ISSN 2706-6282
e-ISSN 2706-6290

Тернопільський національний медичний університет імені І. Я. Горбачевського

# Вісник медичних і біологічних досліджень

Науково-практичний журнал

Заснований у 2019 році
Періодичність випуску: щоквартально

**Том 6, № 4**

Тернопіль – 2024

ISSN 2706-6282
e-ISSN 2706-6290

**Засновник:**
Тернопільський національний медичний університет імені І. Я. Горбачевського

**Рік заснування: 2019**

*Рекомендовано до друку та поширення*
*через мережу Інтернет Вченою радою*
*Тернопільського національного медичного університету імені І. Я. Горбачевського*
*(протокол № 13 від 26 листопада 2024 р.)*

**Ідентифікатор медіа: R30-03952**
(Рішення Національної ради України з питань телебачення і радіомовлення
№ 1187, протокол № 13 від 11 квітня 2024 р.)

**Журнал входить до переліку наукових фахових видань України**
Категорія «Б». Спеціальності: 222 – «Медицина», 223 – «Медсестринство», 091 – «Біологія та біохімія»
(наказ Міністерства освіти і науки України
№ 886 від 02 липня 2020 та № 1188 від 24 вересня 2020)

**Журнал представлено у міжнародних наукометричних базах даних, репозитаріях та пошукових системах**: Національна бібліотека України імені В. І. Вернадського, Фахові видання України, BASE, Ulrich's Periodicals Directory, ScienceOpen, EuroPub, DOAJ, UCSB Library, Dimensions, German Union Catalogue of Serials, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, Open Ukrainian Citation Index, Worldcat, CORE

Вісник медичних і біологічних досліджень / [редкол.: Л. Я. Федонюк (голов. ред.) та ін.]. – Тернопіль : Тернопільський національний медичний університет імені І. Я. Горбачевського, 2024. – Т. 6, № 4. – 89 с.

**Адреса редакції**:
Тернопільський національний медичний університет імені І. Я. Горбачевського
46001, майдан Волі, 1, м. Тернопіль, Україна
E-mail: info@bmbr.com.ua
https://bmbr.com.ua/uk

ISSN 2706-6282
e-ISSN 2706-6290

I. Horbachevsky Ternopil National Medical University

# Bulletin of Medical and Biological Research

Scientific-Practical Journal

Founded in 2019
Frequency: quarterly

**Volume 6, No. 4**

Ternopil – 2024

ISSN 2706-6282
e-ISSN 2706-6290

**Founder:**
I. Horbachevsky Ternopil National Medical University

**Year of foundation: 2019**

*Recommended for printing and distribution*
*via the Internet by the Academic Council*
*I. Horbachevsky Ternopil National Medical University*
*(Minutes No. 13 of November 26, 2024)*

**Media identifier: R30-03952**
(Decision of the National Council of Television and Radio Broadcasting of Ukraine
No. 1187, Minutes No. 13 of April 11, 2024)

**The journal is included in the list of scientific professional publications of Ukraine**
Category "B". Specialties: 222 – "Medicine", 223 – "Nursing", 091 – "Biology and Biochemistry"
(Order of the Ministry of Education and Science of Ukraine
No. 886 as of July 02, 2020 and No.1188 of September 24, 2020)

**The journal is presented international scientometric databases, repositories and scientific systems**: National Library of Ukraine named after V. I. Vernadskyi, Professional publications of Ukraine, BASE, Ulrich's Periodicals Directory, ScienceOpen, EuroPub, DOAJ, UCSB Library, Dimensions, German Union Catalogue of Serials, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, Open Ukrainian Citation Index, Worldcat, CORE

Bulletin of Medical and Biological Research / Ed. by L. Fedoniuk. (Editor-in-Chief) et al. Ternopil: I. Horbachevsky Ternopil National Medical University, 2024. Vol. 6, No. 4. 89 p.

**Editors office address**:
I. Horbachevsky Ternopil National Medical University
46001, 1 Maidan Voli, Ternopil, Ukraine
E-mail: info@bmbr.com.ua
https://bmbr.com.ua/en

# Вісник медичних і біологічних досліджень

Том 6, № 4. 2024



## Редакційна колегія

**Головний редактор**

| | |
|---|---|
| **Тімо Ульріхс** | Доктор медичних наук, професор, керівник відділу досліджень міжнародної допомоги при катастрофах, Акконський університет гуманітарних наук, Німеччина |

**Заступник головного редактора**

| | |
|---|---|
| **Лариса Федонюк** | Доктор медичних наук, професор, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |

**Відповідальний секретар**

| | |
|---|---|
| **Неля Гливка** | Кандидат біологічних наук, асистент, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |

**Національні члени редколегії**

| | |
|---|---|
| **Ігор Господарський** | Доктор медичних наук, професор, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |
| **Надія Деміхова** | Доктор медичних наук, професор, Сумський державний університет, Україна |
| **Інна Криницька** | Доктор медичних наук, професор, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |
| **Людмила Мазур** | Кандидат медичних наук, доцент, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |
| **Лілія Мартинюк** | Доктор медичних наук, професор, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |
| **Марія Марущак** | Доктор медичних наук, професор, Тернопільський національний медичний університет імені І. Я. Горбачевського, Україна |
| **Дмитро Дмитрієв** | Доктор медичних наук, професор, медичний директор, Комунальне некомерційне підприємство «Подільський регіональний центр онкології Вінницької обласної Ради», Україна |
| **Олександр Назарчук** | Доктор медичних наук, доцент, Вінницький національний медичний університет ім. М. І. Пирогова, Україна |
| **Гаяне Акопян** | Доктор медичних наук, професор, Державна установа «Інститут спадкової патології національної академії медичних наук України», Україна |
| **Борис Зіменковський** | Доктор фармацевтичних наук, професор, Львівський національний медичний університет імені Данила Галицького, Україна |
| **Ростислав Стойка** | Доктор біологічних наук, професор, Інститут біології клітини Національної академії наук України, Україна |

**Міжнародні члени редколегії**

| | |
|---|---|
| **Параскеві Папаіонаідоу** | Доктор медичних наук, професор, Університет Арістотеля в Салоніках, Греція |
| **Рамаз Чіхладзе** | Доктор медичних наук, професор, Тбіліський медичний університет, Грузія |
| **Киялбек Сакибаєв** | Кандидат медичних наук, доцент, Ошський державний університет, Киргизстан |
| **Ілона Гартмане** | Кандидат медичних наук, лікар-дерматолог, Ризький університет імені Страдіня, Латвія |
| **Джузеппе Фабіо Парізі** | Доктор медичних наук, лікар-педіатр пульмонолог, Лікарня Сан-Марко, Університет Катанія, Італія |
| **Кретою Драгош** | Доцент, Університет медицини та фармацевтики Кароля Довіла, Румунія |

# Bulletin of Medical and Biological Research

Volume 6, No. 4. 2024



## Editorial Board

**Editor-in-Chief**

| | |
|---|---|
| **Timo Ulrichs** | Doctor of Medical Sciences, Professor, Head of Studies on International Disaster Relief, Akkon University of Human Sciences, Germany |

**Deputy Editor-in-Chief**

| | |
|---|---|
| **Larysa Fedoniuk** | Doctor of Medical Sciences, Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |

**Executive Secretary**

| | |
|---|---|
| **Nelia Hlyvka** | PhD in Biological Sciences, Assistant Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |

**National Members of the Editorial Board**

| | |
|---|---|
| **Ihor Hospodarskyy** | Doctor of Medical Sciences, Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |
| **Nadiya Demikhova** | Doctor of Medical Sciences, Professor, Sumy State University, Ukraine |
| **Inna Krynytska** | Doctor of Medical Sciences, Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |
| **Lyudmyla Mazur** | PhD in Medical Sciences, Associate Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |
| **Liliya Martynyuk** | Doctor of Biological Sciences, Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |
| **Mariya Marushchak** | Doctor of Medical Sciences, Professor, I. Horbachevsky Ternopil National Medical University, Ukraine |
| **Dmytro Dmytriiev** | Doctor of Medical Sciences, Professor, Medical Director, Communal Nonprofit Enterprise "Podilsky Regional Center of Oncology of the Vinnytsia Regional Council", Ukraine |
| **Oleksandr Nazarchuk** | Doctor of Medical Sciences, Associate Professor, National Pirogov Memorial Medical University, Vinnytsya, Ukraine |
| **Hayane Akopyan** | Doctor of Medical Science, Professor, State University "Institute of Hereditary Pathology of the National Academy of Medical Sciences of Ukraine", Ukraine |
| **Borys Zimenkovsky** | Doctor of Pharmaceutical Sciences, Professor, Danylo Halytsky Lviv National Medical University, Ukraine |
| **Rostyslav Stoika** | Doctor of Biological Sciences, Professor, Institute of Cell Biology National Academy of Sciences of Ukraine, Ukraine |

**International Members of the Editorial Board**

| | |
|---|---|
| **Paraskevi Papaioannidou** | Doctor of Medical Sciences, Professor, Aristotle University of Thessaloniki, Greece |
| **Ramaz Chikhladze** | Doctor of Medical Sciences, Professor, Tbilisi State Medical Univertitery, Georgia |
| **Kyialbek Sakibaev** | PhD in Medical Sciences, Associate Professor, Osh State University, Kyrgyzstan |
| **Ilona Hartmane** | PhD in Medical Sciences, Dermatologist, Riga Stradins University, Latvia |
| **Giuseppe Fabio Parisi** | Doctor of Medical Sciences, Pediatric Pulmonologist, San Marco Hospital, University of Catania, Italy |
| **Cretoiu Dragos** | Associate Professor, Carol Davila University of Medicine and Pharmacy, Romania |

# ЗМІСТ / CONTENTS

**С. Коновалов**
Кореляційні особливості між морфометричними та показниками проточної цитометрії
у соматосенсорній корі та гіпокампі щурів із церебральною ішемією-реперфузією та її корекція ..... 8
**S. Konovalov**
Correlation peculiarities between morphometric and flow cytometric indexes
in the rat hippocampus and somatosensory cortex afterward cerebral ischemia-reperfusion and its correction ..... 8

**О. Матяш, Н. Волощук**
Роль гідроген сульфіду в біохімічних механізмах ураження міокарду за алкогольної кардіоміопатії ..... 16
**A. Matiash, N. Voloshchuk**
Role of hydrogen sulphide in biochemical mechanisms of myocardial damage in alcoholic cardiomyopathy ..... 16

**С. Мостовий**
Ішемічна хвороба серця та динаміка показників внутрішньосерцевої гемодинаміки
після госпіталізації через COVID-19: результати 12-місячного спостереження ..... 23
**S. Mostovyi**
Coronary heart disease and dynamics of intracardiac haemodynamics
after hospitalisation for COVID-19: Results of a 12-month follow-up study ..... 23

**П. Омецинський**
Кореляція між прогресуванням цефалгії і хронічного стресу в умовах війни ..... 34
**P. Ometsynskyi**
Correlation between cephalgia and chronic stress progression in wartime ..... 34

**А. Стюарт**
Вплив антиоксидантів на морфологію та функцію яєчників щурів
за умов запального процесу (вагітності) в рамках аналізу оцінки їх дії та ефективності
на ядерне та цитоплазматичне дозрівання ооцитів ..... 45
**A. Stewart**
The effect of antioxidants on the morphology and function of ovaries
in rats inflammatory process (pregnancy) within the framework of the analysis
of the assessment of their action and effectiveness on nuclear and cytoplasmic maturation of oocytes ..... 45

**В. Юр, О. Лебедєв**
Основна методика лікування міофасціального больового синдрому лікувальною фізкультурою та масажем ..... 55
**V. Yur, O. Lebediev**
General procedures for treating myofascial pain syndrome with therapeutic exercises and massage ..... 55

**Є. Федорець, О. Голубовська, Л. Пінський**
Оптимізація лікування хронічних ран при бульозному епідермолізі
та вплив мікрофлори: огляд літератури ..... 64
**Ye. Fedorets, O. Golubovska, L. Pinsky**
Optimisation of treatment of chronic wounds in bullous epidermolysis
and the influence of microflora: A literature review ..... 64

**Б. Кравченко, К. Лихота**
Наслідки вживання нікотинових виробів для тканин ротової порожнини: підходи до профілактики ..... 76
**B. Kravchenko, K. Lykhota**
Health effects of nicotine products on oral tissues: Prevention approaches ..... 76

