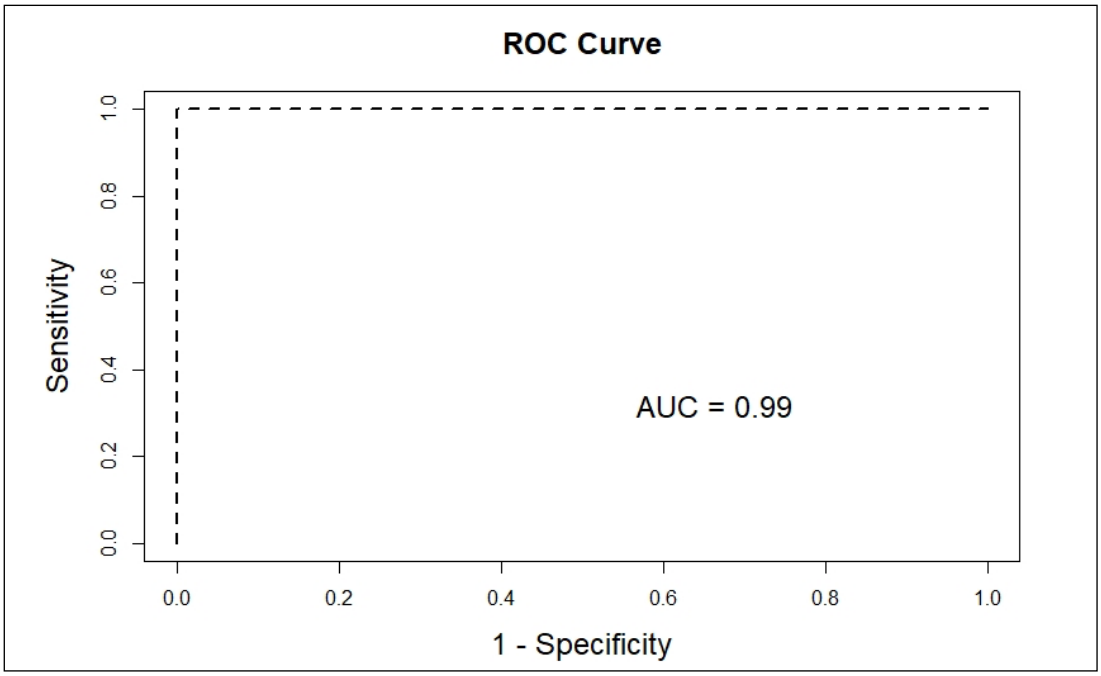

Fig. 2. ROC curve of the diagnostic capability of the logistic regression model for oligosymptomatic choledocholithiasis (9-factor model)
Picture taken by the authors

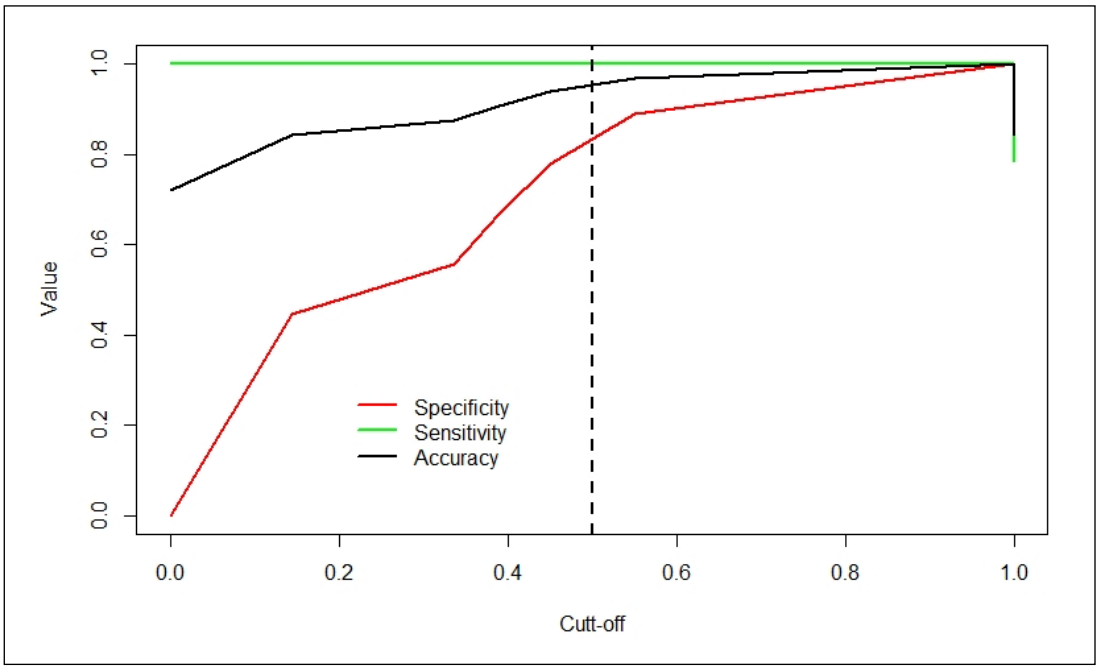

Fig. 3. Optimal threshold for decision-making in determining the presence of oligosymptomatic choledocholithiasis (9-Factor Model)
Picture taken by the authors

based on TAUS, alkaline phosphatase (ALP) activity, AST activity, ALT activity, total bilirubin level, and the expression levels of miR-122 and miR-21 in blood serum and bile (Table 1).

The overall probability **(*P*)** of OCL in ACC patients can be expressed using the regression equation:

$P=1/(1+e^z)$

$Z=1.03\times V1-73.04\times V2+13.38\times V3+772\times V4+58.53\times V5-3.39\times V6+0.82\times V7-0.54\times V8+15.05\times V9-128.41$

where: *e* is the Euler's number (exponent) equal to 2.718; *Z* is the value of the associated criterion; *V1* is alkaline phosphatase activity (U/L); *V2* is common bile duct diameter (mm); *V3* is total bilirubin level (μmol/L); *V4* is AST activity (U/L); *V5* is ALT activity (U/L); *V6* is miR-21 expression level in serum (RU); *V7* is miR-122 expression level in serum (RU); *V8* is miR-21 expression level in bile (RU); *V9* is miR-122 expression level in bile (RU).

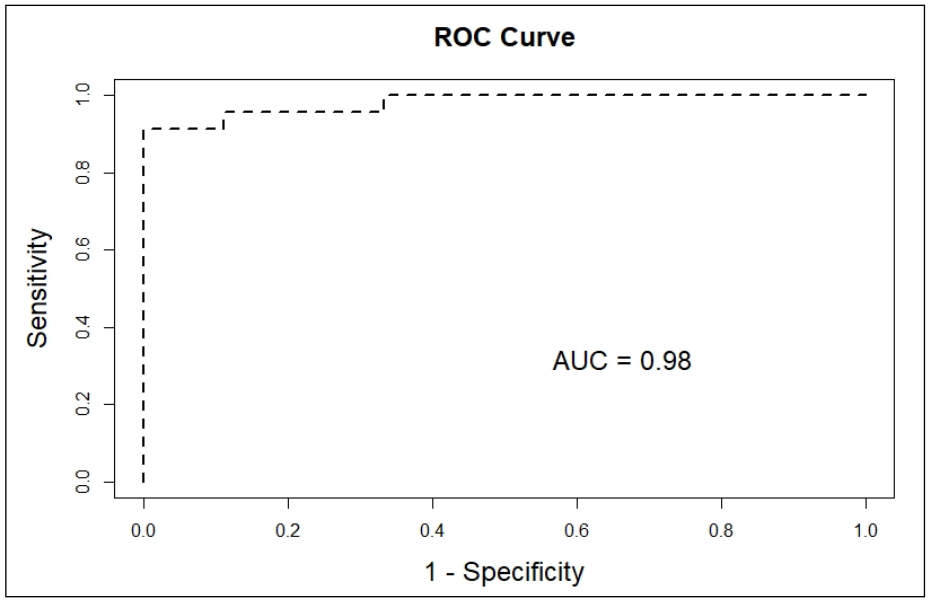

Fig. 4. ROC curve of the diagnostic capability of the logistic regression model for oligosymptomatic choledocholithiasis (6-factor model)
Picture taken by the authors

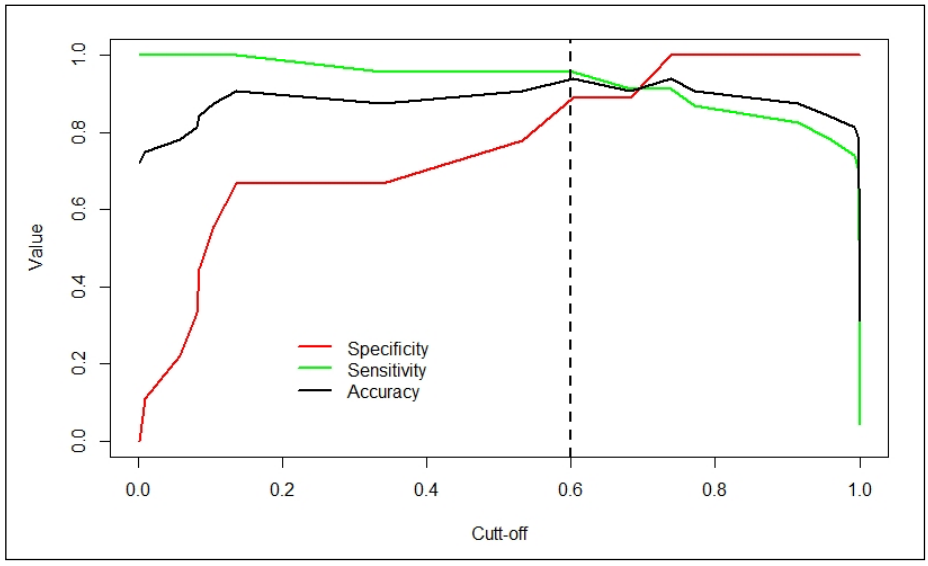

Fig. 5. Optimal threshold for decision-making in determining the presence of oligosymptomatic choledocholithiasis (6-factor model)
Picture taken by the authors

The associated criterion values were determined for all patients and then entered into ROC analysis to predict the OCL presence (Fig. 2). A high predictive quality for OCL was found (AUC=0.99).

The optimal threshold for decision-making in determining the presence of OCL was 0.5 (Fig. 3). Values above this threshold indicate the presence of OCL, while lower values suggest its absence. Sensitivity at the established threshold was 100%, specificity was 88.90%, and accuracy was 96.88% ($p<0.001$).

From the perspective of broader application in clinical practice for OCL prediction in ACC patients, a multivariate regression model was also developed using six factors: the activity of ALP, ALT, and AST, total bilirubin level, common bile duct diameter according to TAUS, and serum miR-122 expression level (Table 2).

Based on these data, the probability (*P*) of OCL in ACC patients can be expressed using the regression equation:

$$P=1/(1+e^{z})$$

$$Z=0.02\times V1+0.745\times V2+0.23\times V3+10.80\times V4+1.51\times V5+0.018\times V6-17.44$$

where: *e* is the Euler's number (exponent) equal to 2.718; *Z* is the value of the associated criterion; *V1* is ALP (U/L); *V2* is common bile duct diameter (mm); *V3* is total bilirubin level (μmol/L); *V4* is AST activity (U/L); *V5* is ALT activity (U/L); *V6* is miR-122 expression level in serum (RU).

Associated criterion values were determined for all patients and then entered into ROC analysis for OCL prediction. A high-quality prediction of OCL (AUC=0.98) was observed, compared to the 9-factor model (Fig. 4).

The threshold for decision-making in determining the presence or absence of OCL was 0.6 (Fig. 5). The sensitivity of the model was 95.65%, specificity was 88.90%, and accuracy was 93.75%. From a practical perspective, this means that in 95.6% of cases, the test will be positive if the patient has OCL.

DISCUSSION

The evaluation of the probability of choledocholithiasis in ACC patients remains a relevant issue in clinical practice and continues to be a subject of scientific debate to this day [1-3]. According to clinical treatment protocols, patients with symptomatic choledocholithiasis require treatment to remove stones from the bile ducts and ensure adequate bile drainage. However, the clinical management of patients with asymptomatic choledocholithiasis remains controversial, as any intervention, including endoscopic procedures, carries a certain risk of side effects [5].

It is well known that the presence of stones in the common bile duct is associated with a risk of complications. Therefore, during surgical treatment for ACC, it is advisable to simultaneously address choledocholithiasis if present. Diagnosing this condition before surgical intervention is crucial, as removing stones from the bile ducts along with cholecystectomy helps avoid additional future invasive procedures, reducing the risk of complications, lowering treatment costs, and lessening the burden on the healthcare system.

ERCP, previously considered the gold standard for diagnosing common bile duct stones, is associated with a risk of serious complications and is not widely recommended as a diagnostic technique in this clinical scenario [4, 6, 7]. Additionally, the benefit-risk ratio must be determined for therapeutic ERCP to be performed.

The limited availability of other imaging techniques for diagnosing choledocholithiasis (MRCP, CT, EUS) and their practical unavailability in some cases necessitate the development of simple and effective predictive models for OCL diagnosis. Although some sources highlight the prognostic value of biochemical and instrumental parameters in assessing OCL risk and choosing patient management methods, no universally accepted evaluation system currently exists [6-8, 17].

Choledocholithiasis is typically suspected in patients with signs of cholestasis (elevated bilirubin levels and alkaline phosphatase activity in serum) and confirmed by TAUS. Indeed, in patients with gallstone disease, a total bilirubin level of 3–4 mg/dL (51.3–68.4 μmol/L) is closely associated with choledocholithiasis. Serum ALT, AST, and alkaline phosphatase activity levels are also elevated in bile duct obstruction. However, in cases of OCL in ACC, the prognostic value of elevated liver enzyme levels is limited, as other factors may also contribute to these abnormalities [4, 18].

TAUS is the initial test for patients with suspected choledocholithiasis. Although abdominal ultrasound often reveals only dilation of the bile ducts, its sensitivity for detecting common bile duct stones is relatively low.

Consequently, biochemical tests and TAUS cannot reliably predict the presence of common bile duct stones, and ERCP is not a viable screening tool due to its associated complications.

Recent studies demonstrate the important role of microRNAs in the pathogenesis of liver diseases. The levels of these molecules increase in bile and blood serum in various hepatobiliary disorders. Based on this, miR-122 and miR-21 are suggested as markers for diagnosing cholestatic liver injury and bile duct pathology [14-16].

Our previous research demonstrated the potential of quantifying serum and bile miR-122 and miR-21 expression levels to predict the probability of choledocholithiasis in ACC patients [19]. Additionally, we determined the diagnostic significance of some liver function tests and ultrasound indicators for detecting common bile duct stones. Notably, combining different indicators in multivariate analysis improved the predictive accuracy compared to using individual components.

In this study, we combined nine different indicators into a multivariate model, which demonstrated a high prognostic value in ROC analysis (AUC=0.99), with a decision-making threshold for OCL presence at 0.5. The sensitivity of the method was 100%, and the specificity was 88.9%. From a practical perspective, this method allows for a highly reliable risk assessment of OCL in patients with ACC when the calculated probability exceeds 0.5.

However, the mentioned model includes bile miR-122 and miR-21 expression levels, which cannot be routinely measured in clinical practice. Previous findings indicated that the most sensitive microRNA indicator (according to ROC analysis) is the bile miR-122 level, and increased serum miR-122 and miR-21 expression levels significantly ($p<0.05$) correlated with those in bile [19].

To enhance practical applicability, we developed a regression model based on six widely accessible parameters in routine surgical practice: the activity of alkaline phosphatase, AST, and ALT, total bilirubin level, common bile duct diameter based on TAUS, and serum miR-122 expression.

The analysis of this model showed its high prognostic accuracy (AUC=0.98) compared to the 9-factor model, with a sensitivity of 95.65% and specificity of 88.90%

allowing for a highly confident assessment of OCL risk in ACC patients.

The simplicity and accessibility of determining each indicator make this model feasible for use in routine clinical practice. Since this evaluation system has high prognostic accuracy and broad applicability, it can help avoid unnecessary referrals for MRCP, EUS, and ERCP. This model can serve as a diagnostic tool for predicting OCL in ACC patients.

CONCLUSIONS

1. The diameter of the common bile duct, the activity of ALT, AST, and alkaline phosphatase, total bilirubin levels, as well as the expression levels of miR-122 and miR-21 in serum and bile, serve as reliable diagnostic criteria for the presence of oligosymptomatic choledocholithiasis.
2. A multivariate model based on six factors (the activity of alkaline phosphatase, AST, and ALT, total bilirubin level, common bile duct diameter assessed via TAUS, and serum miR-122 expression level) demonstrates comparable predictive accuracy and is more practical for routine clinical application. This method can be recommended as a diagnostic test for predicting the presence of OCL in patients with ACC.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 03.07.2025
ACCEPTED: 02.03.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Explanaton symptoms into mechanisms: functional gastrointestinal disorders in the children

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ABSTRACT

Aim: To investigate and analyze clinical and laboratory data in children with functional gastrointestinal disorders and the role of phytotherapeutic correction in the treatment.

Materials and Methods: Clinical and follow-up observation was conducted on 131 children aged 4 to 22 months (72 boys and 59 girls) with manifestations of functional gastrointestinal disorders.

Results: 53 (40,5%) children received artificial milk formulas from birth, 20 children (15,3%) - from 2 months of age, 22 (16,8%) - from 4 months of age, 36 (27,4%) - from 6 months of age or older. Complementary feeding in most cases was introduced earlier than the WHO recommended terms - 85 children (64,8%) began to receive complementary feeding before 6 months of age. The most frequent clinical manifestations were: flatulence (97 children/74,2±3,8%), pain syndrome (92 patients/70,4±3,9%), decreased appetite (64 children/48,6±4,4%) and refusal to eat (47 children/36,0±4,2%), constipation (45 patients/34,8±4,2%). Children with functional gastrointestinal disorders were characterized by a decrease in the degree of physical development: physical development below average was observed in 23,6±3,7% of the examined children, low physical development - in 18,3±3,4%, very low - in 22,9±3,7%. Liver ultrasound revealed no abnormalities in children with FGID. Coprological findings reflected impaired gastric and intestinal digestion and impaired pancreatic secretion.

Conclusions: The most significant risk factors for the development of FGID in young children are certain inconsistencies of nutrition with the functional capabilities of the child's organism in the form of artificial feeding and early introduction of complementary feeding.

KEYWORDS: functional gastrointestinal disorders, functional dyspepsia, homeostasis disorders, children

Wiad Lek. 2026;79(7):1615-1624. doi: 10.36740/WLek/225984 DOI

INTRODUCTION

Functional gastrointestinal disorders (FGIDs), also recently referred to as gut-brain interaction disorders, are common in children and vary according to age groups, i.e., FGIDs in neonates/toddlers and children/adolescents. Pediatric FGIDs typically develop and persist into adulthood, causing financial and psychological distress. Despite several decades of progress and advances in molecular biology and medical sciences, the exact pathophysiology remains unknown, although genetic, psychosocial, gut dysbiosis, visceral hypersensitivity, and neuroimmune causes have been considered [1]. The Rome IV criteria facilitate easier and earlier diagnosis of FGIDs by excluding organic causes and minimizing unnecessary investigations. There are dietary, psychosocial, neurostimulating and pharmacological treatment methods, although fewer studies have focused on drug treatment in children. Early diagnosis and appropriate treatment have the potential to improve recovery and quality of life. Functional gastrointestinal disorders (FGIDs) are a common pathology among children. According to statistics, they occupy the largest percentage in the structure of digestive pathology in young children and occur with a frequency of 85-90% [1,2]. Numerous studies conducted in Western Europe and North America allow us to consider this pathology the most frequent among gastroenterological diseases in children. During the first year of life, about half of children have at least one symptom of functional disorders of the gastrointestinal tract, and often a combination of several symptoms [3]. The treatment of children with functional dyspepsia syndrome is quite complex, since no clear schemes have been developed for this, and the literature on the treatment of children with digestive pathologies, in particular with functional dyspepsia, is scarce [4]. When choosing a treatment strategy for young children, preference should be given to drugs that have a mild effect and cause the least number of side effects [5].

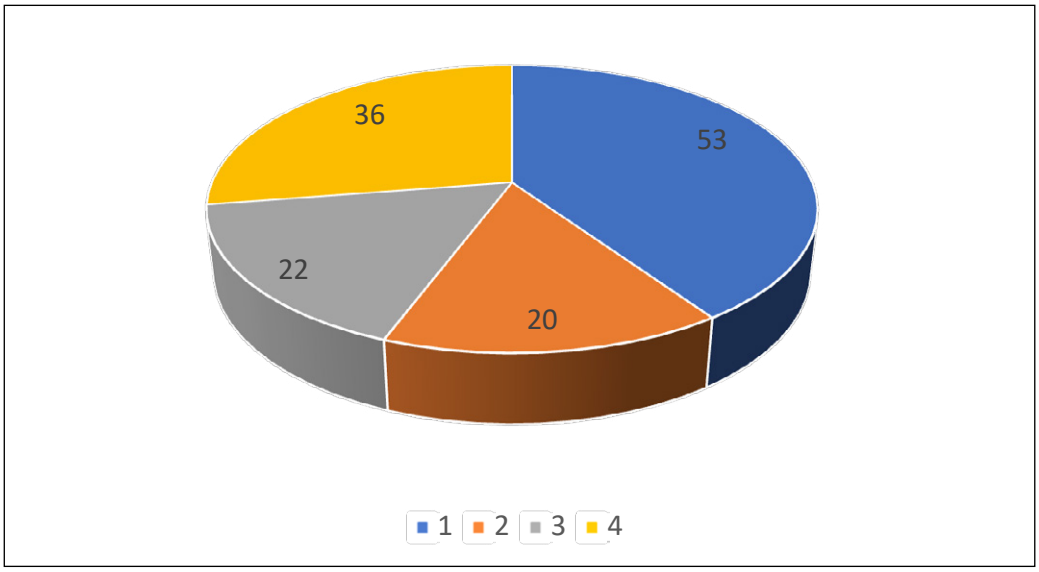

Fig. 1. Type of feeding of the surveyed contingent of children
1- artificial feeding from birth
2- artificial feeding from 2 months of age
3- artificial feeding from 4 months of age
4- artificial feeding from 6 months of age or older
Picture taken by the authors

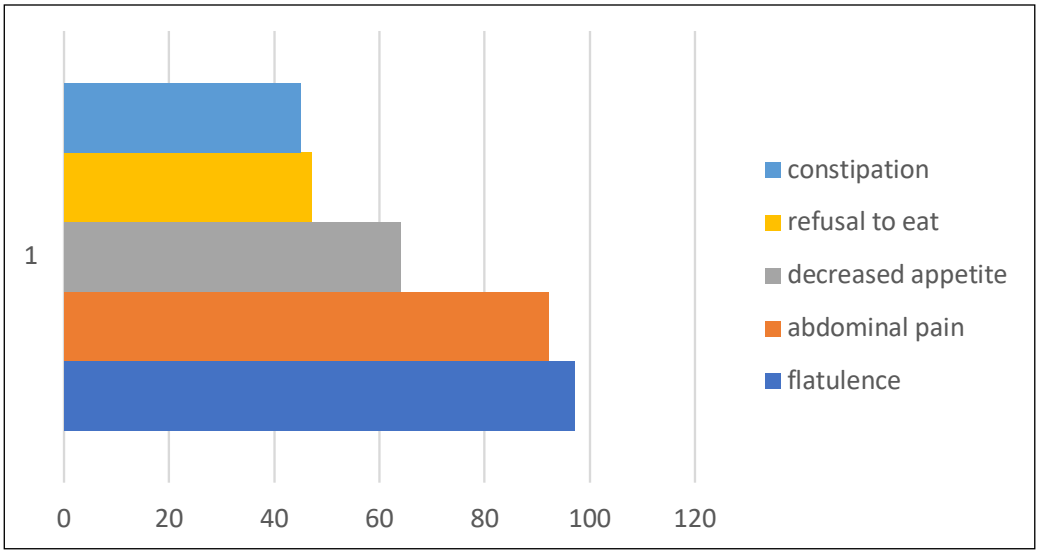

Fig. 2. Main clinical manifestations of functional gastrointestinal disorders in the examined children
Picture taken by the authors

AIM

To investigate and analyze clinical and laboratory data in children with functional gastrointestinal disorders and the role of phytotherapeutic correction in the treatment.

MATERIALS AND METHODS

Clinical and follow-up observation was conducted on 131 children aged 4 to 22 months (72 boys and 59 girls) with manifestations of functional gastrointestinal disorders who were inpatients in the early childhood department of the Uzhhorod Clenecal Children hospital. Diagnoses were verified according to the diagnostic criteria developed by the Ministry of Health of Ukraine. The studies were carried out in compliance with the basic provisions of GCP, the Council of Europe Convention on Human Rights and Biomedicine, and the World Medical Association Declaration of Helsinki on the ethical principles of conducting scientific medical research involving human subjects (2000).

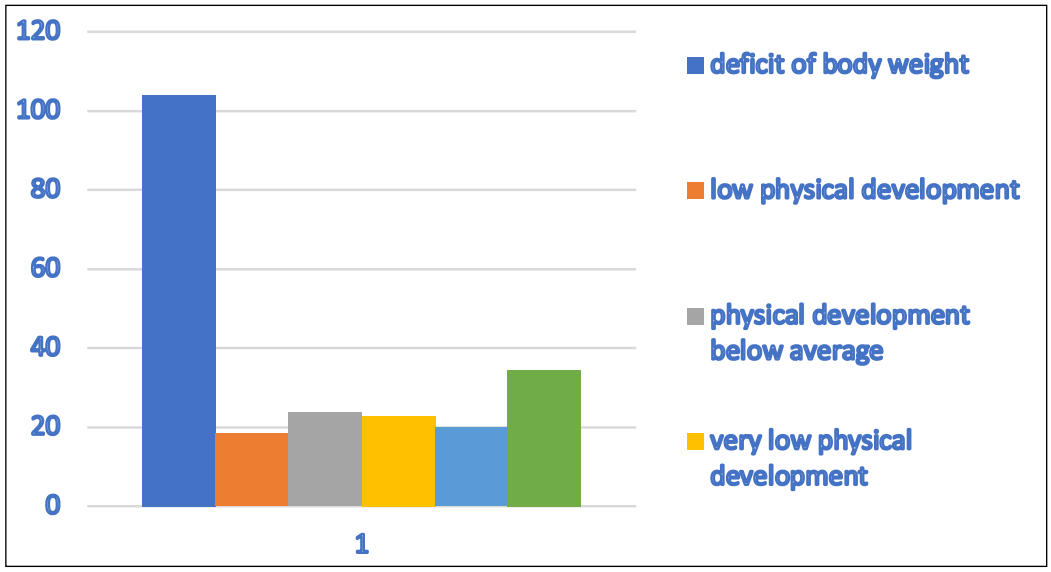

Fig. 3. Deviations in physical development indicators in children with functional gastrointestinal disorders
Picture taken by the authors

RESULTS

The examined children with functional gastrointestinal disorders were on artificial feeding, which confirms the important role of breastfeeding in the prevention of the development of pathological processes in children. Children did not receive human milk at all or its amount was less than 1/5 of the daily food intake, and the main food product were artificial milk formulas. 53 (40,5%) children received artificial milk formulas from birth, 20 children (15,3%) - from 2 months of age, 22 (16,8%) - from 4 months of age, 36 (27,4%) - from 6 months of age or older (Fig. 1).

As a substitute for breast milk, the examined children received highly adapted milk formulas. In all cases, the formulas corresponded to the age of the examined children.

Complementary feeding in most cases was introduced earlier than the WHO recommended terms - 85 children (64,8%) began to receive complementary feeding before 6 months of age (55 children received porridge starting from 4 months of age, 10 from 5 months of age; 12 children were introduced to vegetable puree from the 4th month of life; 8 children received meat puree as complementary feeding from the 5th month). From the beginning of the examination, complementary feeding products were introduced to the studied contingent of children in accordance with age standards.

The obtained data make it possible to state that the nature of feeding, violation of the timing of the introduction of complementary feeding are significant risk factors for diseases of the digestive system, the development of clinical manifestations of functional gastrointestinal disorders.

The examined children received food 5 times a day both before, during and after the course of treatment. The diet was calculated so that the amount of proteins constituted on average 3,5 g/kg of body weight per day for children aged 4-6 months and 4,0 g/kg of body weight per day for older children (of which 65-70% were animal proteins); fats – 6,0 and 5,5 g/kg of body weight per day, respectively (of which 85-90% were of animal origin); carbohydrates – 12-14 g/kg of body weight per day for both age groups (special attention was paid to the presence in the diet of a sufficient amount of plant fibers that stimulate the motor activity of the digestive system and normalize peristalsis). The energy value of the daily diet for 4-6 months old infants was on average 120 kcal/kg of body weight per day, for older children – 115 kcal/cell per day.

Due to the presence of a fairly large difference in the age of the examined children (4-22 months), the study of clinical manifestations of functional gastrointestinal disorders was carried out in two age categories: children under 12 months and children aged 13-22 months.

It was established that regardless of the age of the examined contingent, the most frequent clinical manifestations of functional gastrointestinal disorders were flatulence, pain syndrome, decreased appetite and even refusal to eat, constipation (Fig. 2). Based on the conducted studies, it was found that age differences in the clinical picture of the disease are not observed, the prevalence of the dyskinetic form of functional dyspepsia was established.

Flatulence was diagnosed on the basis of superficial palpation of the anterior abdominal wall, during which 97 children (74,2±3,8%) had abdominal distension.

Table 1. The content of total protein and protein fractions in the blood serum of children with functional gastrointestinal disorders (n = 131)

Indicator	Indicator level (M±m)	Reference values
Total protein, g/l	57.8±0.7*	57.0-73.0
Albumines, %	47.8±0.8**	56.5-66.5
Globulines, %	52.2±0.6**	33.5-43.5
α1-globuline, %	9.3±0.2**	2.5-5.0
α2-globuline, %	12.0±0.5**	5.1-9.2
β-globuline,%	12.5±0.7*	8.1-12.2
γ-globuline, %	19.3±0.6*	12.8-19.0

Notes: *– significance of differences between indicator values p>0,05; **– significance of differences between indicator values p<0,05
Source: compiled by the authors of this study

Table 2. Serum biochemical tests in children with functional gastrointestinal disorders (n = 131)

Indicator	Indicator level (M±m)	Reference values
Creatinine, mmol/l	71.2±1.8*	50-115
Urea, mmol/l	4.4±0.2*	4.2-8.3
Thymol test, un.	0.25±0.04*	до 4.0
ALT, Mkat/l	0.20±0.04*	0.1-0.68

Notes: *– significance of differences between indicator values p>0,05; **– significance of differences between indicator values p<0,05
Source: compiled by the authors of this study

Due to the age characteristics of the examined contingent, the equivalent of pain syndrome, which is one of the main manifestations of functional gastrointestinal disorders in older children, was considered to be restlessness and crying of the child along with tension of the muscles of the anterior abdominal wall. Such phenomena were observed in 92 (70,4±3,9%) of the examined children.

Decreased appetite was manifested by reluctant eating or undereating of a portion of food appropriate for the child's age and was found in 64 of the examined children (48,6±4,4%).

Refusal to eat was recorded in 47 children (36,0±4,2%) with functional gastrointestinal disorders. In this case, the children refused not a specific type of food, but one or more meals.

Constipation was manifested by a prolonged (48 hours or more) delay in bowel movements, accompanied by difficulty in defecation, as well as a small amount or increased density of feces, and was diagnosed in 45 examined children (34,8±4,2%) (Fig. 2).

ASSESSMENT OF PHYSICAL DEVELOPMENT OF CHILDREN WITH FUNCTIONAL GASTROINTESTINAL DISORDERS

Regarding physical development, as the main indicator of health in young children, certain deviations were found in the examined children with functional gastrointestinal disorders.

104 (79,4±3,5%) children had a deficit of body weight in relation to the normative indicators, which reflects the main clinical manifestations of functional gastrointestinal disorders in young children in the form of reduced appetite and refusal to eat. Low levels of other anthropometric indicators, such as height, chest circumference, etc. can be considered a consequence of the above manifestations of the disease. During the assessment of physical development using centile tables, changes in the degree and harmony of physical development were observed [6]. Children with functional gastrointestinal disorders were characterized by a decrease in the degree of physical development: physical development below average was observed in 23,6±3,7% of the examined children, low physical development - in 18,3±3,4%, very low - in 22,9±3,7%. Average physical development was observed in 26,7±3,8% of children, and physical development above average - in 9,2±2,5%.

Deviations were found in the harmony of physical development: extremely disharmonious physical development was diagnosed in 34,4±4,1% of children with functional dyspepsia, disharmonious - in 19,9±3,5%. Harmonious physical development was in 45,8±4,4% of the examined children. The most frequent deviations in physical development indicators are presented in Fig. 3.

Based on the analysis of anthropometric data, it was found that the main deviations in physical development in children with functional gastrointestinal disorders are: deficit of body weight in relation to the normative indicators for children of this age, low levels of physical development and its disharmony.

Before the initiation of treatment and after its completion, the examined contingent of children underwent a general blood test, the main average indicators of which were as follows: ESR – 12,6±1,2 mm/h, hemoglobin – 111,0±6,5 g/l, erythrocytes – 3,5±0,1×10^{12}/l, leukocytes – 10,2±0,2×10^{9}/l, with no significant deviation from the age norm.

However, there were isolated cases of decreased hemoglobin and erythrocyte levels - in 27 (20,6%) children, which, in our opinion, was associated with malnutrition in the form of reduced appetite and, as a consequence, insufficient intake of iron with food products. Being in close contact with proteins of various tissues, blood proteins react very subtly to changes in chemical and physicochemical processes occurring in the body, and disorders of protein metabolism in tissues have a significant impact on the composition of blood proteins [7]. Therefore, the determination of protein fractions is an important component of assessing the child's condition, in particular in functional gastrointestinal disorders. The total content of plasma proteins does not have significant age-related changes. In newborns, especially in premature infants, the protein content is somewhat lower, and increases during the first year of life. The albumin content in children approaches that of adults by the end of the first year of life, while globulins reach this level somewhat later. No significant difference in the content of blood plasma proteins in girls and boys was found [8].

Table 1 presents the main indicators of proteinogram in young children with functional gastrointestinal disorders.

It was found that children with functional gastrointestinal disorders have changes in the level of total protein and protein fractions of blood serum. In particular, there was a tendency to a low level of total protein – 57,8±0,7 g/l (at the lower limit of reference values) and albumin fractions – 47,8±0,6% ($p<0,05$), while globulin fractions exceeded the upper limit of the norm – 52,2±0,6% ($p<0,05$). Such indicators indicate the presence of dysproteinemia in young children with functional gastrointestinal disorders [9].

In addition to determining the content of total protein and its fractions, other biochemical indicators of blood serum were studied as well (table 2):

The serum creatinine level in the examined contingent corresponded to the reference values and was 74,5±1,8 μmol/l with a norm of 50-115 μmol/l. There were no cases with low or high creatinine levels among the examined children. As for urea, the average values were 4,4±0,2 mmol/l with a norm of 4,2-8,3 mmol/l, however, there were cases (12 children) when the urea level was lower than 4,2 mmol/l; since the urea content in the blood reflects the level of protein load of the body, in our opinion, this is associated with insufficient intake of proteins as a result of reduced appetite in the examined children [9].

The thymol test, a biochemical test that assesses the protein-synthetic function of the liver and reflects the nonspecific inflammatory reaction of the liver tissue, did not deviate from the reference values in any child and was at the level of 0,25±0,04 units. The content of alanine aminotransferase (ALT) - an enzyme that is present in many tissues of the body, in particular in the liver and the release of which occurs when the internal structure of hepatocytes is disrupted and the permeability of cell membranes increases - also was within the normative limits at the level of 0,20±0,04 μmol/l. Such data confirm the absence of organic changes, in particular of the liver in functional gastrointestinal disorders in young children.

In children who had clinical manifestations of functional gastrointestinal disorders, a study of pigment metabolism indicators was conducted. The state of pigment metabolism was studied by the content of total bilirubin and its fractions: indirect bilirubin (free, unconjugated) and direct bilirubin (conjugated, bound). Under physiological conditions, bilirubin is accumulated by the liver and excreted in the bile. No deviations in the indicators of pigment metabolism were detected, the levels of total bilirubin and its fractions were within the age norm. The level of total bilirubin was on average 7,5±0,7 μmol/l, direct bilirubin – 3,3±0,4 μmol/l, indirect bilirubin – 4,1±0,9 μmol/l.

No significant deviations were detected in the electrolyte metabolism of the examined children. The level of calcium ions was slightly reduced at the level of 2,02±0,21 mmol/l with reference values of 2,2-2,4 mmol/l. The content of potassium ions constituted 4,27±0,34 μmol/l (normal 4,1-5,7 μmol/l), sodium ions – 139,6±0,86 mmol/l with a norm of 133-142 mmol/l.

Children with functional gastrointestinal disorders had their pituitary and thyroid hormone levels determined by enzyme-linked immunosorbent assay.

The relevance of their determination is explained by the fact that the leading role in controlling the growth processes belongs to neuroendocrine regulation with the participation of the hypothalamic-pituitary system. In addition, determining the level of thyroid hormones is of interest in regions endemic for iodine deficiency, such as Transcarpathia [10].

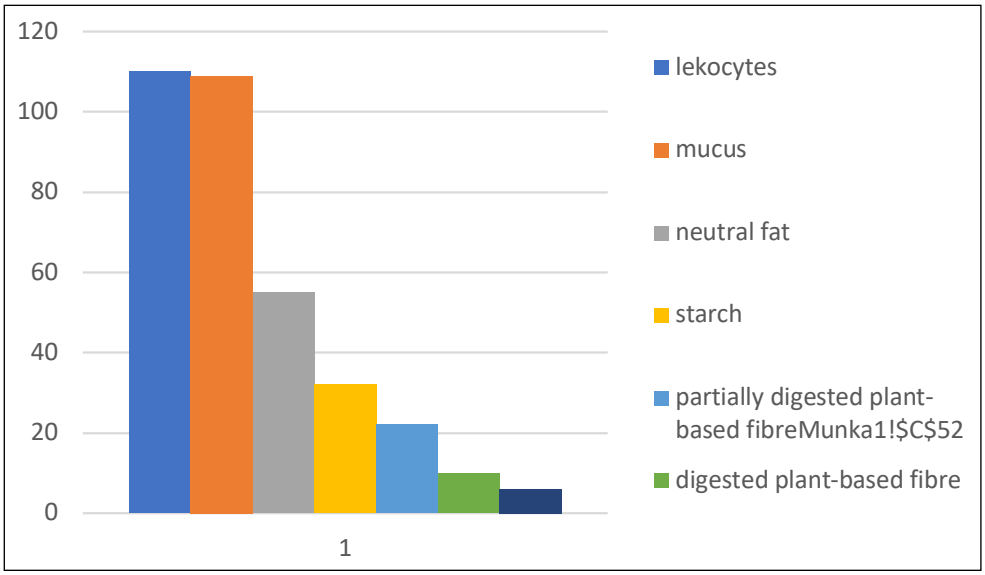

Fig. 4. Coprological findings in children with FGIR
Picture taken by the authors

It is known that in the first five years of life, the greatest growth effect of thyroid hormones is manifested (stimulation of osteogenesis and skeletal maturation). Later, the role of thyroxine is reduced to participation in the synthesis of somatomedins, which are mediators in the implementation of the action of somatotropic hormone. The activity of the anterior pituitary gland increases after two years, the secretion of somatotropic hormone increases, which from the age of five becomes decisive in the stimulation of chondrogenesis and linear bone growth. In addition, somatotropic hormone (STH) stimulates gluconeogenesis, protein synthesis, increases muscle mass and strength, absorption of calcium and phosphorus in the intestine and bone ossification, which is also of great importance during the period of intensive growth of the child [11].