Article's History: Received: 15.08.2024; Revised: 02.11.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.08

Journal homepage: https://bmbr.com.ua/en

UDC 61.82:616.831-005.4:611.013.395:569.323.4

# Correlation peculiarities between morphometric and flow cytometric indexes in the rat hippocampus and somatosensory cortex afterward cerebral ischemia-reperfusion and its correction

**Serhii Konovalov***

PhD in Medical Sciences, Associate Professor
National Pirogov Memorial Medical University, Vinnytsya
21018, 56 Pyrohov Str., Vinnytsia, Ukraine
https://orcid.org/0000-0002-9729-7204

**Abstract.** The increase in the ischaemic stroke incidence has become one of the main problems in the world due to high its disablement and mortality. The purpose of the study was to establish and analyse the correlations between the number of affected neuronal nuclei in the somatosensory cortex and hippocampus and flow cytometric indexes in rats having model cerebral ischaemia-reperfusion. The effects of mesenchymal stromal cells (acquired from human umbilical cord, rat and human adipose tissue, and rat embryonic fibroblasts and their lysate) on morphometric and flow cytometric parameters in the hippocampus and somatosensory cortex of adult Wistar rats (at the age – 3-4 months old, with a body weight – 160-190 g, after model cerebral ischaemia-reperfusion) were explored. The neuronal nuclei total numbers per 1 mm and the ratio of the intact neuronal nuclei number to the pathologically affected neuronal nuclei number (having karyopyknosis or karyorrhexis) were counted in the somatosensory cortex and hippocampal CA1 area. Nonparametric Spearman's correlation rank analysis was used to determine relationships between individual parameters. When using mesenchymal stromal cells having different ancestry and their lysate as therapy for ischaemia-reperfusion damage to brain structures, multidirectional correlations (both direct and inverse) were found between flow cytometry parameters and the affected neuronal nuclei number on day 7 and 14 after the ischaemia-reperfusion modelling, both in the somatosensory cortex and in the hippocampus. Thus, the results of the correlation analysis demonstrated that mesenchymal stromal cells of different ancestry have a distinct neuroprotective effect aimed at restoring neurogenesis in brain structures and suppressing the intensity of neuroapoptosis in post-perfusion injuries. The data obtained from the correlation analysis will be used to determine the most effective stem cells' class as a neuroprotectant and further develop an injectable drug based on it for the ischaemic stroke therapy

**Keywords:** mesenchymal stromal cells; cerebral ischaemia-reperfusion; morphometry; neuroapoptosis; correlation analysis

## INTRODUCTION

The progressive increase in the number of brain disease cases accompanied by cerebrovascular disorders makes this problem of modem medicine increasingly relevant around the world. The most common of these is a stroke, a neurological disease with high mortality and disablement [1]. Ischaemic stroke amounts for about 70% of strokes, and this figure is growing every year due to ageing of a population and other causes [2]. An acute ischaemic stroke is a pathological process evoked by an abrupt cessation of cerebral blood circulation leading to the neuronal death and causing an irreversible damage to brain tissue. Up to 2025, the main treatment for ischaemic stroke was thrombolysis and mechanical thrombectomy. However, C.T. Primiani *et al*. [3] showed that problems with accessibility due to the narrow time interval and the bleeding risk make these treatments possible in only 5%

**Suggested Citation**:
Konovalov S. Correlation peculiarities between morphometric and flow cytometric indexes in the rat hippocampus and somatosensory cortex afterward cerebral ischemia-reperfusion and its correction. Bull Med Biol Res. 2024;6(4):8–15. DOI: 10.63341/bmbr/4.2024.08

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

of patients. M. Li *et al*. [4] emphasised that that perfusion restoration in the ischaemic zone contributes to the aggravation of metabolic disorders in the brain and development of reperfusion injury.

Moreover, as W.J. Powers *et al*. [5] showed, further search for effective and safe treatments is still relevant. As for 2022, according to M. Kawabori *et al*. [6] therapy with stem cells has shown encouraging results in the ischaemic stroke treatment. N.G. Toman [7] analysed that numerous preclinical studies in animal models have shown that therapy by stem cells transplantation reduces ischaemic brain injury through targeting nerve cells proliferation and differentiation. Amongst different stem cells types, mesenchymal stromal cells (MSCs) were proven by J. Li *et al*. [8] to be the best choice for ischaemic stroke treatment due to such their characteristics as availability, ease of isolation and cultivation, sufficient immune tolerance, few complications during treatment. The study by J.W. Chung *et al*. [9] showed that autologous MSCs administration with autologous serum intravenously was permissible and safe in persons having a chronic stroke.

B. Brooks *et al*. [10] discovered that they also possess plasticity and the ability to undergo multidirectional differentiation, have anti-inflammatory and immunomodulatory actions, which may contribute to regeneration in the ischaemic brain tissue. Thus, the study by Y. Li *et al*. [11] demonstrated that human umbilical cord MSC therapy in combination with curcumin had anti-inflammatory and antioxidant efficacy and contributed to the neurological function improvement after an acute ischaemic stroke. H. Cao *et al*. [12] found that the combined therapy with umbilical cord MSCs and tetramethylpyrazine improved neurogenesis, suppressed inflammation, and reduced histological damage after cerebral ischaemia in rats. Therefore, the purpose of this study was to find out and analyse correlations between the affected neuronal nuclei number in the somatosensory cortex and hippocampus and flow cytometry indexes in rats subjected to model cerebral ischaemia-reperfusion (IR) and therapeutic MSCs transplantation (of different genesis) or MSCs transplantation of cell lysate from human Wharton's jelly (hWJ).

## MATERIALS AND METHODS

The research was conducted at the Educational and Research Laboratory for Preclinical Evaluation of New Medicinal Products and Biologically Active Compounds "Farmadar" (Certificate for Technic Competence No. 031/18 suitable for 31.10.2023) of Vinnytsya National Pirogov Memorial Medical University (for 2021-2023 years) on rats (adult Wistar rats at the age – 3-4 months old, with a body weight – 160-190 g). The rats were held on the standard water-food diet under normal vivarium conditions with controlled lighting (12-hour light cycle), 20-26°C room temperature, 40-70% air humidity, with unlimited food and water access. The study protocol was confirmed by the Bioethics Committee of Vinnytsya National Pirogov Memorial Medical University (protocol No. 2 of January 31, 2024). All experimental studies were carried out in accordance with modern methodological approaches and in compliance with relevant requirements and standards, in particular, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [13]. The animals were kept and all manipulations were carried out in accordance with the provisions of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" [14].

MSCs and cell lysate from hWJ-MSCs were obtained from the Institute of Molecular Biology and Genetics at the Ukrainian National Academy of Sciences. The cells were transferred on the basis of the Agreement on Scientific Cooperation between the Institute of Molecular Biology and Genetics and Vinnytsya National Pirogov Memorial Medical University of September 22, 2017. Brain cortex morphology of rats and humans is similar, making rats ideal experimental animals. The therapeutic effect and cytoprotective properties of MSCs (of different ancestry) and cell lysate from hWJ-MSCs were studied in rats right after the model transient ischaemia-reperfusion of a rat brain (the internal carotid arteries were legated for 20 minutes bilaterally, Propofol-Novo medication was used for the central anaesthesia, produced by Novopharm-Biosynthesis, Ukraine, at the dose 60 mg/kg). The distribution of rats into experimental groups in the study was as follows (Fig. 1).

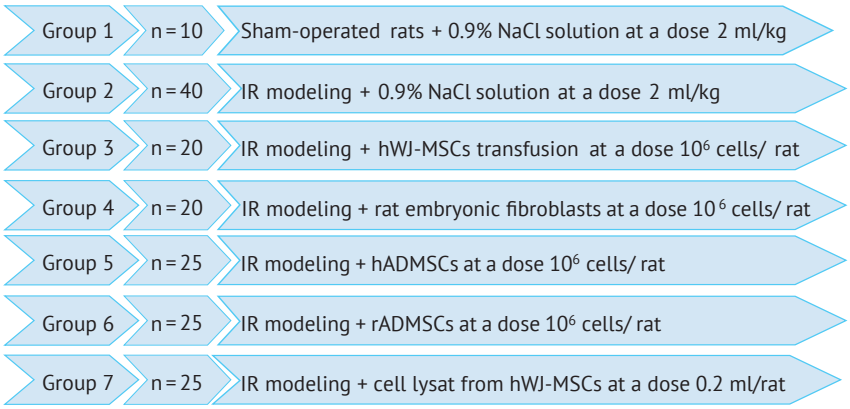


**Figure 1.** Distribution of rats into experimental groups in the study
**Source:** compiled by the author

MSCs and cell lysate from hWJ-MSCs were administered into the femoral vein right after IR, as an early transplantation had a better cerebroprotective effect and needed smaller donor MSCs number ($1\times10^6$) [15]. On Day 7

(subacute ischaemic period) and Day 14 (recovery ischaemic period) days after the IR, animals were euthanised by the humane method (decapitation) using central pentobarbital anaesthesia (Penbital, produced by Bioveta, Czech Republic, 100 mg/kg) [16]. Rat brains were extracted, morphological studies were performed, and the DNA fragmentation level in the neuronal nuclei of the hippocampus and somatosensory cortex was determined using by flow cytometry method [17]. Nuclear suspensions of rat sensorimotor cortex and hippocampus biopsies, made according to SyStain DNA Step 1 (Partec apparatus, Germany) were prepared immediately after sampling and washing with cold (temperature +4 to +8°C) phosphate-salt buffer solution having pH 7.4 (Sigma). Flow cytometry was performed on the Partec PAS multifunctional flow cytometer (Partec, Germany). UV rays were used to excite DAPI fluorescence. 20,000 events were analysed from each nuclear suspension sample. Cyclic cell analysis was performed using the FloMax software suite (Partec, Germany) in a full digital accordance to the mathematic pattern, which determined: G0G1% as the ratio (in %) of the G0G1 phase cells to all the cells in the cell cycle (DNA content – 2s); S% as the ratio (in %) of the DNA synthesis phase cells to all the cells in the cell cycle (DNA content – between 2s and 4s); G2+M% as the ratio (in %) of the G2+M phase cells to all the cells in the cell cycle (DNA content – 4s);

DNA fragmentation (i.e. apoptosis) was determined through SUB-GOGI region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s. For morphological studies, the extracted rat brains were fixed in 4% formaldehyde solution for 24 hours at the appropriate time points. After fixation, the brains were washed in running water, passed through ascending alcohols and xylenes, and after the standard histological wiring, embedded in Paraplast Plus (Leica Scientific (McCormicke), USA). 5 μm thick sections were made in a rotary microtome. Deparaffinised sections were dyed with haematoxylin-eosin (ordinarily) as well as by the Nissl method. Morphometric analysis was performed in an automatic mode.

Digital images of the frontal brain slices obtained on the BX-51 microscope (Olympus, Japan) were analysed using the ImageJ computer programme (1.48v, free license, Rasband, USA, 2015). The total number of neuronal nuclei in 1 mm of the somatosensory cortex and hippocampal CAl area, and the ratio of the intact neuronal nuclei number to the pathologically changed neuronal nuclei number (i.e. with karyorrhexis and karyopyknosis) were counted. Statistical processing of the research results was carried out using the Statistica 7.0 software suite (StatSoft Inc., USA) by the methods of non-parametric (Wilcoxon-Mann-Whitney U-test) statistics. Differences in the average values of indicators between comparison groups were considered significant at $p \leqslant 0.05$. Spearman's non-parametric correlation rank analysis was used to determine the relationship between individual parameters. Correlation dependence reflected the statistical relationship of two (or more) random variables, while changes in the values of one (or more) of these variables accompany a systematic change in the values of another (or other) variables.

## RESULTS

A correlation relationship reflects a statistical relationship between two (or more) random variables, whereby changes in the values of one (or more) of these variables accompany a systematic change in the values of another (or other) variables. When analysing the significant correlations between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex of rats with IR (Group 2), a single strong inverse ($r = -0.90$, $p < 0.05$) relationship between the G0G1% in the hippocampus and the damaged nuclei number in the hippocampus on Day 7 after IR was discovered; including a direct ($r = 0.90$, $p < 0.05$) relationship between the G2 + M% in the hippocampus on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the hippocampus on Day 14 after IR (Table 1).