Thyroid-stimulating hormone (TSH) is the main regulator of thyroid function, which, by the principle of feedback, ensures compliance between the activity of the thyroid gland and the needs of the body [10].

According to our data, the level of somatotropic hormone in children with functional gastrointestinal disorders was at the level of 7,41±0,40 mIU/l (normally its level fluctuates within 2-20 mIU/l), i.e. the average values of the indicators did not exceed the age norm. However, in 5 (3,8%) children, the level of STH was lower than the reference values and ranged from 0,31-0,9 mIU/l.

The level of thyroid-stimulating hormone in children with functional gastrointestinal disorders was on average 1,19±0,08 μIU/l with a norm of 0,23-3,4 μIU/l.

The thyroid hormone levels in the examined children were also within the normal limits: the level of triiodothyronine (T3) was 1,63±0,06 nmol/l, thyroxine (T4) – 146,9±0,9 nmol/l with a norm of 1,01-2,01 nmol/l and 53-158 nmol/l, respectively.

Based on the data obtained, it can be stated that in functional gastrointestinal disorders in the examined children there are no significant deviations in the level of pituitary and thyroid hormones.

During the work, a study of humoral immunity indicators was conducted. The main serum immunoglobulins - immunoglobulin E (Ig E), immunoglobulin A (Ig A), immunoglobulin G (Ig G) were determined using enzyme-linked immunosorbent assay.

The indicators of humoral immunity were as follows: the content of immunoglobulin E (Ig E) was on average 19,5±1,2 IU/ml, immunoglobulin A (Ig A) – 0,31±0,07 mg/ml, immunoglobulin G (Ig G) – 12,4±3,4 mg/ml. The reference values of immunoglobulins are up to 100 IU/ml, 0,3-1,2 mg/ml and 3,1-13,8 mg/ml, respectively. It was established that in children with functional dyspepsia there is a slight increase in the level of immunoglobulin G, and the level of immunoglobulin A is at the lower limit of the age norm.

The level of cytokines such as interleukin-2 and interleukin-4 was determined in the examined contingent of children.

The relevance of studying the level of cytokines in this contingent of patients is due to their participation in the formation and regulation of the body's protective reactions. At the body level, cytokines establish a connection between the immune, nervous, endocrine, hematopoietic and other systems and involve them in the organization and regulation of protective reactions [12].

It was found that the level of IL-2 in the examined children was 35,5±4,8 pg/ml, which is within the reference limits of indicators for children of this age.

As for the level of IL-4 in the examined contingent, the indicators also corresponded to the age reference values and were 13,1±1,0 pg/ml.

Table 3. Results of the ultrasound examination of the liver in healthy children and in children with functional gastrointestinal disorders (n=131)

Indicator	The value of indicators in children with FGID	Reference values
Liver	Right lobe up to 12 months: 66.5±0.4 mm 13-22 months: 72.5±0.5mm Left lobe up to 12 months: 33.6±0.3 mm 13-22 months: 36.1±0.3 mm	up to 12 months: 65-70mm 13-22 months: 70-75mm up to 12 months: 32-35 mm 13-22 months: 35-38mm
	V. porta: 5.2±0.1mm	5-6 mm
	V. hepatica dextra: 2.5±0.1mm	2-3 mm
	V. hepatica sinistra: 2.4±0.1mm	2-3 mm
	D. hepaticus communis: 3.6±0.1mm	до 4 mm
	D. choledochus: 4.9±0.1mm	до 6 mm

Source: compiled by the authors of this study

The obtained data confirm that the examined children, despite the existing clinical symptoms, did not have organic pathology and there were no signs of inflammatory reactions.

INSTRUMENTAL EXAMINATION OF THE GASTROINTESTINAL TRACT IN FUNCTIONAL GASTROINTESTINAL DISORDERS IN YOUNG CHILDREN

Considering that the fibroesophagogastroduodenoscopy (FGDS) method of the study is quite traumatic for the examined contingent of patients (examining the mucous membrane of the esophagus, stomach and duodenum using an endoscope, which is inserted through the root of the tongue into the listed parts of the digestive tract), in our opinion, the diagnosis of functional gastrointestinal disorders in young children can be made without FGDS and the study can be used only if there are indications.

Therefore, FGDS was performed on 10 children from each examined group, the clinical course of the disease in whom was the most severe. The need for examination of the entire group was not justified, since no pathological changes were observed during endoscopic examination of the esophagus, stomach, and duodenum in children with functional gastrointestinal disorders, which confirms the absence of organic changes in the mentioned parts of the gastrointestinal tract.

The results obtained during the study in children with functional gastrointestinal disorders were as follows (extract from the medical history No. 102 of child R., 14 months):

The esophagus without pathological findings, the cardia is located at a distance of 21 cm from the front teeth. The stomach contains a small amount of secretory fluid with mucus. The gastric mucosa is pale pink, unremarkable. The pylorus is centrally located, passable. The duodenal mucosa is pale pink, without visible changes.

Children with dyspepsia underwent ultrasound examination of the abdominal cavity as a safe and highly informative diagnostic method for diseases of the digestive tract.

The study revealed the following ultrasound picture (table 3):

Ultrasound examination of the liver revealed no abnormalities in children with functional gastrointestinal disorders. The sizes of the liver and its vessels, bile ducts corresponded to reference values.

There were isolated cases of irregular shape of the gallbladder due to bends in the neck area - in 15 examined children (11,5±2,8%). No changes of the gallbladder walls were detected, their thickness did not exceed 3 mm.

The pancreas had no pathological changes in any case during ultrasound examination - a homogeneous echostructure and smooth contours of the gland were detected.

The examined contingent also underwent a coprological examination, which was carried out in three stages: macroscopic, chemical and microscopic examination.

Macroscopic examination revealed no abnormalities – the consistency of the stool was mushy in 91,2% of cases, the color was yellow (80,3% of children), less often light yellow or yellowish-brown.

During chemical examination, blood in the stool was not detected in any case.

The most characteristic changes in the coprogram of children with functional gastrointestinal disorders were:

1. the presence of small amounts of leukocytes in the stool in 110 children (83,9±3,2%).
2. the presence of mucus in the stool in 109 (83,2±3,3%) of the examined children.
3. the presence of neutral fat in 55 (42,0±4,3%) children, while it is not normally detected in healthy children.
4. presence of intracellular starch in the feces in 32 (24,5±3,8%) cases.
5. presence of plant-based fiber: partially digested - in 22 children (16,8±3,3%), digested - in 10 children (7,7±2,3%). Undigested plant-based fiber was found in 49 cases (37,5±4,2%), it may be present in the feces of healthy children.
6. a small amount of muscle fibers (+) - in 6 examined children (4,6±1,8%).

Isolated cases of detection of connective tissue fibers in the feces were observed, which may be the result of impaired gastric and intestinal digestion and impaired pancreatic secretion.

Elastic fibers or erythrocytes were not found in any examined child with functional gastrointestinal disorders.

The findings of coprological examination in children with FGIR are presented in fig. 4:

Thus, based on the analysis of clinical characteristics of the course of functional gastrointestinal disorders in young children, the following conclusions can be drawn.
- in the examined children, the leading clinical symptoms were decreased appetite or even refusal to eat, flatulence, constipation and pain syndrome.
- in terms of physical development, deviations were detected in the form of body weight deficiency, low levels of physical development and its disharmony.
- in the examined children with FGIR, changes in laboratory parameters were noted: hypoproteinemia, dysproteinemia, hypocalcemia.
- during instrumental studies, the following changes were detected: flatulence; the presence of leukocytes, mucus, neutral fat, intracellular starch, partially digested and digested plant-based fiber, muscle fibers in the feces.

DISCUSSION

"Functional gastrointestinal disorders are a diverse combination of gastrointestinal symptoms without structural or biochemical abnormalities." A new definition was created and agreed upon in the Rome IV criteria, which considers functional gastrointestinal disorders as disorders of gut-brain interaction [13,14]. FGID are associated with any combination of certain functions: motility, impaired visceral hypersensitivity, altered mucosal and immune functions, altered gut microbiota, and altered central nervous system (CNS) function.

The functional nature of the changes is relative and depends on the level of research used: the absence of changes at the organ, tissue and cellular levels does not exclude the presence of molecular and subcellular changes [15]. Morphological changes can be observed only at the ultrastructural level - when using electron microscopic histochemistry, immunocytochemistry, autoradiography. The approach to diagnosing FGID should include a careful history taking with special emphasis on red flags - signs of potentially dangerous pathological conditions. Currently, there is no unique laboratory marker that confirms the presence of FGID, so clinical examination remains the basis for diagnosis [16].

Given the prevalence of functional digestive disorders among young children, it is recommended that in the presence of typical complaints (flatulence, abdominal pain, appetite disorders, constipation) a thorough examination of children be carried out in order to identify disorders that occur in this pathology (indicators of physical development, protein and mineral metabolism). In the presence of clinical signs of functional gastrointestinal disorders in young children, the principles of "step-up" therapy should be followed in the examination and treatment: invasive examination methods should be used only in case of ineffectiveness of the proposed correction methods.

CONCLUSIONS

1. The most significant risk factors for the development of FGID in young children can be considered to be a certain inconsistency of nutrition with the functional capabilities of the child's organism in the form of artificial feeding (100% of children) and early introduction of complementary feeding (64,8%), which can serve as the basis for developing measures to prevent the development of functional gastrointestinal disorders.

2. It has been established that the clinical manifestation of functional gastrointestinal disorders in both infants and children over 1 year of age is dominated by the phenomena of flatulence (74,2%), abdominal pain syndrome (70,4%), decreased appetite (48,6%) or temporary refusal to eat (36,0%), impaired intestinal motility in the form of functional constipation (34,8%), which are accompanied by impaired physical development (in the form of body weight deficiency (79,4%) and/or low level of physical development and its disharmony). These changes typically occur against the background of impaired digestion of food ingredients and are combined with hypoproteinemia, dysproteinemia, hypocalcemia, characteristic changes in the coprogram (moderate

number of leukocytes (83,9%), the presence of mucus (83,2%), neutral fat (42,0%), starch (24,5%) and plant-based fiber (partially digested – 16,8%, digested – 7,7%).

3. For young children with functional gastrointestinal disorders, physiological values of the level of immunoglobulins, inflammatory cytokines are characteristic, no significant deviations in the level of pituitary and thyroid hormones from the reference values were found.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 17.03.2026
ACCEPTED: 30.06.2026

CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Structural characteristics of the kidney in conditions of experimental hypothermia

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ABSTRACT

Aim: To investigate histological, ultrastructural, and morphometric changes in the wall of arterial vessels, glomerular and peritubular capillaries, and convoluted tubules of the kidney under the influence of a cold factor in an experiment.

Materials and Methods: The experiment was conducted on 80 mature white Wistar male rats, 10 of which served as controls. Animals were divided into 7 groups of 10 animals each according to experimental time points: at the peak of cooling and at 1, 3, 7, 14, 21, and 30 days of the recovery period. All procedures with animals were performed according to bioethical standards. Experimental-morphological analysis was conducted using histological, electron microscopic, and morphometric research methods.

Results: It was established that hypothermia causes vasoconstriction of the kidney's arterial vessels. From day 1 of the post-hypothermic period, edematous changes develop, which increase maximally by day 3 and persist through day 7 of the experiment. Starting from day 14, edematous changes decrease and structural organization of kidney parenchyma is restored. Reparative processes are completed by day 30.

Conclusion: Profound renal hypothermia causes a complex process: early vascular spasm leading to ischemia, subsequent inflammatory enhancement of damage with maximum effect at days 3-7, followed predominantly by reversible recovery with isolated foci of irreversible remodeling.

KEYWORDS: kidney parenchyma, nephron, intrarenal arteries, podocytes, endotheliocytes.

Wiad Lek. 2026;79(7):1625-1632. doi: 10.36740/WLek/225986 DOI

INTRODUCTION

The effect of extreme temperatures on the human body is an important problem in modern medicine, with special attention deserved to the effects of low temperatures [1,2]. In a World Health Organization report, it was noted that under conditions of war in Ukraine, destruction of infrastructure, population displacement, and limited access to heating significantly increase the risk of low temperature exposure affecting the health of the population: extreme cold in such conditions can lead to increased morbidity [3]. Furthermore, military personnel during combat operations remain in low-temperature conditions for extended periods, which increases the risk of frostbite and cold injuries, especially during prolonged outdoor exposure.

Special attention should be given to the effects of low temperatures on organs of the urinary system, particularly the kidneys, which play a key role in maintaining homeostasis. The vascular network and kidney parenchyma are highly sensitive to changes in temperature regime, as even brief cold exposure can cause disruption of microcirculation, vascular spasm, ischemic changes, and development of dystrophic and inflammatory processes in nephrons.

AIM

To investigate histological, ultrastructural, and morphometric changes in the wall of arterial vessels, glomerular and peritubular capillaries, and convoluted tubules of the kidney under the influence of a cold factor in an experiment.

MATERIALS AND METHODS

The experiment was conducted on 80 sexually mature white Wistar male rats weighing 170-190 g, 10 of which served as controls. Animals were divided into 7 groups

Table 1. Lumen of arterial vessels at various levels following cold factor exposure (μm)

Day	Interlobar arteries	Arcuate arteries	Interlobular arteries	Afferent arterioles
Control	116.26 ± 14.39	32.91 ± 0.56	11.66 ± 1.21	6.95 ± 0.24
Cold	69.32 ± 7.91*	23.01 ± 0.95*	7.22 ± 0.71*	4.25 ± 0.56*
1	75.88 ± 8.71*	24.54 ± 0.81*	7.23 ± 0.52*	4.85 ± 0.84*
3	79.14 ± 7.31*	26.55 ± 1.31*	7.44 ± 0.44*	4.83 ± 0.55*
7	95.52 ± 10.05	27.96 ± 1.35*	7.54 ± 0.51*	4.76 ± 0.67*
14	98.37 ± 16.81	28.41 ± 1.87*	7.89 ± 0.61*	5.66 ± 0.75*
21	133.96 ± 11.73	32.43 ± 1.27	10.43 ± 1.31	5.66 ± 0.75
30	125.26 ± 10.43	32.49 ± 1.32	11.36 ± 0.75	6.23 ± 0.95

*Note: * indicates statistically significant difference from control animals; for each experimental time point n = 10; data presented as Mean ± SD.*

Table 2. Area of the middle coat of arterial vessels at various levels following cold factor exposure (μm^2)

Day	Interlobar arteries	Arcuate arteries	Interlobular arteries	Afferent arterioles
Control	10432.9 ± 2122.7	2194.8 ± 459.9	625.6 ± 16.5	144.3 ± 16.5
Cold	15955.3 ± 1234.2*	4365.6 ± 341.3*	930.6 ± 17.1*	146.6 ± 22.4*
1	20544.6 ± 2123.4*	8990.1 ± 650.6*	890.3 ± 12.3*	216.9 ± 12.3*
3	20822.6 ± 2115.6*	8069.4 ± 812.5*	805.5 ± 15.1*	340.3 ± 19.1*
7	14966.3 ± 1928.1	6120.9 ± 840.9*	791.9 ± 13.2*	346.5 ± 19.1*
14	14985.8 ± 2671.4	3965.7 ± 251.7*	725.3 ± 19.9*	256.2 ± 14.3*
21	10740.1 ± 1280.1	2450.7 ± 370.8	635.6 ± 25.6	185.2 ± 19.6
30	10856.3 ± 1130.7	2196.3 ± 265.5	603.1 ± 25.3	115.2 ± 12.3

*Note: * indicates statistically significant difference from control animals; for each experimental time point n = 10; data presented as Mean ± SD.*

of 10 animals each according to experimental time points: at the peak of cooling and at 1, 3, 7, 14, 21, and 30 days of the recovery period.

Modeling of deep hypothermia in animals with achievement of rectal body temperature of +12°C was performed as follows. Animals were placed in a cold chamber that did not restrict their movement or breathing. The temperature in the chamber was gradually lowered to -30°C to -33°C. Continuous observation of animal behavior was maintained. Initially, the behavior was calm; after a short time, excitement appeared followed by increased defecation and urination. Subsequently, inhibitory processes began, which ended with a sharp decrease in motor activity. At this time, rectal body temperature was measured in the animals, and upon reaching +12°C, the experiment was concluded. Animal care, all procedures with them, and euthanasia were performed in accordance with general bioethical requirements and rules for animal experimentation.

Material sampling for light optical and electron microscopic examination, block preparation, section fabrication, and their staining were conducted using generally accepted methods [4]. To objectively characterize the damaging effects on components of the glomerular filter and tubular reabsorption, histometry of their constituents was performed [5] with modification [6].

RESULTS

REMODELING OF ARTERIAL VESSELS OF THE KIDNEY AT VARIOUS LEVELS UNDER THE INFLUENCE OF THE COLD FACTOR AND AT VARIOUS TIMES OF THE POST-HYPOTHERMIC PERIOD

Morphometric data indicating the state of vasoconstriction or vasodilation of arterial vessels are presented in Table 1, and edematous changes are presented in Table 2.

At the peak of hypothermia, vasoconstriction of arterial vessels of various caliber occurs (Table 1). The internal elastic membrane becomes unevenly undulating; sometimes its folds deeply penetrate the muscular layer. In the lumen of individual arcuate arteries, exfoliated endotheliocytes are observed. The most pronounced morphological changes in the structural components of arterial vessel walls occur on day 3 of the post-hypothermic period. The vessel lumen somewhat increases and becomes filled with formed blood elements. Edematous changes increase significantly, resulting in an increased area of the muscular coat (Table 2). In arcuate arteries, the internal elastic membrane is locally destroyed and sometimes significantly penetrates the muscular coat, whose area also increases. In the lumen of interlobular

arteries, significant accumulation of erythrocyte masses is observed. Myocytes have vacuolated sarcoplasm and deformed nuclei. The lumen of afferent arterioles has an irregular oval shape and is also filled with erythrocytes. In the perivascular space, numerous formed blood elements are observed.

Morphometric parameters on day 7 of the post-hypothermic period differ little from those of previous time points. Edematous-dystrophic changes in arterial vessels were still present to a significant degree. Starting from day 14 after cold exposure, reparative processes increase at more rapid rates. Numerical values on day 21 of the post-hypothermic period occupy an intermediate position between days 14 and 30. Reparative processes are largely completed by day 30 of the experiment.

Changes in kidney parenchyma following cold factor exposure and at various times of the recovery period. It was established that immediately after cold factor exposure, kidney corpuscles (glomeruli) have normal shape, increase slightly in size, and loops of glomerular capillaries are somewhat constricted. Edematous-dystrophic changes develop in the kidney parenchyma, and the glomerular capsule dilates. Accumulation of formed blood elements is noted. In convoluted tubules, granular dystrophy is observed.

On day 3 of the post-hypothermic period, there is significant deepening of the above-described phenomena. The volume of kidney corpuscles increases. Concurrently, reduced kidney corpuscles are also observed. In the cortical substance, massive accumulation of formed blood elements sometimes occurs, indicating hemorrhage into the kidney parenchyma. Convoluted tubules of nephrons are locally dilated. In nephrocytes of convoluted tubules, there is pronounced cloudy swelling. In the medullary substance, dilated venous vessels are present, which are significantly engorged with blood.

By day 7 of the post-hypothermic period, the above-described changes persist at the same level. Significant granular dystrophy of nephrocytes in convoluted tubules is preserved; in certain locations they are dilated. In the medullary substance, dilated portions of nephron loops and collecting ducts are revealed.

From day 14 onward, significant reduction of edematous processes is observed. In some cases, adhesive processes between the layers of the glomerular capsule occur, with necrotic processes in kidney corpuscles accompanied by significant deformation of the vascular glomerulus. The lumen of convoluted tubules of nephrons is dilated with layers of exfoliated epithelium. In the medullary substance, dilated portions of nephron loops are filled with exfoliated epithelium.

By day 21 of the study, the processes become even more pronounced. Kidney corpuscles decrease in size. In the lumen of convoluted tubules of nephrons, exfoliated cells are encountered. In the cytoplasm of nephrocytes, granular and vacuolar dystrophy are observed.

On day 30 of the experiment, reparative processes in the kidneys are largely completed. The volume of kidney corpuscles returns almost to normal. Convoluted tubules of nephrons locally have dilated lumens. In certain areas, connective tissue proliferation is found at the site of damaged kidney parenchyma, and vascular glomerulus wrinkling is observed.

ULTRASTRUCTURE OF THE FILTRATION BARRIER, GLOMERULAR CAPILLARIES, AND KIDNEY PARENCHYMA UNDER COLD FACTOR EXPOSURE

At the peak of hypothermia, the nuclei of endotheliocytes acquire an oval shape, often with invaginations. The cytoplasm contains a small number of oval mitochondria with occasional disrupted cristae and a cleared matrix. In the cytoplasm, large quantities of free ribosomes and polysomes are frequently encountered. The lumen of glomerular capillaries is narrowed due to endotheliocyte edema and filled with erythrocytes. The basal membrane maintains its trilaminar structure. In podocytes, nuclei are rounded or oval with clear outlines; chromatin is distributed in clumps. The cytoplasm contains a large quantity of cisternae and vacuoles. The podocyte processes (foot processes) are dilated and deformed (Fig 1A). In nephrocytes of proximal convoluted tubules, the microvilli of the brush border lose their basal-apical orientation and orderliness. The quantity of lysosomes and vacuoles increases. Mitochondria are elongated and swollen, with damaged cristae in certain areas (Fig 1B, 1C).

By day 1, edematous-dystrophic changes increase and reach their maximum on day 3 of the study (Fig 1D). The cytoplasm of endotheliocytes of glomerular capillaries becomes edematous, significantly narrowing their lumen. The nuclei of cells are also edematous, with irregular outlines; nucleoplasm has a granular cleared matrix. Mitochondria in endotheliocytes are predominantly rounded in shape, with relatively clear outlines and occasional preserved cristae. Individual glomerular capillaries are densely packed with erythrocytes. Their basal membrane at bending sites is significantly thickened; its trilaminar nature is poorly expressed. In podocytes, nuclei are enlarged with invaginations of the karyolemma. Mitochondria have unclear outlines and occasional cristae. The Golgi complex is dilated. The cytoplasm is light with a large quantity of ribosomes, polysomes, vesicles, and vacuoles (Fig 2A). The most

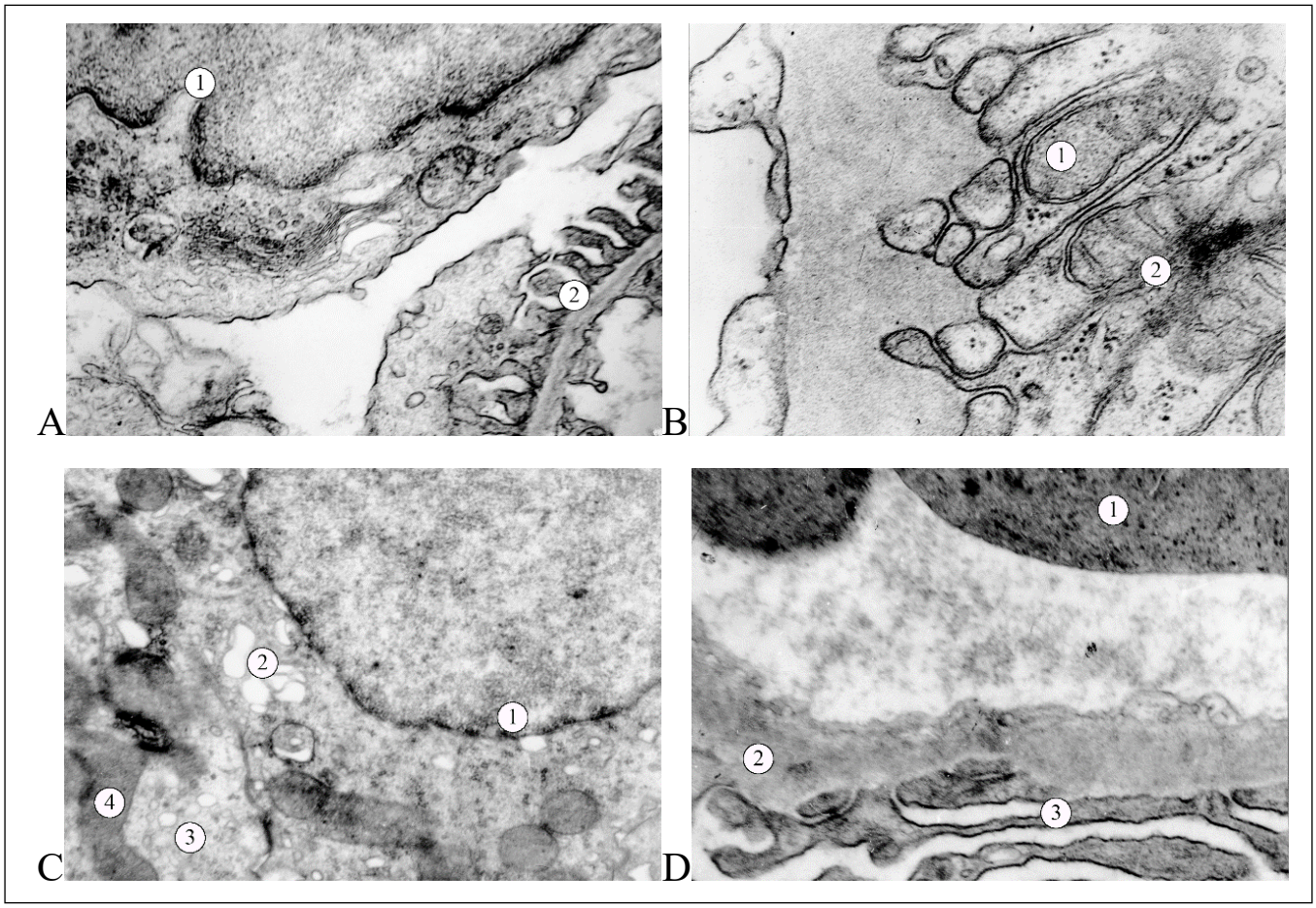

Figure 1. Submicroscopic changes in renal structures at the peak of hypothermia and on day 1 of the post-hypothermic period. A. Podocyte ultrastructure at the peak of hypothermia: (1) nuclear envelope invaginations; (2) podocyte foot process effacement. B. Basal domain of a proximal renal tubular epithelial cell at the peak of hypothermia (1) mitochondria; (2) plasma membrane folds. C. A distal renal tubular epithelial cell at the peak of hypothermia: (1) nucleus; (2) Golgi apparatus; (3) electron-lucent cytoplasm; (4) mitochondrion. D. Glomerular filtration barrier on day 1 of the post-hypothermic period: (1) erythrocyte sludging; (2) basement membrane; (3) podocyte foot processes. Electron micrographs. Magnification: A ×6,000, B ×32,000, C ×24,000, D ×8,000.

pronounced ultrastructural changes are present in nephrocytes of proximal and distal convoluted tubules (Fig 2B, 2C). Dilation and desquamation of microvilli of the brush border is observed. Frequently, there is destruction of the cell membrane with extrusion of organelles into the tubule lumen. The nucleus is swollen with deep invaginations of the karyolemma. The Golgi complex is represented by dilated cisternae. The endoplasmic reticulum appears as dilated, interconnected microtubules. The cytoplasm of nephrocytes is light, with a large quantity of free ribosomes and large round lysosomes. The basal portion of nephrocytes contains swollen, dilated mitochondria. Peritubular capillaries are engorged with formed blood elements. Endothelial cells have swollen nuclei. The cytoplasm is light with the presence of free ribosomes and vacuolization.

On day 7 of the post-hypothermic period, damage to the filtration barrier and nephrocytes of proximal and distal convoluted tubules decreases (Fig 2D). Starting from day 14 of the experiment, reparative processes intensify, and by day 30 of the recovery period, they are largely completed. However, in some cases at the site of damaged podocytes, myelin-like formations are found, which significantly deform the capsular space.

DISCUSSION

The data obtained in this work show that deep hypothermia causes changes not in isolated structures, but in a complex of vascular, glomerular, and tubulointerstitial damage. These results correspond to current understanding of cold-induced kidney injury, which is associated simultaneously with vasoconstriction, hypoxia, mitochondrial dysfunction, inflammatory reaction, and the peculiarities of subsequent organ rewarming [7-9].

Worth noting is the established fact that narrowing of the lumen of arterial vessels at all levels, deepening of the folds of the internal elastic membrane, and increase in the area of the middle coat of arterial walls indicate dominance of a vasoconstrictor response in the early

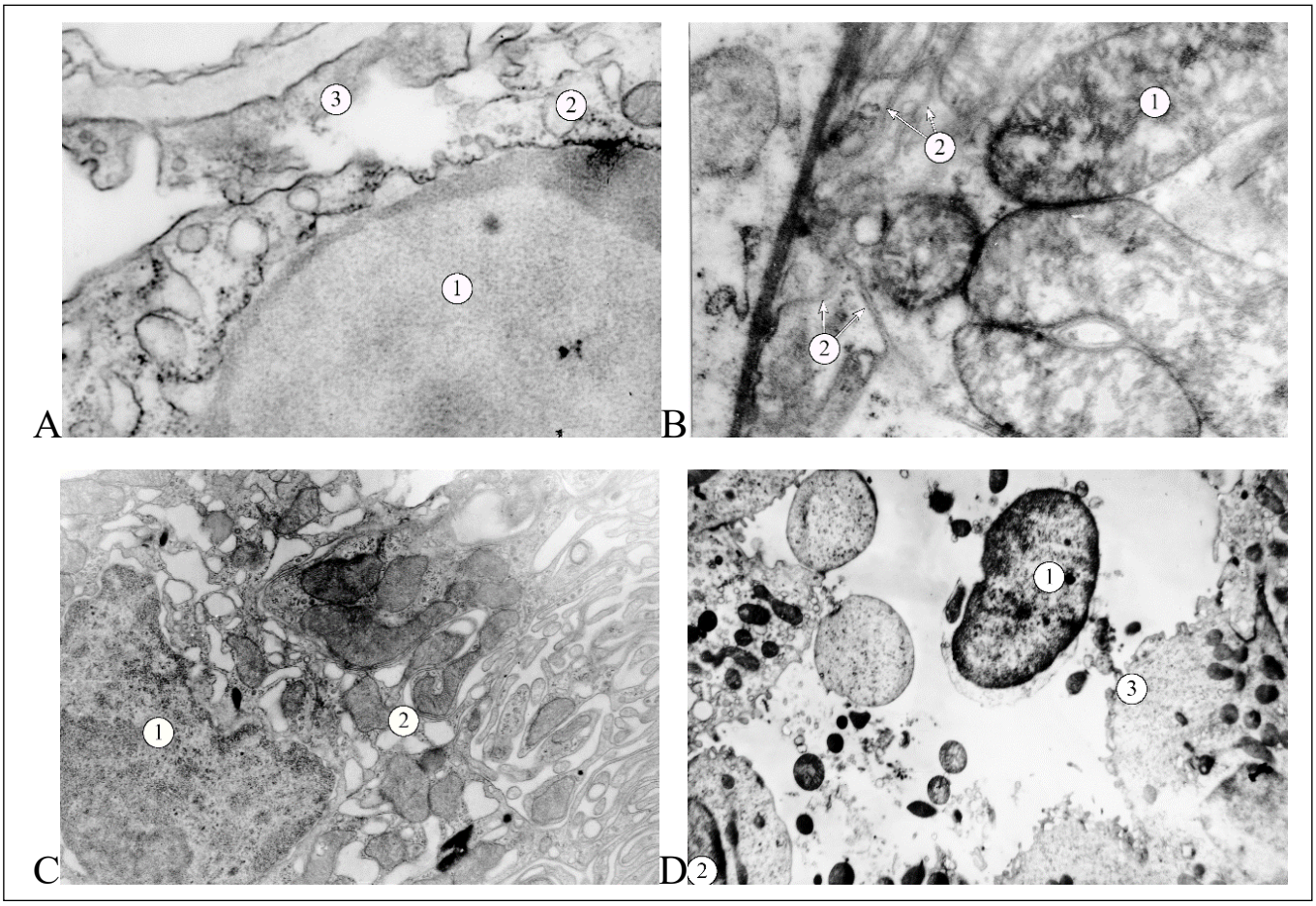

Figure 2. Kidney ultrastructure on days 3 and 7 of the post-hypothermic period. A. A podocyte on day 3 of the post-hypothermic period: (1) nucleus; (2) cytoplasmic vacuoles; (3) blurred plasma membrane boundaries. B. Basal domain of a proximal renal tubular epithelial cell on day 3 of the post-hypothermic period: (1) mitochondria; (2) plasma membrane folds. C. A distal renal tubular epithelial cell on day 3 of the post-hypothermic period: (1) nucleus; (2) cytoplasmic vacuoles; (3) mitochondrion. D. A distal tubule on day 7 of the post-hypothermic period: (1) nucleus of a desquamated epithelial cell in the lumen; (2) distal renal tubular epithelial cell; (3) apical domain of the epithelial cell lining. Electron micrographs. Magnification: A, B ×16,000; C ×5,000; D ×4,000.

phase of cold exposure, which is consistent with current data showing that cold and warm ischemia are among the key factors in subsequent injury to transplanted or ischemized kidney[7,8]. This is explained by the fact that even a cooled kidney requires energy support, particularly in the cortico-medullary zone and in areas with high ATP requirements [8,9].

Importantly, the maximum severity of injury in our study was observed not at the peak of cooling, but predominantly at days 3-7 of the post-hypothermic period, indicating that cold is only an initiating factor, while damaging factors include excessive production of reactive oxygen species, accumulation of succinate, disruption of oxidative phosphorylation, and activation of pro-inflammatory pathways[10-13], which develop after restoration of blood flow and temperature. Therefore, the increase in volume of kidney corpuscles, swelling of vessel walls, desquamation of endothelium, venous congestion, and perivascular hemorrhages in later time periods should be interpreted as manifestations of the reperfusion component of injury [10-12, 14-15].

Particularly revealing are the ultrastructural changes we identified: endotheliocyte edema, capillary lumen narrowing, disruption of the clarity of the trilaminar structure of the basal membrane, and deformation and approximation of podocyte foot processes. These demonstrate that cold factor disrupts not only hemodynamics but also the very architecture of the filtration barrier itself.

Our findings on endothelial desquamation, capillary overfilling with erythrocytes, and perivascular hemorrhages can be considered morphological confirmation of the leading role of endothelial dysfunction in the genesis of cold-induced kidney injury, as indicated by other authors [7,14,16].

The deformation of foot processes and narrowing or dilation of the capsular space we identified indicate that hypothermia can significantly alter the barrier properties of the visceral layer of the capsule. Podo-

cytes are cells with high structural specialization that are extremely sensitive to oxidative stress [11,12,17].

In our study, nephrocytes of the proximal and distal convoluted tubules proved to be the most sensitive to cold exposure. Granular dystrophy, vacuolization, destruction of the brush border, swelling and destruction of mitochondria, and desquamation of cells into the tubule lumen were observed, which fully corresponds to current understanding that tubular epithelium is the primary target of ischemia-reperfusion injury of the kidney [7,10-12,18].

Mitochondria play an important role in cold and ischemic kidney injury. Mitochondrial dysfunction initiates ROS-dependent damage to proteins, lipids, and DNA, activates apoptosis, necrosis, ferroptosis, and pathological or insufficient mitophagy [11-13,19]. Therefore, the swelling and destruction of mitochondria we identified in nephrocytes should be viewed not simply as an associated sign, but as a central link in pathogenesis.

In our work on days 3 and 7, leukocyte infiltrates, perivascular accumulation of blood cells, venous congestion, hemorrhages, and progressive dystrophic changes in parenchyma were observed. Current experimental data show that following cold storage and reperfusion, pro-inflammatory signaling axes are activated in the kidney, which maintain injury even after elimination of the primary ischemic factor [14]. Hypothermic machine perfusion, conversely, can reduce inflammation [20]. This indicates that in our experiment, late morphological changes were due to secondary inflammatory reaction.

At the same time, the results of our work do not contradict the concept of potentially protective role of controlled hypothermia. Current clinical and experimental studies show that properly dosed cooling or hypothermic perfusion can improve early graft function and limit metabolic requirements of the organ [21-24]. Thus, clinical studies confirm the advantages of continuous hypothermic machine perfusion compared to static cold storage [21,22], while optimization of oxygenation and controlled rewarming further reduce the consequences of reperfusion stress [23,24].

It is notable that by day 30, there was almost complete restoration of the structure of kidney corpuscles, vessels, and convoluted tubules. This indicates the significant reparative potential of the kidney following a single hypothermic episode and explains why a significant portion of acute tubular kidney injury following ischemia is reversible [7,11,17,18]. However, the presence in some animals of shrunken glomeruli, adhesions between capsular layers, and connective tissue proliferation indicates incomplete recovery of tubular epithelium. This, combined with persistent mitochondrial dysfunction, inflammation, and activation of pro-fibrotic systems, forms the transition from acute injury to chronic kidney disease [7,12,13,17].

In the clinic, hypothermia is neither harmful nor beneficial; its effect is determined by the balance between metabolic reduction and the risk of vascular-reperfusion injury [9, 21-27]. This is precisely why the structural changes we describe have not only experimental-morphological but also practical significance. They clarify that the most vulnerable links are the arterial wall, vascular endothelium, podocytes, and epithelium of convoluted tubules. Accordingly, promising directions for correction may include agents that affect microcirculation, mitochondrial metabolism, oxidative stress, and inflammatory processes [11-13, 28-29].

Deep hypothermia of the kidney causes a complex process: early vascular spasm and ischemia, subsequent inflammatory enhancement of injury with maximum effect at days 3-7, followed predominantly by reversible recovery with isolated foci of irreversible remodeling. The epithelium of convoluted tubules and components of the glomerular filtration barrier show the greatest vulnerability, which is explained by their high energy dependence and sensitivity to endothelial-mitochondrial dysfunction. From a practical standpoint, this determines the advisability of using controlled hypothermia as an anti-ischemic agent, but only with optimization of cooling duration, adequate oxygenation, protection of microcirculation, and controlled rewarming of the organ [12-13, 20-26, 28-29].

CONCLUSIONS

1. Morphometrically, it was established that deep hypothermia causes vasoconstriction of arterial vessels at all levels, which weakens after one day. The lumen of interlobular arteries is restored by day 7 following cold exposure, but vasoconstriction of arcuate and interlobular arteries and afferent arterioles persists through day 21 of the study.
2. The most pronounced edematous changes are determined from days 3 to 7 of the recovery period, as evidenced by an increase in both the area of the middle coat of arterial vessels and the volume of kidney corpuscles. During this period, focal desquamation of endothelial cells of the intima of arterial vessels, microthromboses, significant dilation of venous vessels, and perivascular leukocyte infiltrates are observed.
3. Reparative processes begin to intensify significantly from day 14 and are completed by day 30 of the recovery period, as indicated by normalization of the lumen of arterial vessels, decrease in the area of

their middle coat and volume of kidney corpuscles, absence of vacuolization of smooth muscle tissue of the vascular wall, and restoration of normal structure of kidney corpuscles.

4. Among the components of the nephron, the most sensitive to the effects of deep hypothermia are epitheliocytes of proximal and distal convoluted tubules, with the most sensitive sites for cold exposure being the microvilli of the brush border, expressed in loss of their proper orientation and orderliness in the basal-apical direction, desquamation of microvilli, and mitochondrial disorganization.
5. By the end of the experiment, kidney corpuscles with signs of persistent damage are frequently encountered, manifested as collapsed glomerular capillaries, dilation of the glomerular capsular space, adhesions between the parietal and visceral layers of the capsule, and connective tissue proliferation at sites of leukocyte infiltrates, indicating the beginning of sclerotic processes. At the same time, enlargement of cell nuclei, invagination of the nuclear envelope, dilation of endoplasmic reticulum microtubules and Golgi cisternae, and thickening of mitochondrial cristae are observed, indicating compensatory hypertrophy.

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The work is a fragment of the research work of the Department of Human Anatomy at Ivano-Frankivsk National Medical University, as part of the project "Possibilities of Radiological and Morphological Methods for Diagnosing Diseases Caused by Endothelial Dysfunction of Multifactorial Origin" (0124U004858).