**Table 1.** Correlation between the damaged neuronal nuclei number in the hippocampus and somatosensory cortex and flow cytometry indexes in rats with model brain IR and in the background of correction

| Parameters | The number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex | | | | The number of damaged nuclei (karyopyknosis, karyorrhexis) in the hippocampus | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 7 | | Day 14 | | Day 7 | | Day 14 | |
| | r | p | r | p | r | p | r | p |
| IR (control pathology) | | | | | | | | |
| G0G1 | -0.21 | 0.7406 | 0.30 | 0.6238 | **-0.90** | **0.0374** | -0.60 | 0.2848 |
| S | 0.21 | 0.7406 | -0.30 | 0.6238 | 1.00 | | 0.30 | 0.6238 |
| G2+M | 0.82 | 0.0886 | 0.50 | 0.3910 | 0.10 | 0.8729 | **0.90** | **0.0374** |
| Sub-G0G1 | 0.82 | 0.0886 | -0.10 | 0.8729 | 0.10 | 0.8729 | -0.60 | 0.2848 |
| IR + human umbilical cord Wharton's jelly MSCs | | | | | | | | |
| G0G1 | 0.50 | 0.3910 | 0.00 | 1.0000 | 0.60 | 0.2848 | -0.50 | 0.3910 |
| S | -0.40 | 0.5046 | 0.30 | 0.6238 | 0.10 | 0.8729 | **0.90** | **0.0374** |
| G2+M | -0.30 | 0.6238 | -0.40 | 0.5046 | **-0.90** | **0.0374** | -0.50 | 0.3910 |
| Sub-G0G1 | -0.10 | 0.8729 | 0.20 | 0.7471 | -0.10 | 0.8729 | **0.90** | **0.0374** |
| IR + rat embryonic fibroblasts | | | | | | | | |
| G0G1 | 0.10 | 0.8696 | **-0.90** | **0.0374** | 0.53 | 0.3615 | -0.82 | 0.0886 |
| S | -0.36 | 0.5528 | 0.40 | 0.5046 | -0.37 | 0.5411 | 0.67 | 0.2189 |
| G2+M | -0.10 | 0.8696 | **0.90** | **0.0374** | -0.53 | 0.3615 | **0.97** | **0.0048** |

*Table 1. Continued*

| Parameters | The number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex | | | | The number of damaged nuclei (karyopyknosis, karyorrhexis) in the hippocampus | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 7 | | Day 14 | | Day 7 | | Day 14 | |
| | r | p | r | p | r | p | r | p |
| Sub-G0G1 | -0.82 | 0.0886 | 0.70 | 0.1881 | -0.53 | 0.3615 | 0.15 | 0.8048 |
| IR + human adipose tissue MSCs | | | | | | | | |
| G0G1 | 0.30 | 0.6238 | -0.21 | 0.7406 | -0.80 | 0.1041 | 0.20 | 0.7471 |
| S | -0.20 | 0.7471 | -0.21 | 0.7406 | **0.90** | **0.0374** | -0.10 | 0.8729 |
| G2+M | -0.70 | 0.1881 | 0.87 | 0.0539 | 0.30 | 0.6238 | -0.20 | 0.7471 |
| Sub-G0G1 | 0.40 | 0.5046 | -0.21 | 0.7406 | 0.50 | 0.3910 | -0.50 | 0.3910 |
| IR + rat adipose tissue MSCs | | | | | | | | |
| G0G1 | 0.10 | 0.8696 | **0.90** | **0.0374** | 0.10 | 0.8729 | 0.20 | 0.7471 |
| S | -0.21 | 0.7406 | -0.80 | 0.1041 | 0.00 | 1.0000 | 0.10 | 0.8729 |
| G2+M | 0.21 | 0.7406 | -0.50 | 0.3910 | -0.60 | 0.2848 | -0.80 | 0.1041 |
| Sub-G0G1 | 0.15 | 0.8048 | -0.30 | 0.6238 | -0.20 | 0.7471 | 0.50 | 0.3910 |
| IR + cell lysate from hWJ-MSCs | | | | | | | | |
| G0G1 | -0.30 | 0.6238 | **0.90** | **0.0374** | **-0.95** | **0.0138** | -0.60 | 0.2848 |
| S | 0.30 | 0.6238 | **-0.90** | **0.0374** | 0.32 | 0.6042 | 0.70 | 0.1881 |
| G2+M | -0.10 | 0.8729 | -0.30 | 0.6238 | **0.95** | **0.0138** | 0.60 | 0.2848 |
| Sub-G0G1 | 0.30 | 0.6238 | **-0.90** | **0.0374** | 0.53 | 0.3615 | **0.90** | **0.0374** |

**Notes:** r – rank correlation coefficient; p – probability of the expected result
**Source:** compiled by the author

Correlations analysis made between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex in the rats with IR and consequent immediate intravenous transplantation of human umbilical cord MSCs (Group 3) revealed strong direct ($r = 0.90$, $p < 0.05$ in both cases) relationships between the S% and the number of damaged nuclei (having karyopyknosis and karyorrhexis) in the hippocampus on Day 14 after IR and corresponding therapy, as between the SUB-G0G1 areas in the DNA histograms – RNI before the G0G1 peak, which indicates cell nuclei with DNA content less 2s and the damaged nuclei number in the hippocampus on Day 14 after IR and corresponding therapy. An inverse ($r = -0.90$, $p < 0.05$) relationship was found between the G2 + M% and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus on Day 7 after IR and corresponding therapy (Table 1).

In rats of the Group 4 (i.e. in the rats having IR followed by rat embryonic fibroblasts transplantation) a strong direct ($r = 0.90$, $p < 0.05$) relationship between the G2 + M% on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the somatosensory cortex on Day 14 after IR and corresponding therapy was found. In addition, an inverse ($r = -0.90$, $p < 0.05$) relationship between the G0G1% on Day 7 and the damaged nuclei number in the somatosensory cortex on Day 14 after IR and corresponding therapy was observed. A direct ($r = 0.97$, $p < 0.01$) relationship between the G2 + M% in the hippocampus on Day 7 and the damaged nuclei number also in the hippocampus on Day 14 after IR and corresponding therapy was found (Table 1).

In the Group 5 (where rats were undergone IR and subsequent MSCs administration having human adipose tissue origin) a strong direct ($r = 0.90$, $p < 0.05$) relationship between the S% in the hippocampus and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the hippocampus on Day 7 after IR and corresponding therapy was (Table 1) was found. The correlations analysis made in the Group 6 (rats with IR and consequent MSCs of the rat adipose tissue origin administration) showed a significant strong direct correlation ($r = 0.90$, $p < 0.05$) between the G0G1% in the somatosensory cortex on Day 7 and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex on day 14 after IR and corresponding therapy (Table 1).

Last group (Group 7) of rats (which had IR and immediate cell lysate from hWJ-MSCs transplantation) demonstrated significant correlations between flow cytometry parameters and the damaged neuronal nuclei number, such as strong direct ($r = 0.90$ to $0.95$, $p < 0.05$) relationships between the GOG1% in the somatosensory cortex on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the somatosensory cortex on Day 14 after IR and corresponding therapy, between the G2 + M% and the damaged nuclei number in the hippocampus on Day 7 after IR corresponding MSCs therapy, between the SUB-G0G1 region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s in the hippocampus and the damaged nuclei number also in the hippocampus on Day 14 after IR and corresponding therapy. Strong inverse ($r = -0.90$ to $-0.95$, $p < 0.05$) relationships were found in this group between the S% in the somatosensory cortex and DNA fragmentation ibid on Day 14; between the SUB-G0G1 region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s in the somatosensory cortex and the damaged nuclei number also in the somatosensory cortex on Day 14 after IR and corresponding therapy; between the G0G1% in the hippocampus on Day 7 and the damaged nuclei number in the hippocampus on Day 7 after IR and corresponding therapy (Table 1).

When analysing the correlations between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex in rats with experimental acute IR lesions and their MSC therapy, the principle of data independence was strictly adhered, which implies that the values of variables in one sample are not related to the values of variables in another sample with which the comparison is made. The study found that intravenous allogeneic and xenogeneic transplantations of MSCs having different origins and the cell lysate from hWJ-MSCs introduction at once after IR modelling in rats reduce the volume of ischaemic brain damage. The demonstrated cerebroprotective properties of MSCs transplantations may indicate the prospect of their use in cell therapy of acute cerebral ischaemia.

## DISCUSSION

Numerous preclinical studies in animal models have shown that stem cell transplantation therapy reduces ischaemic brain damage and neurological deficits through directed proliferation and differentiation of nerve cells [7, 18]. In the group of rats with model IR (without treatment), when analysing the correlations between flow cytometry parameters and the damaged neuronal nuclei number (both in the hippocampus and somatosensory cortex) during the studied periods, there were no correlations in the somatosensory cortex. The author found mainly single linear relationships in the hippocampus between the damaged nuclei number (with karyopyknosis and karyorrhexis) and the G0G1% in the hippocampus (on Day 7 after IR, $r = -0.90$, $p < 0.05$), indicating an increase in neuroapoptosis intensity in the rat hippocampus. There is a direct correlation between the G2 + M% in the hippocampus and the damaged nuclei number in the hippocampus (on Day 14 after IR, $r = 0.90$, $p < 0.05$), which may indicate neuronal recovery beginning in the hippocampus on Day 14 after IR.

In rats from the Group 3 (i.e., rats with a model of acute subtotal cerebral IR injury and subsequent therapeutic MSCs transplantation of umbilical cord hWJ), there was no correlation in the somatosensory cortex between the flow cytometry parameters and the number of damaged neuronal nuclei, both on Day 7 and Day 14 after IR. Attention should be drawn to the reliable linear multiple dependencies between the G2 + M% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus on Day 7 after IR, $r = -0.90$, $p < 0.05$), thus, there is a feedback indicating the regenerative potential of the umbilical cord hWJ MSCs after experimental IR, which may be explained not by the substitution of affected cells in the ischaemic zone, but by their release of bioactive substances that facilitate neurogenesis and defend brain tissue from ischaemic damage [19]. Mechanisms underlying favourable outcomes in MSC transplantation include "bystander" effects, paracrine mechanisms, or restorative effects mediated by extracellular vesicles [10].

Thus, the study by A.A. Taei *et al*. [20] found that MSC secretome therapy reduced inflammation, apoptosis, and neuronal loss in the ischaemic brain. On Day 14 day after IR, strong direct correlations were found between the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus and the S% in the hippocampus ($r = 0.90$, $p < 0.05$) and DNA fragmentation level also in the hippocampus ($r = 0.90$, $p < 0.05$). Thus, under the influence of umbilical cord hWJ MSCs in rats on Day 14 after IR, positive correlations were observed in the hippocampus towards endogenous neuroregeneration and switching of necrotic neuronal death to apoptotic. It is known from literary sources that programmed cell death by apoptosis occurs in the late stages of ischaemic stroke progression and in neurons of the ischaemic penumbra. During apoptosis, DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, development of apoptotic bodies, which are subsequently subjected to phagocytosis, occur in the cell. That is why the apoptotic death of neurons has certain advantages over necrosis and is a "lesser evil" for the brain, although the total number of cells will be reduced [21]. The results of the study are consistent with those obtained by H. Cao *et al*. [12]. The authors found that treatment with umbilical cord MSCs can stimulate neurogenesis while reducing the degree of damage and inflammation, and improve neuroprotection after the ischaemic brain lesion.

In Group 4 rats having rat embryonic fibroblasts transplantation just after IR modelling, a direct correlation was found between the G2 + M% in the somatosensory cortex and the damaged nuclei number in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$). Analogous correlations were observed in the hippocampus between the G2 + M% and the damaged nuclei number (on Day 14 after IR, $r = 0.97$, $p < 0.01$). Thus, after intravenous transplantation of rat embryonic fibroblasts on Day 14 after IR, positive correlations were found in the brain damage in rats due to paracrine effects on neurogenesis in both the somatosensory cortex and hippocampus.

In rats from the Group 5 (i.e., rats with a model of brain IR followed by human adipose tissue MSCs transplantation), there was no correlation in the somatosensory cortex between the flow cytometry parameters and the damaged neuronal nuclei number in the periods studied after IR. There was a direct correlation between the S% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = 0.90$, $p < 0.05$), which may indicate the activation of endogenous neurogenesis in the hippocampus by paracrine signalling of human adipose tissue MSCs through trophic factors. The results of the current research are consistent with the data of K. Yatsenko *et al*. [21], according to which the described effects are intermediated by the secretome of human adipose tissue MSCs, since these cells do not have direct contact with the brain of experimental animals when injected intravenously.