CONFLICT OF INTEREST

The Author declare no conflict of interest

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RECEIVED: 19.03.2026
ACCEPTED: 29.06.2026

CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Tibiofibular stabilization in patients with ankle and posterior malleolus fractures: A systematic review and meta-analysis

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ABSTRACT

Aim: To compare the functional outcomes and the frequency of use of distal tibiofibular stabilization (TFS) in patients with ankle fractures involving the posterior malleolus (PM).

Materials and Methods: A systematic literature search was conducted in MEDLINE (PubMed), Scopus and the Cochrane Library databases in accordance with the PICOS and PRISMA guidelines. Studies included in the meta-analysis were analyzed using the Cochrane RevMan software. 20 studies were selected for qualitative analysis and 18 for quantitative analysis, and included 2179 patients. Functional outcomes according to the AOFAS scale in patients with ankle fractures and PM fractures treated with TFS were not statistically different from the control group (MD = 1.00; 95% CI [-4.70, 6.70]; p = .073, I^2=87%). A significant decrease in the use of TFS was found in patients after fixation of the PM (OR = 3.77; 95% CI [2.13, 6.69]; p < 0.00001, I^2=78%) and in patients who had PM fixation with a buttress plate (OR = 0.44; 95% CI [0.25, 0.79]; p = 0.006, I^2=0%).

Conclusions: The use of additional TFS after fixation of the PM does not affect the functional outcomes of treatment of patients with ankle fractures. Fixation of the PM fragment significantly reduces the need for TFS. Open repositioning and osteosynthesis of the PM by a buttress plate reduces the need for TFS as compared to screws.

KEYWORDS: ankle fracture, posterior malleolus, distal tibiofibular syndesmosis, osteosynthesis, meta-analysis

Wiad Lek. 2026;79(7):1633-1645. doi: 10.36740/WLek/222249 DOI

INTRODUCTION

Distal tibiofibular syndesmosis (DTFS) injury occurs in 20% of malleoli fractures [1] and is a component of complex ankle joint (AJ) injuries. Over the past decades, a number of studies have demonstrated the involvement of the posterior malleolus (PM) in ensuring congruence, restoring of anatomy and stability of the DTFS, which are important conditions for the successful treatment of ankle fractures [2]. At the same time, there is controversial data regarding the need for additional stabilization of the DTFS after full restoration of the skeletal anatomy of the AJ. Thus, some authors emphasize that fixation of broken malleoli and the PM fragment may be sufficient to ensure proper stability of the DTFS [3,4], while others argue for the need for routine use of additional tibiofibular stabilization (TFS), taking into account the nature of the injury [5,6].

There are controversial studies on the use of TFS for PM fractures. Some authors emphasize the mandatory use of TFS, justifying this by the need to restore the syndesmosis and prevent its instability [7,8]. Others are critical for TFS due to a number of complications: implant instability and associated osteolysis and infection, implant fractures, tibiofibular synostosis, insufficient restoration of syndesmotic anatomy, the need for repeat operation in a case of complication [9,10]. Another part of the authors believe that TFS does not affect the outcome of surgical treatment of malleoli fractures at all [11,12]. At the same time, as noted by Kocan et al, despite the relatively stable level of fixation of the PMF, the frequency of TFS has increased significantly over the past 10 years [13].

Thus, there is a need for a study comparing the necessity and effectiveness of TFS in patiens with malleoli and PM fractures, which was the basis of this systematic review and meta-analysis.

AIM

The aim is to compare the functional outcomes and the frequency of use of distal tibiofibular stabilization (TFS) in patients with ankle fractures involving the posterior malleolus (PM).

MATERIALS AND METHODS

The systematic literature review and meta-analysis were prepared in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [14]. A search for original studies was conducted in the MEDLINE (PubMed), Scopus, and Cochrane Library databases/registers: Cochrane reviews published from the beginning to May 2025. The search strategy was developed for MEDLINE (PubMed) and adapted for other search systems (databases), respectively. Search strategy for PubMed (search formula): ("Ankle Fractures"[MeSH Terms] OR "posterior malleolus*"[All Fields] OR "trimalleolar*"[All Fields] OR "Volkmann's triangle"[All Fields] OR"posterior pilon"[All Fields] OR "posterior ankle"[All Fields]) AND ("Ankle Joint"[MeSH Terms] OR "syndesmos*"[All Fields] OR "tibiofibular syndesmosis"[All Fields] OR "syndesmotic injury"[All Fields] OR "syndesmotic screw"[All Fields] OR "distal tibiofibular joint"[All Fields] OR "distal tibiofibular syndesmosis"[All Fields] OR "positioning screw"[All Fields] OR "syndesmotic fixation"[All Fields]) AND ("Patient Reported Outcome Measures"[MeSH Terms] OR "Treatment Outcome"[MeSH Terms]). We also reviewed the "gray literature", references to bibliographies, reviews, and studies that may have been missed during the systematic search. Relevant articles were added after reading the full text and identifying the necessary parameters. The inclusion criteria for the review were developed in accordance with the PICOS criteria [15] and are listed in Table 1.

Exclusion criteria: 1) isolated PMF, fractures of the ankle without the involvement of the PM and "pylon" type fractures; 2) reviews, abstracts, or articles that did not include sufficient data; 3) biomechanical, anatomical, or computer modeling studies; 4) studies with fewer than 20 patients; 5) articles published in non-English language.

DATA EXTRACTION

Each database was searched separately; the datasets were exported to Mendeley Reference Manager (version 1.12.1; Elsevier) and duplicates were subsequently removed. Two independent experts (T.M.O., O.A.T. – scientific degree of doctor of medical sciences in the specialty of "traumatology and orthopedics", more than 15 years of experience as an orthopedic surgeon) independently checked the search results by title and/or abstract and full text. Any contradictions or discrepancies were resolved by consensus or by the fourth author (A.P.L. – scientific degree of doctor of medical sciences in the specialty of "traumatology and orthopedics", 40 years of experience as an orthopedic surgeon). Each article included in the review was entered into a standardized data extraction table. The data obtained included: author, year of publication, study design, demographic data, classification of ankle injury, classification of PM fractures, size and type of PM fixation, type of TFS, and mean follow-up time.

QUALITY ASSESSMENT OF INCLUDED STUDIES

The risk of bias in the publications included in the analysis was assessed using the tool Newcastle-Ottawa Quality Assessment Scale for Cohort Studies and Case-Control Studies (NOS) [16]. This scale assesses three domains: Selection, Comparability, and Outcome/Exposure. The maximum score is 8 for cohort studies and 9 for case-control studies. Studies with a score of 8 to 9 are considered to be of high quality, those with a score of 7 to 5 are of moderate quality, and those with a score below 4 are of low quality. The methodological quality of publications was assessed using the "funnel plot" tool. The symmetrical distribution of studies assessing the pooled effect indicates a low publication bias.

DATA SYNTHESIS AND ANALYSIS

The publications included in the review were analyzed for comparability and combined into the final database. In case 3 or more studies had sufficient comparability (inclusion criteria, outcome parameters), a meta-analysis was performed. In the case of insufficient comparability but similarity of groups and outcome parameters, a quantitative analysis was performed to illustrate the current trend of results. The meta-analysis was performed using the Cochrane RevMan software (version 5.4.1; The Cochrane Collaboration, 2020). The method of inverse variance was used for continuous data (assessment of functional outcomes - PROMs) based on the calculation of mean differences (MD); to estimate the frequency of use of TFS, a dichotomous analysis model (OR - odds ratio) with 95% confidence intervals (CI) was used. The I^2 method was used to test the heterogeneity of the results of each study. When $I^2 \leq 50\%$, which indicates a small heterogeneity among studies, a meta-analysis with a fixed effects model was used; when $I^2 > 50\%$, which indicates a large heterogeneity among studies, a meta-analysis with a random effects model was used.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

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

Table 1. PICOS Criteria

Participants	Patients (>18 years) with malleoli and posterior malleolus fractures (OTA/AO types 44 - B and 44 - C)
Intervention	Using of distal tibiofibular stabilization (positional screw(s), elastic systems)
Comparison	The absence of distal tibiofibular stabilization
Outcomes	Patient reported outcome measurements (PROMs); frequency of TFS
Study design	Original articles, cohort studies (retrospective and prospective)

Source: compiled by the authors of this study

Table 2. Characteristic data of the study

Author, year	Countries	Design	Total subjects	Male/ Female	Age,years- mean (range)
Neumann AP, Rammelt S, 2022 [29]	Germany	Retrospective	100	31\69	60 (20-83)
*Saygılı et al., 2017 [17]	Turkey	Retrospective cohort	73	34\39	(18-75)
*Erdem et al., 2014 [18]	Turkey	Prospective case series	40	20\20	(24-77)
Behery et al.,2021 [24]	USA	Retrospective	85	32\53	51
Li et al.,2017 [34]	USA	Retrospective rewiev	97	42\55	48.0 ± 15.47
Tosun et al.,2018 [33]	Turkey	Retrospective rewiev	49	21\28	47 (20-82)
*Bingol et al.,2023 [19]	Turkey	Retrospective	55	29\26	(18-83)
Marques Ribeiro et al., 2021 [4]	Portugal	Retrospective comparative study	64	20\44	58.6 ± 17.8 (23–75)
Aravindan et al.,2023 [5]	USA	Retrospective review	79	30\49	44 (IQR: 28–56)
Mertens et al., 2020 [32]	Belgium	Coparative prospective cohort vs retrospective cohort	135	60\75	(21–84)
Baumbach et al., 2019 [25]	Germany	Retrospective registry study	236	99\137	53.0 ± 18.3 (18-100)
*Miller et al., 2018 [20]	USA	Retrospective comparative series	198	69\129	(18-92)
Karaismailoglu et al., 2023 [30]	Turkey	Prospective randomized controlled study	40	20\20	(18-65)
Stringfellow et al., 2021 [31]	UK	Retrospective	160	57\103	50.2 (19–92)
Pilskog et al.,2020 [26]	Norway	Retrospective comparative study	86	23\63	57 (IQR 41-67)
*O'Connor et al.,2015 [21]	USA	Retrospective case series	27	11\16	(21.1-67.1)
*Kalem et al.,2018 [22]	Turkey	Retrospective-multicenter comparative	67	27\40	(40.8-48.3)
Miller et al.,2010 [9]	USA	Prospective	31	11\20	48 (21–83)
*Cho et al., 2025 [23]	Republic of Korea	Retrospective	472	182\290	45.8 ± 14.1 (18–70)
Liu et al., 2020 [35]	China	Retrospective cohort study	85	38\47	(42.92±12.26)
Total 20			2179	856\1323	

*Notes: * - calculated using data from original article*
Source: compiled by the authors based on [4, 5, 9, 17-26, 29-35]

REVIEW AND DISCUSSION

The process of selecting studies in accordance with PRISMA recommendations is presented in Fig. 1. The results of the systematic review and the characteristics of each study are summarized in Table 2 and Table 3.

20 studies met the criteria and were included for qualitative and 18 for quantitative analysis with 2179 patients. The studies were published between 2010 and 2025. Fractures of the PM were presented as a component of trimalleolar or equivalent ankle injuries

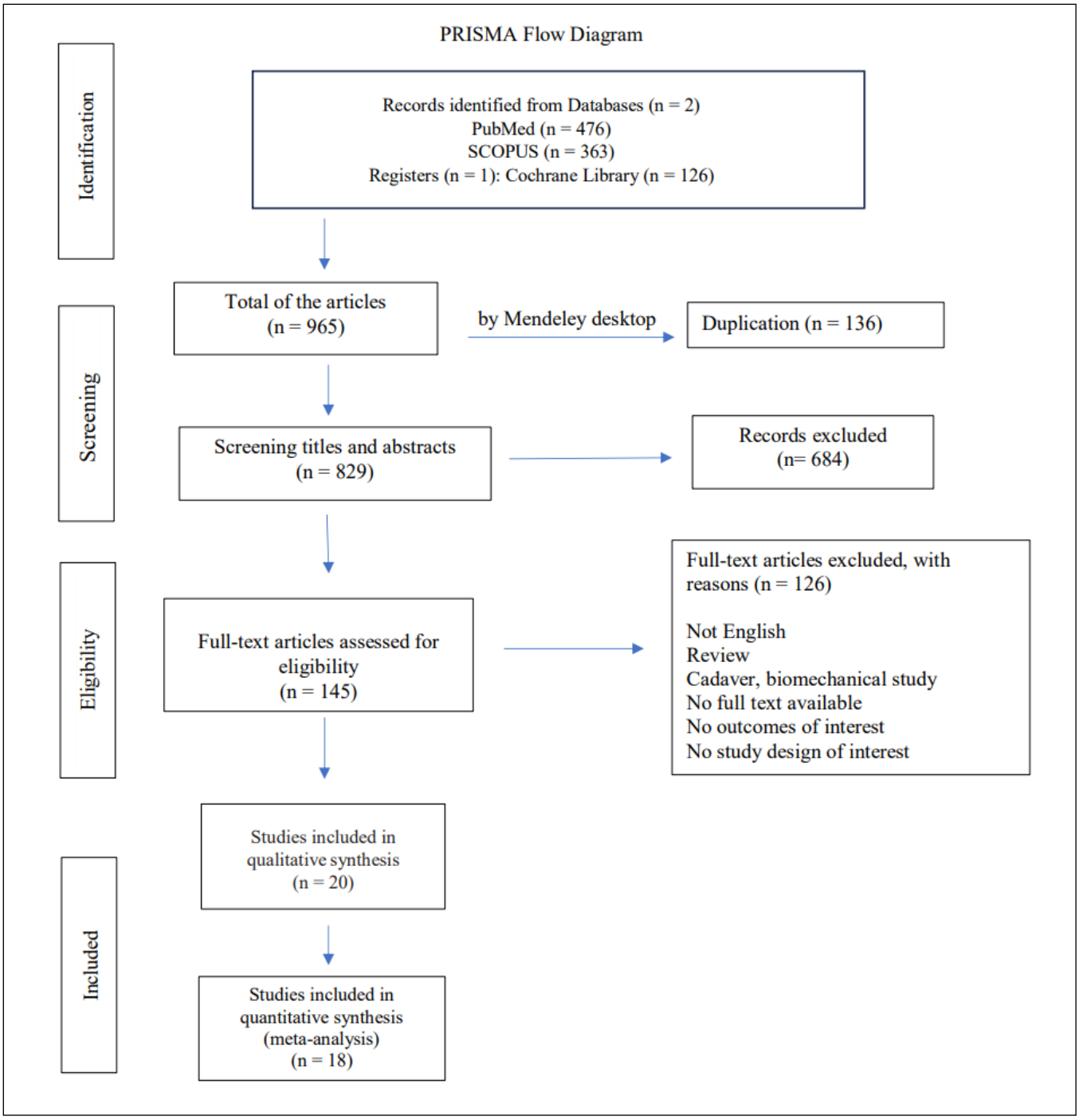

Fig. 1. The PRISMA flow diagram
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(single malleolus and bimalleolar fractures involving the posterior malleolus). Retrospective study design was performed in 16 studies, prospective in 3 studies (1 case series; 2 randomized controlled studies) and 1 comparative prospective-retrospective cohort study. Quantitative indicators in 7 studies [17-23] were obtained by self-reported calculations, as they provided data for each patient or group separately.

The most frequent method of intraoperative determination of DTFS instability in the analyzed publications was the external rotation stress test and hook test or their combination. TFS was performed using one or two positioning screws tri- and quadricortically in 12 publications, in 4 studies [23-26] TFS was performed using positioning screws or elastic systems (suture button, tight rope, internal brace) in 3 studies [5,20,21] the specific method of TFS was not specified. Miller et al. [9] performed TFS using two blocked quadricortical positioning screws through a 1\3 tubular plate to prevent excessive compression of the DTFS.

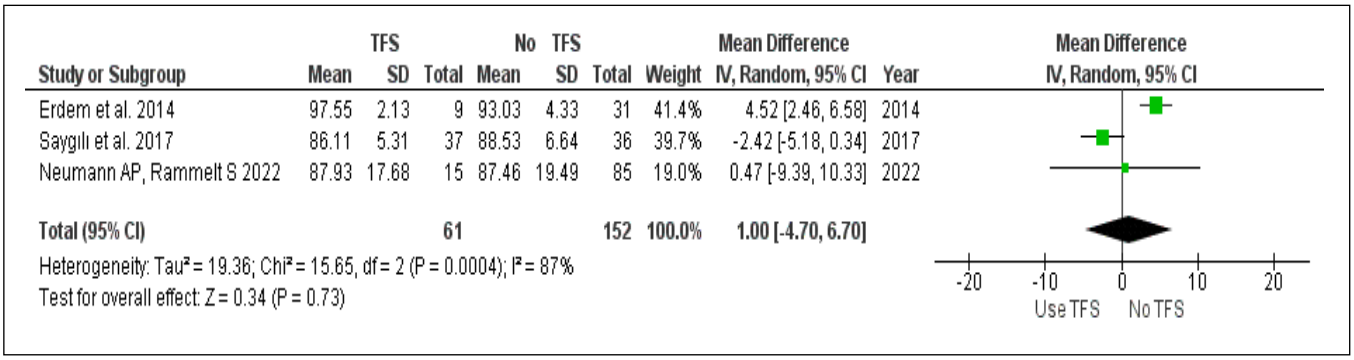

Fig. 2. Forest plot showing AOFAS scores for malleoli and PM fractures treated with and without TFS
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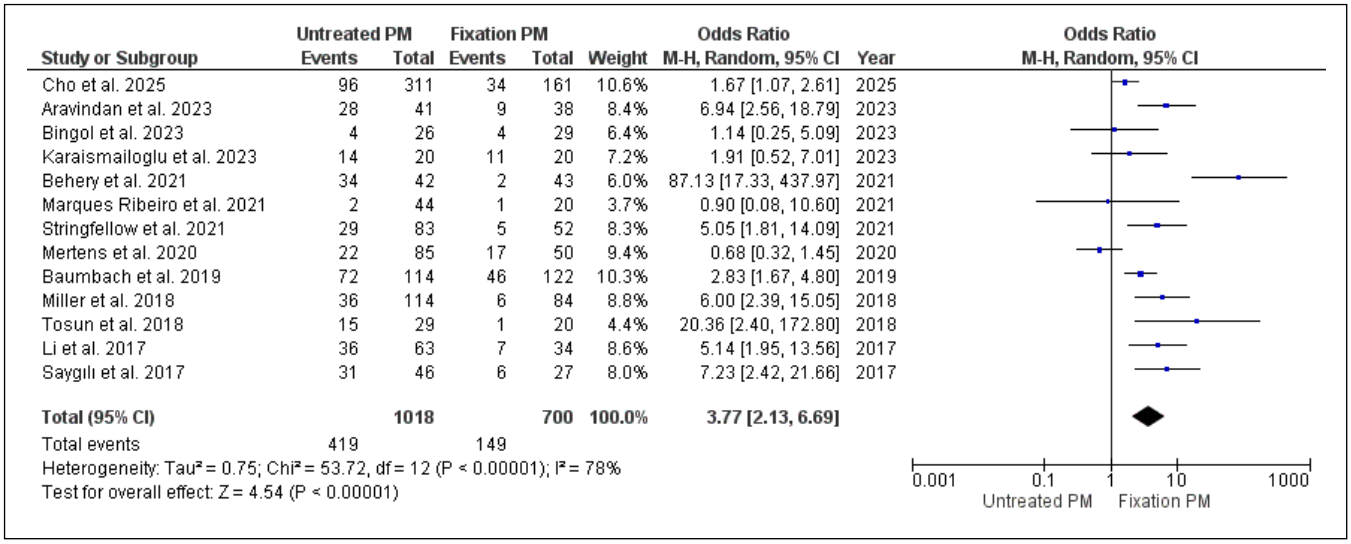

Fig. 3. Forest plot comparing the frequency of TFS use after osteosynthesis versus untreated PM fractures
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In order to determine the morphology of the PM fractures, the Bartoníček-Rammelt [27] and Haraguchi [28] classifications were used in the publications found. Morphologic verification of the PM fractures was used only in 9 studies found (47%), while the size of the fractured PM was reported in 14 studies (74%). In all these studies, osteosynthesis of the PM was performed by 3 methods (posterior-to-anterior (PA) screws; anterior-to-posterior (AP) screws, buttress plate), and the restoration of the lateral and medial malleolus was performed in accordance with the principles of the Arbeitsgemeinschaft für Osteosynthesefragen (AO).

FUNCTIONAL RESULTS

Three studies [17,18,29] included in the systematic review reported data assessing functional outcomes of patient-reported outcome measures (PROMs). The AOFAS scale, in comparison with other PROMs, was the only objective measure of quality of life that was defined in the same way in all studies, which was used for the cumulative analysis. A meta-analysis was performed in terms of functional outcomes in patients who received TFS after malleoli and PM osteosynthesis and patients who did not receive TFS. The results of the meta-analysis showed no statistical difference between the two groups (MD = 1.00; 95% CI [-4.70, 6.70]; p = .073). A high level of heterogeneity was found in this comparison (I^2 = 87%, p = 0.0004) (Fig. 2).

PMF FIXATION VS UNTREATED

13 studies [4,5,17,19,20,23–25,30–34] included in the analysis compared the frequency of TFS use in patients who underwent PM fixation and patients who had their PM injuries treated conservatively. In a quantitative analysis, the results of 1018 patients treated conservatively were compared with 700 patients who underwent PM osteosynthesis. The results of the meta-analysis demonstrate that fixation of the PM significantly reduces the need for additional use of TFS (OR = 3.77; 95% CI [2.13, 6.69]; p < 0.00001). The

Table 3. Publication outcome measurement

Author, year	Syndesmosis test	TFS	Size of PM,%	PM classification	Type of PM fixation	Danis–Weber \AO classification	Lauge–Hansen classification	Time of follow up (months)
Neumann AP, Rammelt S, 2022 [29]	hook test	syndesmotic screw	N\R	Bartoníček-Rammelt classification Type 1 - 7%, Type 2 - 35%, Type 3- 35%, Type 4- 23%	No fixation - 37%, AP screw- 14%, PA screw- 13%, Plate - 36%	Weber B- 76%; C - 24%	SE 3 type - 4%, SE 4 type - 26%, PA 3 type - 46%, PE 4 type - 24%	83,6 (33-171)
*Saygılı et al., 2017 [17]	fluoroscopic external rotation stress test	syndesmotic screw	PMF no fix: 19.8% ± 4.54% (10-25) PMF fix: : 20% ± 4.66% (15-25)	Haraguchi classification Type 1 -57 %; Type 2 - 38 %; Type 3 - 5%	No fixation - 46 (63%) Posterior plate - 17 (63%) Percutaneous screw - 10 (34%)	44B3 - 70 %; 44C1 - 20.5%; 44C2 -9.5 %.	N\A	< 24
*Erdem et al., 2014 [18]	fluoroscopy control	syndesmotic screw	>20%	Haraguchi classification Type 1 -70%; Type 2 - 25 %; Type 3 - 5%	Reconstruction locking plates with either 4 or 5 screw holes - 20 (50%) 1 or 2 or 3 screws -20 (50%)	44B3 - 90 %; 44C 1.3 - 5%; 44C 2.3 -5%.	N\A	38,2 (24-51)
Behery et al., 2021 [24]	external rotation stress test under fluoroscopy	screws - 94% suture button - 6%	PMF no fix: 19% ± 8% PMF fix: : 27% ± 9%	N\R	T or 1/3 tubular plates -39 (91%) AP screws - 4 (9%)	N\R	N\R	N\R
Li et al.,2017 [34]	external rotation stress test under fluoroscopy	2 trans-syndesmotic screws	27% - AP screw group 27.67% - plate group	N\R	AP screw -15 Posterolateral plating - 19	N\A	SER 97 (67.8) PER 35 (24.5) PAB 6 (4.2) SAD 2 (1.4) Not classifiable or previous fracture 3 (2.0)	5.47 ± 3.87
Tosun et al., 2018 [33]	lateral translation stress test and external rotation stress	3.5 mm cortical or 4.5 mm malleolar screw tricortically	PMF no fix : 21.3+7.5 (6-32) PMF fix : 28.9+ 10.5 (12-45)	N\R	Screw -9 Plate - 11	N\A	44-B/SER - 67.3% 44-C/PER - 32.7%	15 (12-51)
*Bingol et al., 2023 [19]	hook test	syndesmotic screws	N\R	Haraguchi classification Type 1 -34,6%; Type 2 - 23,6 %; Type 3 - 41,8%	Posterior buttress plating AP one or two 4.0-mm cannulated screws	N\R	N\R	12-42
Marques Ribeiro et al., 2021 [4]	manual external rotation test	trans-syndesmotic screw	14.7 ± 5.3% (5–24)	N\R	70% plate and screws 25% AP screw 5% PA screw.	44-B - 87% (B3.2: 39%; B3.3: 48%) 44-C - 13% (C1: 2%; C2: 8% C3: 3%).	N\A	43.1 ± 22.2 (24–96)

Table 3. Cont.

Aravindan et al., 2023 [5]	external rotation stress test	N\R	PMF no fix: 16.50% (25.6–32.1%) PMF fix: : 28.90% (25.6–32.1%)	Haraguchi classification Type 1 -50,6%; Type 2 - 31,6 %; Type 3 - 17,8%	48.1% ORIF 48.1% no fixation 3.8% percutaneous screw fxation	N\R	N\R	> 3
Mertens et al., 2020 [32]	hook test	syndesmotic screw	N\R	Bartoníček-Rammelt classification Type 1 - 2, Type 2 - 17, Type 3- 13, Type 4- 14	No fixation - 76 2.7 mm plate -50 AP screw -9	N\R	N\R	> 12
Baumbach et al., 2019 [25]	hook test or external rotation test	trans-syndesmotic screw (56.8%) tight rope (36.4%) internal brace (6.8%)	21.4 ± 10.4% (2.7-55.9%)	Haraguchi classification Type 1 -47,9%; Type 2 - 33,9 %; Type 3 - 18,2%	No fixation - 48,3% CRIF using AP screws - 18.6% ORIF using a plate or screw(s) - 33.1%	1.7% - 44-A (A2: 0.4%; A3: 1.3%) 77.5% - 44-B (B1: 0.8%; B2: 0.4%; B3.1: 20.3%; B3.2: 19.5%; B3.3: 36.4%) 11.9% - 44-C fracture (C1: 1.3%; C2: 6.8% C3: 3.8%) 8.9% - not classified	N\A	N\R
*Miller et al., 2018 [20]	hook test	N\R	PMF fix : 22.2 ± 10.2 PMF no fix : 15.2 ± 7.4	N\R	Plate fixation - 48 Other - 36	Weber A - 1 B -159 C - 36	Maissoneuve - 2 ISO - 2 PER - 22 PAB - 13 SER - 159	N\R
Karaismailoglu et al., 2023 [30]	external rotation stress or hook test	one or two trans-syndesmotic screws	25.8% ± 5.9% X-ray 19.1% ± 5.2% CT	Haraguchi classification Type 1 -60%; Type 2 - 40%	buttress plate or screws	Weber B - 72,5% C -27,5%	SER - 72,5% PER - 27,5%	>24
Stringfellow et al., 2021 [31]	N\R	cortical screws	17.8% X-ray 24.5% CT	N\R	42 (80.7%) plate fixation 4 cortical and 6 cancellous screw	N\R	N\R	N\R
Pilskog et al., 2020 [26]	cotton test or external rotation test	1 quadricortical screw 2 tricortical 3.5-mm screws or a suture button	17% (12%-24%)	N\R	3.5-mm screws with or without a one-third tubular plate AP partially threaded, 3.5-mm cancellous screws	Weber B - 30 C - 56	SER - 55 PER - 31	26 (IQR 19-35)
*O'Connor et al., 2015 [21]	N\R	N\R	12.8-41.2%	N\R	AP 1 or 2 percutaneous 3.5-mm lag screws – 41% T plate or 1/3 tubular plate – 59%	N\R	N\R	Plate - 54.9 (19.5–99.5) AP screws - 32 (14.9–48.8)

Table 3. Cont.

*Kalem et al., 2018 [22]	N\R	one screw	<10% - 25 10-25% - 31 >25% - 11	N\R	20 AP\ 13 PA - one or two partially threaded 4.5 mm canullated screws 35 plate (3.5 mm. 1/3 tubular plate)	N\A	SER - 32.8% PER - 11.9% PA - 47.8% SA - 7.5%	12-24
Miller et al.,2010 [9]	placing a narrow Hohmann retractor in the interosseous space to apply an external rotation stressor	two locked quadricortical syndesmotic screws through a separate three-hole one-third tubular plate	N\R	N\R	six-hole 2.0 or 2.4 reconstruction or T-plate	N\A	SER 4 - 80% PER 4 - 10% Unclassifiable - 10%	15 (12–31)
*Cho et al., 2025 [23]	external rotation or hook test	trans-syndesmotic screw or suture button	N\A	Haraguchi classification Type 1 -50.2 %; Type 2 - 42.2 %; Type 3 - 7.6%	one or two 3.5-mm screws -134 (83,2%) or a posterior buttress plate - 27 (16,8%)	Weber B -335 C - 137	PER - 132 PAB - 19 SER - 321	51 (36–120)
Liu et al., 2020 [35]	cotton test	tricortical screw	≥25%	N\R	Plate fixation – 47 Screws - 38	N\A	SER 4 – 100%	(13.11±1.12) months

Notes : * - calculated using data from original article; N\A - not applicable; N\R- not reported.
PM – posterior malleolus; TFS – distal tibiofibular stabilization; SAD - Supination – Adduction; SER - Supination - External Rotation; PER - Pronation - External Rotation; PAB - Pronation – Abduction; PA – posterior-to-anterior screws; AP – anterior-to-posterior screws ; ORIF – open reduction and internal fixation; CRIF – closed reduction and internal fixation.
Source: compiled by the authors based on [4, 5, 9, 17-26, 29-35]

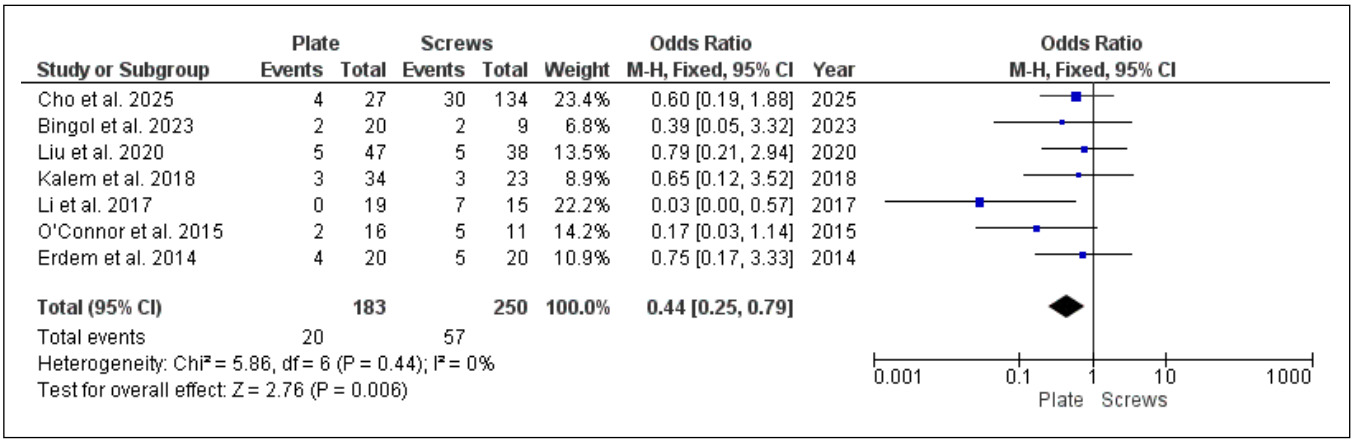

Fig. 4. Forest plot showing the frequency of TFS application after PM fixation with a buttress plate compared to screws
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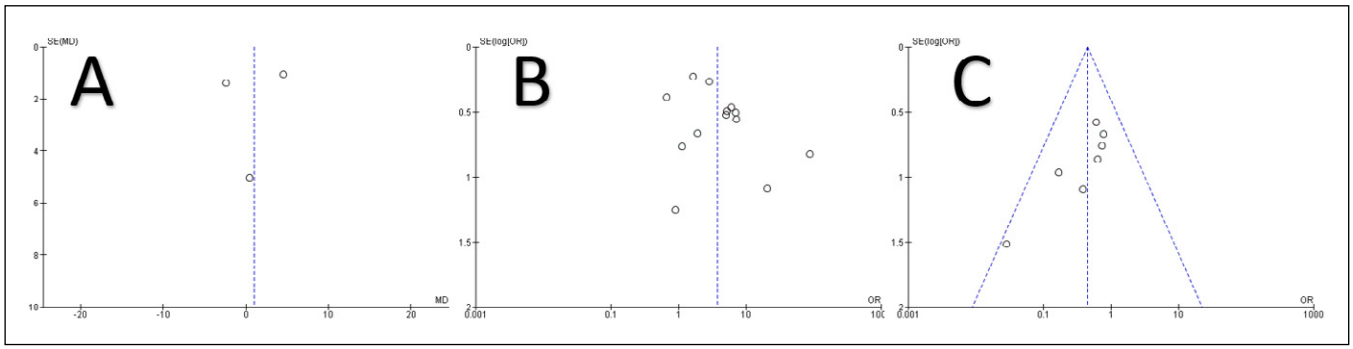

Fig. 5. Funnel plots showing: A — comparison of AOFAS scores with versus without TFS use; B — frequency of TFS use after osteosynthesis versus untreated PM fractures; C — frequency of TFS use after osteosynthesis of the PM with a buttress plate versus screws
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analysis revealed a high level of heterogeneity (I^2=78%; p<.00001) (Fig. 3).

PLATE VS SCREWS

In 7 studies [18,19,21–23,34,35], data are presented that demonstrate the frequency of TFS use after osteosynthesis of the PM with a buttress plate and after the use of screws in the anterior-to-posterior (AP) and posterior-to-anterior (PA) directions. The results of the quantitative analysis demonstrate that fixation of the PM with a buttess plate significantly reduces the need for additional TFS (OR = 0.44; 95 % CI [0.25, 0.79]; p = 0.006). The model showed a low level of heterogeneity (I^2 = 0%; p = 0.44) (Fig. 4).

PUBLICATION BIAS

The methodological quality of all included studies was assessed using the Newcastle-Ottawa Quality Assessment Scale. 9 studies were classified as having high quality, and 11 studies were classified as having moderate quality (Table 4). The funnel plots show some asymmetry, which may indicate the presence of publication bias (Fig. 5).

In our study, we comprehensively collected the relevant literature and analyzed the clinical efficacy and frequency of TFS use in patients with ankle fractures involving PM. The results of our systematic literature review and meta-analysis demonstrate that restoration of the bone anatomy of the AJ reduces the frequency of TFS use, and its additional use may not affect the functional outcomes of treatment.

From an anatomical point of view, the modern principle of treatment of AJ injuries is "directly fix what is broken and repair what is torn" [36]. The advantages of fixation of the posterior malleolus are to ensure anatomical repair of the incisura fibularis tibiae and the posterior part of the DTFS, which may reduce the need for stabilization of the syndesmosis or be its equivalent[37]. This is probably due to the extensive attachment of the posterior inferior tibiofibular ligament to the posterior malleolus, which provides up to 42% of the stability of the DTFS [38]. Miller et al [20] showed that open reduction and internal fixation (ORIF) of PM fracture restores syndesmosis stability in

Table 4. Risk of Bias Assessment per the Newcastle-Ottawa Quality Assessment Scale

Author,year	Selection				Comparability	Outcome			Total	Quality
	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study		Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts		
Neumann AP, Rammelt S, 2022	1	0	1	1	2	1	1	1	8	High
Saygılı et al., 2017	1	1	1	1	1	1	1	0	7	Moderate
Erdem et al., 2014	1	1	1	1	1	1	1	1	8	High
Behery et al.,2021	1	1	1	1	2	1	1	0	8	High
Li et al.,2017	1	1	1	1	2	1	1	0	8	High
Tosun et al.,2018	1	1	1	1	1	1	1	1	8	High
Bingol et al.,2023	1	1	1	1	1	1	1	0	7	Moderate
Marques Ribeiro et al., 2021	1	1	1	1	2	1	0	1	8	High
Aravindan et al.,2023	1	1	1	1	1	1	0	1	7	Moderate
Mertens et al., 2020	1	1	1	1	1	1	0	1	7	Moderate
Baumbach et al., 2019	1	1	1	1	2	1	1	0	8	High
Miller et al., 2018	1	1	1	1	1	1	1	0	7	Moderate
Karaismailoglu et al., 2023	1	1	1	1	2	1	1	1	9	High
Stringfellow et al., 2021	1	1	1	1	1	1	1	0	7	Moderate
Pilskog et al.,2020	1	1	1	1	1	1	0	1	7	Moderate
O'Connor et al.,2015	1	1	1	1	1	1	1	1	8	High
Kalem et al.,2018	1	1	1	1	1	1	1	1	8	High
Miller et al.,2010	1	1	1	1	1	1	1	0	7	Moderate
Cho et al., 2025	1	1	1	1	1	1	1	0	7	Moderate
Liu et al., 2020	1	0	1	1	2	1	1	1	9	High

*High quality: 8-9 stars, Moderate quality: 7-5 stars, Low quality: < 4 stars

Source: compiled by the authors of this study

more than 97% of cases. Neumann and Rammelt [29] report the use of TFS after osteosynthesis of the PM in only 15% of cases, mainly with pronation - external rotation 4 type injuries, Weber C and Bartonicek-Rammelt type 2 injuries. Saygili et al [17] noted that TFS was necessary in 22% of patients after fixation of PM, while in the group of conservative treatment of PM fractures, TFS was 68%. The authors did not note a correlation between the frequency of TFS and the morphology of the PM. Çağlar et al [3] showed that anatomical repair of the PM provides high stability of the syndesmosis, which is confirmed by the radiological parameters of the AJ after 1 year of observation, and does not require additional fixation of the syndesmosis with screws.

On the other hand, other studies emphasize that fixation of the PM may not be sufficient to fully restore the stability of the DTFS. For example, Erdem et al [18] reported that the presence of TFS led to better results on the AOFAS scale regardless of the type of fixation of the PM at 6 and 24 months after surgery and at the final follow-up. The authors attribute this phenomenon to possible undiagnosed intraoperative damage to the DTFS ligaments, which affected clinical outcomes in this cohort of patients. Aravindan et al [5] emphasize that fixation of the PM reduces (23.7% vs. 68.3%) but does not completely eliminate the need for TFS, especially in the case of small PM fragment, where 40% of patients still required TFS after fixation of the PM. Baumbach et al [25] found that TFS was required in 50% of cases, despite a significant reduction in its use after ORIF of the PM fragment. TFS was required significantly less frequently in patients who underwent ORIF of the PM fragment (25%) compared with closed reduction and osteosynthesis (61%) and the group of conservative treatment of PM fracture (63%). The authors emphasize that the size of the fragment of the PM did not have a significant effect on the incidence of TFS in all three groups. J. Bartoníček and S. Rammelt[27] and L.W. Mason[39] recommend TFS as an alternative to fixation of the PM fragment, for a certain type of PM fragment, given the lack of advantages of surgical fixation of avulsive (type 1 - extraincisional) fragments of the PM, and the existing damage to the DTFS.

Although anatomical repositioning and fixation of the PM fragment may sufficiently stabilize the DTFS, residual instability is due to concomitant damage to other structures of the DTFS, including the interosseous ligament and the anterior inferior tibiofibular ligament, which may require additional use of TFS. Intraoperative verification of residual instability of the DTFS after fixation of malleoli fractures should remain a necessary criterion for the use of TFS. However, its routine use, in our opinion, may not have additional benefits, given the potential risks and the need for a second visit to the operating room for removal of hardware in static scenario of stabilizing the DTFS.

One of the results obtained in our study is a decrease in the use of TFS when using a buttress plate. The probable reason that may explain this phenomenon is that ORIF of the PM with the help of a buttress plate not only promotes anatomical restoration of the articular surface of the ankle joint and stable fixation of the PM fragment, but also can provide (compared to AP and PA screws) reapproximation of the posterior inferior tibiofibular ligament, given its wide attachment to the posterior part of the distal tibial plateau, especially in rotationally unstable ankle fractures where damage (delamination) of the ligament can manifest itself as its detachment without visible tearing [40].

LIMITATIONS

This meta-analysis has certain limitations. Firstly, the use of TFS is a common technique in the treatment of Maisonneuve fractures and complex "logsplitter injuries" of the AJ, where restoration of the DTFS is a recommended procedure, interpreted by the nature of the injury, which makes it difficult to assess the need for TFS in this group of patients. Secondly, we did not separately evaluate the difference between TFS in fixation of PM with PA and AP screws. Thirdly, we did not analyze the complication rate in patients treated with TFS, which may be the basis for future studies. Methodological and clinical heterogeneity in the included studies limited the conclusions drawn from this analysis. Thus, our results should be interpreted with caution, as we believe that large and rigorous RCTs are needed to improve the evidence.

CONCLUSIONS

A systematic review of the literature and meta-analysis revealed that fixation of the PM in the treatment of patients with ankle fractures reduces the frequency of TFS use. No effect of additional TFS on functional outcomes was found. Osteosynthesis of PM with a buttress plate reduces the frequency of TFS use compared to AP and PA screws.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 26.06.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

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REVIEW ARTICLE

CONTENTS

Hopes and doubts about zilebesiran, a new antihypertensive drug

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ABSTRACT

The aim of this work is to critically evaluate the benefits and risks of zilebesiran. A comprehensive literature search was conducted in PubMed and Google Scholar databases to identify relevant studies on zilebesiran and other small-interfering RNA (siRNA)-based therapies for hypertension. The search covered publications from January 2010 to December 2025. The following keywords and combinations were used: "zilebesiran", "siRNA therapy", "angiotensinogen inhibition" "hypertension". Hypertension is one of the most prevalent cardiovascular diseases worldwide**.** The effectiveness of the therapy is still unsatisfactory, which is caused, among other things, non-adherence to pharmaceutical treatment. A long-acting drug could reduce this non-adherence to some extent. The mechanism of action of zilebesiran is based on silencing the angiotensinogen gen by activation the RNA-induced silencing complex (RISC). In animal studies and then in patients with hypertension (KARDIA I and KADRIA 2), zilebesiran after a single subcutaneous administration reduced angiotensinogen concentrations in blood by 90% and a systolic blood pressure by 10-20 mm Hg compare to placebo for a period of 3-6 months. In the studies conducted so far, apart from a few cases of orthostatic hypotension and a slight increase in creatinine in the blood, no serious side effects were found. A large study, KARDIA 3, is currently underway to assess the antihypertensive efficacy and reduction of cardiovascular complications. Despite the promising results of previous studies further observation are still necessary regarding long-term hypotensive effectiveness, reduction of risk cardiovascular complication and side effects.

KEYWORDS: zilebesiran, small interfering RNA, low level of angiotensinogen, new drugs on hypertension

Wiad Lek. 2026;79(7):1646-1653. doi: 10.36740/WLek/221451 DOI

INTRODUCTION

EFFECTIVENESS OF TREATMENT AND ADHERENCE TO THERAPY OF ARTERIAL HYPERTENSION

Numerous studies indicate that over 1.3 billion to 1.4 billion people worldwide suffer from hypertension, but less than 16% of them have blood pressure below 140/90 [1-3].

It is estimated that hypertension is a leading factor in cardiovascular disease and death [4]. As the population of developed countries ages, the number of people with hypertension is expected to increase further. This will also be facilitated by increased obesity, air pollution, exposure to noise, and poor diet [3, 4]. The higher the blood pressure, the higher the risk of serious cardiovascular events [4, 5]. Intensive treatment of hypertension with a reduction in blood pressure from 140/81 to 133/76 mm Hg significantly reduces the risk of serious cardiovascular complications by 10%. Each drop in blood pressure by 5 mm Hg reduces this risk by 10% [6].

Therefore, the latest guidelines of the European Society of Hypertension recommend lowering blood pressure to below 130/80 mm Hg, but not below 120/80 mm Hg [7]. However, the beneficial effects of hypertension therapy described above depend on long-term, sustained blood pressure reduction. Not only discontinuation of effective hypertension therapy but also poor adherence to antihypertensive therapy leads to a significant increase in the risk of cardiovascular complications and increased mortality [8-10]. However, large meta-analyses covering tens of thousands of patients with hypertension have shown that the rate of patients discontinuing hypertension therapy or low adherence to antihypertensive medications is high, ranging from 31-83%, and usually 30% greater in men than in women [11-13].

Overall, nearly two-thirds (62.5%) of the medication nonadherence was noticed in Africans and Asians. A particularly high rate of discontinue therapy is observed in young people [5, 11].

Many years ago, it was noted that the simultaneous use of several antihypertensive drugs leads to more

frequent, irregular use of these medications, or even complete discontinuation of these medications. This is one reason why guidelines for the treatment of hypertension recommend the use of several antihypertensive medications in a single tablet as a fixed-dose combination pill (FDC) [7]. This therapeutic approach has improved the effectiveness of hypertension therapy, but in many patients, satisfactory therapeutic efficacy has still not been achieved [14]. Low effectiveness of hypertension therapy is still observed not only in Africa but also in Europe and the United States [11, 13, 14]. According to the research by Bruner et al. [13] the unsatisfactory effectiveness of therapy is influenced by stress, emotions, insomnia and multimorbidity. The lowest effectiveness of therapy related to non-adherence to treatment recommendations is observed in low- to middle-income and non-income countries and non-Western countries. No significant positive trend in adherence was detected between 2010 and 2020 [11].

SEARCHING FOR NEW DRUGS FOR HYPERTENSION

Here is an intensive search for new pharmacological preparations that would improve the effectiveness of hypertension therapy [15-17] . The pharmaceutical industry is conducting in-depth research on compounds that potentially influence the mechanisms leading to hypertension. They are currently focusing on the use of, among others, activation of the AT2 receptor agonist, angiotensin (1-7) analogs., vasoactive intestinal peptide receptor agonists, intestinal L-type calcium channel blocker/ endothelin A/B2 receptor antagonist, antihypertensive vaccines. These studies did not lead to the introduction of any new antihypertensive drug into therapy, except for Baxdrostat, which inhibits aldosterone synthesis (similarly to the already known spironolactone), and Aprocitentan, a drug that inhibits endothelin receptors and is already used in hypertension resistant to antihypertensive therapy. Increased activity of the renin-angiotensin-aldosterone system (RAS) in the brain of patients with hypertension has also been noted [18]. Stimulation of this system increases the activity of the sympathetic nervous system, causes vasoconstriction, and increases the release of arginine vasopressin, which leads to an increase in blood pressure. Aminopeptidase (APA), located in the brain, converts angiotensin II to angiotensin III, responsible for the increase in blood pressure [18]. The drug firibastat, which inhibits aminopeptidase, lowers blood pressure by reducing the release of arginine vasopressin from the posterior pituitary gland into the bloodstream, which increases diuresis, reduces the activity of the sympathetic nervous system, and enhances baroreceptor function [18]. Firibastat, previously named QGC001 or RB150, is an orally active brain-penetrating prodrug of EC33, a selective and specific inhibitor of APA. Upon entry into the brain, it is cleaved by brain reductases to generate two active molecules of EC33, which inhibit brain APA activity, block brain angiotensin-III formation, and decrease BP. The decrease in blood pressure following firibastat administration depends not only on the drug's inhibitory effect on aminopeptidase but also on the conversion of angiotensin II to angiotensin 1-7, which lowers blood pressure. Initially, high hopes were placed on this drug. The first clinical trial, designated NEW-HOP, on the effects of firibastat included 256 overweight or obese patients with hypertension [19, 20]. After 8 weeks of firibastat administration, systolic blood pressure in the study patients decreased statistically significantly by 9.5 mm Hg and diastolic blood pressure by 4.2 mm Hg. In the black patients participating in this study, systolic blood pressure decreased by 10.5 mm Hg, and in the remaining patients by 8.9 mm Hg ($P<0.0001$). Furthermore, it was found that the higher the baseline hypertension, the more the study drug lowered blood pressure [19,20]. However, subsequent studies in patients with resistant hypertension did not demonstrate a significant reduction in blood pressure, and the study was discontinued [20, 21]. The low effectiveness of hypertension therapy may also be due to a small extent to patients who are resistant to antihypertensive therapy. However, it has been shown that only 10% of patients with hypertension are resistant to the inhibitors ACEIs, calcium antagonists, diuretics [22]. However, the lack of effectiveness of hypertension therapy in society is primarily due to irregular use of antihypertensive medications or temporary or even permanent discontinuation of antihypertensive therapy. This situation could be prevented by a drug that, after a single administration, would work for much longer than a day and would not cause adverse effects. It is possible that such a drug would significantly improve regular and long-term treatment of hypertension, and therefore the effectiveness of this therapy. For this reason, recent clinical trials of a new drug called zilebesiran have generated considerable interest and hope.

AIM

The aim of this work is to critically evaluate the benefits and risks of zilebesiran.

MATERIALS AND METHODS

A comprehensive literature search was conducted in PubMed and Google Scholar databases to identify relevant studies on zilebesiran and other small-inter-

fering RNA (siRNA)-based therapies for hypertension. The search covered publications from January 2010 to December 2025. The following keywords and combinations were used: "zilebesiran", "siRNA therapy", "angiotensinogen inhibition" "hypertension".

REVIEW

ZILEBESIRAN - MECHANISM OF ACTION

This drug inhibits the release of angiotensinogen in the liver. It is administered by subcutaneous injection once every 3 months or once every 6 months and produces a significant and long-lasting reduction in blood pressure. It is the first antihypertensive drug to modify the function of the gene responsible for angiotensinogen synthesis in the liver [23, 24].

Angiotensinogen secreted by the liver is converted into angiotensin-1 under the influence of renin, moreover small amounts angiotensinogen are also synthesized in adipose tissue, the kidney and the brain [24]. Angiotensin-1 is in turn converted into angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II exerts a powerful vasoconstrictor effect, stimulates the sympathetic nervous system, and stimulates the action of aldosterone and vasopressin. Both of these compounds also significantly increase blood pressure. Drugs such as ACE inhibitors or sartans significantly lower blood pressure and are frequently used in clinical practice. However, after prolonged use, due to the so-called escape phenomenon, a compensatory increase in renal renin secretion occurs, which weakens the antihypertensive effect of ACE inhibitors [24]. The relatively frequent side effects of ACE inhibitors and the insufficient antihypertensive effect of these drugs (due to the so-called escape mechanism) discourage many patients from long-term use of these drugs [25]. As indicated above, angiotensinogen plays a key role in the mechanism of action of the RAAS system., Andrew Fire and Crayg Mello discovered a method for gene silencing, for which they were awarded in 2006 the Nobel Prize. This discovery enabled the development of two methods for silencing the gene: antisense oligonucleotides (ASos) and small interfering RNAs (siRNAs). The latter method was used to develop the drug zilebesiran that silences the angiotensinogen gene [24]. These methods were used to synthesize several drugs successfully used in clinical practice. These include patisiran and vutrisiran, used for polyneuropathy associated with hereditary transthretin-mediated amyloidosis; givosiran, used for acute hepatic porphria; lumasiran and nedosiran, used for primary hyperoxaluria type 1; and inclisiran, used for primary hyperlipidemia [24].

Zilebesiran – a combination of a small RNA molecule composed of approximately 20 pairs of nucleosome binds to N-actylgalactosam. The latter compound ensures connection with liver cell receptors. The drug is then transported into the liver cell *via* clathrin-mediated endocytosis and is stored in endosomes, from where it slowly penetrates into the cytoplasm. As a result of further complex processes, the anti-sense strand binds to the RNA-inducing complex (RISC) molecule, thus forming an active RISC. The active RISC with the quide strand is recycled [24]. Thus, the modified RNA (siNRA) long-term silences the activity of the gene responsible for the production of angiotensinogen by liver cells, thanks to which a single administration of zilebesiran lowers blood pressure for a period of many weeks. The details of the operation of zilebesiran are, among others described, in Morosan's work [24].

ANIMAL STUDY

The first studies on the effect of zilebesiran on blood pressure were conducted in spontaneously hypertensive male rats fed a standard HIFH-sodium diet. SiRNA was administered by injection every two weeks at a dose of 10 mg/kg body weight. Following such administration, the drug is absorbed within approximately 8-16 hours. The half-life of the drug in blood serum is 3-5 days, while the half-life of pharmacological action is 3-6 months due to the slow return of RISC to its normal activity [24] .This drug was shown to induce a 98% reduction in serum angiotensinogen concentration. Zilebesiran at a dose of 400-800 mg also significantly reduces angiotensin I and angiotensin II concentrations and moderately reduces aldosterone concentrations, proportionally to the drug dose. All doses of zilebesiran moderately increase plasma renin activity [24]. The rats' blood pressure decreased by 14 mm Hg. In a similar study, where the animals received only valsartan, blood pressure decreased by 10 mm Hg [24].

Unlike angiotensin-converting enzyme inhibitors, zilebesiran does not interfere with bradykinin degradation and therefore does not cause dry cough or angioedema [26].

In the group of animals co-administered with zilebesiran and valsartan, blood pressure decreased by 70 mm Hg. As expected, the decreases in blood pressure following zilebesiran administration were greater in animals receiving the low-sodium diet than the high-sodium diet [24]. After zilebesiran the pressure drop was constant, no escape effect was observed [27].

The effect of zilebesiran can be reversed by subcutaneous administration of an siRNA fragment that reverses the drug's action (the REVERSIR effect). Such

studies were conducted in animals [28]. As a result of this approach, blood pressure returned to baseline values after 4-7 days. Another study demonstrated that acute administration of norepinephrine rapidly raised blood pressure. Other methods for terminating prolonged hypotension following zilebesiran administration include administration of fludrocortisone, a high-sodium diet, or administration norepinephrine. However, administration of midodrone did not increase the reduced blood pressure [29].

Zilebesiran also reduced cardiac hypertrophy [30]. Bovee et al. observed the effect of zilbesiran on the behavior of blood pressure in rats in which profound renal failure was induced by removing one kidney and in the other one a partial nephrectomy was performed. Administration of zilebesiran prevented the increase in blood pressure in the course of uremia [31]. Crus-Lopez et al. also noted the preventive effect of zilebesiran on the increase in blood pressure in rats with experimentally induced diabetes [32].

CLINICAL STUDY

The first clinical trials were conducted between 2019 and 2022 [33]. The study was conducted at four sites in the United Kingdom. The study participants were divided into several groups from group A, B and E. In Part A, eligible patients (12 per cohort) were randomly assigned in a 2:1 ratio to receive a single subcutaneous dose of zilebesiran (10, 25, 50, 100, 200, 400, or 800 mg) or placebo. Zilebesiran reduced angiotensinogen concentrations by more than 90% from a 100 mg dose. The decrease in blood pressure correlated with decreasing angiotensinogen levels (r=0.52). A single dose of 20 mg of zilebesiran reduced systolic blood pressure by more than 10 mm Hg and diastolic blood pressure by more than 5 mm Hg.

In Part B, after sequential administration of a low-salt diet (0.23 g per day) and a high-salt diet (5.75 g per day) from days − 21 through − 8, patients were randomly assigned in a 2:1 ratio to a single dose of 800 mg zilebesiran or placebo. After administration of 800 mg of zilebesiran, the corresponding changes systolic blood pressure after a low-salt diet were − 18.8±4.3 mm Hg and only -8,4 mm Hg on high-salt diet [33].

In part E all the patients received a single 800 mg dose of zilebesiran. Six patients had a mean decrease from baseline in systolic blood pressure of 21.8±2.9 mm Hg at week 6 after administration of 800 mg of zilebesiran and at week 24 change in the mean systolic and diastolic blood pressure was - 22,5 mm Hg and - 10,8 mm Hg respectively, among the eight patient [33].

No hypotension or worsening of renal function was observed. The increase in blood pressure observed due to the escape phenomenon in patients treated with RAAS inhibitors was not observed after zilebesiran administration [33].

The second phase of the randomized, placebo-controlled trial, KARDIA-1 was conducted between 2021 and 2023 in 78 centers in the USA, Canada, Great Britain, and Ukraine [33]. People aged 18-75 years with moderate hypertension were invited to participate in the study. The study included 394 patients, including 167 women and 93 Black patients. The study participants were divided into 5 groups. Patients in one group received only a placebo injection once every 3 months. Patients in the other groups received zilebesiran in doses of 150, 300, 600 mg every 6 months, or 300 mg every 3 months. The study lasted ½ year. In the first, third, and sixth months of the study, ABPM was performed. Automatic office BP measurement was performed on the first day of the study, then weekly, and finally monthly until the end of the study. In the group of patients receiving only placebo, blood pressure increased significantly, while in the groups treated with zilebesiran, it decreased. Redness (erythema) accompanied by pain at the injection site was observed in 5 patients. Mild hyperpotassemia (above 5.2 mmol/l) was noted in 5 percent of patients and hypotension in 13 patients. In four patients discontinued the drug due to orthostatic hypotension [33].

The randomized KARDIA-2 study evaluated the efficacy and safety of zilebesiran administered in combination with other antihypertensive drugs in hypertensive patients with uncontrolled hypertension [34, 35]. Specifically, the study included patients with previously untreated hypertension (systolic blood pressure 155-180 mm Hg) and patients treated with one or two antihypertensive drugs whose systolic blood pressure was 145-180 mm Hg. The study was conducted between 2022 and 2023 in 150 centers in Canada, Estonia, Germany, Latvia, Lithuania, Poland, England, and the USA. During the run-in period, previously used medications were discontinued and monotherapy with indapamide, amlodipine, or olmesartan was initiated once daily. A total of 667 patients were recruited, divided into indapamine groups (n=127), amlodipine groups (n=239), and olmesartan groups (n=301). Indapamine was administered at a dose of 2.5 mg once daily, amlodipine 5 mg once daily, and olmesartan 40 mg once daily. Additionally, patients in each group were randomly assigned to receive either zilebesiran or placebo. After three months of zilebesiran therapy systolic blood pressure decreased by 12,1 mm Hg in indapamide group ($p< 0,0001$), 9,7 mm Hg in amlodipine group ($p<0,0001$)

and 4,0 mm Hg in olmesartan group (p>0,006). Greater reductions in office SBP were observed. They were 18.5 mm Hg, 10.2 mm Hg, and 7 mm Hg, respectively. After 6 months, the reductions were 13.6 mm Hg in the indapamide group, 8.6 mm Hg in the amlodipine group, and 4.6 mm Hg in the olmesartan group (p<0.001). No serious complications were observed; only a transient trend toward hyperkalemia and increased serum creatinine was noted, which spontaneously resolved during further follow-up. A decrease in GFR of more than 30% was observed in 4.8% of patients in the indapamide and zilbesiran group, 0.8% in the amlodipine and zilebesiran group, and 2.7% in the olmesartan and zilebesiran group [34, 35].

Preliminary results of the KARDIA 3 study, currently comprising 270 patients with a mean age of 67 years, 77% of whom were at high cardiovascular risk, and 23% of whom had already been diagnosed with cardiovascular disease [36]. Baseline blood pressure in the study group was 142/70 mm Hg. Three months after subcutaneous administration of 300 mg of zilebesiran, blood pressure decreased by 5 mm Hg, and after 600 mg of zilebesiran, by 3.3 mm Hg (ABPM). Six months after administration of 300 mg of zilebesiran, blood pressure decreased by 3.9 mm Hg, and after 600 mg of zilebesiran, by 3.6 mm Hg. The decrease in blood pressure in ABPM measurements after 3 months was more pronounced: 5.5 mm Hg and 7.4 mm Hg. The nocturnal blood pressure decrease was greater, reaching 6.6 mm Hg and 8.2 mm Hg, respectively [36]. The drug's manufacturers plan to conduct another Phase III ZENIT CVT study involving 11,000 patients with uncontrolled hypertension despite the use of at least two antihypertensive drugs and cardiovascular disease or relatively high cardiovascular risk. The study is scheduled to be completed in 2030. The aim of this study will be not only to assess the drug's antihypertensive effect but also to assess the reduction in the number of heart attacks and strokes compared to placebo [36].

Lemine et al. conducted a meta-analysis of available studies on zilebesiran

that have been published until 2024 [37]. The study included 1,145 randomized patients whose blood pressure and serum angiotensinogen levels were assessed after treatment with zilebesiran and placebo. In all patients included in the meta-analysis, blood pressure was measured using ABPM. This meta-analysis showed that in patients treated with zilebesiran at doses greater than 250 mg and less than 500 mg, reduction of systolic blood pressure was the greatest (the difference compared to placebo was 16.40 mmHg.) (p<0.00001). Higher doses of zilebesiran above 500 mg of zilebesiran were found to be less effective [37].

Another meta-analysis conducted by Raza et al. showed that zilebesiran significantly lowers blood pressure after 12 weeks of treatment with ABPM (MD 11.9 nn Hg p<0.00000001 and after 24 weeks MD is 19.68 (p<0.00001). [38]. In office SBP measurements the difference compared to placebo after 12 weeks was 12.21 mmHg and after 24 weeks 8.49 mmHg p<0.00001. Zilebesiran caused mild side effects (OR 1.37) p<0.01, but with no serious side effects reported [38]. Changes in creatinine concentration and glomerular filtration rate proved statistically insignificant. A significant decrease in blood pressure was observed in both ABPM and office SBP [38]. Another meta-analysis by Singh et al. confirmed the results of the previous meta-analyses [39]. Twelve weeks after zilebesiran administration, a decrease in systolic blood pressure of 15.12 mm Hg and diastolic blood pressure of 7.34 mm Hg was observed in ABPM. The most common side effect were erythema (at the site of drug administration) and headache. A slight increase in potassium and creatinine levels was observed. Low titers of anti-zilebesiran antibodies in blood were detected in 5% of patients.

DISCUSSION

Despite the current availability of numerous antihypertensive medications, the effectiveness of hypertension therapy remains unsatisfactory. This is largely due to poor adherence to medical recommendations regarding hypertension therapy. The advantages of the possible introduction of zilebesiran to the treatment of hypertension were recently presented by Addison et al. [40] Will zilebesiran therapy solve this problem? Whether zilebesiran effectively reduces hypertension resistant to previously used medications has not been studied. There are also no studies of zilebesiran combined with beta-blockers and aldosterone antagonists. It is currently known that zilebesiran exerts its antihypertensive effect for at least a year. However, it is unknown whether longer-term administration (a few years) of this drug will result in a surrogate increase in angiotensinogen synthesis by adipose tissue or the kidneys. Zilebesiran only inhibits the synthesis of angiotensinogen produced in the liver. The promising observations that zilebesiran reduces cardiac fibrosis and slows the progression of chronic renal failure in experimental animals have not yet been verified in humans. If therapy with this drug significantly reduces hypertension and reduces the risk of cardiovascular complications we will only know after the completion of the KARDIA 3 study. Many issues related to adherence to medical recommendations will remain unresolved. How to convince patients to change their lifestyle, such

as a high-sodium diet and low physical activity, which also may lead to hypertension.

The smaller reduction in blood pressure following zilebesian administration in patients with cardiovascular disease in the first period of the KARDIA 3 study may already raise some concerns [36]. A similar situation occurred in the Firibastat study, when it was found that this drug demonstrated only marginal antihypertensive efficacy in patients with more treatment-resistant hypertension, and further study of the drug was discontinued. However, the final efficacy of zilebesiran cannot be determined based on the several-month duration of the KARDIA 3 study. The coming years will reveal the extent of the reduction in blood pressure and whether any adverse events are more frequent than previously reported. Special attention will be paid to the potential occurrence of chronic, irreversible renal failure, significant hyperkalemia, or uncontrolled hypotension. If zilebesiran proves to be free of these dangerous symptoms, it would be worthwhile to consider which patients with hypertension this drug would be most useful for. For many patients, zilebesiran may improve the effectiveness of hypertension therapy, but its limitations should be remembered. Zilebesiran lowers blood pressure 4-7 days after administration. Until zilebesiran takes effect, the patient must receive rapid-acting antihypertensive medications. An increase in blood pressure during therapy with zilebesiran at a dose of 500 mg requires the addition of another antihypertensive drug, not an increase in the dose of zilebesiran [37].

In patients with mild hypertension who were previously adequately treated with a single daily antihypertensive medication but subsequently were treated very irregularly, zilebesiran appears to be a good alternative, provided that no more severe hypotension develops. This situation may occur suddenly, for example in dehydration, diarrhea, sepsis. Therefore, the patient should be instructed to frequently monitor their blood pressure. A more profound drop in blood pressure will require emergency norepinephrine and a high-sodium diet [29]. This, in turn, may be more burdensome for the patient than taking the previous medication orally. This example indicates that zilebesiran will not always be readily used by patients and may even worsen the lack of patient compliance with the physician. In situations where several medications are used simultaneously, it would be advisable to use zilebesiran in combination with a diuretic or calcium channel blocker. This combination of drugs, as shown by the KARDIA 2 study [34,35] will provide greater blood pressure reduction than combining zilebesiran with an angiotensin-converting enzyme inhibitor or sartans. Furthermore, concomitant use of zilebesiran with an angiotensin-converting enzyme inhibitor can more easily lead to excessive serum potassium levels. The above examples indicate that even if zilebesiran proves to be an effective antihypertensive in everyday medical practice, it may not always be the ideal treatment for patients who do not adhere to treatment recommendations.

The large cardiology study KARDIA 3 will be completed in 2030. Only then will we know whether zilebesiran not only effectively lowers blood pressure but also reduces the risk of cardiovascular complications and whether it does not cause serious organ damage in some clinical situations. If zilebesiran successfully completes all these trials, we will have a drug that will expand our treatment options for hypertension.

CONCLUSIONS

The number of patients with hypertension is increasing worldwide, causing increasing cardiovascular complications and premature deaths. Despite numerous antihypertensive medications, the effectiveness of hypertension treatment has not improved sufficiently and remains unsatisfactory, primarily due to a lack of adherence to antihypertensive medications. Zilebesiran, administered as a subcutaneous injection twice a year, can improve adherence and effectiveness of hypertension therapy. We await the results of further studies with concern and hope to see whether zilebesiran will reduce the risk of cardiovascular complications and avoid serious side effects.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 30.01.2026
ACCEPTED: 05.05.2026

CREATIVE COMMONS 4.0

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REVIEW ARTICLE

CONTENTS

The role of collagen types I and II in the regeneration of temporomandibular joint and bone tissues - from molecular mechanisms to modern treatment protocols: Literature review

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ABSTRACT

Aim: The aim of this study is to analyze current data on the molecular and cellular mechanisms underlying the development of degenerative-dystrophic diseases of the temporomandibular joint (TMJ). The article explores the potential of collagen therapy as a pathogenetically substantiated approach to the comprehensive treatment of this disease.

Materials and Methods: A systematic review of the scientific literature over the past 20 years was conducted, indexed in PubMed/MEDLINE, Scopus, Web of Science, the Cochrane Library, RCSB Protein Data Bank until March 2026.

A comprehensive literature search identified 14,865 publications. Following a sequential analysis of titles, abstracts, and full texts based on predefined inclusion criteria (original experimental and clinical studies, systematic reviews and meta-analyses that revealed the molecular and cellular mechanisms of TMJ osteoarthritis, the therapeutic impact of types I and II collagen on joint and bone regeneration) and exclusion criteria (irrelevant papers, duplicates, TMJ rheumatoid arthritis, septic arthritis, systemic vasculitis). Ultimately, 37 primary sources were selected for the final review.

Conclusions: The authors summarized current data on the pathogenetic mechanisms of TMJ osteoarthritis, analyzed data on the use of collagen in the treatment of TMJ. The biological role of collagen types I and II as key structural components of fibrocartilage and subchondral bone is examined.

Special attention is given to the feasibility of using injectable tropocolagen to stimulate reparative processes in the TMJ.

KEYWORDS: temporomandibular joint, bone, osteoarthritis, treatment, cartilage regeneration, collagen type I, collagen type II, tropocollagen.

Wiad Lek. 2026;79(7):1654-1660. doi: 10.36740/WLek/225981 DOI

INTRODUCTION

The temporomandibular joint (TMJ) is a synovial joint that ensures jaw movement and function. Its complex anatomical structure and unique biomechanics, combined with the multifactorial nature of degenerative diseases, pose significant challenges for clinicians [1,2]. Osteoarthritis (OA) of the TMJ affects all joint structures, including articular cartilage, synovial membrane, subchondral bone, capsule, ligaments, periarticular muscles, sensory nerves, and innervating tissues [3,4].

OA develops primarily due to disruption of the metabolic homeostasis of cartilage and bone tissue. Deficient collagen and glycosaminoglycan synthesis leads to matrix dehydration, loss of cartilage elasticity, and the appearance of microcracks, erosions, and defibrosis [4,5].

Since collagen accounts for up to 70% of the dry mass of articular cartilage, it acts as a structural framework that provides three-dimensional strength and holds aggregates together. With age, collagen synthesis decreases, and the ratio of collagen types in tissues changes. When advanced glycation end products accumulate in the body, collagen fibers become rigid and lose their ability to stretch and cushion. This worsens the condition of the cartilage and subchondral bone. A decrease in the supply of nutrients and minerals to chondrocytes leads to their functional depletion and apoptosis, making the pathological process irreversible without external therapeutic support [6, 7]. It should be noted that, unlike the hyaline cartilage of large joints, the surfaces of the TMJ are covered with fibrous cartilage which contains both type I and type II collagen [8]. This underscores a critical demand not only for type II collagen but also for the structural reinforcement of the type I collagen scaffold.

AIM

The purpose of this study is to analyze current data on the molecular and cellular mechanisms underlying the

development of degenerative-dystrophic diseases of the TMJ. The article explores the potential of collagen therapy as a pathogenetically substantiated approach to the comprehensive treatment of this disease.

MATERIALS AND METHODS

A systematic review of the scientific literature over the past 20 years was conducted, indexed in PubMed/MEDLINE, Scopus, Web of Science, the Cochrane Library, and the RCSB Protein Data Bank until November 2025. Google Scholar and other sources were also used. The search utilized MeSH terms, keywords ("temporomandibular joint," "TMJ," "osteoarthritis," "treatment," "collagen type I," "collagen type II," "collagen supplement," "collagen therapy," "tropocollagen"), and the logical operators AND and OR.

Initially, 14,865 publications were identified. The inclusion criteria were original experimental and clinical studies, systematic reviews and meta-analyses that revealed the molecular and cellular mechanisms of TMJ osteoarthritis, as well as the effect of collagen types I and II on joint regeneration. Studies with poor methodology, irrelevant studies (rheumatoid arthritis, septic arthritis, systemic vasculitis), and unreviewed publications were excluded.

The papers were excluded by sequential analysis of titles, abstracts, and then full texts based on established selection criteria. After full text analysis, 37 primary sources that fully met the inclusion criteria were included in the final analysis.

The authors summarized current data on the pathogenetic mechanisms of TMJ OA and the potential therapeutic effects of collagen therapy. The results of in vitro and clinical trials of collagen preparations of various origins: bovine, porcine, marine and recombinant were studied. Particular attention was given to studies that shed light on the pathogenesis of inflammatory and degenerative diseases of the TMJ and the characteristics of its fibrocartilage.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

The following artificial intelligence tools were used in this work: [Gemini 3 Flash]. They were used for [linguistic editing, translation refinement, reference editing]. All sections using artificial intelligence were reviewed and edited personally by the author(s). The scientific statements, conclusions, and results are the author(s)'s own intellectual contribution.

REVIEW AND DISCUSSION

COLLAGEN TYPE I

More than 90% of collagen in the human body is type I collagen due to its widespread distribution in almost all connective tissues. The molecular formula of type I collagen is $[\alpha 1(I)]_2\alpha 2(I)$ ($\alpha 1$, $\alpha 2$ are proteins encoded by the COL1A1 and COL1A2 genes (Fig.1).

Type I collagen is the main component of calcified tissue in teeth and bones; it is present in skin, tendons, blood vessels, lungs, and the heart. Type I collagen plays a critical role in maintaining the integrity of subchondral bone, as it constitutes approximately 90% of its organic matrix and forms a highly ordered fibrillar network, which provides bone with the ability to withstand tensile loads and serves as the basis for hydroxyapatite mineralization. Impaired type I collagen synthesis leads to a decrease in the mechanical stability of the condylar process of the mandible, which accelerates the destruction of the cartilage above it. Modern protocols consider the use of bioactive collagen peptides type I as a means of stimulating osteoblasts and restoring subchondral bone density, which can slow the progression of osteoarthritis [6].

OA develops as an acute inflammatory process with a tendency to chronic development: chondrocytes enter the joint cavity, the osmotic pressure in the articular cartilage changes, and proteoglycans progressively migrate into the joint space, interfering with the natural stages of healing. Proinflammatory factors (synoviocyte tumor necrosis factor (TNF-β), interleukins: IL-6, IL-1α and IL-1β) are released, stimulating enzymes that degrade cartilage: disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and matrix metalloproteinases (MMP) [9]. These enzymes cause degradation of the extracellular matrix (ECM), including collagen. For this reason, exogenous collagen administration has been investigated as a possible symptomatic adjuvant or independent method of treating osteoarthritis [10].

COLLAGEN TYPE II

Collagen type II is a common matrix molecule of cartilage, with the molecular formula $[\alpha 1(II)]3$ (Fig.2).

Collagen type II is associated with many diseases, such as skeletal dysplasia, rheumatoid arthritis, osteoarthritis, and OA. The degradation of type II collagen (COL2A1) induces chondrocyte hypertrophy via the bone morphogenetic protein (BMP) signaling pathway,

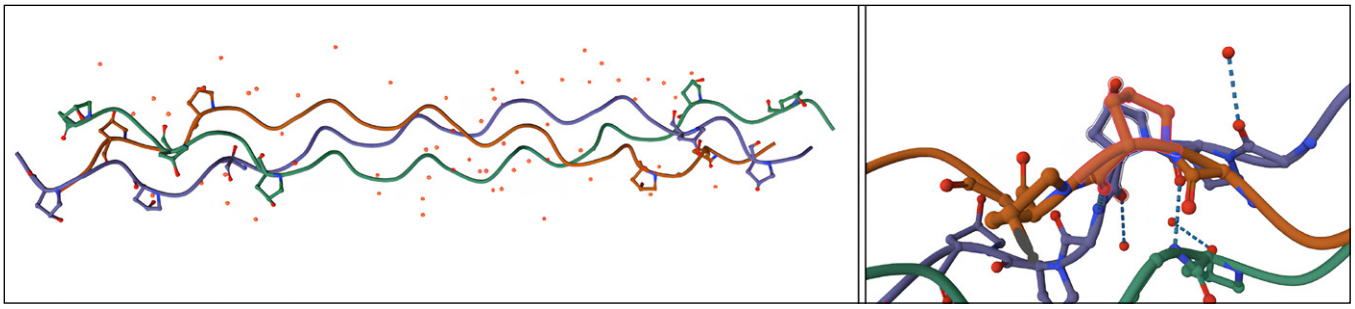

Fig. 1. Structure of the triple helical region of human collagen type I by Trautec from RCSB protein data bank
Source: compiled by the authors based on [https://www.rcsb.org/structure/8K4W]

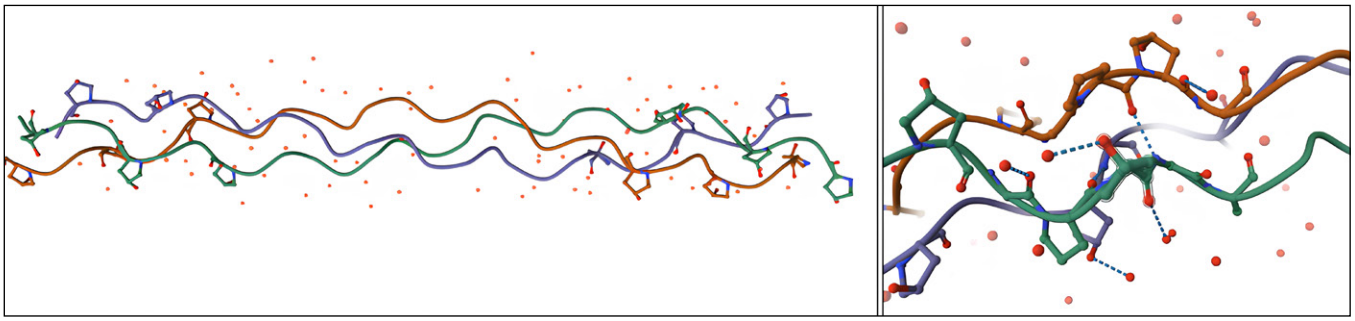

Fig. 2. Structure of the triple helical region of human collagen type II by Trautec from the RCSB protein data bank
Source: compiled by the authors based on [https://www.rcsb.org/structure/9J23]

consequently accelerating the progression of TMJ OA [11]. A significant number of preclinical, experimental, and clinical studies over the past 20 years have demonstrated the effectiveness of type II collagen preparations (including its native form UC-II®) in the treatment of joint inflammation [12,13].

UCII® is a triple helix with bioactive regions called epitopes. These promote binding of undenatured type II collagen to Peer's plaques in the small intestine, which underlies its unique mechanism of action inducing oral tolerance. After binding, a Peer's plaque dendritic cell determines that this antigen is harmless to the body and initiates the activation of immune cells, specifically regulatory T-lymphocytes. The importance of regulatory T-cells stems from their ability to reach sites of inflammation and cartilage destruction and secrete anti-inflammatory cytokines such as IL10 and transforming growth factor β (TGF-β). IL10 inhibits the synthesis of IL1, IL6, and TNFα, preventing the latter from stimulating chondrocytes to release aggressive enzymes and nitric oxide. TGF-β stimulates the synthesis of cartilage matrix components by chondrocytes: collagen, chondroitin sulfate, and hyaluronic acid (HA). Therefore, the use of UC-II® helps reduce inflammation, renew cartilage, and improve joint movement [13].

SOURCES OF COLLAGEN

Nowadays, collagen is obtained using a variety of methods, including natural sources and specially developed recombinant systems based on bacteria, yeast, insects, or mammalian cells [14]. Chemical or enzymatic hydrolysis is typically used to process raw materials, resulting in hydrolyzed collagen. This group of peptides dissolves better than their original (native) form. Extraction is easy, so peptides are actively used in production [15].

Traditionally, the most common sources of animal collagen remain the tissues of cattle, pigs, poultry, and marine organisms [16]. Such products may carry a risk of transmitting spongiform encephalopathy. Clinical observations of patients receiving bovine collagen implants indicate that immune reactions are almost nonexistent and subside when the material is completely absorbed [14]. Furthermore, this collagen is well suited as a precursor to peptides that reduce the activity of angiotensin-converting enzyme, helping to correct blood pressure [17,18].

In parallel, porcine collagen is widely used in medical practice; due to its high similarity to human protein, it causes virtually no allergic reactions. Its peptides demonstrate significant potential in the prevention and treatment of osteoporosis [19], and collagen membranes based on porcine tissue have confirmed their biocompatibility in bone regeneration processes [20]. Chicken feet and sheep and goat tendons are rich in beneficial nutrients and have therefore proven themselves to be alternative collagen sources. Collagen obtained from goat tendons has been shown to be similar in its physical and chemical properties to calf collagen and has demonstrated better cytocompatibility [21].

At the same time, religious restrictions on the consumption of pork or veal products, as well as epidemiological threats, are stimulating the development of the marine collagen segment [14]. Collagen from fish or marine invertebrates yields a high yield and does not transmit zoonotic diseases. Although marine collagen has somewhat lower thermal stability and mechanical strength due to its lower amino acid content, the use of cross-linking methods allows its successful use as a biomaterial in tissue engineering [22]. Over the past twenty years, marine organisms and their derivatives have increasingly been used in the development of new drugs [23,24].