In rats with a model of brain IR followed by the therapeutic MSCs transplantation of rat adipose cells, there was no correlation in the hippocampus between flow cytometry parameters and the damaged neuronal nuclei number in the subacute and recovery periods of IR. A strong direct correlation was found between the G0G1% in the somatosensory cortex and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$), which reflects the protective potential of MSCs of the rat adipose tissue on nervous cells present in the somatosensory cortex.

A study by C.T. Van Velthoven *et al*. [22] found that MSCs reduce ischaemic damage that occurs during experimental reversible occlusion of the middle cerebral artery in newborn rats, protect white matter in a brain, and make better long-term functional curing after a focal stroke. They found a positive correlation between the white matter integrity (which was affected by MSCs) and functional performance analysed in ischaemic newborn rats. In Group 7 rats with a model cerebral insult followed by the MSCs transplantation of cell lysate from hWJ multiple linear correlations between flow cytometry parameters and the damaged neuronal nuclei number in the period after the insult in both the somatosensory cortex and hippocampus are noteworthy.

The author found multiple negative correlations between the S% in the somatosensory cortex and DNA fragmentation level in the somatosensory cortex with the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = -0.90$ in both cases, $p < 0.05$), which may indicate that the MSCs transplantation of cell lysate from hWJ has no effect on neurogenesis and neuroapoptosis in the somatosensory cortex after IR in rats. In addition, the transition from an inverse correlation (on Day 7 after IR) to a direct correlation (on Day 14) between the G0G1% in the somatosensory cortex and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$), in authors' opinion, may indicate the neuroprotective effect of cell lysate from hWJ MSCs on the neurons of the somatosensory cortex.

However, MSCs of the cell lysate from hWJ had no protective action on hippocampal neurons against ischaemia-reperfusion injury on Day 7 after IR. This was evidenced by a strong inverse correlation between the G0G1% in the hippocampus on Day 7 and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = -0.95$, $p < 0.05$). There were strong direct correlations between the G2 + M% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = 0.95$, $p < 0.05$) and between DNA fragmentation level in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 14 after IR, $r = 0.90$, $p < 0.05$), indicating activation of endogenous neurogenesis in the hippocampus and switching of necrotic neuronal death to apoptotic one under the influence of cell lysate from hWJ. The obtained results of the correlation analysis between morphometric and cytometric indicators in the hippocampus and somatosensory cortex of rats with cerebral ischaemia-reperfusion confirm that MSC transplantation effectively protects ischaemic neurons of the penumbra zone.

## CONCLUSIONS

The correlation analysis revealed strong direct and inverse line dependencies between the flow cytometry parameters and the damaged neuronal nuclei number on Day 7 and Day 14 after IR modelling in rats, both in the somatosensory cortex and hippocampus. It was confirmed that subtotal reversible brain IR in rats caused early damage to hippocampal neurons by activating neuroapoptosis. MSCs usage having different ancestry and cell lysate from hWJ as a therapy for ischaemia-reperfusion injury of brain structures showed different correlations (both direct and inverse) between the flow cytometry parameters and the damaged neuronal nuclei number during the experimental period. Thus, transplantation of umbilical cord hWJ MSCs after IR modelling caused positive correlations directed to the endogenous neuroregeneration and switching of necrotic neuronal death to apoptotic.

Transplantation of rat embryonic fibroblasts in rats revealed strong direct correlations with neurogenesis in both somatosensory cortex and hippocampus on Day 14 after model IR. Transplantation of human adipose tissue-derived MSCs caused strong direct correlations aimed at activation of neurogenesis in the hippocampus on Day 7 after model IR in rats. In general, MSCs transplantation from rat adipose tissue for IR therapy caused a strong direct correlation aimed at protecting somatosensory cortex neurons from ischaemia-reperfusion injury in rats. MSCs administration of cell lysate from hWJ caused strong direct correlation dependencies aimed at protecting somatosensory cortex neurons from ischaemia-reperfusion injury and activation of endogenous neurogenesis in the hippocampus.

Thus, the results demonstrated that MSCs having different ancestry exerts a distinct neuroprotective effect aimed at restoring neurogenesis in brain structures and suppressing the intensity of neuroapoptosis in post-perfusion injuries. The data obtained during the correlation analysis will be used to determine the most effective MSC class as a neuroprotective agent, in order to further develop an injectable medication for the cure of patients with the ischaemic stroke. The results experimentally substantiate the expediency of clinical trials of an injectable drug based on human Wharton's umbilical cord MSCs for a new purpose, namely, as a cytoprotector in patients with the ischaemic stroke.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Feigin VL, Stark BA, Johnson CO, Roth GA, Bisignano C, Abady GG, et al. Global, regional, and national burden of stroke and its risk factors, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet Neurol. 2021;20(10):795–820. DOI: 10.1016/S1474-4422(21)00252-0

[2] Phipps MS, Cronin CA. Management of acute ischemic stroke. BMJ. 2020;368:16983. DOI: 10.1136/bmj.l6983

[3] Primiani CT, Vicente AC, Brannick MT, Turk AS, Mocco J, Levy EI, et al. Direct aspiration versus stent retriever thrombectomy for acute stroke: A systematic review and meta-analysis in 9127 patients. J Stroke Cerebrovasc Dis. 2019;28(5):1329–37. DOI: 10.1016/j.jstrokecerebrovasdis.2019.01.034

[4] Li M, Tang H, Li Z, Tang W. Emerging treatment strategies for cerebral ischemia-reperfusion injury. Neuroscience. 2022;507:112–24. DOI: 10.1016/j.neuroscience.2022.10.020

[5] Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Correction to: Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: A guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2019;50(12):440–1. DOI: 10.1161/STR.0000000000000215
[6] Kawabori M, Shichinohe H, Kuroda S, Houkin K. Clinical trials of stem cell therapy for cerebral ischemic stroke. Int J Mol Sci. 2020;21(19):7380. DOI: 10.3390/ijms21197380
[7] Toman NG, Grande AW, Low WC. Neural repair in stroke. Cell Transplant. 2019;28(9–10):1123–6. DOI: 10.1177/0963689719863784
[8] Li J, Zhang Q, Wang W, Lin F, Wang S, Zhao J. Mesenchymal stem cell therapy for ischemic stroke: A look into treatment mechanism and therapeutic potential. J Neurol. 2021;268(11):4095–107. DOI: 10.1007/s00415-020-10138-5
[9] Chung JW, Chang WH, Bang OY, Moon GJ, Kim SJ, Kim SK, et al. Efficacy and safety of intravenous mesenchymal stem cells for ischemic stroke. Neurology. 2021;96(7):1012–23. DOI: 10.1212/WNL.0000000000011440
[10] Brooks B, Ebedes D, Usmani A, Gonzales-Portillo JV, Gonzales-Portillo D, Borlongan CV. Mesenchymal stromal cells in ischemic brain injury. Cells. 2022;11(6):1013. DOI: 10.3390/cells11061013
[11] Li Y, Huang J, Wang J, Xia S, Ran H, Gao L, et al. Human umbilical cord-derived mesenchymal stem cell transplantation supplemented with curcumin improves the outcomes of ischemic stroke via AKT/GSK-3β/β-TrCP/Nrf2 axis. J Neuroinflammation. 2023;20(1):49. DOI: 10.1186/s12974-023-02738-5
[12] Cao H, Cheng Y, Zhang J, Xu M, Ge L. The effect of umbilical cord mesenchymal stem cells combined with tetramethylpyrazine therapy on ischemic brain injury: A histological study. J Stroke Cerebrovasc Dis. 2020;29(12):105298. DOI: 10.1016/j.jstrokecerebrovasdis.2020.105298
[13] Council of Europe. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [Internet]. 1986 [cited 2025 Jan 10]. ETS No. 123. 1986 Mar 18. Available from: https://rm.coe.int/168007a67b
[14] Law of Ukraine. On the Protection of Animals from Cruelty [Internet]. 2006 [cited 2025 Jan 10]. Order No. 3447-IV. 2006 Feb 21. Available from: https://zakon.rada.gov.ua/laws/show/3447-15#Text
[15] Chen Y, Peng D, Li J, Zhang L, Chen J, Wang L, et al. A comparative study of different doses of bone marrow-derived mesenchymal stem cells improve post-stroke neurological outcomes via intravenous transplantation. Brain Res. 2023;1798:148161. DOI: 10.1016/j.brainres.2022.148161
[16] Mehta A, Mahale R, Buddaraju K, Javali M, Acharya P, Srinivasa R. Efficacy of neuroprotective drugs in acute ischemic stroke: Is it helpful? J Neurosci Rural Pract. 2019;10(4):576–81. DOI: 10.1055/s-0039-1700790
[17] Toyoshima A, Yasuhara T, Kameda M, Morimoto J, Takeuchi H, Wang F, et al. Intra-arterial transplantation of allogeneic mesenchymal stem cells mounts neuroprotective effects in a transient ischemic stroke model in rats: Analyses of therapeutic time window and its mechanisms. PLoS One. 2015;10(6):e0127302. DOI: 10.1371/journal.pone.0127302
[18] Yu SP, Tung JK, Wei ZZ, Chen D, Berglund K, Zhong W, et al. Optochemogenetic stimulation of transplanted iPS-NPCs enhances neuronal repair and functional recovery after ischemic stroke. J Neurosci. 2019;39(33):6571–94. DOI: 10.1523/JNEUROSCI.2010-18.2019
[19] Tuo QZ, Zhang ST, Lei P. Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. Med Res Rev. 2022;42(1):259–305. DOI: 10.1002/med.21817
[20] Taei AA, Dargahi L, Khodabakhsh P, Kadivar M, Farahmandfar M. Hippocampal neuroprotection mediated by secretome of human mesenchymal stem cells against experimental stroke. CNS Neurosci Ther. 2022;28(9):1425–38. DOI: 10.1111/cns.13886
[21] Yatsenko K, Lushnikova I, Ustymenko A, Patseva M, Govbakh I, Kyryk V, et al. Adipose–derived stem cells reduce lipopolysaccharide-induced myelin degradation and neuroinflammatory responses of glial cells in mice. J Pers Med. 2020;10(3):66. DOI: 10.3390/jpm10030066
[22] Van Velthoven CT, Dzietko M, Wendland MF, Derugin N, Faustino J, Heijnen CJ, et al. Mesenchymal stem cells attenuate MRI-identifiable injury, protect white matter, and improve long-term functional outcomes after neonatal focal stroke in rats. J Neurosci Res. 2017;95(5):1225–36. DOI: 10.1002/jnr.23954

# Кореляційні особливості між морфометричними та показниками проточної цитометрії у соматосенсорній корі та гіпокампі щурів із церебральною ішемією-реперфузією та її корекція

**Сергій Коновалов**
Кандидат медичних наук, доцент
Вінницький національний медичний університет ім. М.І. Пирогова
21018, вул. Пирогова, 56, м. Вінниця, Україна
https://orcid.org/0000-0002-9729-7204

**Анотація.** Зростання захворюваності на ішемічний інсульт стало однією з головних проблем у світі через високу інвалідність та смертність. Метою роботи стало проведення аналізу кореляцій між ушкодженими нейронами та показниками проточної цитометрії у соматосенсорній корі та гіпокампі щурів в умовах терапії церебральної ішемії-реперфузії. Досліджено вплив мезенхімальних стромальних клітин (отриманих із пуповини людини, жирової тканини щура та людини, а також ембріональних фібробластів щурів та їх лізату) на показники морфометрії та проточної цитометрії в гіпокампі та соматосенсорній корі статевозрілих щурів лінії Вістар (вік – 3-4 міс, маса тіла – 160-190 г, яким проведено модельну ішемію-реперфузію головного мозку). У соматосенсорній корі та СА1 ділянці гіпокампа підраховано загальну кількість ядер нейронів на 1 мм$^2$, а також визначено співвідношення кількості неушкоджених ядер нейронів та ядер із патологічними змінами (каріорексис та каріопікноз). Для визначення зв'язку між окремими параметрами використано непараметричний кореляційний ранговий аналіз Спірмена. При застосуванні мезенхімальних стромальних клітин різного походження та їх лізату при терапії ішемічно-реперфузійного ураження структур головного мозку виявлено різноспрямовані кореляції (як прямі, так і зворотні) між параметрами проточної цитометрії та кількістю ядер уражених нейронів на 7 та 14 добу після ішемії, як у соматосенсорній корі, так і в гіпокампі. Отже, результати кореляційного аналізу демонструють наявність у мезенхімальних стромальних клітин різного походження виразної нейропротекторної дії спрямованої на відновлення нейрогенезу в структурах головного мозку та пригнічення інтенсивності нейроапоптозу при постреперфузійних пошкодженнях. Отримані дані кореляційного аналізу будуть використані з метою визначення найбільш ефективного класу мезенхімальних стромальних клітин у якості нейропротектора та подальшого створення на його основі ін'єкційного препарату для лікування хворих з ішемічним інсультом