TMJ AND COLLAGEN

Modern collagen preparations, given their wide range of sources and high biocompatibility, open up new perspectives in the treatment of degenerative-dystrophic joint diseases. This issue is particularly relevant in the treatment of TMJ, as the structural integrity of its cartilage and subchondral bone directly depends on collagen metabolism.

Unlike the large joints of the limbs, the TMJ has a unique histological structure. Its articular surfaces are covered not by hyaline cartilage, but by fibrous cartilage—a dense fibrous connective tissue where type I collagen predominates [25]. This influences the choice of treatment, since in TMJ OA, the collagen framework is destroyed due to progressive degradation of the collagen matrix. Consequently, the joint space narrows, crunching occurs, and jaw mobility is limited [5].

The use of collagen hydrolysates in the complex therapy of degenerative-dystrophic diseases of the TMJ not only inhibits the activity of proinflammatory cytokines but also stimulates chondrocytes and fibroblasts to restore damaged structures. Furthermore, given the complex etiology of degenerative-dystrophic processes in the TMJ, modern protocols include a combination of collagen peptides with B vitamins and other cofactors. This helps build new modified, mechanically stable collagen fibers [26].

Collagen can help with osteoarthritis of the TMJ not only by replenishing protein deficiency. Its effects are complex, influencing molecules and cells. A key aspect in the pathogenesis of this disease is changes in metabolism in fibrocartilage. The matrix then degrades faster than it can be restored, as metalloproteinases begin to break it down more rapidly. Therefore, oral administration of hydrolyzed collagen initiates anabolic processes in the joint due to the accumulation of specific peptides, namely proline-hydroxyproline, in the synovial fluid. These bioactive molecules act as ligands for receptors on the surface of chondrocytes and fibroblasts of the TMJ, stimulating them to increase the expression of genes responsible for the synthesis of endogenous collagen types I and II, as well as aggrecan [5,25].

Of particular importance for the TMJ is the ability of collagen peptides to influence the local immune response through the mechanism of oral tolerance. The interaction of native collagen molecules with intestinal lymphoid tissue promotes the differentiation of specific T-regulatory cells, which migrate to the site of inflammation in the TMJ area and secrete anti-inflammatory cytokines [26]. This helps stop secondary synovitis and slow the destruction of the collagen framework of the articular disc. At the same time, restoration of the structural integrity of the subchondral bone of the condylar process of the mandible with the participation of type I collagen provides the necessary biomechanical support to the articular surfaces, preventing further deformation of bone structures. Optimization of the rheological properties of synovial fluid by stimulating the synthesis of its own HA complements this complex multicomponent mechanism, providing the necessary lubrication and reducing mechanical friction during articulation [27].

Therefore, both type I and type II collagen help ensure pathogenetically based restoration of all parts of the temporomandibular joint as a unified system. This allows for a synergistic effect, as the combination of different collagen types allows for the joint to be treated as a holistic biomechanical structure.

When planning the treatment of TMJ osteoarthritis, the clinical picture, the degree of joint destruction, and the intensity of the pain syndrome are primarily taken into account. Although many different methods exist for achieving stable treatment, individualized and comprehensive treatment is essential. Timely and comprehensive treatment is essential for slowing the pathological process [4, 28]. Early diagnosis and treatment strategies for osteoarthritis can improve outcomes and reduce disability and social burden associated with this disease [29].

COLLAGEN AND BONE METABOLISM

In vitro studies of collagen preparations with varying degrees of hydrolysis or polymerization have demonstrated their positive effects on synovial and cartilage cells in animals and humans. Collagen stimulated increased chondrocyte proliferation and the synthesis of cartilage extracellular matrix proteins, increased HA synthesis, and reduced the release of inflammatory mediators [30]. Purified atelocollagen of porcine ori-

gin is a soluble form of type I collagen. This collagen is readily absorbed by the body and has little immune response. Purified atelocollagen has a long half-life and is resistant to enzymatic degradation. This collagen has demonstrated the ability to directly reduce the inflammatory response and promote tissue repair, serving as a scaffold matrix for regenerative processes in the area of injury [31].

Recent meta-analyses show that collagen supplementation helps alleviate the symptoms of osteoarthritis. They found an overall significant improvement in knee joint mobility and pain reduction [32]. Following oral administration of collagen, an improvement in bone mineral density was observed in individuals with early bone loss, particularly in postmenopausal women with osteopenia [33].

Massicotte F. et al. (2002) investigated intra-articular injection of type I collagen as a possible symptomatic adjuvant or standalone treatment for OA. The results showed that collagen injection stimulates chondrocytes to produce hyaline cartilage and inhibits the normal inflammatory response, leading to the formation of fibrous tissue, reducing symptoms, and improving functionality [34].

In studies by Tsai K.S. et al. (2010) of different types of biomaterial coatings, collagen type I has proven to be the best extracellular matrix protein for enhancing osteogenesis, as it promotes the proliferation and osteogenesis of human mesenchymal stem cells (hMSC) by activating the extracellular signal-regulated kinase (ERK), protein kinase B (Akt) pathways [35].

In vivo studies by Tarantino D. et al. (2023) of intra-articular administration of collagen type II solution demonstrated therapeutic efficacy and cartilage regeneration efficiency similar to that of HA in rats with osteoarthritis induced by meniscectomy of the knee (MNX). In this study, treatment of articular cartilage with collagen type II solution attenuated the loss of collagen type II expression, thereby maintaining chondrocyte and cartilage density, similar to HA. Treatment with a solution of collagen II and HA increased aggrecan, which acted as a protective mechanism in osteoarthritis, and expression of SRY-box transcription factor 9 (SOX-9) reduced cartilage damage, thereby preventing significant destruction of chondrocyte collagen in the articular cartilage of rats with MNX-induced OA, similar to that of HA [30].

Porcine collagen, obtained by tangential filtration and sterilization with controlled molecular weight, is contained in an injectable preparation of tropocollagen type I with a molecular weight of 300 kDa (MD-Collagen (GUNA SpA, Milan, Italy)). The obtained injectable tropocollagen type I is a pure product that has standardized physicochemical characteristics with high clinical safety. In addition to biochemical and phylogenetic similarity with collagen of human tissues, it contains high concentrations of the main collagen-forming amino acids: glycine (22.8%), proline (13.8%), hydroxyproline (13%). The average content of other amino acids is only 3% (maximum glutamic acid 9.5%, minimum tyrosine 0.4%). The MD-Collagen line of products includes MD-TISSUE, which, in addition to tropocollagen type I, contains other biologically active substances in its formula, namely a complex of vitamins C, B1, B2, B6, and Mg gluconate, essential for collagen synthesis [31]. This tropocollagen normalizes metabolic processes in the joint at the cellular level, stimulates biorevitalization and protects against the effects of free radicals, promoting tissue regeneration in the event of damage. It has proven itself in orthopedics and traumatology for the treatment of degenerative-dystrophic joint diseases, lumbago, osteoporosis, and rheumatoid arthritis. In dentistry, it is used to improve tissue regeneration in the alveolar bone of the jaws and the oral mucosa, particularly during oral surgery on both soft and hard tissues.

Given the pronounced regenerative therapeutic effect of tropocolagen type I, its use is advisable for the treatment of inflammatory, degenerative-dystrophic processes in the TMJ, osteoporosis, and degenerative-dystrophic changes in bone structure.

The effectiveness of collagen therapy for the treatment of degenerative-dystrophic diseases of the TMJ is determined by the presence of specific cofactors. Vitamin C helps hydroxylate proline and lysine residues. This maintains the stability of the collagen triple helix. B vitamins reduce neurogenic pain, which often occurs during the treatment of TMJ diseases, and improve bone structure by promoting the metabolic processes necessary for collagen formation and metabolic regulation [37].

CONCLUSIONS

The conducted systematic review showed that, taking into account the above-mentioned anti-inflammatory effect of collagen, slowing down the destruction of the collagen skeleton of the articular disc, restoring the structural integrity of the subchondral bone, the bone of the mandible head, ensuring biomechanical support of the articular surfaces, improving the rheological properties of the synovial fluid, etc. in the tissues of the joint, it is advisable to use collagen preparations of types I and II for the treatment of degenerative-dystrophic diseases of the TMJ, osteoporosis, and degenerative changes in the bone structure.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 10.03.2026
ACCEPTED: 30.06.2026

CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

General structure of scorpion venom and its influence on morphological parameters of the mammalian liver

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ABSTRACT

Aim: To determine the general patterns of the structure of scorpion venom and its influence on the morphological parameters of the mammalian liver.

Materials and Methods: A thorough literature analysis was conducted on the basis of PubMed, Google Scholar, Web of Science, and Scopus databases. When searching for information of the general patterns of the structure of scorpion venom and its influence on the morphological parameters of the mammalian liver, we used the following combinations of keywords: "scorpions", "toxins", "liver", "hepatotoxicity", "hepatocytes". When processing the search results, we chose the newest publications up to 5 years old or the most thorough publications that vividly described the essence of our topic. After conducting a detailed review of the abstracts of the articles and getting acquainted with their full content, 32 sources were selected that fully corresponded to the results of the request.

Conclusions: Scorpion venom causes the development of local, cardiotoxic, neurotoxic effects and effects of autonomic nervous system. Depending on the predominance of a particular component in the venom, a wide range of clinical signs and symptoms can be observed from local reactions (hyperemia, pain, edema) to serious consequences, including respiratory, gastrointestinal, cardiac or neurological complications. The influence of toxic components on the structural and functional parameters of the mammalian liver is currently at the stage of comprehensive and thorough study. It has been established that under the conditions of administration of scorpion venom to experimental animals, hydropic degeneration and karyorrhexis of hepatocytes, fibrinoid necrosis, blood stasis in the vessels, an increase in the level of enzymes - ALT, AST, LDH were noted.

KEYWORDS: liver, hepatotoxicity, toxins, hepatocytes, scorpion

Wiad Lek. 2026;79(7):1661-1666. doi: 10.36740/WLek/222244 DOI

INTRODUCTION

Scorpion stings are a serious threat to human health and life in almost all countries of the world. The amount and toxicity of the venom that enters the body of the victims depends on the interspecific variability of these animals. Scorpion venom usually causes the development of local, cardiotoxic, neurotoxic and effects of autonomic nervous system. Depending on the predominance of a particular component in the venom, a wide range of clinical signs and symptoms can be observed from local reactions (hyperemia, pain, edema) to serious consequences, including respiratory, gastrointestinal, cardiac or neurological complications. The severity of poisoning depends on the size and type of scorpion, the amount of venom injected, as well as the body weight of the victim and sensitivity to the venom. Studies in recent decades have reported damage of the kidneys, liver, pancreas, heart, as well as the appearance of hemolytic disorders as a result of poisoning with toxins of some species of scorpions [1, 2].

The evolution of scorpions is approximately 400 million years, during which they have spread throughout the world. To date, more than 2,700 extant species of these animals have been recorded, numbering about 20 families. The extraordinary resilience, adaptability to changing climate conditions and high survival rates of scorpions have contributed to their colonization in tropical, subtropical and regions of almost all continents, except Antarctica and several Pacific islands. However, in recent years, the expansion of human civilization and the growth of the human population have led to a sharp reduction in the usual habitats of scorpions, but have significantly increased the frequency of poisonings due to their bites. In several regions of the world, including Central America, southern Latin America, Western Asia, North Africa and the Middle East, scorpionism has become an important health problem [3]. More than 1.2 million cases of scorpion stings are reported worldwide each year, with consequences ranging from localized pain or inflammation to serious clinical complications

or death, depending mainly on the types and amounts of neurotoxins present in the scorpion venom. In particular, most species of scorpions in the Buthidae family are more venomous than those in the Scorpionidae and Hemiscorpiidae families. A scorpion sting usually causes local pain that lasts for a few minutes. Meanwhile, the neurotoxins released by venomous scorpions cause sympathetic excitation and the release of catecholamines into the blood plasma, leading to severe clinical manifestations, including high blood pressure, cardiac arrhythmias, pulmonary edema, loss of consciousness, and sometimes death. [4, 5].

AIM

The aim of the study was to determine the general patterns of the structure of scorpion venom and its influence on the morphological parameters of the mammalian liver.

MATERIALS AND METHODS

A thorough literature analysis was conducted on the basis of PubMed, Google Scholar, Web of Science, and Scopus databases. When searching for information of the general patterns of the structure of scorpion venom and its influence on the morphological parameters of the mammalian liver, we used the following combinations of keywords: "scorpions", "toxins", "liver", "hepatotoxicity", "hepatocytes". When processing the search results, we chose the newest publications up to 5 years old or the most thorough publications that vividly described the essence of our topic. After conducting a detailed review of the abstracts of the articles and getting acquainted with their full content, 32 sources were selected that fully corresponded to the results of the request.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

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

REVIEW AND DISCUSSION

For the purpose of hunting and protection from predators, in the process of long evolution, scorpion venoms have acquired extraordinary molecular diversity, acquiring high activity, specificity, structural variability and a wide range of clinical effects. Among the main non-protein components of scorpion venom, lipids, nucleotides, free amino acids, organic acids, etc. are distinguished. However, the high viability and extraordinary adaptability of scorpion venom are explained precisely by the complex heterogeneous proteins contained in them, including neurotoxins, long-chain cationic antimicrobial peptides, cytolytic peptides, phospholipases, serine proteases, mucoproteins, lysozymes. Among them, short-chain neurotoxic peptides, which constitute 10–70% of the venom content, are the subject of interest to many scientists, since their study is important for assessing the harmful effects on cells and tissues of the victim's body, as well as the potency and selectivity of neurotoxins for certain ion channels. At the same time, progressive achievements in biotechnology have facilitated the identification of a wide range of scorpion venom peptides, some of which have found use in the manufacture of medicines. [6, 7].

According to their structural and functional properties, the peptide components of scorpion venom can be divided into two large groups: disulfide-bridged peptides (DBPs) and non-disulfide-bridged peptides (NDBPs). DBPs usually consist of 13–70 amino acids with three or four disulfide bridges. They include a number of toxins, such as sodium channel toxins (NaTx), potassium channel toxins (KTx), calcium channel toxins (CaTx), chloride channel toxins (CLTx), and TRP channel toxins (TRPTx). These toxins are capable of affecting the permeability of ion channels. NDBPs contain 13–56 amino acids and do not have specific targets among ion channels. However, they have attracted increasing attention in recent years due to their diverse biological and pharmacological effects, such as antimicrobial, antiviral, and antitumor effects. [8].

NaTx are major components of scorpion venom that target voltage-gated Na^+ channels and cause paralysis or death of the victim. They are long-chain venom toxins consisting of 13–56 amino acid residues, most of which contain four disulfide bridges, although some may have three. Structurally, all NaTx share a conserved cysteine-stabilized α-helix and β-sheet (CSαβ) that are cross-linked by 3 or 4 intramolecular disulfide bonds. Depending on the binding site to the ion channel and whether it affects the opening or closing dynamics of Na^+ channels, NaTx can be further divided into α-NaTx and β-NaTx. α-NaTx stimulate inhibition or delay of Na^+ channel inactivation by binding to receptor site 3 (located in domain IV of the ion channel), whereas β-NaTx alters the dynamics of activation of these channels by binding to receptor site 4 (located in domain II of the ion channel). It has been established

that α-NaTx are able to prolong the action potential of excitatory cells and cause paralysis or cardiac arrhythmias, while β-NaTx mainly induce a response in the body of the victims, mediated by monoclonal antibodies or muscle spasms [9, 10].

KTx are among the most common types of scorpion venom neurotoxins that inhibit the activity of potassium ion channels. Due to their unique interaction with K^+ channels, KTx have become indispensable molecular tools for assessing the structural and functional characteristics of various K^+ channels. KTx are typically composed of 23–64 amino acids and contain 2–4 disulfide bridges. Depending on their amino acid sequence, these peptides can be classified into α-KTx, β-KTx, γ-KTx, κ-KTx, δ-KTx, λ-KTx, and ε-KTx [11].

α-KTx is the largest subgroup of KTx, typically includes peptides of 23–43 amino acid residues with 3 or 4 disulfide bridges that share a conserved CSαβ framework. In addition, more than 31 α-KTx subfamilies (α-KTx1 to α-KTx31) have been identified to date, most of which exert their inhibitory activity through interactions between a functional domain located on their β-sheets and amino acid residues surrounding the K1.x pore filters. In contrast to α-KTx, β-KTx (also known as orphan toxins) are approximately 60 amino acid residues longer. α- and β-KTx share a CSαβ framework with three disulfide bridges. β-KTx is mainly found in scorpion species belonging to the genera Androctonus, Mesobuthus, and Tityus. These toxins are characterized by an α-helical N-terminus and a tightly coiled C-terminal region with a CSaβ motif [12, 13]. They exhibit potent antibacterial and hemolytic activity, as well as selective inhibition of potassium channels. γ-KTx contain 3 or 4 disulfide bridges and can selectively block Kv11.x. In contrast to the above three groups of KTx, κ-KTx with two disulfide bridges contain a CSαα framework consisting of two short α-helices connected by a β-sheet; this structural difference results in low-affinity inhibition of potassium channels. δ-KTx constitute a new subcategory of KTx that have Kunitz-type peptides in their structure and exhibit protease and potassium channel inhibition properties. Both λ-KTx with three disulfide bridges and ε-KTx with four disulfide bridges inhibit potassium channels with low affinity, similar to κ-KTx. In vitro experiments have demonstrated that voltage-gated K^+ channels play an important role in immune reactivity. Inhibition of their activity leads to inhibition of T-cell activation and the appearance of delayed-type hypersensitivity. In particular, butanthoxin, which is present in the venoms of three Brazilian scorpions T. serrulatus, T. bahiensis and T. stigmurus, reversibly blocks K^+ channels and inhibits T-cell proliferation and IL-2 production. [14, 15, 16].

Experimental studies of various scorpion families, including Vaejovidae, Euscorpiidae, Hemiscorpidae, Scorpionidae and Buthidae, have identified only a few calcium channel-specific toxins. The CaTx group typically contains 33–36 amino acids with 2–3 disulfide bridges. Most CaTx activate ligand-gated Ca^{2+} channels. A variety of calcins (hemicalcin, gadrocalcin, maurocalcin and opicalcin) have affinity for ryanodine receptors (RyRs), which regulate intracellular Ca^{2+} dynamics from the endoplasmic and sarcoplasmic reticulum. RyRs play an important role in the excitation-contraction processes of the heart and skeletal muscle. Calcins, acting as RyR agonists, regulate elevated intracellular Ca^{2+} levels and induce systolic paralysis. In addition, most calcins are amphiphilic and highly alkaline, which gives them the ability to penetrate inside the cell. [17, 18, 19].

ClTx is a class of peptides that can inhibit the influx of Cl^- ions into the cell membrane. Most members of this group consist of 35–38 amino acids with four disulfide bridges. The classic representative of ClTx is chlorotoxin (CTX), which consists of 36 amino acids and was first isolated from the venom of the giant yellow Israeli scorpion Leiurus quinquestriatus and exhibits a wide range of biological and pharmacological properties. ClTX has been shown to bind to neuroectodermal glioblastoma (GBM) due to its ability to penetrate the blood-brain barrier while showing minimal cross-reactivity with normal brain cells, which plays an important role in the diagnosis and treatment of glioma. [20, 21].

NDBPs are peptides of 13–56 amino acid residues with an extremely heterogeneous composition. In comparison to scorpion venom peptides containing disulfide bridges, NDBPs do not exhibit a conserved or predictable structure-function relationship. Most of these peptides are cationic molecules that exhibit remarkable structural flexibility. In aqueous solutions, these peptides are capable of conformational changes. However, in environments that mimic cell membranes, they readily adopt an amphipathic α-helical structure. This characteristic allows them to interact with a wide range of biological targets, but they do not have any specific molecular targets known to date. [22-24].

The vast majority of scientific sources contain data on the damage of the cardiovascular, respiratory, nervous and excretory systems by scorpion toxins [25-29]. A careful analysis of recent studies has shown a significant limitation of information on histological and biochemical changes in the liver under conditions of poisoning with scorpion toxins.

Fetaih H. A. et al. [30] in experiments on mice studied histopathological changes in the structure of the liver under the influence Androctonus amoreuxi scorpion

venom. 6 hours after the introduction of ¼ LD50, significant blood stasis was detected in the vessels of the organ. On the 4th day, hydropic degeneration of hepatocytes, karyolysis and karyorrhexis of nuclei were noted. An increase in the concentration of the venom, namely ½ LD50, after 9 hours of observation showed the appearance of extramedullary hematopoiesis islands in the liver and dilation of sinusoidal capillaries. Already on the 4th day of the study, at the indicated dose of venom, dilation of blood vessels, fibrinoid degeneration, and deposition of weakly basophilic homogeneous material in the portal zones of the organ were detected.

The bites of the scorpion Leiurus quinquestriatus have been proven to cause changes in the morphological organization of the rat liver. Histological examination of the organ after injection of the venom in a dose of 0.03 mg/kg revealed marked edema of hepatocytes, which led to a change in the shape of the cells and the disappearance of most sinusoidal capillaries. The cytoplasm of hepatocytes underwent vacuolization and the appearance of areas that were not stained with hematoxylin and eosin. Hypochromia of the nuclei and their pyknotic changes were noted. Degenerative changes in individual nuclei and chromatin margination were also observed. In some fields of view, hepatocyte necrosis and congestion in the portal vein branch were present. [31].

Clinical observations of patients after scorpion stings demonstrate cases of toxic hepatitis and coagulopathy. Biochemical studies under these conditions record an increase in ALT, AST, LDH, rarely hyperbilirubinemia. The mechanisms of liver damage caused by scorpion stings remain unclear, although the hypothesis of a direct hemolytic and cytotoxic effects of the venom prevails. In addition, stimulation of mediators (neurotransmitters, catecholamines) and the release of cytokines and inflammatory mediators are usually associated with hepatotoxicity and hematological disorders. In addition, intravascular hemolysis, coagulopathy, thrombocytopenia are characteristic, which may act as indirect factors of liver damage. [32].

CONCLUSIONS

Scorpion venom causes the development of local, cardiotoxic, neurotoxic effects and effects of autonomic nervous system. Depending on the predominance of a particular component in the venom, a wide range of clinical signs and symptoms can be observed from local reactions (hyperemia, pain, edema) to serious consequences, including respiratory, gastrointestinal, cardiac or neurological complications. The influence of toxic components on the structural and functional parameters of the mammalian liver is currently at the stage of comprehensive and thorough study. It has been established that under the conditions of administration of scorpion venom to experimental animals, hydropic degeneration and karyorrhexis of hepatocytes, fibrinoid necrosis, blood stasis in the vessels, an increase in the level of enzymes - ALT, AST, LDH were noted.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 02.03.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Dysregulation of immune and stress responses in post-traumatic stress disorder: Increased inflammation and altered stress signalling pathways

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ABSTRACT

Aim: The aim of this review is to summarize current knowledge on the interplay between immune activation and stress-response dysregulation in post-traumatic stress disorder, with particular emphasis on molecular and neurobiological mechanisms.

Materials and Methods: This narrative review was based on a structured search of the PubMed, Scopus, and Web of Science databases, including studies published between 2010 and 2025. Keywords related to post-traumatic stress disorder, inflammation, cytokines, stress response, and neuroendocrine regulation were used. Clinical and experimental studies addressing stress–immune interactions were included.

Post-traumatic stress disorder is increasingly conceptualized as a systemic condition involving persistent dysregulation of neuroendocrine and immune pathways. Disturbances of the hypothalamic–pituitary–adrenal axis, including altered glucocorticoid receptor sensitivity and impaired negative feedback regulation, coexist with increased sympathetic nervous system activity. These abnormalities promote activation of pro-inflammatory transcription factors such as nuclear factor kappa B and increased production of cytokines, including interleukin-1 beta, interleukin-6, tumour necrosis factor alpha, and interleukin-18. Additional mechanisms include activation of the NLR family pyrin domain containing 3 inflammasome, oxidative stress, mitochondrial dysfunction, and microglial activation, which contribute to neuroinflammation and impaired synaptic plasticity. These processes are associated with both psychiatric symptoms and increased risk of cardiovascular and metabolic comorbidities.

Conclusions: Post-traumatic stress disorder may be understood as a disorder of maladaptive stress–immune interaction. Targeting inflammatory pathways and stress-response mechanisms may improve diagnostic approaches and support the development of personalized therapeutic strategies.

KEYWORDS: allostasis, cytokines, glucocorticoid receptors, hypothalamo-hypophyseal system, psychoneuroimmunology

Wiad Lek. 2026;79(7):1667-1675. doi: 10.36740/WLek/222497 DOI

INTRODUCTION

Post-traumatic stress disorder is a trauma-related psychiatric condition that develops after exposure to a life-threatening or extremely distressing event. It is characterised by intrusive memories, avoidance, negative alterations in mood and cognition, and persistent hyperarousal. Although traditionally conceptualised as a disorder of psychological processing, increasing evidence indicates that post-traumatic stress disorder is also associated with persistent biological alterations involving neuroendocrine and immune systems. These changes may contribute not only to psychiatric symptoms but also to increased risk of somatic diseases [1].

A central mechanism in post-traumatic stress disorder is dysregulation of the hypothalamic–pituitary–adrenal axis, the primary system coordinating the stress response. In healthy individuals, activation of this axis leads to cortisol release, which exerts anti-inflammatory effects and restores homeostasis through negative feedback regulation. In post-traumatic stress disorder, altered glucocorticoid receptor sensitivity and impaired feedback inhibition have been observed. Some patients present with relatively reduced cortisol levels combined with increased receptor responsiveness, which may weaken immunosuppressive control and promote sustained inflammatory activity [2].

Patients with post-traumatic stress disorder also demonstrate increased activity of the sympathetic nervous system. Chronic sympathetic activation elevates catecholamine levels, particularly norepinephrine, contributing to hyperarousal. Immune cells express adrenergic receptors, and prolonged adrenergic stimulation enhances pro-inflammatory signalling. Together, dysregulation of the hypothalamic–pituitary–adrenal axis and sympathetic

overactivity create a state of persistent stress signalling and reduced inflammatory control [3].

At the molecular level, activation of nuclear factor kappa B promotes production of pro-inflammatory cytokines, including interleukin-1 beta, interleukin-6, tumour necrosis factor alpha, and interleukin-18 [4, 5]. Activation of the NLR family pyrin domain containing 3 inflammasome further amplifies inflammation through caspase-1–mediated cytokine maturation. These processes contribute to neuroinflammation, impaired synaptic plasticity, and increased cardiovascular and metabolic risk.

AIM

The aim of this review is to synthesise current evidence on the interplay between stress-response dysregulation and immune activation in post-traumatic stress disorder, with particular emphasis on neuroendocrine, inflammatory, and molecular mechanisms.

MATERIALS AND METHODS

This narrative review was conducted using a structured literature search in PubMed, Scopus, and Web of Science. Publications from 2010 to 2025 were included. The search strategy comprised combinations of the following keywords: post-traumatic stress disorder, hypothalamic–pituitary–adrenal axis, sympathetic nervous system, cytokines, inflammation, nuclear factor kappa B, NLR family pyrin domain containing 3 inflammasome, oxidative stress, mitochondrial dysfunction, microglia, and neuroinflammation.

Studies were included if they addressed neuroendocrine, inflammatory, or molecular mechanisms of post-traumatic stress disorder. Clinical studies in human populations were prioritised, while selected experimental studies, review articles, and meta-analyses were included when relevant. Studies not directly related to post-traumatic stress disorder or not addressing stress–immune mechanisms were excluded.

REVIEW

DYSREGULATION OF THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS IN POST-TRAUMATIC STRESS DISORDE

The hypothalamic–pituitary–adrenal axis is the main neuroendocrine system coordinating the physiological response to stress. Activation begins with the release of corticotropin-releasing hormone from the hypothalamus, which stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone. This hormone promotes cortisol release from the adrenal cortex. Cortisol mobilizes energy resources, supports cardiovascular adaptation, and exerts anti-inflammatory effects. Under normal conditions, cortisol binds to glucocorticoid receptors in the brain and peripheral tissues, activating negative feedback mechanisms that suppress further hypothalamic and pituitary stimulation, thereby restoring homeostasis [6, 7].

In PTSD, regulation of the HPA axis is altered. In contrast to major depressive disorder, which is often associated with elevated cortisol levels, PTSD frequently presents with relatively reduced basal cortisol concentrations in some patient groups. At the same time, increased sensitivity of glucocorticoid receptors has been reported. Enhanced receptor responsiveness may strengthen negative feedback inhibition, resulting in lower cortisol secretion despite persistent stress-related activation. This pattern reflects maladaptive regulation rather than simple endocrine hyperactivity [8, 9].

Genetic and epigenetic factors contribute to this dysregulation. Variations and epigenetic modifications in the gene encoding the glucocorticoid receptor and in the gene encoding FK506 binding protein 5 influence receptor sensitivity and stress reactivity. These alterations may arise following trauma exposure and contribute to long-term biological vulnerability by maintaining altered stress responsiveness [10, 11].

Impaired HPA axis function has direct consequences for immune regulation. Cortisol normally suppresses inflammatory gene expression and limits production of pro-inflammatory cytokines. When cortisol secretion is reduced or glucocorticoid signalling is dysregulated, anti-inflammatory control weakens. This may permit increased activation of NF-κB, a transcription factor that promotes expression of inflammatory mediators.

Consequently, elevated levels of IL-1β, IL-6, TNF-α, and IL-18 are observed in many individuals with PTSD [12, 13]. Altered HPA axis activity also affects brain function. Cortisol modulates hippocampal plasticity, amygdala reactivity, and prefrontal cortical regulation of emotional responses. Persistent dysregulation may impair fear extinction and cognitive control. Importantly, HPA axis dysfunction interacts with SNS activation and immune pathways, creating a physiological state characterized by sustained stress signalling and low-grade inflammation. These mechanisms link trauma exposure with chronic immune activation and increased risk of psychiatric and somatic complications in PTSD [14].

SYMPATHETIC NERVOUS SYSTEM HYPERACTIVITY AND IMMUNE MODULATION IN POST-TRAUMATIC STRESS DISORDER

The SNS is a core component of the stress response and functions in coordination with the HPA axis. Activation of the SNS leads to release of catecholamines, primarily

norepinephrine and epinephrine, which increase heart rate, blood pressure, and metabolic activity. Under physiological conditions, this response is transient. In PTSD, persistent sympathetic overactivity is frequently observed [15].

Patients with PTSD often demonstrate elevated resting heart rate, increased blood pressure variability, enhanced startle responses, and increased circulating norepinephrine levels.

Sustained adrenergic activation contributes to hypervigilance, sleep disturbances, and autonomic instability. Importantly, chronic SNS activation has significant effects on immune regulation [16].

Immune cells express adrenergic receptors, particularly beta-adrenergic receptors, which enable catecholamines to modulate immune function. Prolonged adrenergic stimulation enhances intracellular signalling pathways that activate NF-κB, promoting increased production of cytokines such as IL-6 and TNF-α. As a result, sympathetic overactivity contributes directly to a pro-inflammatory state [17].

A bidirectional interaction develops between stress signalling and immune activation. Elevated cytokines influence central nervous system (CNS) function and may further stimulate stress-responsive neural circuits. This interaction occurs through neural and humoral pathways and may sustain heightened arousal and emotional dysregulation [18].

Chronic catecholamine exposure also affects vascular and metabolic systems. Endothelial dysfunction, oxidative stress, and increased platelet activation contribute to elevated cardiovascular risk. Adrenergic effects on glucose metabolism and adipose tissue may increase susceptibility to insulin resistance and metabolic syndrome.

In the brain, excessive noradrenergic signalling alters activity in the amygdala, hippocampus, and prefrontal cortex, impairing fear extinction and cognitive control. Together with HPA axis dysregulation, sympathetic overactivity promotes sustained stress signalling and reduced anti-inflammatory control, contributing to persistent low-grade inflammation and systemic comorbidity in PTSD [19, 20].

PRO-INFLAMMATORY SIGNALLING PATHWAYS AND CYTOKINE DYSREGULATION IN POST-TRAUMATIC STRESS DISORDER

Persistent activation of inflammatory pathways is a central biological feature of PTSD. Trauma exposure and chronic stress are associated with sustained upregulation of pro-inflammatory signalling in both the peripheral immune system and the central nervous system. These alterations are closely linked to dysregulation of stress-response systems and contribute to psychiatric symptoms and systemic complications [21].

A key molecular regulator of inflammation in PTSD is NF-κB. Nuclear factor kappa B is a transcription factor that controls genes involved in immune activation and cytokine production.

In response to stress signals, oxidative stress, or adrenergic stimulation, NF-κB translocates to the nucleus and promotes transcription of pro-inflammatory mediators. Increased NF-κB activity enhances production of IL-1β, IL-6, TNF-α, and IL-18. Elevated circulating levels of these cytokines are frequently observed in individuals with PTSD, reflecting persistent low-grade inflammation [22].

Pro-inflammatory cytokines affect brain function through humoral and neural pathways. They influence neurotransmitter systems and impair synaptic plasticity. Increased IL-1β and TNF-α levels have been associated with reduced long-term potentiation, decreased hippocampal neurogenesis, and altered glutamatergic transmission. These changes may contribute to memory disturbances, heightened fear responses, and emotional dysregulation.

Inflammatory signalling interacts with oxidative stress. Chronic stress increases production of reactive oxygen species, which further activate NF-κB and damage cellular structures. Release of danger-associated molecular patterns sustains immune activation. Microglia respond by adopting a pro-inflammatory phenotype, maintaining neuroinflammation and impairing synaptic integrity [23]. Systemic inflammation also contributes to endothelial dysfunction and metabolic disturbances, increasing cardiovascular risk. Thus, NF-κB activation and cytokine overproduction link psychological trauma with neuroinflammation and somatic disease in PTSD.

THE ROLE OF THE NLRP3 INFLAMMASOME IN POST-TRAUMATIC STRESS DISORDER

The NLRP3 inflammasome is a multiprotein complex central to innate immune activation. It functions as a sensor of cellular stress and promotes maturation of pro-inflammatory cytokines. Evidence indicates that NLRP3 activation links chronic psychological stress with persistent inflammation in PTSD [24].

The complex consists of NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain, and caspase-1. Activation occurs in two stages. The priming stage involves transcriptional upregulation of inflammasome components through pathways such as NF-κB. The activation stage is triggered by intracellular danger signals, leading to assembly of the complex

and caspase-1 activation. Caspase-1 cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18 [25, 26].

Chronic stress promotes inflammasome activation through increased reactive oxygen species production, mitochondrial dysfunction, and release of mitochondrial deoxyribonucleic acid (DNA). HPA axis dysregulation and persistent SNS activation enhance priming via NF-κB signalling. In the CNS, microglia expressing NLRP3 produce IL-1β and IL-18, contributing to neuroinflammation and synaptic dysfunction. Inflammasome activation may also induce caspase-1–dependent pyroptosis, increasing neuronal vulnerability [27, 28]. Systemically, elevated IL-1β and IL-18 promote endothelial dysfunction and metabolic disturbances, linking NLRP3 activation with cardiovascular risk in PTSD.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION IN POST-TRAUMATIC STRESS DISORDER

Oxidative stress is an important mechanism linking chronic psychological stress with persistent inflammation in PTSD. It reflects an imbalance between the production of reactive oxygen species and the capacity of antioxidant systems to neutralize them. Although reactive oxygen species participate in physiological signalling, their excessive accumulation damages lipids, proteins, and nucleic acids, leading to cellular dysfunction [29].

Chronic activation of stress-response systems promotes increased generation of reactive oxygen species. Sustained stimulation of the sympathetic nervous system enhances metabolic demand and activates enzymes such as nicotinamide adenine dinucleotide phosphate oxidase, which produces superoxide radicals. Dysregulation of the HPA axis may further impair antioxidant defenses, including reduced activity of superoxide dismutase and glutathione peroxidase. Together, these mechanisms create a pro-oxidative state [30].

Mitochondria are a major source and target of reactive oxygen species. Under chronic stress, impaired oxidative phosphorylation increases electron leakage and reactive oxygen species production. Mitochondrial DNA is particularly vulnerable to oxidative damage due to limited repair capacity. Damage to mitochondrial DNA disrupts respiratory chain function, generating a self-perpetuating cycle of oxidative stress and mitochondrial dysfunction.

Neurons are especially sensitive to mitochondrial impairment because of high energy requirements. Disrupted mitochondrial dynamics may impair long-term potentiation and synaptic stability, particularly in the hippocampus and prefrontal cortex. These alterations may contribute to cognitive dysfunction and impaired emotional regulation [31].

Oxidative stress amplifies inflammation. Reactive oxygen species activate NF-κB, increasing transcription of pro-inflammatory cytokines. Mitochondrial damage promotes activation of the NLRP3 inflammasome, partly through thioredoxin-interacting protein–dependent mechanisms. This interaction sustains immune activation. Systemically, oxidative stress contributes to endothelial dysfunction and impaired insulin signalling, increasing cardiovascular and metabolic risk. Thus, oxidative stress and mitochondrial dysfunction represent central components of stress-related immune dysregulation in PTSD [32].

NEUROINFLAMMATION, MICROGLIAL ACTIVATION, AND IMPAIRED SYNAPTIC PLASTICITY IN POST-TRAUMATIC STRESS DISORDER

Neuroinflammation links stress-related immune dysregulation with brain dysfunction in PTSD. It reflects persistent activation of immune processes within the CNS and results from interactions between peripheral cytokines, glial cells, and neural circuits regulating emotion and cognition.

Microglia play a central role. Under physiological conditions, they support synaptic remodeling and homeostasis. In response to chronic stress, elevated cytokines, and danger-associated molecular patterns, microglia shift toward a pro-inflammatory phenotype characterized by increased production of IL-1β, TNF-α, and IL-6, largely regulated by NF-κB. Sustained microglial activation disrupts synaptic structure and function [33].

Pro-inflammatory cytokines impair long-term potentiation and reduce hippocampal neurogenesis. IL-1β interferes with synaptic plasticity, while TNF-α alters glutamate receptor trafficking, disturbing excitatory and inhibitory balance and promoting heightened reactivity within fear circuits.

The amygdala, hippocampus, and prefrontal cortex are particularly vulnerable. Increased amygdala activity contributes to exaggerated threat perception, whereas reduced hippocampal and prefrontal function impairs contextual processing and fear extinction. Neuroinflammation may underlie these abnormalities by affecting neuronal survival and synaptic connectivity.

Astrocytes further amplify inflammatory signalling. Activated astrocytes release cytokines and regulate glutamate uptake and blood–brain barrier integrity. Their dysfunction may enhance excitotoxicity and facilitate peripheral immune signalling into the brain [34].

Neuroinflammation also reduces proliferation of neural progenitor cells and may alter brain-derived

neurotrophic factor signalling. Interactions between inflammatory pathways, the HPA axis, and the SNS create a self-reinforcing cycle that sustains immune activation. Persistent glial activation therefore impairs neuroplasticity and contributes to cognitive and emotional symptoms in PTSD [35].

SYSTEMIC CONSEQUENCES OF STRESS–IMMUNE DYSREGULATION IN POST-TRAUMATIC STRESS DISORDER

Post-traumatic stress disorder is associated with increased risk of somatic diseases in addition to psychiatric symptoms. Persistent dysregulation of the hypothalamic–pituitary–adrenal axis, chronic activation of the sympathetic nervous system, and sustained inflammatory signalling contribute to systemic alterations. These mechanisms affect cardiovascular, metabolic, and immune function, supporting the concept of PTSD as a multisystem disorder [36].

Elevated cardiovascular risk is one of the most consistent findings. Individuals with PTSD show higher incidence of hypertension, coronary artery disease, and myocardial infarction. Chronic sympathetic activation increases heart rate, vasoconstriction, and blood pressure variability. Concurrently, persistent inflammation marked by elevated interleukin-6 and tumour necrosis factor alpha promotes endothelial dysfunction. Reduced nitric oxide bioavailability and increased oxidative stress impair vascular relaxation and accelerate atherosclerotic processes. Platelet activation further enhances thrombotic risk [37].