**Ключові слова:** мезенхімальні стромальні клітини; церебральна ішемія-реперфузія; морфометрія; нейроапоптоз; кореляційний аналіз

Article's History: Received: 21.07.2024; Revised: 22.10.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.16

Journal homepage: https://bmbr.com.ua/en

UDC 546.221.1: 616.61:616.379-008.64:612.08

# Role of hydrogen sulphide in biochemical mechanisms of myocardial damage in alcoholic cardiomyopathy

**Alexander Matiash***

Postgraduate Student
National Pirogov Memorial Medical University, Vinnytsya
21018, 56 Pyrohov Str., Vinnytsia, Ukraine
https://orcid.org/0000-0002-9797-1588

**Nataliia Voloshchuk**

Doctor of Medical Science, Professor
National Pirogov Memorial Medical University, Vinnytsya
21018, 56 Pyrohov Str., Vinnytsia, Ukraine
https://orcid.org/0000-0002-0166-9676

**Abstract.** Excessive alcohol consumption, which is observed in many patients, causes cardiovascular diseases, in particular alcoholic cardiomyopathy, but the role of changes in $H_2S$ metabolism in heart damage in this pathology remains understudied. Therefore, the study aimed to determine the role of hydrogen sulphide in the mechanism of heart damage in alcoholic myocardial injury in rats. The study was carried out on 35 white male Wistar rats weighing 260-290 g, divided into two groups. The study determined that the development of alcoholic cardiomyopathy in rats is accompanied by the development of pathobiochemical processes, including cardiomyocyte apoptosis, inflammation, development of oxidative and nitrosative stress, as evidenced by a significant increase in the content of caspase-3 (5.23 times) of tumour necrosis factor α (70.2%), malondialdehyde of carbonyl groups of proteins (2.7 and 2.5 times), respectively, as well as imbalance in the system of nitric oxide synthases (increase in inducible and decrease in endothelial isoform (+94.4 and -40.0%, respectively, $p < 0.05$). All these changes were associated with a decrease in myocardial hydrogen sulphide content (-36.0%, $p < 0.05$). The correlation and permutation analysis confirmed the relationship between the content of hydrogen sulphide and markers of cardiac damage in alcoholic cardiomyopathy, namely, a low content of the gas transmitter was associated with more severe disorders, while a higher level of hydrogen sulphide, on the contrary, was associated with moderate or minimal signs of cardiac damage under conditions of chronic alcoholism in animals. Assessment of the level of hydrogen sulphide in the body can predict the development of alcohol-induced heart damage and is an experimental basis for the development of $H_2S$-releasing drugs to improve the pharmacological management of patients

**Keywords:** alcoholic myocardial injury; hydrogen sulphide; inflammation; apoptosis; oxidative stress; NO synthase; rats

## INTRODUCTION

Excessive alcohol consumption is observed in a wide range of patients worldwide. The World Health Organization (WHO) defines alcohol as toxic, psychoactive and addictive [1]. Alcohol consumption remains the third most important risk factor for poor health worldwide; excessive alcohol consumption is one of the four most common modifiable risk factors for major non-communicable diseases [2]. In Ukraine, according to a WHO sociological survey conducted in 2023, 77.4% of the adult population consumed alcohol during the year (80.1% and 75.2% of men and women, respectively), and in 2021, the average per capita consumption of pure alcohol per year in Ukraine was 8.1 litres [3]. Although the number of people who reduced alcohol consumption exceeded the number of those who increased it since the outbreak of full-scale war, this problem remains severe, as it usually causes health and social problems, Alcohol consumption is responsible for 3 million annual deaths worldwide in addition to disability

**Suggested Citation**:
Matiash A, Voloshchuk N. Role of hydrogen sulphide in biochemical mechanisms of myocardial damage in alcoholic cardiomyopathy. Bull Med Biol Res. 2024;6(4):16–22. DOI: 10.63341/bmbr/4.2024.16

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

and related diseases affecting millions of people, with a global burden of disease estimated at 5.1% (7.1% for men and 2.2% for women) [1]. Alcohol is the main risk factor for premature mortality and disability among people aged 15-49 years, accounting for 10% of all deaths in this age group; in the 20-39 age group, ~13.5% of all deaths are alcohol related.

Among the targets of the negative impact of chronic alcohol consumption, the cardiovascular system is distinguishable, since alcoholic cardiomyopathy (ACM), which is a progressive cardiac dysfunction, dilated cardiomyopathy, causes heart failure and other heart diseases, often with fatal consequences. M. Shahid *et al.* [4] analysed mortality rates in the United States for 1999-2020 and estimated the Social Vulnerability Index (SVI), which determined the impact of a range of external stressors on the infrastructure and community well-being. The study demonstrated that the SVI index is directly correlated with alcohol abuse and mortality from ACM. The literature describes the negative changes that occur in the myocardium under conditions of direct and indirect toxic effects of alcohol in a thorough manner. However, according to C. Andersson *et al.* [5], some issues remain insufficiently studied due to the lack of systematic population-based studies and a small number of described clinical cases. The data on the reversibility of negative morphological changes in the heart after alcohol abstinence also appear controversial. There is no definitive evidence of sex differences in the development of this pathology, although some results indicate that, despite the generally higher number of men who abuse alcohol, alcohol-induced heart damage occurs more rapidly in women.

The pathobiochemical mechanisms of ACM development and progression are also far from being fully elucidated, which significantly hinders the effective prevention and treatment of this pathology. The protective role of $H_2S$ in the treatment of ischemia-reperfusion injuries of various organs (heart, brain, liver, intestines, lungs, spinal cord, etc.) was described by X. Sun *et al.* [6], which became the basis for testing $H_2S$-releasing molecules in the treatment of these conditions. F.M. Payne *et al.* [7] demonstrated that hydrogen sulphide, along with two other gas molecules – nitrogen monoxide (NO) and carbon monoxide (CO), provide anti-inflammatory effects on the myocardium in experimental myocardial infarction due to inhibition of the proinflammatory inflammasome NLRP3, which reduces the regulation of cellular inflammation as a pathobiochemical link in myocardial damage. Clinical confirmation of the protective effect of the donor hydrogen sulphide – sodium thiosulfate in patients with myocardial infarction was also obtained by M.L. de Koning *et al.* [8]. The pleiotropic effects of $H_2S$ (antioxidant, vasodilator, antiapoptotic, anti-inflammatory, etc.) are correlated with the pathogenesis of other myocardial lesions, such as diabetic cardiomyopathy, as demonstrated by S. Zhao *et al.* [9]. To date, there is no literature data on changes in $H_2S$ metabolism and their role in heart damage in alcoholic cardiomyopathy. The study aimed to determine the role of hydrogen sulphide in the mechanisms of heart damage in alcoholic myocardial damage in rats.

## MATERIALS AND METHODS

During the experimental studies, the standards of current legislation and international guidelines for conducting medical and biological research using animals were maintained [10]. The experiment included male Wistar rats weighing 260-290 g of mature age, kept in the vivarium of the National Pirogov Memorial Medical University, Vinnytsya (the room temperature was maintained in the range of 20-22°C, and the relative humidity was within 50 ± 5%) and maintained on a standard diet. The experiments were conducted with consideration of daily and seasonal rhythms, and all manipulations were performed under standard conditions from 9:00 to 10:00. Following the aim and objectives of the study, all experimental animals were divided into 2 groups. Animals in the experimental group (20 rats) were modelled for 90 days with alcoholic cardiomyopathy by intragastric administration of a 20% aqueous ethanol solution (8 g/kg/day) [11]. Animals in the control group (15 rats) were administered freshly boiled water in equivalent volumes. A 20% ethanol solution and water were administered daily. After 90 days, the animals were euthanised by dislocation of the cervical vertebrae, and then the hearts were removed for further studies. The rat hearts were perfused with 1.15% potassium chloride solution and then homogenised in 1.15% potassium chloride (1:3 ratio) at 3,000 rpm. The resulting homogenate was centrifuged for 30 min at 600 g and aliquots of the supernatant were taken into Eppendorf microtubes. The $H_2S$ content in the heart was determined by the method of B. Wiliński *et al.* [12]. The levels of the apoptosis marker caspase-3 and the proinflammatory cytokine TNF-α in the rat heart were determined by enzyme-linked immunosorbent assays using the corresponding kits "Rat Caspase-3 ELISA Kit" (Elabscience Biotechnology Inc., USA) and "Rat TNF-α ELISA Kit" (Elabscience Biotechnology Inc., USA) on a STAT-FAX 303+ analyser (USA).

The protein level in the postnuclear supernatant of the heart was determined by the spectrophotometric method according to the Lowry method [13], the content of malondialdehyde (MDA) was estimated in the reaction of formation of a coloured complex with 2-thiobarbituric acid [14], and the carbonyl groups of proteins (CG) – in the reaction of hydrazone formation with the participation of 2,4-dinitrophenylhydrazine [15]. The total NO synthase activity, as well as the activity of its isoforms (endothelial and inducible), was determined by spectrophotometric methods by increasing the amount of nitrite anion after incubation in the corresponding incubation mixtures, as described by N. Hula *et al.* [16].

Statistical processing of the primary material was performed using Statistica 18.0 (StatSoft Inc., USA). The results were presented as M ± m. To assess the probability of differences in indicators, parametric and nonparametric criteria (Student and Mann-Whitney U) were used. The relationship between the indicators was determined by Pearson and Spearman correlation analysis. The critical level of significance was assumed to be 5%.

## RESULTS AND DISCUSSION

The data obtained in this study demonstrated that chronic alcoholisation of experimental animals caused significant changes in biochemical parameters of heart function. Thus, the content of hydrogen sulphide in the myocardial homogenate of rats of the experimental group was statistically significantly reduced (by 36.02 %) compared to that of control animals (Table 1). At the same time, signs of

apoptosis and inflammation were recorded, as evidenced by a significant increase in the corresponding markers – caspase-3 (5.23 times) and TNF-α (70.2 %) compared to animals without experimental pathology.

**Table 1.** Influence of alcoholic cardiomyopathy on the content of hydrogen sulphide, apoptosis and inflammation in the rat heart (M ± m)

| Biochemical parameters of the heart | Control, n = 15 | Alcohol cardiomyopathy, n = 20 |
|---|---|---|
| $H_2S$, nmol/mg protein | 3.22 ± 0.13 | 2.06 ± 0.08* |
| Caspase-3, ng/mg protein | 0.237 ± 0.020 | 1.24 ± 0.04* |
| TNF-α, pg/mg protein | 161 ± 5.69 | 274 ± 6.17* |

**Notes:** * – p < 0.05 compared to the control group of animals
**Source:** compiled by the authors

These disorders were also accompanied by other signs of myocardial damage by alcohol and its derivatives, such as the development of oxidative and nitrosative stress and endothelial dysfunction. The data presented in Table 2 show that alcoholic cardiomyopathy is accompanied by an imbalance in redox balance with a significant increase in prooxidant processes. This is evidenced by a significant increase in the content of markers of oxidative stress (products of fat and protein peroxidation) – malondialdehyde and carbonyl groups of proteins (2.7 and 2.5 times, respectively (p < 0.05).

The results demonstrated that prolonged alcohol use is accompanied by an imbalance in the vasodilator/vasoconstrictor system towards the predominance of vasoconstrictor molecules, which is accompanied by dysfunction in the NO synthase system – decreased expression of the endothelial NOS isoform and decreased sensitivity of blood vessels to the vasodilating effect of NO and increased expression of the inducible NOS isoform, which causes the development of nitrosative stress, since under these experimental conditions the content of endothelial NO synthase decreased by 40.0% and inducible NO synthase increased by 94.4%, respectively, compared to the animals without experimental pathology (p < 0.05).