Endothelial dysfunction represents a central link between inflammation and vascular pathology. Pro-inflammatory cytokines increase adhesion molecule expression, facilitating leukocyte recruitment and vascular inflammation. Oxidative stress promotes lipid oxidation and structural vascular changes, impairing tissue perfusion over time.

Metabolic disturbances are also common. Chronic inflammation and altered stress hormone dynamics interfere with insulin receptor signalling, increasing the risk of insulin resistance and type 2 diabetes. Changes in cortisol regulation may promote visceral adiposity, which further amplifies inflammatory activity. This interaction between metabolic and inflammatory pathways sustains systemic dysfunction [38].

Chronic immune activation may also contribute to accelerated biological aging and increased susceptibility to autoimmune and neurodegenerative disorders. Sleep disturbances frequently observed in PTSD further enhance sympathetic activity and inflammatory signalling, increasing cardiovascular and metabolic risk.

Overall, persistent stress–immune dysregulation extends beyond the central nervous system. Chronic inflammation, autonomic imbalance, and metabolic alterations collectively increase somatic disease burden and may contribute to reduced life expectancy in PTSD [39].

POTENTIAL BIOMARKERS AND THERAPEUTIC IMPLICATIONS IN POST-TRAUMATIC STRESS DISORDER

Identification of biological markers in post-traumatic stress disorder (PTSD) may improve diagnostic precision and support individualized treatment. Persistent dysregulation of stress-response and immune pathways provides several candidate biomarkers reflecting underlying mechanisms [40].

Markers related to the HPA axis include basal cortisol levels, diurnal cortisol rhythm, and glucocorticoid receptor sensitivity. Altered cortisol dynamics and enhanced negative feedback responses have been described in subgroups of patients. Epigenetic modifications in genes regulating glucocorticoid signalling may indicate long-term stress vulnerability.

Pro-inflammatory cytokines are among the most studied biomarkers. Elevated concentrations of IL-1β, IL-6, TNF-α, and IL-18 have been reported in many patients with PTSD. C-reactive protein has also been associated with symptom severity. Despite variability across studies, a pattern of low-grade systemic inflammation is frequently observed [5].

Components of the NLRP3 inflammasome may represent more specific indicators of innate immune activation. Increased expression of inflammasome-related genes, elevated caspase-1 activity, and higher levels of mature IL-1β and IL-18 suggest enhanced inflammasome signalling. Markers of oxidative stress, including reduced antioxidant capacity and mitochondrial DNA alterations, further reflect cumulative cellular stress [7, 12].

These biological findings have therapeutic implications. Standard treatment includes psychotherapy and pharmacotherapy targeting neurotransmitter systems. However, persistent inflammation may reduce treatment responsiveness in some individuals. Anti-inflammatory agents, inhibitors of IL-1 signalling, and compounds targeting oxidative stress are being investigated. Modulation of SNS activity and lifestyle interventions improving sleep and metabolic health may also reduce inflammatory burden. Stratification of patients based on biological profiles may enable more targeted therapeutic approaches. Integration of neuroendocrine, inflammatory, and oxidative markers could

Table 1. Integrative stress–immune axis in post-traumatic stress disorder

Pathophysiological Level	Mechanism	Mediators	Functional Consequences
Stress exposure	Severe psychological trauma	Acute stress signaling	Activation of HPA axis and SNS
Neuroendocrine regulation	HPA axis dysregulation	Cortisol, glucocorticoid receptor	Impaired anti-inflammatory feedback
Autonomic activation	SNS hyperactivity	Norepinephrine, adrenergic receptors	NF-κB activation, cytokine production
Transcriptional control	NF-κB activation	IL-1β, IL-6, TNF-α, IL-18	Persistent low-grade inflammation
Innate immune activation	NLRP3 inflammasome	Caspase-1, IL-1β, IL-18	Amplified inflammatory signaling
Oxidative mechanisms	Mitochondrial dysfunction	Reactive oxygen species	Inflammasome activation, cellular damage
Central nervous system effects	Neuroinflammation	Microglia, astrocytes	Impaired synaptic plasticity
Systemic consequences	Endothelial dysfunction, metabolic alterations	Cytokines, oxidative stress	Cardiovascular and metabolic risk

Source: Compiled by the authors of this study

Table 2. Comparative neuroendocrine and inflammatory features across stress-related conditions

Feature	PTSD	Major Depressive Disorder	Chronic Stress
Basal cortisol	Often reduced	Often elevated	Variable
Glucocorticoid receptor sensitivity	Increased	Decreased	Mild alteration
SNS activity	Markedly increased	Moderately increased	Transient
NF-κB activation	Increased	Increased	Mild–moderate
NLRP3 inflammasome activation	Present	Partial evidence	Limited evidence
IL-1β / IL-6 / TNF-α	Elevated	Elevated	Slight increase
Neuroplasticity	Impaired	Impaired	Reversible changes
Cardiovascular risk	Increased	Increased	Usually low

Source: Compiled by the authors of this study

support development of precision strategies aimed at reducing both psychiatric symptoms and systemic risk in PTSD [18].

INTEGRATIVE MODEL, LIMITATIONS, AND FUTURE DIRECTIONS

PTSD can be conceptualized as a disorder of persistent interaction between stress-response systems and immune regulation. Severe stress activates the HPA axis and the SNS. In PTSD, altered glucocorticoid receptor sensitivity and sustained sympathetic overactivity reduce anti-inflammatory control and enhance immune activation [12–16].

Chronic stress promotes NF-κB activation, increasing transcription of pro-inflammatory cytokines and inflammasome components. Subsequent activation of the NLRP3 inflammasome, driven by oxidative stress and mitochondrial dysfunction, leads to maturation of IL-1β and IL-18, amplifying systemic and central inflammation [7, 8].

Neuroinflammation mediated by microglia and astrocytes disrupts synaptic plasticity and connectivity in the amygdala, hippocampus, and prefrontal cortex. Impaired neuroplasticity links molecular inflammation with hyperarousal, intrusive memories, and cognitive dysfunction. Persistent inflammation and endothelial dysfunction increase cardiovascular and metabolic risk [10, 15, 17–20].

To provide a structured summary of the sequential and interacting mechanisms linking stress exposure with immune activation and systemic consequences in PTSD, the principal components of the stress–immune axis are presented below (Table 1).

Current evidence is limited by population heterogeneity, methodological variability, and predominance of cross-sectional studies. Lack of standardized biomarker panels restricts translation. Future research should include longitudinal designs integrating molecular and neuroimaging data. Identification of inflammatory phenotypes may support development of biologically informed therapeutic strategies in PTSD [23].

For comparative context and to distinguish shared and disorder-specific patterns of neuroendocrine and inflammatory dysregulation, selected features of PTSD, major depressive disorder, and chronic stress are summarized below (Table 2).

DISCUSSION

Post-traumatic stress disorder may be conceptualised as a condition involving persistent interaction between stress-response systems and immune regulation. Available evidence suggests that dysregulation of the hypothalamic–pituitary–adrenal axis and sustained sympathetic nervous system activation are associated with increased inflammatory activity; however, it remains unclear whether these alterations represent primary mechanisms or secondary consequences of chronic stress exposure.

Activation of nuclear factor kappa B and increased cytokine production have been reported across multiple studies, although findings are not entirely consistent. Variability may reflect differences in study design, patient populations, comorbidities, and timing of biomarker assessment.

Similarly, activation of the NLR family pyrin domain containing 3 inflammasome has been proposed as a link between stress signalling and innate immune activation. While several studies indicate increased inflammasome activity, its causal role in disease progression remains to be fully established.

Evidence regarding cortisol dynamics is also heterogeneous. Some studies report reduced basal levels, whereas others show normal or variable concentrations. These discrepancies may be related to trauma type, chronicity, or methodological differences.

From a clinical perspective, integration of neuroendocrine and inflammatory markers may support identification of biologically distinct subgroups. However, current evidence is insufficient for routine clinical application.

LIMITATIONS

Current evidence is limited by the predominance of cross-sectional studies, heterogeneity of study populations, and lack of standardised biomarker assessment. Comorbid conditions may further influence neuroendocrine and inflammatory markers, complicating interpretation.

CONCLUSIONS

Post-traumatic stress disorder may be understood as a condition involving dysregulated interaction between stress-response systems and immune pathways. Although increasing evidence supports the involvement of neuroendocrine and inflammatory mechanisms, their exact contribution to disease onset and progression remains incompletely defined.

Future research should focus on longitudinal study designs, standardisation of biomarker assessment, and integration of molecular and clinical data. Identification of biologically defined subgroups may improve diagnostic precision and support the development of targeted therapeutic strategies.

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Funding
This study was supported by the National Center for Research and Development, a Polish executive agency as defined by the Act of 27 August 2009, on Public Finance (Journal of Laws of 2022, item 1634, as amended). The agency is responsible for implementing tasks related to the state's scientific, technical, and innovation policy. Funding was provided through the ERA-Net Neuron International Grant No. 20/2019.

CONFLICT OF INTEREST
The Author declares no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 01.03.2026
ACCEPTED: 11.05.2026

CREATIVE COMMONS 4.0

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REVIEW ARTICLE

CONTENTS

The right to die: Legal analysis of end-of-life decisions in Ukraine

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ABSTRACT

Aim: To analyze medical and legal aspects of end-of-life decision-making in Ukraine, focusing on euthanasia, assisted suicide, withdrawal of life-sustaining treatment, and advance directives. The objective was to evaluate arguments for and against legalization and to examine their relationship with patient autonomy and human rights.

Materials and Methods: An interdisciplinary qualitative approach was applied, combining medical, ethical, and legal perspectives. A systematic review of publications was conducted in PubMed, Scopus, and Web of Science. Ukrainian legislation, including the Civil Code, Criminal Code, and Health Law provisions, was analyzed alongside International Human Rights instruments and relevant jurisprudence of the European Court of Human Rights. When searching for information for this study, the following combinations of keywords were used: "euthanasia", "assisted suicide", "end-of-life decisions", "palliative care", "terminal illness", "patient rights", "right to life", "right to die", "advance directives", "withdrawal of life-sustaining treatment", "DNAR/DNR", "legal regulation in Ukraine", "European Court of Human Rights", "human rights and medicine". 37 sources were selected that fully corresponded to the results of the request.

Conclusions: Ukrainian law prohibits active euthanasia, while assisted suicide and withdrawal of life-sustaining treatment in unconscious patients remain unregulated and may fall under criminal liability. Withdrawal of futile therapy and opioid-based symptom management are permitted forms of terminal care. Comparative analysis shows that judicial practice of European court of human rights increasingly recognizes patient non-property rights and self-determination in end-of-life decision-making. Clarifying the legal status of assisted suicide and advance directives, with appropriate safeguards, may enhance patient rights and harmonize national law with European standards and will also make the legal status of medical workers more certain.

KEYWORDS: Euthanasia, Assisted Suicide, Terminal Care, Patient Rights, Advance Directives

Wiad Lek. 2026;79(7):1676-1684. doi: 10.36740/WLek/220855 DOI

INTRODUCTION

Currently, most diseases are treatable even if they cannot be completely cured, therefore patients can continue their lives even with some compromises. However, some functional conditions can significantly impact on a patient's life quality, moreover, eventually resulting in death, that is called terminal illness. The term "terminal illness" is defined as an irreversible or incurable disease from which death is expected in the foreseeable future [1]. Terminal illness is associated with wide variety of complications such as physical and psychological symptoms of current disease which are significantly impairing and gradually disrupting life of patients [2]. The most common complications affecting quality of life are anorexia and cachexia, constipation, anxiety and depression, dyspnea, fatigue, nausea and vomiting, pain and pain-related symptoms [3]. Moreover, patients with terminal illness can experience not only physical pain but also emotional and spiritual, thus severe and multifaceted pain is one of the greatest fears among such patients [4]. Systematic studies have shown that pain is the most prevalent increasing distressing symptom during last period of life, moreover, a "good death" among most of respondents is considered as a being pain-free [5]. It is crucially important to manage pain, other symptoms, psychosocial and psychiatric problems by end-of-life care which is a part of palliative care [6]. For all that, as WHO estimated that about 56,8 million patients need end-of-life care, but only 14% received it, thus such patients met poor quality of life and even traumatic death [7]. On the one hand, achievements in medicine made it possible to improve treatment of seriously ill patients and to prolong their life, however, on the other hand, as number of untreatable diseases shows that prolongation of life might not be a goal for all cases of terminal illnesses, thus the goal must be pointed at improvement of life quality and prevention and relief of suffering. In palliative care physicians may sometimes get a request for nontreatment decisions, therefore, such decisions lead to a life end. Request for life end is quite complicated including personal, psychological, social, legal, cultural, religious

and demographic factors, moreover, each request needs individual approach to patient and family with respect, attention, and sensitive communication [8].

The legal framework of the topic under consideration includes the personal non-property rights of an individual, in particular the right to life, which is a natural and inalienable human right. The right to medical care is also the subject of this study. Another important aspect of the issue of ending the life of a terminally ill persons at their request is the aspect of legal consequences that may arise for individuals who are participants in relations connected with the termination of life. First and foremost, this refers to medical professionals, but the subjects of the aforementioned relations may also include the relatives of the terminally ill person or other individuals. Thereby, end-of-life decisions and ways of death hastening are challenging topics, which clearly receives varying degrees of acceptance or lack of acceptance in different countries and relevant legal regulation.

AIM

The aim of the study was to provide an overview of the common end-of-life decisions with primary and secondary intentions, including actions and omissions, to analyze existed arguments and counterarguments for legalization in Ukraine. The study also aims to develop legal approaches to understanding the legal nature of end-of-life decisions and how they relate to the right to life and the right to medical care.

MATERIALS AND METHODS

This article is based on a comprehensive interdisciplinary analysis involving legal and medical dimensions of end-of-life decision-making in the context of Ukraine. The research methodology included a qualitative review of relevant scientific publications and legal documents. To ensure the objectivity and completeness of the analysis, a systematic search of peer-reviewed literature was conducted using international scientific databases such as PubMed, Scopus, and Web of Science. The search focused on studies addressing euthanasia, assisted suicide, palliative care, and patients' rights related to end-of-life decisions. Additionally, the research involved a doctrinal (normative) analysis of Ukrainian legislation, including the Civil Code, Criminal Code, and relevant health laws, as well as a review of international human rights instruments (e.g., the Convention for the Protection of Human Rights and Fundamental Freedoms) and jurisprudence of the European Court of Human Rights. This methodological approach allowed for a detailed examination of both existing legal frameworks and scientific discourse, contributing to a balanced understanding of the current status and challenges related to the right to die in Ukraine. When searching for information for this study, the following combinations of keywords were used: "euthanasia", "assisted suicide", "end-of-life decisions", "palliative care", "terminal illness", "patient rights", "right to life", "right to die", "advance directives", "withdrawal of life-sustaining treatment", "DNAR/DNR", "legal regulation in Ukraine", "European Court of Human Rights", "human rights and medicine". 37 sources were selected that fully corresponded to the results of the request.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

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

REVIEW AND DISCUSSION

Rights of patients with terminal illness or end-stage disease to make decision about end of life is a debatable question around the world, despite that, a growing number of countries support and legalize assisted dying of such patients [9]. Assisted dying is defined as «The involvement of healthcare professionals in the provision of lethal drugs intended to end a patient's life at their voluntary request, subject to eligibility criteria and safeguards. It includes healthcare professionals prescribing lethal drugs for the patient to self-administer («physician-assisted suicide») and healthcare professionals administering lethal drugs («euthanasia»)» [10]. Assisted suicide and euthanasia are the most discussed among medical end-of-life decisions, however, in this review we examine other ways potentially hastening death by direct or indirect actions or omissions of physician, patient or other individuals. Such end-of-life decisions will be discussed and classified in groups. From the one hand, regarding to a physician, the ending life decision can be based on certain actions or omissions depending on the reasons and intentions hastening the death of patient. From the other hand, relatively to a patient, end-of-life decision can be made on preference of the patient or his family, in this case it is important to assess the competence of patient or family to clarify the awareness in making such decision.

Table 1. End-of-life decisions

Ways of enactment	**1. Administration of life-terminating drugs with primary intention to end life** **2. Withdrawal or withholding of life-sustaining therapy** **3. Opioid-based therapy** **4. Assisted suicide** **5. Disconnection from life-support devices** **6. Advance refusal of resuscitation**					
Groups	**Euthanasia**		**Assisted suicide**	**Withdrawal of life-sustaining treatment in unconscious patients**		**Advance refusal of resuscitation**
Subgroups	Active	Passive	-	Proxy-directed withdrawal of life-sustaining treatment	Clinician-directed withdrawal of life-sustaining treatment	-
Content of the decision	Administration of life-terminating drugs with primary intention to end life	Withdrawal or withholding of life-sustaining therapy; Opioid-based therapy without primary intention to end life	Provision of life-terminating drugs; Prescriptions of life-terminating drugs	Withdrawal initiated by a legal representative or surrogate decision-maker, based on presumed will or best interests of the patient	Withdrawal initiated by medical personnel due to treatment futility or excessive burden, in line with legal or ethical standards	An individual's pre-determined decision to forgo life-restoring interventions in the event of clinical death, typically motivated by considerations related to organ donation or religious beliefs

Source: compiled by the authors of this study

In our view, end-of-life decisions can be implemented in several ways: administration of pharmacological agents or other substances leading to death, withdrawal or withholding of life-sustaining therapy, opioid-based palliative treatment, physician-assisted suicide, termination of mechanical life support, or advance refusal of resuscitative measures (Do Not Resuscitate order). These approaches can, in turn, be classified into four groups: euthanasia, assisted suicide, withdrawal of life-sustaining treatment in unconscious patients and advance refusal of resuscitation. This classification is grounded in a comprehensive criterion that considers both the decision-making subject and the purpose for which the decision is made (Table 1).

Euthanasia is defined as the intentional termination of life of a terminally ill patient at their own request, with the aim of alleviating suffering. Assisted suicide – in other words provision of life-terminating drugs or prescriptions. Withdrawal of life-sustaining treatment in unconscious patients is the termination of life of an individual who, due to objective medical conditions, is unable to make an independent and conscious decision to end their life (e.g., brain death, irreversible incapacitated states incompatible with life), based on the decision of third parties, in order to prevent existence under conditions deemed incompatible with human dignity. Advance refusal of resuscitation (or Do not attempt cardiopulmonary resuscitation – DNACPR (DNR)) – a preemptive decision made by an individual to forgo resuscitation in the event of clinical death, typically for the purpose of organ donation or based on religious or ethical beliefs [11].

1. Regarding euthanasia, several subtypes can be distinguished: active euthanasia, passive euthanasia.

1.1. Active euthanasia or administration of lethal drugs is commonly defined as an direct action of physician terminating life of a patient with uncurable disease in terminal phase and performed at the voluntary request of the patient with the purpose of ending of situation the patient considers intolerable, important to add, that physician is not only giving the way to end the life but also is an agent of death [12].

1.2. Passive euthanasia – the withholding or withdrawal of life-sustaining treatment, allowing the patient to die naturally; in certain cases, this may also include the administration of opioids aimed at relieving suffering, even if it may potentially hasten death. Considering decisions of withholding/withdrawing treatment before the death in most of cases are made because of the following: firstly, aggressive treatment in terminal stage of disease is often impairing a quality of life, secondly, there is a little or there is no chance of improvement and are commonly met in a very old patients or patient with dementia. However, the time when treatment of current disease or life-sustaining treatment should be cancelled is still debatable with strong intercultural differences. Results of conducted research have shown that treatments with potential high impact on life quality is rarely continued till death [13]. According to the rule of double effect, an action with the intent of benefits achievement

is morally acceptable even if an action brings a harmful side effects, if benefits outweigh the harm. The intent of withholding/withdrawing treatment reflects freedom from unwanted intervention thus hastening the natural death, which will occur because of the current disease. European Association for Palliative Care stated "Withdrawing/withholding ineffective, futile, burdensome, and unnecessary life-prolonging procedures or treatments doesn't constitute euthanasia or assisted suicide because it is not intended to hasten death but indicate the acceptance of death as a natural consequence of the underlying disease progression" [14]. From the other point of view, withdrawing the measures to support the patients' life and thus hastening the death can be considered as a passive euthanasia [15]. Based on the cited statement, it is possible to conclude that withholding/withdrawing treatment is not intended primarily to hasten death but does have secondary intention. Regarding pain alleviating treatment, then opioids are commonly used drugs for this purpose, however the doctrine of double effect should be taken in account as drugs which are possibly shortening the life of patients, in addition, existing court cases draw attention to use of morphine as "slow euthanasia" [16]. Indeed, opioids provide a good symptoms control, but along with that, opioids have many side effects, moreover, opioids use can result in respiratory suppression, analgetic tolerance, hyperalgesia and dependence [17]. Thus, on the one hand, pain and symptoms control using opioids, even if risk for shortening of life exist, is ethical [18], but, from the other hand, on the line with beneficial effect there is, with no intention, death hastening.

With regard to Ukrainian legislation, the definition of euthanasia can be found in Part 7 of Article 52 of the Law of Ukraine "On the Fundamentals of Health Care Legislation of Ukraine" (Law of Ukraine on Health Care) dated November 19, 1992, No. 2801-XII, which states: "Medical workers are prohibited from performing euthanasia – the deliberate acceleration of death or the mortification of a terminally ill person in order to end their suffering." [19].

Firstly, from the wording of the law, it is clear that euthanasia is explicitly prohibited. Secondly, based on the legal norm, the following characteristics of euthanasia can be distinguished:

(a) intentional nature of the act – euthanasia involves a person's awareness that their actions or inaction will result in death or mortification;

(b) purpose – the act is aimed at ending the suffering of a terminally ill person;

(c) subject – euthanasia is performed in relation to a terminally ill individual, i.e., someone whose illness is incurable;

(d) means – involves the acceleration of death or deliberate killing, rather than the natural course of dying;

(e) actors – the law directly refers to medical professionals, but the concept may be broader in practical or ethical discussions.

These elements define euthanasia in Ukrainian law and underscore its current prohibition, irrespective of the patient's consent or request.

2. Assisted suicide – the provision of means or information by a physician or other party, enabling a patient to end their own life voluntarily [20].

In our opinion, assisted suicide could involve several forms of action, first is providing the patient with a prescription for medications intended to end life, or pharmacologically-assisted suicide is generally defined as a practice when physician prescribes a lethal dose of medication, which a patient intends to use to end own life. It is essential to consider that physician only provides prescription to hasten death, but it is the patient who decides to use drug to end the life, therefore voluntary self-administration of drugs producing lethal effect by a patient, but also a patient is free to decide against self-administration, thus the responsibility for the final action lies with the patient who requested assistance [12]. Second, supplying the patient with life-ending medications; and third one refers to cases when a patient with terminal ill has voluntary will to end life but unable to do its own because of certain disability, thus patient requires physician to perform the ending life action [15]. In certain instances, assisted suicide is nearly indistinguishable from active euthanasia.

3. Withdrawal of life-sustaining treatment in unconscious patients includes disconnection from life-support equipment based on the decision of legal representatives and disconnection from life-support equipment based on the decision of medical professionals. This is often regulated through advance directives or substitute decision-making laws. In turn, disconnection by medical professionals may also be classified as withdrawal of life-sustaining treatment in unconscious patients, depending on the intention, for instance, to relieve suffering or to end life. In many jurisdictions, withdrawal of life support in cases of brain death or medical futility is not legally defined as euthanasia, but rather as allowing natural death especially when brain death equals legal death.

4. The last category related to end-of-life decisions is DNACPR – do not attempt cardiopulmonary resuscitation, sometimes called DNAR – do not attempt resuscitation or DNR – do not resuscitate or AND – allow natural death, but all these notions refer to the same purpose, in other words in case of cardiac arrest the physician should not try to restart heart function

or breathing, therefore resulting in death, thus this is about CPR only and it doesn't mean that patients will not get care, treatment and support [21]. DNAR decision can be made by person's own or by physician in case when CPR will cause physical or psychological harm or case history states about polyorganic pathology leading to end of life. In contrast to other end-of-life options, decision about DNAR order can be made at any time either when person is healthy or when approaching the end of life, secondly, DNAR decision can be written either for a short time or with no end date, in this case DNAR decision must be periodically reviewed [22].

Consequently, relatively to action/omission and intention, the end-of-life decisions made by physicians can be with primary and secondary intentions in case of withholding and withdrawing treatment when action or omission will hasten death. In turn, treatment which is directed only to relief pain or other symptoms can be considered as hastening of death with secondary intention, but administration/prescription of lethal drugs and DNAR order considered as with primary intention of death hastening.

The characteristics of the actor's actions as having a primary or secondary intention to hasten death may be taken into account in two distinct legal areas: (1) the legal regulation of relations related to the end-of-life decisions, and (2) the legal qualification of the actor's actions in case of investigation needed.

An analysis of the aforementioned end-of-life decisions in the context of Ukrainian legislation allows the following conclusions: active euthanasia is explicitly prohibited by law, and actions of medical professionals exhibiting characteristics of active euthanasia may be classified as criminal offenses under Articles 137 and 140 of the Criminal Code of Ukraine [23]. However, the situation regarding passive euthanasia is less obvious. Ukrainian law permits both the withholding or withdrawal of treatment and the administration of opioid-based therapy.

According to Part 4 of Article 284 of the Civil Code of Ukraine: 'An adult capable individual who is aware of the significance of their actions and able to control them has the right to refuse medical treatment' [24]. In turn, Part 4 of Article 43 of the Law on Health Care establishes that: 'A patient who has attained full civil capacity and is aware of the significance of their actions and able to control them has the right to refuse medical treatment' [25]. At the same time, a physician bears no liability for the patient's health in the event of the patient's refusal to follow medical instructions or failure to comply with a prescribed regimen [26].

The possibility of using opioids in medical care is provided, for example, by the Order of the Ministry of Health of Ukraine "On Approval of Medical Care Standards for Chronic Pain Syndrome in Adults and Children" dated 06 April 2023, No. 643 [27]. Thus, passive euthanasia, as a form of euthanasia and one of the methods for implementing end-of-life decisions, finds its legal basis within the current legislation of Ukraine.

Assisted suicide is not allowed under the Ukrainian legislation. Provision of life-terminating drugs and prescriptions of life-terminating drugs by medical or pharmaceutical worker can be punished according to Article 140 of the Criminal Code of Ukraine [28].

Withdrawal of life-sustaining treatment in unconscious patients is not permitted in Ukraine. In particular, according to Part 2 of Article 52 of the Law on Health Care: 'Active measures to sustain the life of a patient shall be discontinued if the patient's condition is determined to be irreversible death' [29]. Accordingly, advance refusal of resuscitation is currently outside the scope of Ukrainian law, and the failure to provide, without valid reasons, medical assistance to a patient by a healthcare professional who is obliged, according to established rules, to provide such care, and who is aware that omission may result in serious consequences for the patient, is punishable under Article 139 of the Criminal Code of Ukraine [30].

ARGUMENTS FOR LEGALIZATION END-OF-LIFE DECISIONS

For the specific group of people, a life is not always highest achievable value that should be protected and supported, that underlines the importance of personal autonomy and self-determination of end-of-life decisions. The autonomy of patients with uncurable disease in terminal phase that is conception in living own life, rights of self-determination [18]. An Article 8 of European Convention on Human Rights enshrines the right to bodily autonomy, although this is a rather extended interpretation of the said article, which states: «Everyone has the right to respect for his private and family life, his home and his correspondence. There shall be no interference by a public authority with the exercise of this right except such as is in accordance with the law and is necessary in a democratic society in the interests of national security, public safety or the economic wellbeing of the country, for the prevention of disorder or crime, for the protection of health or morals, or for the protection of the rights and freedoms of others.» [31]. In paragraph 51 of the Haas v. Switzerland case, the European Court of Human Rights emphasized that one of the aspects of the right to respect for private life (Article 8 of the Convention) is the individual's right to decide in what manner and at what point their life will

end, provided that they are capable of freely making such a decision [32]. The favor of such decision is to relief the severe unbearable suffering and when there is no prospect to relief, moreover when avoiding or ending suffering cannot be achieved in any other way that is acceptable to the patient. Important to add, as experience of some countries shows that diagnosis of terminal ill must be confirmed at least by two doctors and life-expectancy of 6 month or less [33].

The lack of moral demarcation between direct actions leading to death as assisted suicide or euthanasia and indirect actions such as withdrawal/withholding treatment, treatment of pain and other symptoms, if these actions are not to be in the best interest of patient and, moreover, shorten life or hastening death.

Ukraine is currently undergoing a reform of its civil legislation, within the framework of which the Concept for the Renewal of the Civil Code of Ukraine (hereinafter referred to as "the Concept") was submitted for public discussion. According to the Concept, specifically § 2.9 of Book Two entitled Personal Non-Property Rights, the potential legalization of euthanasia and assisted suicide is being considered. It is proposed to re-evaluate the issue of legalizing passive euthanasia and assisted suicide, taking into account the jurisprudence of the European Court of Human Rights (ECHR), alongside the introduction of corresponding amendments to the Criminal Code of Ukraine [34]. This indicates a normative trend toward the liberalization of legal relations associated with the conscious termination of life in terminally ill individuals [32]. Such a direction aligns with broader pan-European legal trends and the established case law of the ECHR. Nonetheless, as of the time of this study, the proposed amendments have not yet been enshrined in national legislation.

COUNTERARGUMENTS FOR LEGALIZATION END-OF-LIFE DECISIONS

From the moral point of view, which is based on the religious beliefs, no person can have rights to end life, therefore all actions or omissions with primary or secondary intentions are against human nature and reflect degradation of humanity. An Article 2 of European Convention on Human Rights enshrines the *right to life*, where is stated that: «Everyone's right to life shall be protected by law. No one shall be deprived of his life intentionally save in the execution of a sentence of a court following his conviction of a crime for which this penalty is provided by law. Deprivation of life shall not be regarded as inflicted in contravention of this Article when it results from the use of force which is no more than absolutely necessary: (a) in defense of any person from unlawful violence; (b) in order to effect a lawful arrest or to prevent the escape of a person lawfully detained; (c) in action lawfully taken for the purpose of quelling a riot or insurrection.» [31]. In particular, the European Court of Human Rights has emphasized that the right to die is fundamentally opposed to the right to life and cannot be derived from it. Accordingly, Article 2 of the Convention cannot be interpreted as guaranteeing a right to die. This position was clearly articulated in Pretty v. the United Kingdom case, where the Court stated that "Article 2 cannot, without a distortion of language, be interpreted as conferring the diametrically opposite right, namely a right to die" [35].

Additionally, palliative care provides alternatives to relieve severe suffering in most of cases thus legalization of end-of-life procedures may lead to the lack of development and devaluation of palliative care. Legalization of end-of-life procedures may cause tendency of physicians to choose ending of life more frequently and easier to avoid suffering for unconscious patients, patients with dementia or mental diseases and infants that is a category of patients who are incompetent. Also, scientific data shows that up to half of patients with cancer have symptoms of depression, in turn, depression often manifests somatically, thus enhances symptoms of current disease, hence, it is hard to assess the competence of patient and real degree of suffering from terminal illness with sufficient certainty [36]. The main principle of medical education is the patient as the main value of the medical system; therefore, physicians are trained to protect quality and quantity the lives of patients, thus physicians' involvement in the ending life goes against primary principles and will affect ethics of medical practice. Death certificate requires to indicate the cause of death, thus in case of assisted suicide or euthanasia the cause of death is defined because of lethal dose of drugs but not the underlying illness. As statistical data shows that in countries where assisted suicide is legal the total suicides number has been increased compared to period when such procedures were illegal [37].

The initial point for end-of-life decision making can be a prognosis of the patient's current disease, quality and quantity of remaining life and perspectives of possible treatment, also how much the treatment is harmful and could more affect remaining life, consideration of possibilities of alternative treatment and its impact, analysis of similar clinical cases and statistical data in order to compare the effectiveness of treatment and life expectance, also degree of accompanied suffering. Finally, if patient or physician tent to come to end-of-life decision, thus important to specify the ways of death hastening between possible and certain end of life, which depends on patients' condition, action or omission, primary or secondary intention.

COMMON PROPOSALS FOR REGULATION

From the perspective of regulating relations connected to end-of-life decisions under civil law of Ukraine, it should be noted that certain measures, such as the withholding or withdrawal of treatment and the administration of opioids, are provided for under current Ukrainian legislation. Although these measures are not directly aimed at ending a patient's life, they may, to some extent, hasten death. Such decisions exhibit characteristics of passive euthanasia, which has been recently proposed for legalization in the Draft Concept for the Revision of the Civil Code of Ukraine. The same Draft Concept also proposes the legalization of assisted suicide as encompassed by the right to life, which generally corresponds to the current practice of the ECHR and the philosophical-legal ideas of the perception of natural human rights.

At the same time, it appears appropriate to supplement civil legislation with the right to refuse resuscitation. Clearly, such a refusal must be expressed by a legally competent individual in an unequivocal manner and documented appropriately (potentially with notarization). Unlike withdrawal of life-sustaining treatment in unconscious patients for individuals who, due to their medical condition, are unable to express their end-of-life decision, raising doubts about the validity of such a decision, "Do Not Resuscitate" order allows for a clear and verifiable expression of the individual's prior will regarding the non-application of resuscitative measures. The legalization of DNR would allow individuals to make an autonomous choice in favor of consciously terminating life rather than persisting in a state of helplessness or existence in conditions compromising human dignity, which may result from clinical death, coma, or brain injuries associated with these conditions. Furthermore, the legalization of DNR would define the limits of legal liability for healthcare professionals involved in such practices.

CONCLUSIONS

Through the study of legal regulations and scientific literature, we have delineated the primary methods for the realization of life-termination decisions: 1) administration of life-terminating drugs with the primary intention of ending life; 2) withdrawal or withholding of life-sustaining therapy; 3) opioid-based therapy; 4) assisted suicide; 5) disconnection from life-support devices; 6) advance refusal of resuscitation. The identified methods can be systematized into four groups: (1) euthanasia (active and passive); (2) assisted suicide; (3) Withdrawal of life-sustaining treatment in unconscious patients (Proxy-directed withdrawal of life-sustaining treatment; clinician-directed withdrawal of life-sustaining treatment); (4) advance refusal of resuscitation.

We determined that certain end-of-life decisions are expressly prohibited by the current legislation of Ukraine (active euthanasia), some, although not explicitly prohibited, but fall within the scope of criminal law by creating or constituting elements of specific offences (assisted suicide, withdrawal of life-sustaining treatment in unconscious patients, advance refusal of resuscitation), and others are the part of existing medical practice within the legal framework (passive euthanasia).

In scope of modern philosophical and legal views on natural human rights and prevailing judicial practice, we consider that legalization of assisted suicide and advance refusal of resuscitation is justified, insofar as these measures can be verified in accordance with the valid will of the person and are directed toward a dignified termination of life as an element of the content of the right to life.

Further research within this topic will be focused on the medical, ethical, legal, technological, and organizational prerequisites for the legalization of the aforementioned end-of-life decisions.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 18.10.2025
ACCEPTED: 21.04.2026

CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Emerging therapeutic targets beyond amyloid and tau in Alzheimer's Disease: Implications for lifestyle, metabolism and preventive medicine

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ABSTRACT

Alzheimer's disease (AD) is the leading cause of dementia and remains a major global health challenge. Although current disease-modifying therapies primarily target amyloid-β and tau pathology, their clinical efficacy is limited. Increasing evidence indicates that AD is a multifactorial disorder involving neuroinflammation, mitochondrial dysfunction, impaired autophagy-lysosomal pathways, and dysregulated lipid metabolism. These interconnected mechanisms contribute to disease progression and represent promising therapeutic targets. This narrative review summarizes current evidence on emerging treatment strategies beyond the classical amyloid and tau paradigm, with particular emphasis on microglial activation, mitochondrial function, autophagy, and lipid homeostasis. Additionally, the translational potential of lifestyle-based interventions, including physical activity and metabolic regulation, is discussed. A broader understanding of AD pathogenesis may support the development of integrated therapeutic approaches combining pharmacological and non-pharmacological strategies to improve clinical outcomes.

KEYWORDS: Alzheimer's disease, neuroinflammation, microglia, metabolism, physical activity, prevention

Wiad Lek. 2026;79(7):1685-1691. doi: 10.36740/WLek/226612 DOI

INTRODUCTION

Alzheimer's disease (AD) is one of the most prevalent neurodegenerative disorders. The underlying pathological process is characterized by a progressive and irreversible disruption of cellular homeostasis, leading to gradual neuronal dysfunction and death. These changes are associated with the accumulation of amyloid-β deposits, the formation of neurofibrillary tangles composed of hyperphosphorylated tau protein, and disturbances in calcium (Ca^{2+}) homeostasis. Neuronal loss is further promoted by neuroinflammatory mechanisms, including phagocytic activity and the release of inflammatory mediators such as cytokines, reactive oxygen species, and nitric oxide.

The persistent activation of microglia and astrocytes contributes to neuronal apoptosis and impairment of the blood–brain barrier integrity. Consequently, these alterations facilitate the infiltration of peripheral immune cells into the central nervous system, enhancing and sustaining the local inflammatory response. Dysregulation of inflammatory control mechanisms ultimately contributes to impaired brain function and widespread neurodegeneration [1].

Despite extensive research efforts, therapeutic strategies targeting the classical pathological hallmarks of AD have demonstrated limited clinical efficacy [2]. Recent comprehensive studies indicate that AD pathogenesis involves a complex network of interacting mechanisms extending beyond amyloid and tau pathology [3]. Therefore, AD is increasingly recognized as a multifactorial and systemic disorder involving the interplay of neuroinflammation, metabolic disturbances, mitochondrial dysfunction, and lipid metabolism abnormalities [4-6].

AIM

The aim of this review is to analyze emerging therapeutic targets in Alzheimer's disease beyond the classical amyloid and tau paradigm, with particular emphasis on their translational relevance and potential integration with lifestyle-based and preventive strategies.

MATERIALS AND METHODS

A narrative literature review was conducted using the PubMed, Scopus, Web of Science, and Google Scholar databases. Articles published between 2008 and 2024 were considered, with priority given to systematic reviews, meta-analyses, and high-impact original research studies.

The search strategy included the following keywords and terms: Alzheimer's disease, neuroinflammation, microglia, mitochondrial dysfunction, autophagy, lipid metabolism, and emerging therapies. Only articles published in English were included.

The review was performed in accordance with established methodological principles for narrative literature reviews.

REVIEW

ALZHEIMER'S DISEASE AS A NETWORK DISORDER

Although amyloid-β (Aβ) and tau pathology remain central components of the classical model of Alzheimer's disease (AD), these mechanisms alone do not fully explain disease progression or the limited efficacy of current therapeutic approaches [2,7]. Increasing evidence supports the concept of AD as a network disorder involving multiple interacting biological systems and pathological pathways [4-6].

AD may be considered not solely as a disease of the brain, but as a progressive system-level disorder associated with chronic network stress and disruption of homeostatic mechanisms. Such disturbances may arise from various endogenous and exogenous factors, including chronic inflammatory states, infections, vascular dysfunction, traumatic brain injury, environmental toxicity, and immune dysregulation. Regardless of whether the initiating factors originate within the central nervous system (CNS) or in peripheral tissues, chronic stress, inflammation, and toxic stimuli can be transmitted to the CNS through humoral and neural pathways. These processes may preferentially affect highly interconnected regulatory regions and neuronal circuits, eventually contributing to the development of neurodegenerative changes [8].

NEUROINFLAMMATION AND MICROGLIAL DYSFUNCTION

According to the hypothesis proposed by Krstic and Knuesel [9], neuroinflammation may represent a primary mechanism underlying AD pathogenesis, resulting from excessive activation of astrocytes and microglia. Supporting evidence for this concept has been provided by epidemiological studies suggesting a potential protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) [10]. However, this hypothesis has not been confirmed in randomized clinical trials, meta-analyses, or clinical studies involving AD patients. Nevertheless, ibuprofen, a cyclooxygenase inhibitor and peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist, is currently being investigated in combination with cromolyn, an anti-allergic agent that inhibits mast cell degranulation [11].

Chronic inflammation, metabolic disturbances, hormonal alterations, and immune dysregulation are common features of numerous chronic diseases affecting the CNS, including AD. Recent studies indicate that chronic inflammation, whether originating centrally or peripherally, may act both as a cause and a consequence of progressive disturbances in homeostatic regulation and physiological processes underlying complex diseases [12-15].

Neuroinflammation is considered a major contributor to AD pathogenesis. Microglia exhibit both neuroprotective and neurotoxic properties depending on their activation state and the surrounding microenvironment [16,17]. Genetic studies have identified triggering receptor expressed on myeloid cells 2 (TREM2) and CD33 as important regulators of microglial function and AD susceptibility [18,19]. These discoveries have stimulated the development of therapeutic strategies aimed at modifying microglial activity and restoring immune balance rather than completely inhibiting inflammatory responses [20]. Consequently, neuroinflammation, particularly microglial dysregulation, remains one of the most important mechanisms contributing to disease progression [21].

MITOCHONDRIAL DYSFUNCTION AND METABOLIC ALTERATIONS

Mitochondria are essential cellular organelles involved in ATP production, regulation of apoptosis, calcium homeostasis, and iron metabolism. Due to their high energy requirements and limited regenerative capacity, neurons are particularly dependent on efficient mitochondrial function.

Numerous studies have demonstrated that mitochondrial dysfunction represents an important pathological feature of AD. Metabolic impairment appears early during disease development and contributes to increased neuronal vulnerability [22,23]. Mitochondrial abnormalities result in decreased energy production, impaired cellular metabolism, and enhanced oxidative stress [22]. Furthermore, accumulating evidence indicates bidirectional interactions between mitochondrial dysfunction and pathological protein aggregation.

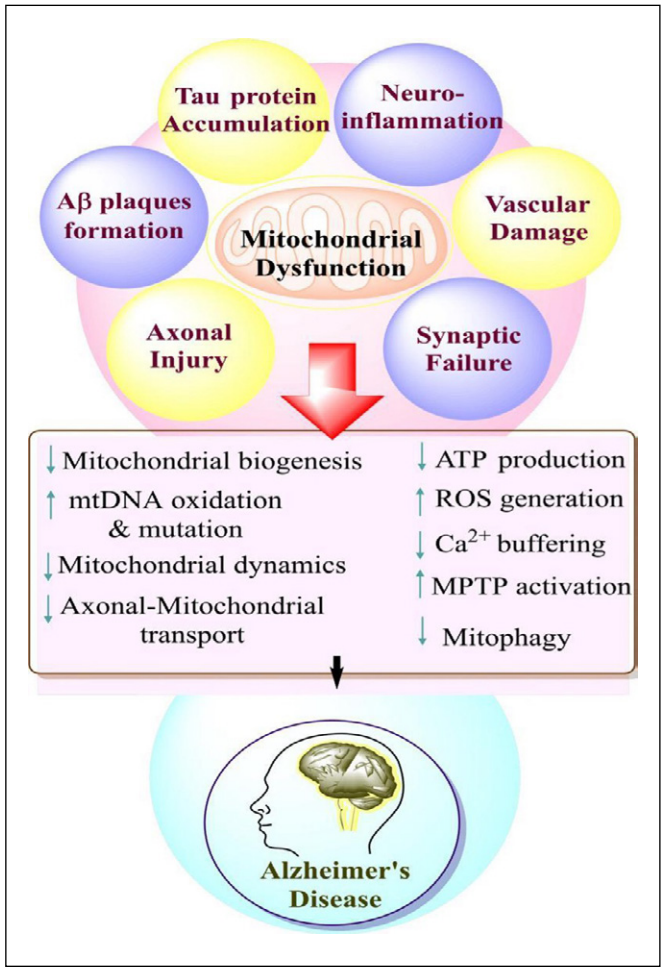

Figure 1. Bhatia S, Rawal R, Sharma P, Singh T, Singh M, Singh V. Mitochondrial Dysfunction in Alzheimer's Disease: Opportunities for Drug Development. Current neuropharmacology, 2022;20(4):675–692. https://pubmed.ncbi.nlm.nih.gov/33998995/ [29]

While impaired mitochondrial activity may promote the accumulation of toxic protein species, restoration of mitochondrial function may potentially reduce these pathological processes. Therefore, elucidating the relationship between mitochondrial bioenergetics and classical AD-related pathways is essential for the identification of novel therapeutic approaches [24].

Experimental studies investigating the association between mitochondrial DNA (mtDNA) alterations and AD have provided important insights into disease mechanisms. In one experimental model, Ntera2D1 (NT2) cells depleted of endogenous mtDNA and repopulated with platelet-derived mtDNA obtained from AD patients demonstrated reduced cytochrome c oxidase activity, increased production of reactive oxygen species (ROS), and enhanced activation of antioxidant defense mechanisms. These findings suggest that mtDNA alterations may contribute to mitochondrial dysfunction and oxidative stress in AD [25].

Additionally, mtDNA deletions have been associated with increased expression of cell cycle arrest-related proteins, including p21 and p53, together with further impairment of cytochrome c oxidase activity, ultimately promoting ROS-mediated neuronal injury [26]. Mitochondrial dysfunction also contributes to amyloid pathology, as increased oxidative stress and ROS generation may enhance amyloid precursor protein (APP) processing, resulting in increased production and accumulation of amyloid-β (Aβ) [27].

Therapeutic strategies targeting mitochondrial pathways and metabolic regulation are currently under active investigation as potential approaches for modifying AD progression [23, 28].

AUTOPHAGY AND PROTEIN CLEARANCE

Dysregulation of autophagy is increasingly recognized as an important contributor to Alzheimer's disease (AD) progression. The accumulation of pathological proteins in AD results not only from their increased production but also from impaired mechanisms of cellular clearance [30,31]. The autophagy–lysosomal system plays a crucial role in the removal of misfolded proteins and damaged cellular organelles, thereby maintaining neuronal homeostasis.

In AD, impairment of autophagic flux, lysosomal dysfunction, and reduced degradative capacity contribute to the accumulation of amyloid-β (Aβ) and hyperphosphorylated tau within neurons, promoting cellular toxicity and neuronal loss. Studies have demonstrated abnormal accumulation of autophagic vacuoles in dystrophic neurites of AD brains, suggesting a defect in efficient clearance mechanisms rather than excessive initiation of autophagy. Moreover, impaired lysosomal acidification disrupts enzymatic degradation processes and further promotes pathological protein aggregation [31].

Importantly, autophagy dysfunction is closely interconnected with other major pathological mechanisms of AD. Oxidative stress and mitochondrial dysfunction may impair autophagic pathways, while accumulated protein aggregates can further compromise lysosomal function, creating a self-perpetuating cycle of neuronal injury.

Current therapeutic approaches aim to enhance autophagic activity and restore lysosomal homeostasis. Pharmacological modulation of pathways involving mechanistic target of rapamycin (mTOR), transcription factor EB (TFEB), and lysosomal biogenesis is under investigation as a potential disease-modifying strategy. Therefore, regulation of autophagy may represent a promising therapeutic direction for reducing pathological protein accumulation and preserving neuronal function in AD.

LIPID METABOLISM AND APOE

Disruption of lipid metabolism represents an important component of AD pathogenesis. The apolipoprotein E (APOE) ε4 allele is the strongest genetic risk factor associated with late-onset AD and plays a critical role in lipid transport, cholesterol metabolism, and neuronal homeostasis [32].

APOE participates in lipid trafficking, neuronal repair mechanisms, and maintenance of synaptic integrity. The ε4 isoform has been associated with impaired lipid transport, reduced synaptic plasticity, and increased amyloid-β (Aβ) aggregation [32]. Alterations in cholesterol homeostasis may influence amyloid precursor protein (APP) processing by promoting amyloidogenic pathways. Furthermore, changes in neuronal membrane lipid raft composition can affect the activity of secretases involved in Aβ generation.

Lipid abnormalities also contribute to neuroinflammatory processes. Disturbed lipid signaling may activate microglia and astrocytes, resulting in increased production of pro-inflammatory mediators. In addition, alterations in phospholipid and sphingolipid metabolism have been associated with synaptic dysfunction and progressive neuronal degeneration.

Therapeutic strategies aimed at restoring lipid homeostasis, including modulation of cholesterol metabolism, APOE-associated pathways, and lipid signaling networks, may provide multidimensional benefits and represent potential approaches for slowing AD progression [33].

RECEPTOR-MEDIATED PATHWAYS

Receptor-mediated mechanisms contribute significantly to AD-related pathology. The receptor for advanced glycation end products (RAGE) is involved in oxidative stress induction, neuroinflammatory signaling, and the transport of amyloid-β across the blood–brain barrier [34].

In addition to receptor-mediated mechanisms, systemic factors such as vascular dysfunction, chronic inflammation, and peripheral metabolic disturbances may influence disease progression [5]. These observations further support the concept of AD as a complex systemic disorder involving interactions between central nervous system mechanisms and peripheral physiological processes.

DISCUSSION

The limited clinical efficacy of amyloid-targeting therapies has emphasized the need for a more comprehensive understanding of Alzheimer's disease (AD) pathogenesis [2]. Current evidence supports a multifactorial model in which several interconnected pathological processes contribute to disease initiation and progression rather than a single dominant mechanism [4,5].

Neuroinflammation and microglial dysfunction are increasingly recognized as key contributors to AD pathophysiology. However, microglial activation represents a complex process that may exert both neuroprotective and neurotoxic effects depending on the cellular environment, disease stage, and specific molecular pathways involved [16,17]. Therefore, therapeutic strategies aimed at restoring immune balance may be more beneficial than complete suppression of inflammatory responses.

Similarly, metabolic disturbances and mitochondrial dysfunction are closely associated with neuronal vulnerability and disease progression. Impaired mitochondrial bioenergetics, increased oxidative stress, and altered cellular metabolism may interact with classical AD-related mechanisms, contributing to progressive neuronal dysfunction and degeneration [22,23].

Defects in protein clearance pathways further promote the accumulation of toxic aggregates and amplify pathological processes. Dysfunction of the autophagy–lysosomal system, together with impaired removal of amyloid-β and tau species, represents an important mechanism linking intracellular homeostasis disruption with neuronal injury [30,31]. In addition, lipid metabolism abnormalities and APOE-associated pathways contribute to disease complexity by influencing amyloid processing, synaptic function, and neuroinflammatory responses [32].

Collectively, these findings support the development of multi-target therapeutic approaches capable of addressing several interconnected pathological mechanisms simultaneously. Such strategies may include modulation of neuroinflammation, restoration of metabolic and mitochondrial function, enhancement of protein clearance pathways, and correction of lipid homeostasis disturbances.

Moreover, non-pharmacological interventions, including regular physical activity, may influence multiple disease-related mechanisms by improving metabolic regulation, reducing inflammatory responses, and supporting mitochondrial function. These observations highlight the importance of integrated approaches that combine pharmacological and lifestyle-based strategies in future AD prevention and treatment.

CONCLUSIONS

The traditional view of Alzheimer's disease (AD) as a disorder primarily driven by amyloid-β and tau pathology is gradually evolving toward a broader concept

of AD as a complex, multifactorial disease involving multiple interconnected biological systems. Current evidence indicates that neuroinflammation, metabolic disturbances, impaired protein clearance mechanisms, mitochondrial dysfunction, and lipid dysregulation represent important contributors to disease progression.

This expanded understanding of AD pathophysiology has important implications for therapeutic development. Given the network-based nature of the disease, interventions targeting a single pathological pathway may have limited efficacy, particularly during advanced stages of neurodegeneration. Future therapeutic strategies should therefore focus on integrated, multi-target approaches aimed at modulating several disease-related mechanisms simultaneously.

Further research should prioritize personalized therapeutic models that combine molecular biomarkers, clinical characteristics, and lifestyle-related factors to optimize prevention strategies and improve individualized patient outcomes.

PRACTICAL IMPLICATIONS

The recognition of AD as a multifactorial disorder has important implications for clinical practice, preventive medicine, and health promotion, emphasizing the need for interdisciplinary cooperation among neurology, internal medicine, rehabilitation, and exercise sciences.

Regular physical activity represents one of the most promising non-pharmacological interventions, as it may positively influence several mechanisms involved in AD progression, including neuroinflammatory regulation, mitochondrial function, vascular health, and cerebral blood flow [16,21]. Preventive strategies should also include lifestyle modifications such as maintaining a balanced diet, optimizing body weight, and improving metabolic health, including insulin sensitivity, which may contribute to reducing the risk of cognitive decline [22,23].

In clinical practice, combining lifestyle-based interventions with pharmacological therapies may provide additional benefits by addressing multiple pathological pathways simultaneously [32]. Furthermore, the development and implementation of reliable biomarkers may facilitate earlier identification of individuals at increased risk and enable timely preventive interventions before extensive neurodegenerative changes occur.

Overall, a holistic approach integrating pharmacological treatment, lifestyle modification, early risk assessment, and personalized care may represent a promising direction for improving long-term outcomes in individuals at risk of or affected by AD.

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CONFLICT OF INTEREST

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RECEIVED: 20.03.2026
ACCEPTED: 30.06.2026

CREATIVE COMMONS 4.0

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REVIEW ARTICLE

CONTENTS

The role of genetic predictors in PTSD development

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ABSTRACT

Aim: To analyse the role of polymorphic sites in genes encoding key regulators of the hypothalamic-pituitary-adrenal axis and the neurotrophic system, which play an important role in determining genetic susceptibility to the development of post-traumatic stress disorder.

Materials and Methods: To prepare this review, a systematic search was conducted in the Scopus, PubMed and ResearchGate databases using keywords relating to the genetic aspects of PTSD development. The analysis included publications from the last ten years that examined molecular-genetic mechanisms, the role of polymorphisms in shaping individual vulnerability to stress, and their impact on the regulation of the hormonal response.

Conclusions: Genetic variability is an important factor determining individual differences in susceptibility to PTSD. Studying polymorphisms in investigated genes helps to explain the difference between resilience and susceptibility to mental disorders following traumatic events. This will help to improve the development of personalised approaches to the diagnosis, prognosis and treatment of PTSD, and opens up prospects for the creation of new therapeutic strategies that take into account patients' individual genetic characteristics.

KEYWORDS: stress, post-traumatic stress disorder, hypothalamic-pituitary-adrenal axis

Wiad Lek. 2026;79(7):1692-1699. doi: 10.36740/WLek/231365 DOI

INTRODUCTION

Post-traumatic stress disorder (PTSD) is an exhausting mental health disorder that develops following exposure to extremely stressful or traumatic events, such as war, accidents, natural disasters or sexual violence. It is characterised by persistent symptoms that go beyond a normal adaptive response to stress and significantly impair the patient's quality of life. The most common symptoms fall into several categories: recurrent experiences related to the traumatic event (flashbacks, nightmares, intrusive thoughts about the event), avoidance (consciously avoiding places, people or situations that remind one of the trauma), hyperarousal (increased anxiety, irritability, sleep disturbances, difficulty concentrating), and negative changes in cognition and mood (feelings of detachment, guilt, and a loss of interest in activities that were previously enjoyed) [1].

PTSD is a complex, multifactorial condition in which numerous pathogenic mechanisms and factors play a role. The involvement of the hypothalamic-pituitary-adrenal axis, as well as dopaminergic, serotonergic, adrenergic, neurotrophic and other systems, has been confirmed [2, 3, 4]. Considering that the pathogenesis of PTSD is quite complex and involves a large number of systems, it was decided in this review to focus on disorders relating to the hypothalamic-pituitary-adrenal axis and the neurotrophic system.

Given that the functional properties of proteins are determined by the structural characteristics of their genes, changes of the DNA nucleotide sequence – such as single-nucleotide polymorphisms – may disrupt regulatory mechanisms and the body's response to stress, ultimately leading to the development of mental health disorders. Sensitivity to stressors depends to a large extent on an individual's personal characteristics. Under similar conditions, when exposed to identical stressors, differences in the body's responses become apparent: some individuals adapt, developing resilience, whilst others are unable to cope with such stress. Disruption occurs in the fine line between physiologically protective compensatory responses to prolonged stressful conditions and the development of persistent pathological changes, which are the cause of mental disorders such as anxiety disorders, depression, aggressive behaviour and PTSD. It is therefore very interesting from a scientific point of view and highly relevant from a medical and so-

cial perspective – given the growing number of patients with such disorders – to investigate the role of genetic variability in susceptibility to the development of mental disorders under the influence of stress.

The study of the genetic mechanisms underlying the development of PTSD, particularly the role of single nucleotide polymorphisms (SNPs), is a promising area of modern psychiatry and neurobiology. The influence of genetic factors may explain individual differences in susceptibility to PTSD and opens up the possibility of personalised treatment.

AIM

The aim was to conduct a systematic analysis of the role of single-nucleotide polymorphisms in genes that regulate the functioning of the hypothalamic-pituitary-adrenal axis and the neurotrophic system in the genetic predisposition to post-traumatic stress disorder development. The study focused on summarising current data on the relationship between gene variants in this system and characteristics of stress reactivity, determining their contribution to the pathogenesis of PTSD, as well as identifying promising areas for further molecular-genetic and clinical research that may contribute to the search for risk biomarkers and the development of personalised strategies for prevention and treatment.

MATERIALS AND METHODS

To prepare this review, a systematic search of scientific sources was conducted in the Scopus, PubMed and ResearchGate databases, which are a leading resources for medical and biological research. The search strategy used keywords related to the genetic aspects of the development of post-traumatic stress disorder, in particular SNPs of genes associated with the functioning of the hypothalamic-pituitary-adrenal axis and neurotrophic system. The focus was on publications released over the past ten years to ensure relevance and alignment with current research directions. The analysis included studies that examined the molecular and genetic mechanisms of PTSD, the role of genetic variations in determining individual vulnerability to stress, and their impact on the regulation of the hormonal response.

This study is linked to the Sustainable Development Goals of Organization of United Nations, in particular Goal 3 – 'Good Health and Well-being'. Its significance lies in helping to create a society where everyone has access to high-quality healthcare, opportunities to maintain their physical and mental health, and the conditions for a long and active life [5].

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

Recent research has confirmed that post-traumatic stress disorder is a complex psychoneurobiological condition involving numerous regulatory systems within the body. Among the most intensively studied are genetic variants relating to the functioning of the hypothalamic-pituitary-adrenal axis. Particular attention has been focused on genes encoding neurotrophic factors, which influence the plasticity of the nervous system and its ability for adaptation. The following sections provide an overview of the main findings from studies investigating associations between single-nucleotide polymorphisms of these systems and the risk of developing PTSD.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

Hypothalamic-pituitary-adrenal (HPA) axis is one of the body's key integrative systems, ensuring coordination between the central nervous and endocrine systems in response to stressful factors. It functions as a complex, multi-level mechanism that regulates adaptation to environmental changes, maintains homeostasis and determines behavioural responses. The HPA axis is a central component of the stress response, enabling the body to mobilise energy resources, modify metabolic processes and modulate the immune response. Its activation enables rapid adaptation to adverse conditions, but prolonged or excessive activation can lead to pathological changes. It is crucial for mental health, as hormonal signals from cortisol influence the functioning of the hippocampus, the amygdala and the prefrontal cortex – structures responsible for memory, emotions and behavioural regulation. According to the conclusion of *Russell et al*., the HPA axis plays a fundamental role in the development of the stress response, as evidenced by the increase in symptoms of depression and anxiety resulting from disturbances in the physiological mechanisms of the hypothalamic-pituitary-adrenal axis [6].

The heritability of post-traumatic stress disorder is estimated to range from 30% to 70%, indicating that genetic factors play a significant role in its development [7]. Much attention has been devoted to genes that encode components of the hypothalamic-pituitary-adrenal axis—a key system regulating the stress response. Polymorphisms of these genes can modify glucocorticoid receptor activity and influence cortisol

secretion, which, in turn, accounts for individual differences in sensitivity to traumatic events and the risk of developing PTSD [8].

Under stress, plasma glucocorticoid (GC) levels rise, leading to the activation of glucocorticoid receptors (GR). This, in turn, triggers specific transcriptional processes that determine functional changes in various tissues and cells. Some scientists suggest that single-nucleotide polymorphisms of the *NR3C1* gene, which encodes glucocorticoid receptors, play a key role in regulating their functional activity and expression levels. Changes in this gene may modulate receptor sensitivity to glucocorticoids, alter transcriptional processes, and account for individual differences in the body's response to stress [9]**.** Another factor that plays a significant role in regulating the action of glucocorticoids is FKBP5. This protein acts as a co-chaperone for HSP90 and regulates glucocorticoid receptor signalling, in particular the process of their translocation from the cytoplasm to the nucleus. When cortisol binds to the glucocorticoid receptor, FKBP5 dissociates, allowing the receptor complex to be transported to the nucleus, where it can influence the transcriptional activity and expression of genes involved in the stress response. Furthermore, FKBP5 provides a negative feedback mechanism in the regulation of glucocorticoid-receptor interaction, as elevated levels of the FKBP5 protein can bind to GR and inhibit its translocation to the nucleus; therefore, polymorphic variants of the *FKBP5* gene may be responsible for the development of PTSD [10, 11].

According to *Carvalho et al*., the BclI polymorphism (rs41423247) of the *NR3C1* gene and four polymorphisms of the *FKBP5* gene (rs1360780, rs3800373, rs9296158, rs9470080) are considered the most significant genetic risk factors for the development of post-traumatic stress disorder, associated with the functioning of the hypothalamic-pituitary-adrenal axis [12]. The results of the studies revealed an association between the BclI polymorphism of the *NR3C1* gene and experiences of childhood trauma in relation to GR levels measured prior to conscription into military service [*13*]. In another study, the BclI polymorphic site of the GR (*NR3C1*) gene has been linked to hypersensitivity to glucocorticoids and the development of PTSD symptoms. Furthermore, a history of traumatic events in childhood and a high GR score, as determined prior, to military conscription, were considered predictors of a more severe course of PTSD. Among patients with PTSD, GR levels remained high during follow-up assessments at one and six months [14].

Sheerin et al. conducted a meta-analysis, the results of which revealed a significant association between polymorphic sites of the *NR3C1* (rs258747) and *FKBP5* (rs9296158) genes and an increased risk of developing PTSD. At the genetic level, significant associations have been identified between post-traumatic stress disorder and the *NR3C1* and *FKBP5* genes. These associations remained significant even after sensitivity analysis, confirming their reliability and potential role in the pathogenesis of PTSD [15]. Given that the *NR3C1* gene is responsible for the synthesis of glucocorticoid receptors, and *FKBP5* encodes a protein that regulates their function, a clear link can be established between these genetic factors and the pathophysiological mechanisms underlying post-traumatic stress disorder. This is consistent with increased sensitivity to glucocorticoids, which is a characteristic feature of PTSD. In this context, it has been shown that the Bcl1 polymorphism of the *NR3C1* gene is associated not only with the severity of PTSD symptoms but also with reduced cortisol levels, confirming its potential role in the dysregulation of the stress response [16, 17].

Lian et al. investigated the association between single-nucleotide polymorphisms of the glucocorticoid receptor gene and the development of PTSD, as well as the combined effect of genetic factors, traumatic events and levels of social support on the risk of developing this condition among the Chinese population. A significant association was found between the rs258747 and rs41423247 variants of the *NR3C1* gene and the risk of developing PTSD. A study of "gene-environment" interactions has confirmed a 3.26-fold increased risk of developing PTSD among individuals in the high-risk group (carriers of the minor allele for the rs258747 and rs41423247 polymorphisms, a high number of traumatic and stressful life events, and a low level of social support). This finding remained statistically significant even after stratification into subgroups based on the presence or absence of traumatic events in childhood [18]. Other studies have also confirmed that the rs41423247 (C/G) polimorphic variant of the *NR3C1* gene is associated with the development of depression and PTSD symptoms [19, 20].

Several research teams have investigated the impact of genetic polymorphisms in the FKBP5 gene on the severity of PTSD, depending on the traumatic experiences endured during childhood and adolescence. It has been found that the polymorphisms rs9296158, rs3800373, rs1360780 and rs9470080 are associated with higher levels of FKBP5 protein expression, leading to glucocorticoid receptor resistance and disruption of negative feedback. An association has been confirmed between polymorphic sites in the *FKBP5* gene and the development of PTSD symptoms over the course of a lifetime in individuals who experienced childhood

abuse [21, 22]. Other researchers have identified a link between the rs1360780 and rs3800373 polymorphisms of the FKBP5 gene and the onset of anxiety, depression, anger and dissociation, which are early predictors of mental disorders, and PTSD in particular [23]. According to *Yeo et al.*, these same polymorphisms of the *FKBP5* gene were studied in relation to the combined influence of genetic factors and a history of childhood trauma on the development of PTSD, and significant findings were obtained confirming this influence [24]. It has been found that the severity of childhood maltreatment is significantly associated with reduced cortisol levels among carriers of the CC genotype of the rs1360780 polymorphism, whereas no such association is observed in individuals with the T allele. The findings suggest that FKBP5 plays a functional role in the development of long-term changes in neuroendocrine stress regulation induced by childhood trauma, which may act as a premorbid risk factor or, conversely, a factor contributing to resilience against the development of stress-related mental disorders [25]. The results of the meta-analysis indicate that carriage of the indicated alleles of the *FKBP5* gene (T – rs1360780, C – rs3800373, T – rs9470080) in combination with traumatic experiences in early childhood is a significant predictor of an increased risk of developing depressive disorders and post-traumatic stress disorder [26].

Other important HPA genes, single nucleotide polymorphisms of which have been studied in relation to the development of PTSD, include the corticotropin-releasing hormone receptor genes (*CRHR1* and *CRHR2*) [27]. A study was conducted among children with accident-related injuries, revealing an association between the rs12944712 polymorphism of the CRHR1 gene and an increased susceptibility to developing PTSD, as well as more severe symptoms in the future [28]. *White et al. investigated the role of other polymorphisms in the same gene among individuals who had experienced trauma as a result of a hurricane. A link was confirmed between the rs12938031 and rs4792887 polymorphisms of the CRHR1 gene and the development of PTSD* [*29*]. Other researchers analysed the impact of polymorphisms of the *CRHR1* (rs110402, rs242924 and rs16940665) and *CRHR2* (rs2190242, rs2284217 and rs2014663) genes on the course and outcomes of childhood trauma. The significant importance of these genetic factors was confirmed, and their association with an increased risk of suicide attempts was identified. In addition, the study examines the interaction between polymorphic loci and environmental factors (G × E) in individuals with childhood trauma [30]. There is evidence that the rs8192496 and rs2190242 polymorphisms of the *CRHR2* gene have a protective effect, as they reduce the risk of developing PTSD in female veterans who have experienced trauma. The presence of a minor allele for these polymorphisms in the genotype is significantly associated with a lower risk and a milder severity of PTSD [31].

NEUROTROPHIC SYSTEM

Neurotrophins are a group of proteins that perform key functions in maintaining neuronal viability, regulating synaptic activity and plasticity, and are also involved in shaping the nervous system's adaptive responses to stressors [32]. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that acts as a key regulator of neuronal growth and plasticity in the brain, ensuring that these processes are maintained throughout a person's lifetime [33]. BDNF is initially synthesised as a 32 kDa precursor – proBDNF – which is capable of interacting with the p75 receptor and the sortilin receptor complex, thereby triggering a cascade of apoptotic reactions. The main part of proBDNF undergoes intracellular and extracellular cleavage involving plasmin, furin and protein convertases, resulting in the formation of the mature form of BDNF with a molecular weight of 14 kDa. The functional role of the mature form of BDNF remained largely unclear for a long time, but there is now evidence that it is involved in modulating physiological processes in neurons [34]. The BDNF protein plays a key role in ensuring the differentiation and maturation of neuronal cells. Its highest level of activity is observed in neurons involved in the cellular signalling required for the transmission of information that determines the mechanisms of synaptic plasticity [35]. People with post-traumatic stress disorder have been found to have lower levels of BDNF both in peripheral blood and in key areas of the brain, particularly the hippocampus and the prefrontal cortex [36, 37]. Reduced levels of BDNF are considered a critical factor that may limit the brain's ability to effectively regulate fear-related memories, hinder the fading of traumatic associations, and disrupt memory consolidation mechanisms. Such neurobiological disturbances, in turn, are associated with the development of persistent nightmares and a deterioration in sleep quality, which has significant clinical implications for individuals with post-traumatic stress disorder [38, 39].

Brain-derived neurotrophic factor is encoded by the *BDNF* gene, which is located on the short arm of chromosome 11p14.1. One of the most widely studied polymorphisms of the *BDNF* gene is Val66Met (rs6265). Its essence lies in the single-nucleotide substitution of guanine for adenine at position 196 of exon 5, which leads to the replacement of the amino acid valine (Val)

for methionine (Met) in codon 66 of the protein [3, 40]. It is known that carriers of the Met allele have a violation of the extracellular level of BDNF, since this polymorphism is able to affect the transition of pro-BDNF from the Golgi apparatus to secretory vesicles with subsequent release into the synaptic cleft. Individuals with the Met allele in the genotype had episodic memory deficits and decreased cognitive function [41, 42]. According to the results of some studies, carriers of the minor allele at the Val66Met polymorphic site of the *BDNF* gene had slower information processing speed and poorer memory than homozygotes for the major allele [43]. Some researchers have concluded that the combination of carrying the Met allele with chronic stress leads to an increase in the formation of fear-related reactions, which makes them more vulnerable to the development of anxiety conditions and disorders such as PTSD [44, 45]. Another study found that the simultaneous carriage of the Met allele at the rs6265 polymorphism with the presence of additional factors, such as reduced prefrontal cortex volume, may cause increased vulnerability to anxiety disorders and exacerbate negative consequences after traumatic events due to increased sensitivity to threats. It is known that the presence of the minor allele in the Val66Met polymorphism of the *BDNF* gene among USA military veterans was associated with a more severe course of PTSD symptoms throughout life [46]. *Nedic Erjavec et al.* investigated the role of polymorphic loci of the *BDNF* gene (rs6265 and rs56164415) and the impact of smoking on the cognitive function properties of military veterans with a diagnosis of PTSD. The study included over six hundred military men with symptoms of PTSD resulting from combat exposure and 120 healthy controls without PTSD. It was found that the rs56164415 polymorphism of the *BDNF* gene was significantly associated with cognitive impairment in individuals with PTSD ($P = 0.011$). The essence of this polymorphism is the replacement of C by T in the 5'-untranslated region of the *BDNF* gene, which affects the transcription of this gene, and therefore, the expression of brain-derived neurotrophic factor. According to the data obtained, in individuals with PTSD who were carriers of the minor allele, a decrease in cognitive functions was observed. The division of patients with PTSD into two subgroups depending on the presence or absence of smoking revealed that carriers of the T-allele had pronounced cognitive impairment and significantly worse short-term visual memory performance, which was confirmed for both subgroups. Thus, the significant role of rs56164415 of the *BDNF* gene in the development of PTSD was confirmed [42].

CONCLUSIONS

Post-traumatic stress disorder is a complex psycho-neurobiological condition in the development of which numerous regulatory systems of the body are involved. According to an analysis of current research, disturbances in the functioning of the hypothalamic-pituitary-adrenal axis and the neurotrophic system play an important role in the pathogenesis of PTSD. It has been established that single-nucleotide polymorphisms in the genes encoding receptors and proteins of these systems can modify the hormonal response, influence neurotransmitter levels and determine individual differences in susceptibility to stress.

Genetic variability is therefore an important factor determining individual differences in the course of PTSD. It explains why some people demonstrate resilience to traumatic events, whilst others develop persistent mental health disorders. Studying these mechanisms opens up prospects for the creation of biomarkers to predict risk and the development of personalised treatment strategies that take into account the patient's genetic profile.

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This paper was supported by the grants from the Ministry of Education and Science of Ukraine: "Investigation of Genetic Predictors of Post-Traumatic Stress Disorder Development and Their Prognostic Value" (State Registration No. 0126U000871) and "Medico-Social Substantiation of the Optimization of the Healthcare Model for Patients with Post-Traumatic Stress Disorder Considering Molecular Genetic Risk Factors" (State Registration No. 0126U000873).

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 22.03.2026
ACCEPTED: 30.06.2026

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CASE STUDY

CONTENTS

Minimally invasive treatment of recurrent varicose veins with thrombotic complications using EVLA: A case report

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ABSTRACT

We present a clinical case of a 59-year-old male who complained of varicose veins and a burning sensation in the right lower limb. His medical history included bilateral great saphenous vein (GSV) removal (2003), type 2 diabetes mellitus, stage 4 chronic kidney disease, gout, and arterial hypertension. Duplex ultrasound revealed varicothrombophlebitis of the small saphenous vein (SSV) and a floating thrombus in the popliteal vein. Initial treatment consisted of oral anticoagulation with rivaroxaban, followed by EVLA of the SSV and ultrasound-guided sclerotherapy of tributaries. After anticoagulant therapy, partial recanalization of the SSV and thrombus lysis in the popliteal vein were observed. EVLA of the SSV was performed without complications. Anticoagulant therapy was continued in coordination with a hematologist. At 6-month follow-up, duplex ultrasound showed the SSV as a fibrotic cord (diameter 3–4 mm), with regression of symptoms of chronic venous insufficiency. Despite the patient's high-risk profile, individualized management and careful monitoring allowed for the successful performance of a minimally invasive procedure. In selected high-risk patients with recurrent varicose disease complicated by thrombosis, EVLA may be considered following appropriate anticoagulant preparation. This case highlights the potential feasibility of an individualized and staged treatment approach in complex clinical settings.

KEYWORDS: recurrence, varicose veins, EVLA, varicothrombophlebitis, floating thrombus, popliteal vein

Wiad Lek. 2026;79(7):1700-1708. doi: 10.36740/WLek/218751 DOI

INTRODUCTION

Varicose veins of the lower extremities are an extremely common condition, affecting a substantial proportion of the adult population worldwide. It is estimated that the prevalence of primary varicose veins in adults ranges up to 40% [1]. According to recent systematic reviews, the global prevalence of chronic venous disease (CVD) among adults ranges between 25 % and 60 %, depending on diagnostic criteria and population characteristics [2,3].

Recurrent varicose disease is associated with a deterioration in patients' quality of life and often necessitates repeated treatment, frequently under more complex clinical conditions than at the initial presentation. Despite advances in surgical management, including standard procedures such as crossectomy and vein stripping, recurrence of varicose disease remains a significant clinical issue. Recent meta-analyses report recurrence rates of varicose veins after endovenous thermal ablation ranging from 10 % to 25 % within five years, which remains significantly lower than after conventional surgery [4,5]. This variability is attributed to differences in surgical techniques, patient characteristics, and postoperative monitoring protocols [6].

In Europe, the prevalence of varicose veins is estimated to be 30–40% among the adult population, with recurrence rates after great saphenous vein stripping reaching 20–40% over 5–10 years [6]. In the United States, the prevalence of chronic venous insufficiency (CVI), including varicose veins, also remains high. Recurrence following surgical treatment has been reported in 20–60% of cases within 10 years. Even with the use of modern minimally invasive procedures such as endovenous thermal ablation, recurrence occurs in 10–20% of cases within 5 years, which is significantly lower than after open surgery [7].

Precise epidemiological data regarding the prevalence of recurrent varicose vein disease in Ukraine remain limited, underscoring the need for further research in this area.

The main causes and types of recurrence are classified according to their anatomical origin and etiology:

· Recurrence at the saphenofemoral or saphenopopliteal junction due to incomplete ligation, neovascularization, or missed tributaries [4,6].

· Recurrence caused by incompetent perforating veins that remained untreated during the initial surgery [5].

· Progression of the underlying disease, with the development of new reflux zones due to the natural course of CVI and genetic predisposition [6].

· Recurrence from new sources or in anatomical areas not addressed during the initial treatment [4].

· Technical errors during the primary procedure, such as incomplete ligation or failure to remove varicose tributaries [5].

Among the most serious complications of varicose vein disease is varicothrombophlebitis, which may progress to deep vein thrombosis (DVT) or lead to pulmonary embolism (PE). According to a Ukrainian study by Chernukha and Shchukin, DVT was identified in 16% of patients with advanced varicose disease, and PE occurred in nearly 4% of cases [8]. Floating thrombi in the small saphenous and popliteal vein systems pose a particular threat and require urgent diagnosis and a structured, staged treatment strategy [8,9].

In modern vascular surgery, minimally invasive techniques are becoming increasingly prioritized, EVLA now regarded as the "gold standard" for the treatment of varicose vein disease, including recurrent cases [10]. EVLA provides effective elimination of pathological venous reflux with minimal trauma and a short recovery period. This method is especially valuable in patients with significant comorbidities, including diabetes, renal insufficiency, obesity, or cardiovascular disease. According to recent international consensus reports, including the UIP 2023 guidelines, modern minimally invasive techniques such as EVLA are considered safe and effective for the treatment of varicose veins, with significantly fewer complications compared with conventional surgery [11].

In clinical practice, EVLA is being increasingly considered in complex scenarios where varicose disease is accompanied by thrombosis and the procedural risk is elevated. In this context, the purpose of this publication is to present a clinical case demonstrating the successful application of EVLA in a patient with recurrent varicose veins of the lower extremities, complicated by a floating thrombus in the popliteal vein, in the presence of multiple comorbidities.

CASE REPORT

PATIENT INFORMATION AND CLINICAL FINDINGS

Patient Ihor S., born in 1964, presented to the our Medical Center on February 19, 2022, with complaints of varicose veins and burning pain in the right lower limb.

Medical history: In 2003, the patient underwent bilateral GSV stripping. Approximately 10 years ago, new varicose veins developed on the right calf, accompanied by swelling and skin hyperpigmentation in the lower third of the leg. One week prior to the visit, he began experiencing burning pain along the varicose veins of the right calf.

Past medical history: In 2000, the patient underwent surgery for a carbuncle of the right kidney. In 2016, he was diagnosed with type 2 diabetes mellitus.

Ultrasound duplex scan (February 19, 2022): Signs of varicose vein disease; status post bilateral GSV stripping. Recurrent varicose disease with sapheno-popliteal reflux in the right lower limb. Varicothrombophlebitis of the right SSV.

Primary diagnosis: Varicose vein disease of the lower limbs.

CEAP classification: C4b, Ep, As, Ap, Pr (recurrent varicose veins after previous GSV surgery).

Status post crossectomy and stripping of both great saphenous veins (2003).

Recurrent varicose veins, varicothrombophlebitis of the right SSV.

Comorbid diagnoses: Stage 4 chronic kidney disease. Mixed nephropathy (gouty and diabetic). Gout. Type 2 diabetes mellitus, severe form, subcompensated stage. Grade III arterial hypertension, risk level 4.

The patient was prescribed phlebotonics, rivaroxaban, and laboratory tests. Due to the onset of the full-scale invasion, the patient returned for consultation one year later.

Duplex ultrasound findings (January 23, 2023): Recanalization of the SSV was observed. EVLA was planned, and a preoperative examination was scheduled.

February 1, 2023: The patient presented with examination results for surgical planning. Duplex ultrasound findings: The GSV had been removed entirely; the stump was not visualized. In the proximal segment of the SSV, the diameter was 12 mm; distally — 9 mm. The SSV walls were thickened, with incomplete compressibility of the lumen. Echogenic masses were visible within the lumen; blood flow was parietal. Fig 1. A protrusion of parietal thrombotic material into the popliteal vein measuring 4 cm was observed. Fig 2. The thrombus in the popliteal vein was bathed in blood flow and floating. Fig 3. An incompetent upper Cockett perforator (4 mm) was also identified.

DISCUSSION

Signs of varicose vein disease; status post bilateral GSV stripping. Recurrent varicose vein disease with sapheno-popliteal reflux in the right lower limb. Varicothrombophlebitis of the SSV. Floating thrombus in the popliteal vein.