The permutation and correlation analyses performed provided evidence of the involvement of hydrogen sulphide in the development of ACM (Table 3). Thus, under conditions of experimental pathology, the content of $H_2S$ in the heart had a positive correlation of a high degree with the content of endothelial NO synthase (r = 0.84, p < 0.05), and in group 3, where the level of hydrogen sulphide was the highest, the level of this vasodilator was 117.6% higher than in group 1, where the $H_2S$ content was the lowest.

**Table 2.** Influence of alcoholic cardiomyopathy on endothelial dysfunction, nitrosative and oxidative stress in rat heart (M ± m)

| Biochemical parameters of the heart | Control, n = 15 | Alcohol cardiomyopathy, n = 20 |
|---|---|---|
| eNOS, pmol/min·mg protein | 5.40 ± 0.30 | 3.24 ± 0.23* |
| iNOS, pmol/min·mg protein | 1.79 ± 0.13 | 3.48 ± 0.20* |
| MDA, μmol/mg protein | 2.54 ± 0.21 | 6.86 ± 0.28* |
| CG, nmol/mg protein | 0.710 ± 0.020 | 1.78 ± 0.03* |

**Notes:** * – p < 0.05 compared to the control group of animals
**Source:** compiled by the authors

**Table 3.** Permutation and correlation analysis between biochemical parameters of rat heart damage and hydrogen sulphide level in alcoholic cardiomyopathy

| Biochemical parameters of the heart | $H_2S$, nmol/mg protein | | | |
|---|---|---|---|---|
| | r | $<P_{25}$ 1.66 ± 0.06 | $P_{25}$-$P_{75}$ 2.01 ± 0.04 | $>P_{75}$ 2.55 ± 0.06 |
| | | Group 1 | Group 2 | Group 3 |
| Caspase-3, ng/mg protein | -0.65& | 1.41 ± 0.06 | 1.24 ± 0.04* | 1.07 ± 0.04*# |
| TNF-α, pg/mg protein | -0.58& | 307 ± 5.84 | 271 ± 6.50* | 248 ± 6.83*# |
| eNOS, pmol/min·mg protein | +0.84& | 2.04 ± 0.19 | 3.24 ± 0.23* | 4.44 ± 0.20*# |
| iNOS, pmol/min·mg protein | -0.78& | 4.54 ± 0.18 | 3.45 ± 0.22* | 2.48 ± 0.10*# |
| MDA, μmol/mg protein | -0.70& | 8.31 ± 0.27 | 6.87 ± 0.26* | 5.38 ± 0.24*# |
| CG, nmol/mg protein | -0.72& | 1.91 ± 0.02 | 1.79 ± 0.03* | 1.63 ± 0.03*# |

**Notes:** * – p < 0.05 relative to the corresponding indicators corresponding to the level of hydrogen sulphide in the percentile interval up to P25; # – p < 0.05 relative to the indicators corresponding to the level of hydrogen sulphide in the percentile interval $P_{25}$-$P_{75}$; & – reliability (p < 0.05) of the correlation coefficient (r)
**Source:** compiled by the authors

Negative correlations of medium strength were established between the content of hydrogen sulphide in the heart and indicators of myocardial damage – levels of caspase-3, TNF-α, MDA, CGP, and inducible NO synthase ($r = -0.65, -0.58, -0.70, -0.72$, and $-0.78$, respectively ($p < 0.05$). The permutation analysis demonstrated that in group 1, where the myocardium of animals had the lowest content of hydrogen sulphide, the elevation of these parameters was the most pronounced and was significantly 24.1, 19.2, 35.2, 14.7 and 45.4% higher than the changes in the corresponding parameters in the myocardium of animals with the highest $H_2S$ content. The obtained results confirm the relationship between the level of hydrogen sulphide and pathobiochemical parameters of heart damage in alcohol-induced heart injury and provide the basis for further research in this area to identify the $H_2S$ system as a target for drug effects.

The diagnosis of ACM can be made in the presence of the following features: heavy alcohol consumption (>80 g/day) for 5 years or more, left ventricular end-diastolic diameter 2 times higher than normal, and a decrease in left ventricular ejection fraction <50% [17]. The incidence of ACM is gradually increasing as a result of widespread alcohol abuse, rendering ACM the leading cause of heart failure and sudden cardiac death in clinical practice [1-3].

The development of ACM is caused by a combination of direct toxic effects of alcohol on the myocardium, oxidative stress, mitochondrial dysfunction, and genetic predisposition. According to S. Wang & J. Ren [18], ethanol and its most toxic derivative acetaldehyde contribute to oxidative stress in the myocardium by promoting the formation of reactive oxygen species (ROS) or activating additional systems, such as the renin-angiotensin system. Experiments demonstrated that acute alcohol consumption in animals leads to mitochondrial dysfunction, impaired myocardial contractility and a decrease in overall peripheral vascular resistance. Thus, J. Tao *et al.* [19] showed that activation of phosphoglycerate mutase 5 (Pgam5) induced by AChP exacerbates alcohol-induced disorders in male mice by inducing dephosphorylation of prohibitin-2 and impairing mitochondrial quality control. Chronic alcohol consumption is toxic to cardiac myocytes, causing cell death, fibrosis and impaired contractility in both animal and human experimental models [20]. In addition, the effect of ethanol on NOX2 activation and subsequent oxidation of Ca/calmodulin-dependent kinase II (CaMKII) has been described, leading to $Ca^{2+}$ release from the sarcoplasmic reticulum, which is one of the reasons for its arrhythmogenic and negative inotropic effects [21]. According to T.A. Manolis *et al.* [22], the arrhythmogenic potential of alcohol consumption leading to cardiac arrhythmia includes the induction of both atrial and ventricular arrhythmias, with atrial fibrillation (AF) being the most common alcohol-related arrhythmia, even with low/moderate alcohol consumption.

The results demonstrated that alcoholic cardiomyopathy in rats is accompanied by the development of such pathobiochemical processes as oxidative stress (increase in prooxidant molecules – MDA and CG), nitrosative stress (imbalance in the system of nitric oxide synthases: increase of inducible and decrease of endothelial isoforms), inflammation and apoptosis of cardiomyocytes (increase of caspase-3 and TNF-α in myocardium), $p < 0.05$). The data obtained are fully comparable with the results of other studies describing pathobiochemical changes in the myocardium under conditions of prolonged alcohol administration [5]. During the onset and development of ACM, various pathological changes in the myocardium may occur, accompanied by inflammation, apoptosis and other types of cell death, oxidative and nitrosative stress, and fibrosis. These changes are often asymptomatic at the initial stages and are detected at the stage of irreversible changes or after the occurrence of fatal complications. Treatment of these lesions is mainly symptomatic, which does not always lead to satisfactory results.

Therefore, the need for in-depth studies of pathobiochemical changes in ACMP and the identification of targeted approaches to modulate this pathology is quite apparent. From this perspective, hydrogen sulphide ($H_2S$), recognised as the third most common endogenous gaseous signalling molecule in mammals (along with NO and CO), has proven to be an important biochemical marker. The distinct cardioprotective effect of hydrogen sulphide and its donors was established by I. Palamarchuk *et al.* [23] on a model of diabetic cardiomyopathy, S.C. Kang *et al.* [24] in myocardial fibrosis, and A. Melnyk *et al.* [25] in post-perfusion cardiac lesions. These results also correlate with the data of N. Li *et al.* [26], according to which endogenous $H_2S$, possessing powerful vasodilatory properties, reduces ischaemic damage to the heart muscle induced by experimental myocardial infarction in mice. Along with the known mechanisms of positive organotropic effects of $H_2S$ (the ability to eliminate mitochondrial and endothelial dysfunction, reduce oxidative and nitrosative stress, antiapoptotic properties, etc. Y. Zhang *et al.* [27] showed the ability of hydrogen sulphide to regulate the processes of pyroptosis (a type of cell death that leads to damage to cell membranes with the subsequent release of proinflammatory cytokines, including IL-1β and IL-18).

Despite the recognised organ-protective role of hydrogen sulphide in many diseases, the involvement of this gas transmitter during ACM and the mechanisms of this effect have been described in only a few publications. In particular, B. Liang *et al.* [28] demonstrated that preconditioning of the body with a hydrogen sulphide donor – NaHS significantly reduces fibrotic changes in the myocardium of mice with experimental ACM by reducing the expression of genes that cause autophagy and collagen 1.

The study demonstrated that the $H_2S$ content in the heart was statistically reduced in the case of alcoholic myocardial damage, and this reduction correlated with the degree of negative changes in the myocardium. The data from the permutation analysis provided additional evidence that low levels of hydrogen sulphide in the myocardium indicate more severe pathological changes in the heart in ACM, while high levels of this gas transmitter, on the contrary, protect the heart from alcohol damage. This suggests that an increase in the content of hydrogen sulphide in the body (including using $H_2S$-releasing drugs or other drugs that increase the level of this gas transmitter) will contribute to more effective cardioprotection in the setting of ACM.

This hypothesis is supported by the results presented in the scientific literature. Thus, B. Tánczos *et al.* [29] demonstrated that an $H_2S$-releasing water-soluble ascorbic

acid derivative improved the function of the isolated cardiomyocytes under ischaemia-reperfusion conditions by providing a potent antioxidant effect, enhanced autophagy, and reduced apoptosis. The study by Q. Zhang *et al.* [30] on animals with myocardial ischaemia or inflammatory injuries determined that $H_2S$ reduces ischaemic injury and suppresses inflammatory reactions in the myocardium by increasing the expression of microRNA-21. Thus, $H_2S$ can reduce the intensity of fibrotic changes in the myocardium in ACM by regulating the levels of microRNA expression and cellular autophagy.

## CONCLUSIONS

Alcoholic cardiomyopathy in rats is accompanied by the development of numerous pathobiochemical disorders in the myocardium, including the induction of cardiomyocyte apoptosis and inflammation, processes of free radical oxidation of lipids and oxidative modification of proteins, as evidenced by a significant increase in the content of caspase-3 (5.23 times), TNF-α (70.2%), MDA (2.7 times) and CG (2.5 times) in the myocardium compared to animals of the control group. Along with this, an imbalance in the NOS system was observed, accompanied by the development of nitrosative stress and endothelial dysfunction, as evidenced by a statistically significant increase in the activity of the inducible NOS isoform in the myocardium by 94.4% against a decrease in the activity of its endothelial isoform by 40.0% compared to the control group. The development of molecular and pathophysiological disorders in the heart against the background of prolonged alcoholisation is associated with a statistically significant decrease in the myocardial content of the gas-transmitter molecule $H_2S$ by 36.0% compared with the control group.