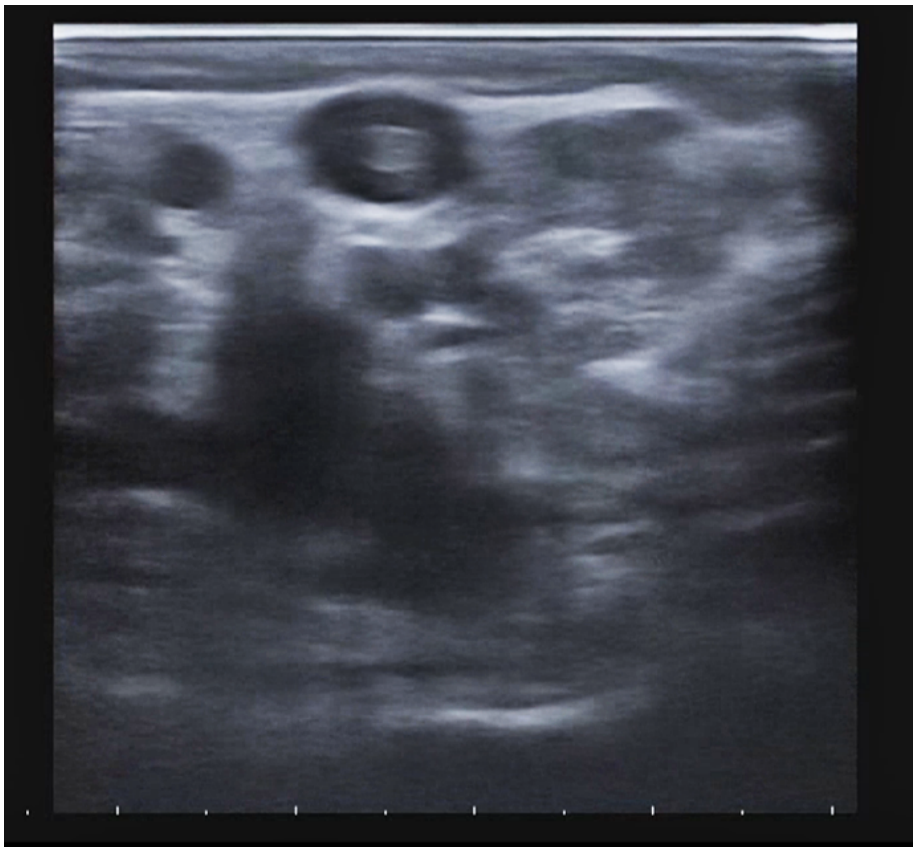

Fig. 1. Thrombosis of the SSV
Picture taken by the authors

Fig. 2. Thrombosis of the sapheno-popliteal junction with spread to the popliteal vein
Picture taken by the authors

Rivaroxaban was prescribed at a dose of 15 mg, one tablet twice daily for 3 weeks, followed by 20 mg once daily for 3 months. After 20 days, recanalization of the SSV and thrombus lysis in the popliteal vein were observed. Fig 4.

On February 21, 2023, EVLA of the SSV was performed. In the early postoperative period, ultrasound-guided foam sclerotherapy of the SSV tributaries was carried out. Rivaroxaban therapy was continued at 20 mg daily for up to 2

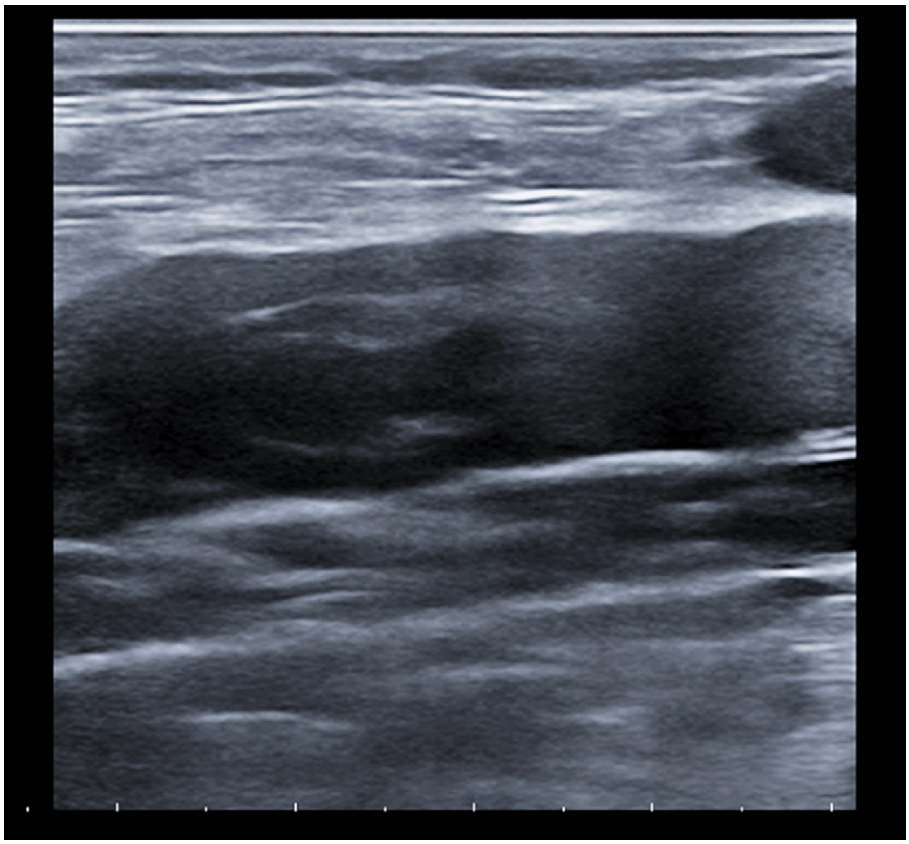

Fig. 3. Floating thrombus in the popliteal vein
Picture taken by the authors

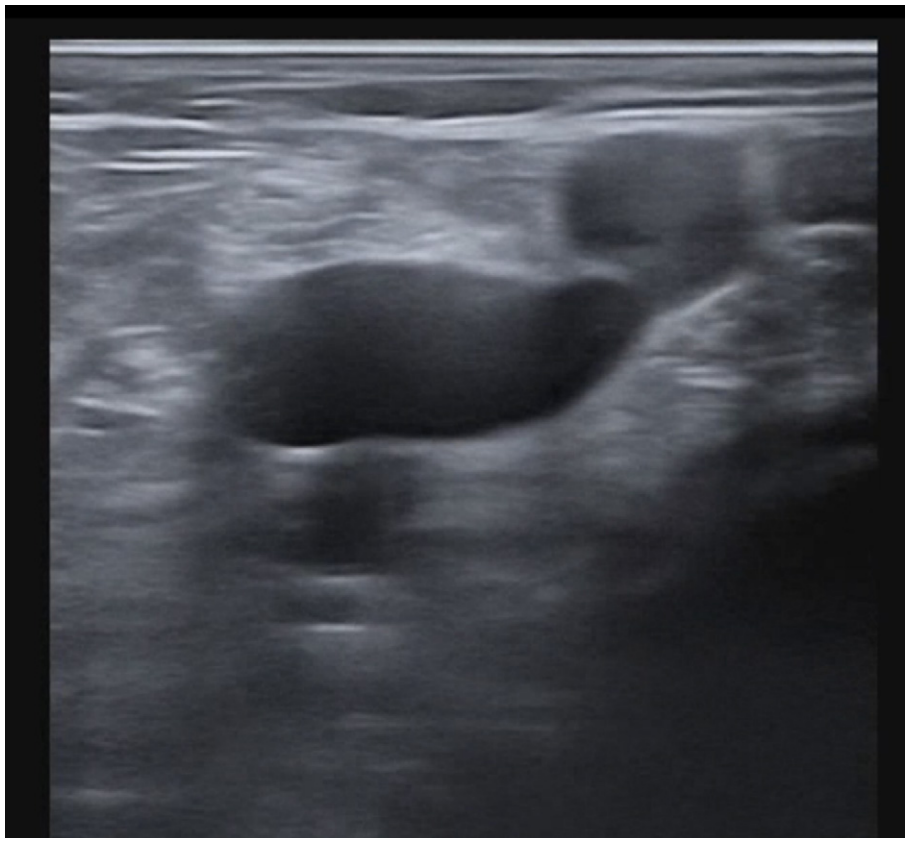

Fig. 4. Sapheno-popliteal junction after resorbtion of thrombotic masses on the background of the anticoagulant therapy
Picture taken by the authors

months, followed by a maintenance dose of 10 mg daily on a long-term basis in coordination with a hematologist.

At the 6-month follow-up, complete regression of CVI symptoms was noted. Duplex ultrasound revealed a fibrous cord in the projection of the MPV with a diameter of 3–4 mm and its partial resorption; no signs of recanalization or thrombotic masses were detected. Fig 5. The patient continues outpatient follow-up care.

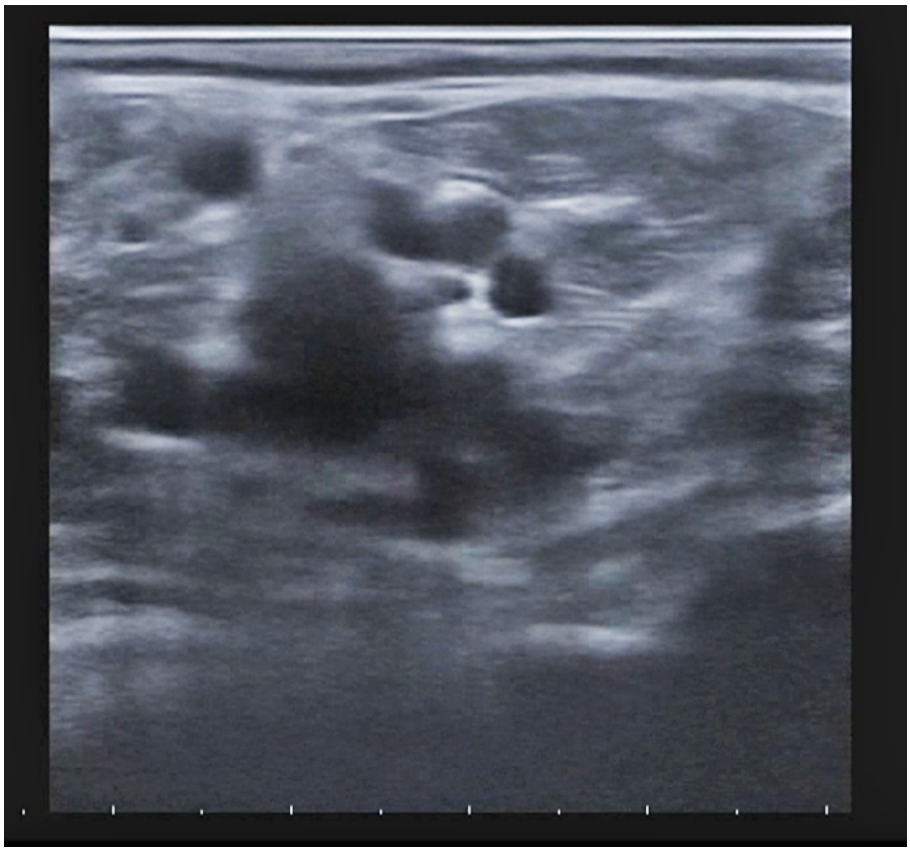

Fig. 5. Resorbtion of the SSV after 6 month after the operation
Picture taken by the authors

DIAGNOSTIC ASSESSMENT

The diagnosis was established based on clinical presentation, medical history, and duplex ultrasound findings. The patient presented with typical signs of recurrent varicose vein disease, including swelling, induration, and pigmentation of the lower leg skin. According to the history, the patient had previously undergone classical surgical treatment (crossectomy and GSV stripping); however, several years later, new symptoms developed, suggesting the formation of compensatory venous hemodynamics and disease recurrence.

Ultrasound examination revealed thrombosis of the SSV at the level of the saphenopopliteal junction, with thrombus extension into the popliteal vein, the presence of floating thrombi, absence of flow in the affected segment, and thickening of the venous wall. The proximal end of the thrombus was identified at the lower margin of the popliteal fossa. The presence of a floating thrombus, without signs of stenosis or occlusion of the deep venous system, indicated a high risk of thromboembolic complications. At the same time, deep veins of the lower extremities remained patent, which allowed consideration of a staged minimally invasive intervention following anticoagulant therapy.

Given the presence of type 2 diabetes mellitus, stage 4 chronic kidney disease, and gout, a decision was made to first stabilize the condition conservatively, followed by surgical intervention. Administration of rivaroxaban at 15 mg twice daily for three weeks, followed by 20 mg once daily, resulted in thrombus lysis in the popliteal vein and partial recanalization of the SSV. This enabled safe performance of EVLA followed by sclerotherapy of tributaries.

THERAPEUTIC INTERVENTION

Following the initial examination and confirmation of the diagnosis of varicothrombophlebitis with a floating thrombus in the popliteal vein, the patient was prescribed rivaroxaban: 15 mg twice daily for 21 days, followed by 20 mg once daily. Dynamic duplex ultrasound monitoring revealed partial recanalization of the thrombosed segment and stabilization of the patient's condition.

In the third week, EVLA of the SSV was performed. The procedure utilized a 1470 nm diode laser (Biolitec) with 2-ring radial fibers. Laser power was set at 9–10 W, with automatic fiber traction at a speed of 0.7 mm/s; the linear endovenous energy density (LEED) was 130 J/cm.

The procedure was performed under tumescent anesthesia and included laser obliteration of the thrombosed SSV segment along with ultrasound-guided sclerotherapy of varicose tributaries. Additional treatments included compression therapy using Class II compression stockings and continued rivaroxaban therapy for another two months under hematologist supervision.

The postoperative period was uneventful. The patient was provided with recommendations for outpatient follow-up, recurrence prevention, and secondary thrombosis prophylaxis.

FOLLOW-UP AND OUTCOMES

At the 6-month follow-up after the intervention, duplex ultrasound demonstrated a fibrotic cord in the projection of the SSV trunk with a diameter of 3–4 mm, without signs of recanalization, thrombotic masses, or venous reflux. All deep veins remained patent. No evidence of recurrent thrombosis or venous hypertension was identified.

Subjectively, the patient reported complete resolution of pain, swelling, nocturnal cramps, and decreased skin pigmentation. During the following 6 months, no episodes of recurrent thrombosis or signs of chronic venous insufficiency were observed.

These treatment outcomes suggest that with a staged approach and close clinical monitoring, EVLA can be safely and effectively applied even in cases of complicated recurrent varicose disease with associated thrombosis.

RELEVANCE OF THE CLINICAL CASE

The presented case of patient Ihor S. is a vivid example of the complexity and multifaceted nature of treating recurrent varicose vein disease of the lower extremities, especially when compounded by severe comorbidities and life-threatening thromboembolic episodes. The significance of this case lies in demonstrating the effectiveness of modern minimally invasive techniques, such as EVLA, in high-surgical-risk patients.

The patient had undergone bilateral great saphenous vein stripping in 2003, indicating a long-standing and progressive course of varicose vein disease. The recurrence, with saphenopopliteal reflux in the right lower limb and subsequent development of small saphenous vein varicothrombophlebitis complicated by a floating thrombus in the popliteal vein, marked a critical clinical turning point. A floating thrombus poses a direct life-threatening risk due to its high potential for pulmonary embolism, rendering this case particularly hazardous.

An additional challenge was the presence of trophic skin changes in the lower third of the leg (hyperpigmentation and edema), which indicated chronic venous insufficiency (C4). These changes further impaired quality of life and complicated the management strategy.

A particularly important aspect of this case is the presence of multiple severe comorbid conditions: stage 4 chronic kidney disease, type 2 diabetes mellitus (severe form), gout, and grade III arterial hypertension. These comorbidities significantly increased both anesthetic and surgical risks, while also complicating perioperative management. They necessitated a careful multidisciplinary approach to treatment.

This case illustrates how timely diagnosis, intensive anticoagulant therapy, and an adapted minimally invasive strategy allowed clinicians to avoid the high risks associated with open surgery and to achieve a favorable clinical outcome.

OUTCOMES OF TREATMENT IN COMORBID PATIENTS IN THE CONTEXT OF LITERATURE DATA

The treatment of varicose vein disease in patients with comorbidities presents a significant challenge for phlebologists and surgeons. According to the literature, the presence of comorbid conditions such as diabetes mellitus, chronic kidney disease, cardiovascular disease, and obesity substantially increases the risks associated with surgical intervention and can negatively impact long-term outcomes of varicose vein treatment.

Patients with diabetes mellitus, for example, have an increased risk of infectious complications, delayed wound healing, and a higher tendency to develop trophic ulcers [5]. Chronic kidney disease (as in the presented case—stage 4 CKD) may alter the metabolism of medications, including anticoagulants, thereby increasing the risk of bleeding or thrombotic events [1]. Arterial hypertension and gout also require close monitoring during any surgical intervention.

Recent literature increasingly emphasizes the advantages of minimally invasive procedures, such as EVLA, for the treatment of varicose vein disease in patients with comorbid conditions. Compared to traditional open surgery, these methods are associated with reduced procedural trauma, the possibility of performing under local anesthesia, shorter recovery times, and a lower risk of complications [7].

For instance, studies have shown that EVLA is associated with lower postoperative pain scores, fewer hematomas, and faster return to normal activities—factors that are especially important in patients with limited physiological reserves [4]. These benefits make EVLA a viable and effective treatment option even in cases where open surgery may be contraindicated or associated with excessive risk.

RISKS OF OPEN SURGERY IN RECURRENT VARICOSE VEIN DISEASE AND COMORBIDITY

Traditional open surgery, such as redo crossectomy and vein stripping, is associated with significantly higher risks and technical challenges in recurrent varicose

vein cases compared to primary interventions. Several factors contribute to this complexity:

1. Anatomical changes and scarring: Following primary surgery, significant scar tissue forms in the inguinal or popliteal regions. This complicates the identification of anatomical structures and increases the risk of nerve injury (e.g., femoral or saphenous nerves) and damage to lymphatic vessels, potentially leading to lymphorrhea, lymphocele, or persistent limb edema [6].
2. Increased risk of bleeding: Scarred tissues are poorly vascularized and do not hold sutures well, which increases the risk of intraoperative and postoperative hemorrhage.
3. Neovascularization: One of the main causes of recurrence after open surgery is neovascularization—the formation of new vessels at the site of previous ligation [4]. These newly formed veins are often fragile and prone to reflux, making them difficult to ligate effectively during redo surgery. Open revision may not fully address this phenomenon.
4. Higher risk of infectious complications: Any repeated surgical intervention in a scarred zone carries an increased risk of wound infection, especially in immunocompromised patients, such as those with diabetes or chronic kidney disease.
5. Longer recovery and more pain: Redo open surgeries are more traumatic, typically associated with greater postoperative pain and longer rehabilitation periods—undesirable in elderly patients or those with significant medical histories.
6. Anesthetic risks: In the case of patient Ihor S., the presence of stage 4 chronic kidney disease, diabetes, gout, and grade III hypertension significantly increased the risks associated with general or even regional anesthesia. These included cardiovascular complications, worsening renal function, glycemic instability, and other systemic issues. Open surgery usually requires deeper anesthesia and prolonged operative time.

In the context of this clinical case, where the patient presented with a floating thrombus in the popliteal vein and multiple severe comorbidities, open surgery would have been associated with an extremely high risk of both local and systemic complications, including potentially fatal pulmonary embolism during or shortly after the procedure. The decision to initiate intensive anticoagulant therapy to stabilize the thrombus, followed by EVLA—a less invasive procedure that can be performed under local anesthesia—was both justified and life-saving.

Our clinical observations are consistent with current literature. For example, a study by Gradman WS demonstrated that EVLA is safe in patients with concomitant thrombophlebitis, provided their condition is stabilized and appropriate anticoagulant preparation is administered [10]. In a randomized trial by Guex et al. [11], the effectiveness of EVLA was compared with vein stripping in patients with varicothrombophlebitis; the EVLA group had a 40% lower complication rate and a 2.5-fold shorter rehabilitation period.

EVLA IN THE TREATMENT OF RECURRENT VARICOSE VEINS

The successful treatment outcome in this case highlights the potential of EVLA as an effective and safe modality for managing recurrent varicose veins, particularly in cases complicated by thrombosis where conventional surgical approaches may carry elevated risks. The mechanism of EVLA involves thermal injury to the venous wall via laser energy, leading to vessel obliteration and subsequent fibrosis. This approach minimizes the risk of neovascularization compared to crossectomy and ensures more durable clinical results [7].

MANAGEMENT OF THROMBOTIC COMPLICATIONS

This clinical case also highlights the importance of adequate preoperative management of thrombotic complications. The presence of a floating thrombus required immediate and effective anticoagulant therapy [12]. The use of high-dose rivaroxaban (Xarelto) followed by a maintenance dose allowed for successful recanalization of the small saphenous vein and lysis of the thrombus in the popliteal vein, thereby eliminating the immediate risk of pulmonary embolism and creating a safe setting for EVLA. This underscores the necessity of close collaboration between phlebologists and hematologists, as well as meticulous monitoring of patients at high risk for thromboembolic events.

COMPARISON WITH OTHER TREATMENT MODALITIES

In the context of recurrent varicose disease and complicated cases, EVLA demonstrates significant advantages over open surgery. Alternative minimally invasive methods such as radiofrequency ablation or cyanoacrylate closure are also effective, but the choice of technique depends on the anatomical characteristics of the recurrence, presence of thrombotic complications, and the patient's comorbid status. In this case, where a floating thrombus was present, controlled thermal ablation became a justified choice after thrombus resolution.

LIMITATIONS

This report describes a single clinical case, which limits the ability to generalize the findings. The favorable

outcome observed in this patient may not be universally applicable. Further controlled studies are needed to evaluate the safety and efficacy of EVLA in similar complex clinical scenarios.

CONCLUSIONS

This clinical case highlights the feasibility of a staged management strategy for recurrent varicose vein disease complicated by a floating thrombus in the popliteal vein in a patient with significant comorbidities.

Anticoagulant therapy with dynamic monitoring allowed us to avoid invasive intervention on the deep venous system. This, in turn, created safe conditions for the subsequent performance of EVLA. Failing to recognize the floating component or delaying anticoagulant therapy could have resulted in potentially fatal complications.

A key factor in the management of such patients is timely diagnosis using duplex ultrasound scanning, a multidisciplinary approach, and early decision-making regarding personalized therapeutic strategy.

This clinical case suggests that EVLA may be considered a feasible and safe option in selected high-risk patients after appropriate anticoagulant preparation and careful monitoring.

However, larger prospective studies are required to confirm the safety and reproducibility of this approach.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 31.07.2025
ACCEPTED: 02.03.2026

CREATIVE COMMONS 4.0

CASE STUDY

CONTENTS

An anesthesiological complication related to bone cement use during reconstructive osteoplasty using the Masquelet technique in a pediatric patient: A case report

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DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

A 16-year-old male (ASA II, BMI 31) underwent reconstructive osteoplasty with insertion of a polymethylmethacrylate (PMMA) spacer. Shortly after cement application, he developed sudden hypotension, tachycardia, and decreased end-tidal CO_2, consistent with Grade II BCIS. Supportive management with fluids, ketamine, and norepinephrine stabilized the patient. Postoperatively, he experienced recurrent hypotension, acute kidney injury, and elevated liver enzymes and creatine kinase. Intensive care treatment resulted in recovery, and he was discharged from the ICU on day five.

Bone cement implantation syndrome, though exceptionally uncommon in pediatric patients, may develop during orthopedic procedures involving polymethylmethacrylate. Early recognition, continuous anesthetic vigilance, and timely multidisciplinary intervention are crucial to reduce morbidity and improve perioperative outcomes.

KEY WORDS: bone cements, anesthesia, hypotension, acute kidney injury, vasopressors, creatine kinase

Wiad Lek. 2026;79(7):1709-1714. doi: 10.36740/WLek/222248 DOI

INTRODUCTION

The use of bone cement (BC), based on polymethyl methacrylate, is widely adopted in orthopedic practice for procedures such as fracture repair and joint arthroplasty. Bone cement facilitates the stabilization and reinforcement of the fracture site, thereby promoting bone healing and functional recovery [1]. Despite the favorable physical properties of BC and its undeniable benefits in orthopedic reconstructive surgery, its use has been associated with a number of complications that may lead to sudden clinical deterioration and even death [2-5].

One of the most severe complications related to bone cement use is Bone Cement Implantation Syndrome — a critical and potentially life-threatening condition that typically occurs during cementation and is characterized by a constellation of clinical features, including hypotension, hypoxemia, cardiac arrhythmias, elevated pulmonary vascular resistance, and, in some cases, cardiac arrest. BCIS usually manifests shortly after cement injection but may also occur in the early postoperative period. The pathophysiology is primarily attributed to the exothermic nature of the cementing process, which leads to local tissue injury, histamine and prostaglandin release, and the formation of microemboli. These events trigger a systemic inflammatory response that may culminate in respiratory and cardiovascular failure [1,2,6].

BCIS most frequently occurs during hip arthroplasty and is closely associated with increased intramedullary pressure following cement injection. However, cases have also been reported during knee and shoulder arthroplasty [7,8].

To assess the severity of BCIS, the grading system proposed by Donaldson et al. is commonly used, which is based on hemodynamic and oxygenation parameters [2]:

Grade 1: Oxygen saturation (SpO_2) < 94% or >20% decrease in systolic blood pressure (SBP);

Grade 2: SpO_2 < 88%, >40% decrease in SBP, or sudden loss of consciousness;

Grade 3: Cardiovascular collapse requiring cardiopulmonary resuscitation.

As for the incidence of BCIS in pediatric patients, only a few isolated reports have been published to date regarding this serious complication [9,10]. We report a clinical case of BCIS in a 16-year-old male patient that occurred intraoperatively and continued into the early postoperative period following orthopedic surgery involving the use of bone cement.

Fig. 1. Initial segmental femoral bone defect following trauma
Picture taken by the authors

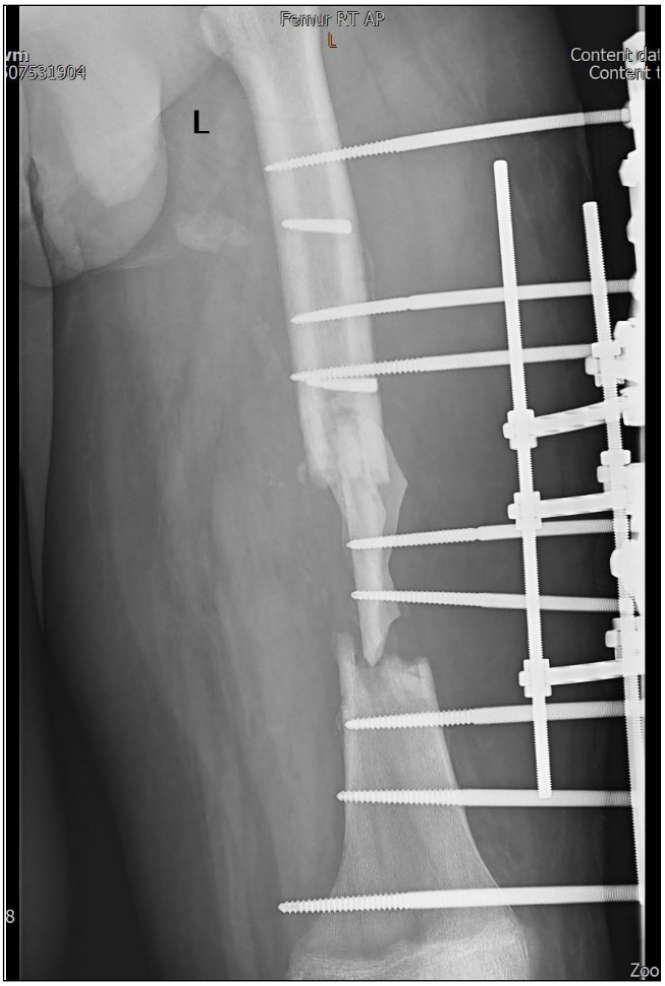

Fig. 2. External fixation and metal osteosynthesis of the femoral fragments
Picture taken by the authors

CASE REPORT

A 16-year-old male pediatric patient with a body mass index (BMI) of 31 presented without significant comorbidities. He was classified as American Society of Anesthesiologists (ASA) Physical Status II. The patient was scheduled for the first stage of surgical treatment using the induced membrane technique (Masquelet technique) to manage a large segmental defect of the femoral shaft.

The patient's history was notable for a high-energy road traffic accident that resulted in an open fracture of the femur with substantial bone loss (Fig. 1). Emergency surgical management was performed, consisting of metal osteosynthesis of the femoral fragments and application of an external fixation device (Fig. 2). Due to delayed bone healing and insufficient incorporation of the autologous bone graft, the first stage of the Masquelet technique was carried out as part of a staged reconstructive approach (Fig. 3).

Course of Anesthesia: anesthesia was induced with intravenous propofol, fentanyl, and atracurium. Endotracheal intubation was performed, followed by mechanical ventilation in pressure-controlled mode (PCV) with the following parameters: FiO_2 50%, peak inspiratory pressure (PIP) 12 cm H_2O, positive end-expiratory pressure (PEEP) 4 cm H_2O, respiratory rate (RR) 15 breaths/min, inspiratory-to-expiratory ratio (I:E) 1:2, and tidal volume (TV) 570 mL. An epidural catheter was inserted at the L2–L3 level. Epidural anesthesia was achieved with 20 mL of 0.25% bupivacaine. Anesthesia was maintained with a continuous propofol infusion at 6 mg/kg/h in combination with epidural anesthesia and intermittent boluses of fentanyl for analgesic augmentation. Tranexamic acid (1 g) was administered intraoperatively to reduce blood loss. Antibiotic prophylaxis was given to prevent

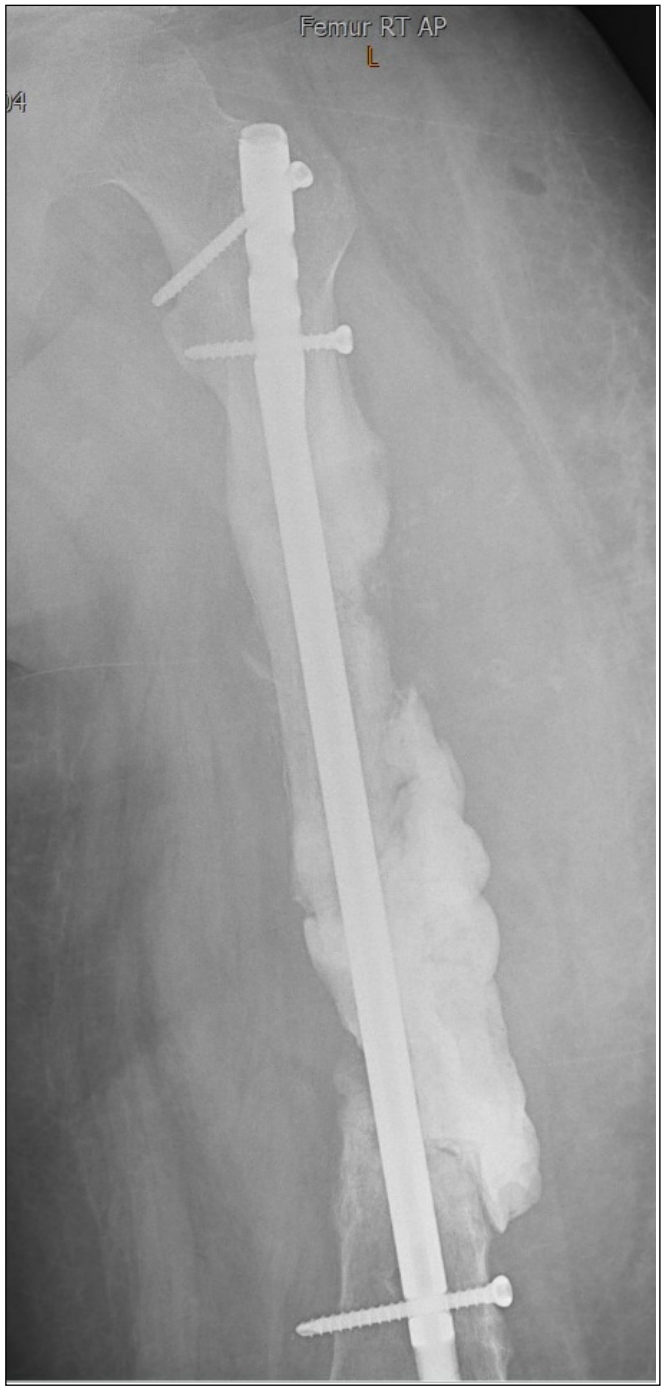

Fig. 3. First stage of reconstruction using the Masquelet technique
Picture taken by the authors

surgical site infections. Intravenous fluid therapy was performed using balanced crystalloids, titrated according to physiological requirements and estimated intraoperative fluid and blood loss.

Intraoperative Monitoring and Complication: vital signs remained stable until the use of bone cement. Baseline monitoring parameters included: SpO_2 97–98%, end-tidal CO_2 ($EtCO_2$) 40–44 mmHg, heart rate (HR) 68–77 bpm, blood pressure (BP) 135/78 mmHg, mean arterial pressure (MAP) 95 mmHg, and core temperature 36.3°C. At the third hour of surgery, shortly after bone cement application, the patient experienced an abrupt hemodynamic deterioration consistent with bone cement implantation syndrome: BP dropped to 62/34 mmHg (MAP 43 mmHg), HR increased to 115 bpm, and $EtCO_2$ decreased to 26 mmHg. A 500 mL bolus of Ringer's lactate was administered, resulting in partial hemodynamic improvement: BP 90/55 mmHg, MAP 66 mmHg. However, hypotension persisted (BP 84/52 mmHg, MAP 62 mmHg). The propofol infusion was reduced to 4 mg/kg/h, and intermittent ketamine boluses (100 mg every 30 minutes) were initiated. A norepinephrine infusion was started at 0.1 µg/kg/min. Blood pressure stabilized in the range of 92/63 to 98/65 mmHg. The total duration of MAP < 60 mmHg was approximately 27 minutes.

By the end of the surgery, the patient was hemodynamically stable with the following parameters: SpO_2 97–98%, $EtCO_2$ 40 mmHg, HR 87 bpm, BP 105/68 mmHg, MAP 79 mmHg, and temperature 36.1°C. The patient was extubated in the operating room and transferred to the pediatric intensive care unit (PICU). Total intraoperative fluid administered: 4300 mL over 7 hours. Estimated blood loss: ~1600 mL. Packed red blood cell (PRBC) transfusion: 1300 mL

Postoperative Period: upon admission to the PICU, the patient developed recurrent hypotension: BP 55/35 mmHg, MAP 39 mmHg, SpO_2 dropped to 88–90%, HR increased to 115 bpm. Norepinephrine was increased to 0.15 µg/kg/min, and supplemental oxygen (10 L/min) was administered via face mask. Hemodynamic stabilization occurred within 2 hours. Norepinephrine infusion was continued for a total duration of 14 hours and then discontinued. Oxygen therapy was ceased 12 hours postoperatively. On postoperative day 1, the patient exhibited oliguria (urine output 0.36 mL/kg/h) and laboratory findings consistent with acute kidney injury (AKI). Additionally, there were significantly elevated levels of AST, ALT, and creatine kinase (CK). Postoperative laboratory results are summarized in Table 1.

Over the following three days, the patient maintained stable hemodynamic and respiratory parameters, and urine output returned to normal. A regression of renal insufficiency was observed, along with a decline in serum levels of creatine kinase, AST, and ALT. On postoperative day five, the patient was transferred from the intensive care unit to the general ward in a satisfactory condition.

To date, complications associated with the use of bone cement are well-documented in the literature. Although the first report of bone cement implantation

Table 1. Laboratory values during the postoperative period

Parameter (Reference range)	Postoperative time (h)							
	12	24	36	48	60	72	96	120
Urea (2.5–8 mmol/L)		9	8.37		7.8		7.1	7.5
Cr (62–115 µmol/L)		227	243		146		127	115
K^+ (3.5–5.0 mmol/L)		5.2	4.3	4.5	4.8		3.8	4.2
AST (0–47 U/L)			599	507		234		144
ALT (0–37 U/L)			135	132		123		115
CK (<270 U/L)				32628				12838
CRP (<5 mg/L)		145		150				28
Ph (7,35-7,45)	7.24	7.33	7.4					
PaO_2 (80–100 mmHg)	59	88	101					
$PaCO_2$ (35–45 mmHg)	34	37	37					
BE N (±2 mmol/L)	-11	-5	-3					
APTT (25–35 s)	47	50	46		45		39	41
INR (0.8–1.2)	1.4	1.5	1.3		1.3		1.0	1.1

Source: compiled by the authors of this study

syndrome (BCIS) was published by Powell J.N et al. in 1970, there remains considerable variation in the reported incidence and associated mortality. This is primarily due to the lack of a standardized diagnostic approach and limited clinical awareness. Furthermore, in earlier studies, BCIS was often misclassified as pulmonary embolism [1,11].

The wide variability in BCIS incidence also stems from the heterogeneity of clinical presentations, which depends on the severity of the syndrome, as described by Donaldson A,J et al. [2]. Reported incidence rates of BCIS in the literature range from 28% to 61.5%, largely due to the absence of a universally accepted definition [12,13]. In a study by Weingärtner K involving 208 patients, 77 (37.0%) developed BCIS. Of these, 53 (25.5%) had Grade I, 17 (8.2%) had Grade II, and 7 (3.4%) had Grade III BCIS [6].

Several theories have been proposed to explain the pathogenesis of BCIS, including embolic, allergic, and toxic responses to polymethylmethacrylate. However, none of these hypotheses fully accounts for the complex and varied clinical manifestations of BCIS, which are more likely to arise from a multifactorial interaction of these mechanisms [1].

BCIS is most commonly associated with cemented arthroplasty procedures of the hip and knee joints, and less frequently with shoulder arthroplasty. Many authors attribute this to the elevated intramedullary pressure that occurs during cement insertion [1,6]. In contrast, the Masquelet technique does not involve increased intramedullary pressure, as the cement spacer is implanted into a bone defect. Its primary purpose is to induce and maintain a biological membrane that facilitates subsequent bone regeneration [14,15].

In the presented case, the acute clinical deterioration following cement application was likely triggered by the exothermic reaction of PMMA affecting adjacent tissues, resulting in the release of histamine, prostaglandins, and other systemic inflammatory mediators, which led to hypotension. Of particular concern was the recurrent episode of hypotension in the postoperative period, suggestive of a prolonged systemic inflammatory response to PMMA.

Also noteworthy was the marked elevation in creatine kinase levels, which may indicate secondary skeletal muscle injury due to the thermal effect of PMMA and cytokine cascade activation. Several clinical and experimental studies have reported similar elevations in CK, ALT, and AST following bone cement use [16,17]. Alternatively, these laboratory abnormalities may have been partially attributable to surgical trauma and transient hypoperfusion of muscle tissue during the intraoperative period [18,19].

The development of acute kidney injury (AKI) in the early postoperative period was likely a consequence

of transient hemodynamic instability. Fortunately, it resolved rapidly following the restoration of effective circulation.

This case of BCIS in a pediatric patient undergoing orthopedic surgery using the Masquelet technique is exceedingly rare in our experience. This may be explained by the generally lower prevalence of comorbid risk factors and the superior hemodynamic compensatory capacity in children compared to adult patients.

CONCLUSIONS

Bone cement implantation syndrome is a multifactorial complication most frequently encountered in elderly patients undergoing cemented hip arthroplasty. Its occurrence in pediatric patients is exceptional. Nevertheless, even rare complications such as BCIS in children warrant further investigation and the development of safety guidelines to improve perioperative outcomes.

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CONFLICT OF INTEREST
The Author declare no conflict of interest

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RECEIVED: 18.08.2025
ACCEPTED: 20.05.2026

CREATIVE COMMONS 4.0