The correlation and permutation analyses provided important evidence of the involvement of the $H_2S$ system in the molecular mechanisms of alcoholic myocardial damage in rats. The study determined that low levels of the gas transmitter under conditions of chronic alcoholisation were associated with more pronounced pathobiochemical disorders in the heart (apoptosis, inflammation, oxidative-nitrosative stress, endothelial dysfunction), while at higher levels of $H_2S$, the intensity of these biochemical disorders was significantly lower. This creates a prospect for further research aimed at clarifying the mechanisms of $H_2S$ action and developing new therapeutic approaches aimed at correcting the dysfunctions associated with ACM. $H_2S$ is considered a promising molecular target for pharmacotherapy of alcoholic cardiomyopathy.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Alcohol [Internet]. World Health Organization [cited 2024 Dec 17]. Available from: https://surl.li/zeavaz
[2] Global strategy to reduce the harmful use of alcohol [Internet]. World Health Organization; 2010 May 31 [cited 2024 Dec 17]. Available from: https://www.who.int/publications/i/item/9789241599931
[3] Alcohol consumption in Ukraine – WHO study results [Internet]. 2024 [cited 2024 Dec 17]. Available from: https://www.apteka.ua/article/693507
[4] Shahid M, Ibrahim R, Arakelyan A, Hassan K, Sainbayar E, Pham HN, et al. Alcoholic cardiomyopathy mortality and social vulnerability index: A nationwide cross-sectional analysis. Int J Cardiol Cardiovasc Risk Prev. 2023;19:200224. DOI: 10.1016/j.ijcrp.2023.200224
[5] Andersson C, Schou M, Gustafsson F, Torp-Pedersen C. Alcohol intake in patients with cardiomyopathy and heart failure: Consensus and controversy. Circ Heart Fail. 2022;15(8):e009459. DOI: 10.1161/CIRCHEARTFAILURE.121.009459
[6] Sun X, Wu S, Mao C, Qu Y, Xu Z, Xie Y, et al. Therapeutic potential of hydrogen sulfide in ischemia and reperfusion injury. Biomolecules. 2024;14(7):740. DOI: 10.3390/biom14070740
[7] Payne FM, Dabb AR, Harrison JC, Sammut IA. Inhibitors of NLRP3 inflammasome formation: A cardioprotective role for the gasotransmitters carbon monoxide, nitric oxide, and hydrogen sulphide in acute myocardial infarction. Int J Mol Sci. 2024;25(17):9247. DOI: 10.3390/ijms25179247
[8] de Koning ML, van Dorp P, Assa S, Pundziute-Do Prado G, Voskuil M, Anthonio RL, et al. Sodium thiosulfate in acute myocardial infarction: A randomized clinical trial. JACC Basic Transl Sci. 2023;8(10):1285–94. DOI: 10.1016/j.jacbts.2023.06.001
[9] Zhao S, Li X, Li X, Wei X, Wang H. Hydrogen sulfide plays an important role in diabetic cardiomyopathy. Front Cell Dev Biol. 2021;9:627336. DOI: 10.3389/fcell.2021.627336
[10] Dobrelia N, Boytsova L, Danova I. The legal basis for realization of ethical assessment of preclinical drug investigations using laboratory animals. Pharmacol Drug Toxicol. 2015;2(43):95–1.
[11] Lieber CS. Medical and nutritional complications of alcoholism. New York: Springer; 1992. 579 p. DOI: 10.1007/978-1-4615-3320-7
[12] Wiliński B, Wiliński J, Somogyi E, Piotrowska J, Góralska M. Amlodipine affects endogenous hydrogen sulfide tissue concentrations in different mouse organs. Folia Med Cracov. 2011;51(1-4):29–35.
[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75. DOI: 10.1016/S0021-9258(19)52451-6
[14] Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86(1):271–8. DOI: 10.1016/0003-2697(78)90342-1
[15] Shevchuk S, Pentiuk O, Musin R, Zaichko N, inventors; Ukrainian State NDI Rehabilitation of the Disabled, Ministry of Health of Ukraine, assignee. Method for assaying carbonyl substances in blood serum proteins. Ukraine patent UA 58110A. 2003 Jul 15.

[16] Hula N, Kosiakova H, Berdyshev A. The effects of n-stearoylethanolamine on the NO-synthase pathway of NO generation in the aorta and heart of streptozotocin-induced diabetic rats. Ukr Biochem J. 2007;79(5):153–8.
[17] Domínguez F, Adler E, García-Pavía P. Alcoholic cardiomyopathy: An update. Eur Heart J. 2024;45(26):2294–5. DOI: 10.1093/eurheartj/ehae362
[18] Wang S, Ren J. Role of autophagy and regulatory mechanisms in alcoholic cardiomyopathy. Biochim Biophys Acta Mol Basis Dis. 2018;1864(6 Pt A):2003–9. DOI: 10.1016/j.bbadis.2018.03.016
[19] Tao J, Qiu J, Zheng J, Li R, Chang X, He Q. Phosphoglycerate mutase 5 exacerbates alcoholic cardiomyopathy in male mice by inducing prohibitin-2 dephosphorylation and impairing mitochondrial quality control. Clin Transl Med. 2024;14(8):e1806. DOI: 10.1002/ctm2.1806C
[20] Fernández-Solà J. The effects of ethanol on the heart: Alcoholic cardiomyopathy. Nutrients. 2020;12(2):572. DOI: 10.3390/nu12020572
[21] Mustroph J, Wagemann O, Lebek S, Tarnowski D, Ackermann J, Drzymalski M, et al. SR $Ca^{2+}$-leak and disordered excitation-contraction coupling as the basis for arrhythmogenic and negative inotropic effects of acute ethanol exposure. J Mol Cell Cardiol. 2018;116:81–90. DOI: 10.1016/j.yjmcc.2018.02.002
[22] Manolis TA, Apostolopoulos EJ, Manolis AA, Melita H, Manolis AS. The proarrhythmic conundrum of alcohol intake. Trends Cardiovasc Med. 2022;32(4):237–45. DOI: 10.1016/j.tcm.2021.03.003
[23] Palamarchuk I, Strutynska O, Melnyk A, Zaichko N. Influence of metformin and its combination with sodium hydrogen sulphide on H2S system and associated biochemical disorders in myocardium and kidney of rats with streptozotocin-induced diabetes. Bull Probl Biol Med. 2020;3(157):133–7. DOI: 10.29254/2077-4214-2020-3-157-133-137
[24] Kang SC, Sohn EH, Lee SR. Hydrogen sulfide as a potential alternative for the treatment of myocardial fibrosis. Oxid Med Cell Longev. 2020;2020:4105382. DOI: 10.1155/2020/4105382
[25] Melnyk A, Zaichko N, Khodakovskyi O, Khodakivska O. Sex characteristics of hydrogen sulfide influence on ischemia reperfusion in myocardium of rats. Fiziol Zh. 2018;1:40–46. DOI: 10.15407/fz64.01.040
[26] Li N, Wang MJ, Jin S, Bai YD, Hou CL, Ma FF, et al. The $H_2S$ donor NaHS changes the expression pattern of $H_2S$-producing enzymes after myocardial infarction. Oxid Med Cell Longev. 2016;2016:6492469. DOI: 10.1155/2016/6492469
[27] Zhang Y, Zhao H, Fu X, Wang K, Yang J, Zhang X, et al. The role of hydrogen sulfide regulation of pyroptosis in different pathological processes. Eur J Med Chem. 2024;268:116254. DOI: 10.1016/j.ejmech.2024.116254
[28] Liang B, Xiao T, Long J, Liu M, Li Z, Liu S, et al. Hydrogen sulfide alleviates myocardial fibrosis in mice with alcoholic cardiomyopathy by downregulating autophagy. Int J Mol Med. 2017;40:1781–91. DOI: 10.3892/ijmm.2017.3191
[29] Tánczos B, Vass V, Szabó E, Lovas M, Kattoub RG, Bereczki I, et al. Effects of $H_2S$-donor ascorbic acid derivative and ischemia/reperfusion-induced injury in isolated rat hearts. Eur J Pharm Sci. 2024;195:106721. DOI: 10.1016/j.ejps.2024.106721
[30] Zhang Q, Zhang Y, Guo S, Wang X, Wang H. Hydrogen sulfide plays an important role by regulating microRNA in different ischemia-reperfusion injury. Biochem Pharmacol. 2024;229:116503. DOI: 10.1016/j.bcp.2024.116503

# Роль гідроген сульфіду в біохімічних механізмах ураження міокарду за алкогольної кардіоміопатії

**Олександр Матяш**

Аспірант
Вінницький національний медичний університет ім. М.І. Пирогова
21018, вул. Пирогова, 56, м. Вінниця, Україна
https://orcid.org/0000-0002-9797-1588

**Наталія Волощук**

Доктор медичних наук, професор
Вінницький національний медичний університет ім. М.І. Пирогова
21018, вул. Пирогова, 56, м. Вінниця, Україна
https://orcid.org/0000-0002-0166-9676

**Анотація.** Надмірне споживання алкоголю, яке спостерігається у багатьох пацієнтів, призводить до серцево-судинних захворювань, зокрема алкогольної кардіоміопатії, однак роль змін обміну $H_2S$ в ураженні серця за цієї патології залишається недостатньо вивченою. Тому метою роботи було визначення ролі сірководню в механізмі пошкодження серця при алкогольному ураженні міокарда у щурів. Дослідження було проведено на 35 білих статевозрілих щурах-самцях лінії Wistar масою 260-290 г, поділених на дві групи. Встановлено, що розвиток алкогольної кардіоміопатії у щурів супроводжується розвитком патобіохімічних процесів, які включають апоптоз кардіоміоцитів, запалення, розвиток оксидативного та нітрозативного стресу, про свідчать вірогідне зростання вмісту в міокарді каспази-3 (в 5,23 рази) фактору некрозу пухлин α (70,2 %), малонового діальдегіду карбонільних груп протеїнів (2,7 та 2,5 рази), відповідно, а також розбалансуванням в системі синтаз оксиду азоту (підвищенням індуцибельної та зменшенням ендотеліальної ізоформи (+94,4 та -40,0 %, відповідно, $p < 0,05$). Всі ці зміни асоціювались із зниженням вмісту в міокарді гідроген сульфіду (-36,0 %, $p < 0,05$). Проведений кореляційний та персентильний аналіз підтвердив зв'язок між вмістом гідроген сульфіду та маркерами ураження серця за алкогольної кардіоміопатії, а саме – низький вміст газотрансміттера асоціювався із більш виразними порушеннями, тоді як більш високий рівень гідроген сульфіду – навпаки, асоціювався із помірними або мінімальними ознаками кардіальних уражень за умов хронічної алкоголізації тварин. Врахування рівня гідроген сульфіду в організмі дасть змогу прогнозувати розвиток алкоголь-індукованих уражень серця та є експериментальним підґрунтям для розробки $H_2S$-вивільняючих препаратів з метою покращення фармакологічного менеджменту пацієнтів

**Ключові слова:** алкогольне ураження міокарда; сірководень; запалення; апоптоз; оксидативний стрес; NO-синтаза; щури

Article's History: Received: 10.08.2024; Revised: 28.10.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.23

Journal homepage: https://bmbr.com.ua/en

UDC 616.12:578.834.1

# Coronary heart disease and dynamics of intracardiac haemodynamics after hospitalisation for COVID-19: Results of a 12-month follow-up study

**Serhii Mostovyi***

PhD in Medical Sciences
Bogomolets National Medical University
01601, 13 T. Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0000-0002-8783-3819

**Abstract.** The study aimed to evaluate the dynamics of intracardiac haemodynamics in patients with coronary heart disease (CHD) after COVID-19 over 12 months. To achieve this goal, patients with COVID-19 and diagnosed with CHD were monitored at the clinical sites of the Bogomolets National Medical University and the cardiology department of the Road Clinical Hospital No. 2, Kyiv Clinical Hospital No. 18, and the Medbud Medical Centre. The main parameters studied were left ventricular ejection fraction (LVEF), end-diastolic volume (EDV), cardiac output, troponins I and T, natriuretic peptide, and C-reactive protein levels. The study determined that LVEF decreased to 47% in the 3rd month after COVID-19 and partially recovered to 52% in 12 months, but did not reach the control values. The EDV increased to 160 ml at month 3 and then decreased to 147 ml at month 12, indicating a volume overload of the left ventricle. Cardiac output at the beginning of the study was 3.8 l/min, and at the end – 4.3 l/min, which indicated positive dynamics, but incomplete recovery. The levels of troponins and natriuretic peptide were initially elevated, but gradually decreased to normal levels, indicating the recovery of cardiac function. The elevated level of C-reactive protein recorded at the beginning of the study indicated the presence of an active inflammatory process, which gradually decreased to normal values over 12 months. This is a substantial indicator of inflammation reduction and improvement of the general condition of patients after COVID-19. The results of the study indicated a partial recovery of heart function in patients but also demonstrated that the recovery process is long and does not always result in a complete return to normal cardiac function

**Keywords:** heart failure; inflammatory process; myocardial recovery; functional heart disorders; cardiac markers

## INTRODUCTION

The COVID-19 pandemic, which as of 2024 has affected more than 240 million people and resulted in more than 4.5 million deaths worldwide, has become one of the biggest crises of 2020s, with serious consequences for public health [1]. The SARS-CoV-2 coronavirus, the causative agent of COVID-19, is a member of the coronavirus family, which is one of the main causes of acute respiratory infections. Unlike other types of coronaviruses, such as HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU1, which cause only mild upper respiratory tract infections, SARS-CoV-2 causes severe respiratory illness with severe complications, which has become a serious threat to human health [2]. An important aspect of the pandemic is that most coronaviruses circulate among animals, and only a few of them can infect humans. One of the reasons for this phenomenon may be contact between different species of animals in specific markets, which creates conditions for genetic recombination between viruses and the emergence of new pathogens such as SARS-CoV-2 [3]. This underscores the importance of research aimed at understanding the mechanisms of virus transmission from animals to humans and finding ways to control them. Since SARS-CoV-2 is a new virus that humanity has never encountered before, most of the population does not have effective immunity against this pathogen. Initial estimates demonstrate that the virus has a high level of transmission, which leads to its rapid spread [4]. This, in turn, underscores the need for effective measures to contain the pandemic, including the introduction of quarantine restrictions to slow the spread of the virus.

**Suggested Citation**:
Mostovyi S. Coronary heart disease and dynamics of intracardiac haemodynamics after hospitalisation for COVID-19: Results of a 12-month follow-up study. Bull Med Biol Res. 2024;6(4):23–33. DOI: 10.63341/bmbr/4.2024.23

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

Clinical manifestations of COVID-19 can range from mild symptoms such as cough, fever and loss of smell to severe complications including respiratory failure, thromboembolism and acute kidney injury [5]. Patients with comorbidities, such as coronary heart disease (CHD), which is one of the main risk factors for severe COVID-19, have particularly difficult cases. CHD is a serious medical problem that causes approximately one-third of all deaths worldwide. According to forecasts, the most dangerous form of coronary heart disease, acute coronary syndrome, may become even more common, increasing the incidence from one in 11 people in 2019 to one in six in 2050 [6]. This trend makes CHD one of the biggest threats to public health in the 21st century. A study by L. Szarpak *et al.* [7] showed that patients with CHD infected with COVID-19 are at increased risk of developing complications and need to be closely monitored. Another study conducted by S. Ganatra *et al.* [8] highlighted the importance of monitoring cardiovascular parameters in patients with COVID-19, those with CHD, to reduce mortality. Given that CHD is a serious health threat, especially in the context of the pandemic, it is critical to assess how COVID-19 affects the course of this disease.

Due to the high prevalence of coronary artery disease and the fact that it is a significant risk factor for the development of severe forms of COVID-19, it is critical to ensure early diagnosis and adequate treatment of this disease. However, this requires an accurate determination of the clinical manifestations of COVID-19 in patients with CHD. Given the significant impact of CHD on the course of COVID-19, there is a need for a more in-depth study of this relationship, which was the main motivation for this study. Despite the high prevalence of CHD and the potential risks associated with COVID-19, the number of studies on this topic remains limited. In particular, studies that systematically evaluate the impact of COVID-19 on the dynamics of intracardiac haemodynamics in patients with CHD after hospitalisation are rare. The research relevance is determined by the lack of sufficient scientific data on the impact of COVID-19 on the dynamics of intracardiac haemodynamics in patients with CHD. Although there are studies that consider general aspects of COVID-19, such as the work of S. Wang *et al*. [9], specific studies on the interaction of COVID-19 with CHD in the long term remain rare. R. Kaddoura & A.M. Salam [10] focused on acute complications, such as thrombosis and acute coronary syndrome, but long-term effects, such as changes in intracardiac haemodynamics, remain poorly understood. This study aimed to fill this gap by assessing changes in intracardiac haemodynamics 12 months after COVID-19 in patients with CHD. The objectives of the study were as follows: firstly, to assess the dynamics of left ventricular ejection fraction (LVEF) in patients with CHD after COVID-19 in 3, 6 and 12 months to identify changes in the functional state of the heart and compare with control values. Secondly, to analyse the impact of COVID-19 on end-diastolic volume (EDV) and cardiac output in patients, in particular, to identify the presence of impaired diastolic cardiac function and reduced pumping efficiency, as well as to note the dynamics of these indicators during the study. Thirdly, to study the levels of troponins I and T, natriuretic peptide and C-reactive protein in patients after COVID-19 to determine the degree of myocardial damage and inflammation, as well as their correlation with the clinical course of the disease.

## THEORETICAL OVERVIEW

In 2023-2024, a significant amount of data was collected on the impact of COVID-19 on the cardiovascular system, in patients with CHD. Studies show that the SARS-CoV-2 virus can cause both acute and long-term cardiac dysfunction, which has significant implications for clinical practice. COVID-19 can cause acute myocardial inflammation and deterioration of myocardial function. Many studies emphasise that infection can result in a decrease in LVEF, increased levels of troponins and natriuretic peptides, and the development of heart failure [11-13]. In addition, evidence suggests that the long-term effects of infection may include residual functional impairment that persists even after the patient's general condition has normalised. These factors are critical in improving treatment and rehabilitation strategies, as the long-term effects of COVID-19 can significantly affect cardiac function and quality of life in patients with CHD [14, 15]. A key aspect of research in this context is changes in intracardiac haemodynamics after COVID-19. LVEF, which is the main indicator of cardiac function, was found to be reduced in patients with CHD after infection [16]. This indicates possible acute myocardial injury, in particular by viral agents such as COVID-19 (Fig. 1). This emphasises the need for careful monitoring of this indicator in the post-COVID period, as a decrease in LVEF may be an indicator of serious cardiac dysfunction caused by the virus [17, 18].

I. Karagodin *et al*. [20] demonstrated that in patients after COVID-19, LVEF decreased to levels below 50%. This decrease is particularly critical, as a score of less than 50% indicates significant impairment in the pumping function of the heart. It is important to note that such a decrease in LVEF can be the result of both direct viral damage to the myocardium and secondary inflammatory processes arising in response to infection [21, 22]. A study by D. Sharma *et al.* [23], which addressed patients with a history of CHD, noted a significant decrease in LVEF to a statistical mean of 45% 6 months after COVID-19. This study highlights that in patients with pre-existing CHD, viral infection can have a particularly negative impact on cardiac function, potentially worsening prognosis and increasing the risk of further complications. The importance of these results is determined by the indication of the long-term negative effects of COVID-19 on cardiac function. This confirms the need for regular monitoring of cardiac parameters in patients who have had COVID-19, especially in those with pre-existing cardiac disease. The results of these studies also highlight the importance of developing rehabilitation and treatment strategies for patients with post-COVID cardiac complications, including both medical treatment and rehabilitation measures aimed at restoring cardiac function and improving patients' quality of life [24-26].

An important aspect to consider in the context of cardiac complications after COVID-19 is the level of troponins I and T. These biomarkers are critical for assessing myocardial damage, as their elevation indicates acute damage to the heart muscle. Troponins I and T are proteins that bind to actin in cardiomyocytes and are released into the bloodstream when myocardial cells are damaged. According to

R. Vazirani *et al.* [27], an increase in the level of these troponins was observed in patients with COVID-19, indicating the presence of acute myocardial injury, which is often accompanied by clinical symptoms of cardiac complications. B-type natriuretic peptide levels are an important indicator of heart function and a diagnostic criterion for heart failure. B-type natriuretic peptide, specifically B-natriuretic peptide, is produced in the heart in response to increased pressure or stretching of the heart chambers (Fig. 2). Increased levels of this peptide are a frequent marker of heart failure and other cardiovascular disease. According to the study by C. Lionte *et al.* [28], in patients with COVID-19, the level of natriuretic peptide increased significantly at 3 months after infection, which may indicate the development of heart failure. This level is higher than normal and indicates an increase in heart load, which is often the result of an inflammatory process or other complication associated with COVID-19.

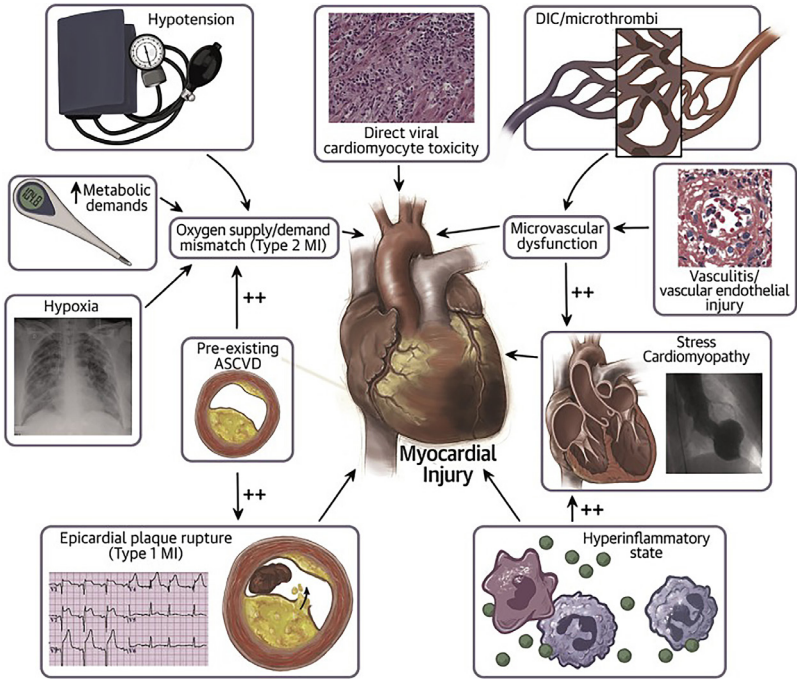


**Figure 1.** Potential mechanism of COVID-19 effect on the myocardium
**Source:** based on D. Atri *et al*. [19]

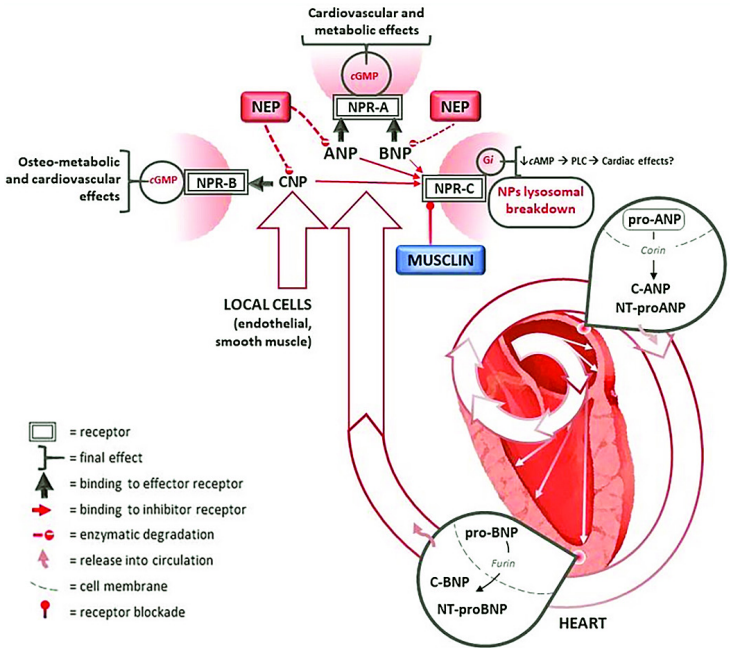


**Figure 2.** B-type natriuretic peptide signalling pathways for the protection of the heart and cardiovascular system
**Source:** based on R. Sarzani *et al*. [29]

Magnetic resonance imaging (MRI) has revealed important findings in the study of patients with COVID-19, especially in those with a history of CHD. As determined, at 3 months after the infection, some patients had structural changes in the myocardium. These changes could include fibrosis, inflammatory infiltrates, or other abnormalities that reflect acute or chronic damage to cardiac tissue, which is often seen in patients with severe COVID-19 [30, 31]. In general, the presence of long-term functional impairment in patients with CHD after COVID-19 emphasises the need for systematic post-COVID monitoring and rehabilitation.

## MATERIALS AND METHODS

The study was conducted at the clinical sites of the Bogomolets National Medical University: the Cardiology Department of the Road Clinical Hospital No. 2, Kyiv Clinical Hospital No. 18, and the Medbud Medical Centre from January 2021 to January 2023. The study covered a 12-month follow-up period, which was used to assess the long-term impact of COVID-19 on the cardiovascular system. The study sample included 200 patients diagnosed with CHD who were treated in the cardiology department of the hospital after hospitalisation for COVID-19. The inclusion criteria were age 45 to 75 years, confirmed diagnosis of coronary heart disease, moderate or severe hospitalisation for COVID-19, and no significant comorbidities (except for hypertension). Exclusion criteria: chronic lung disease, severe renal failure, cancer, and myocardial infarction within the last six months. The sample was formed by random sampling among patients who met the inclusion criteria. The control group included 100 patients with CHD who did not have COVID-19. The control group was used to compare changes in haemodynamic parameters with the main group of patients with COVID-19.

All study participants underwent a comprehensive cardiological examination at three stages: 3, 6 and 12 months after hospital discharge. The examination included a clinical examination, electrocardiography (ECG), echocardiography, and biochemical blood tests to assess the level of troponins, natriuretic peptide, and C-reactive protein. For ECG and echocardiography, Philips HDI 11XE equipment was used, as well as ESAOTE MyLabXE8 XP (USA), which provided a highly accurate assessment of the functional state of the heart. Echocardiography was performed to determine the main parameters of intracardiac haemodynamics, such as LVEF, EDV and cardiac output. In addition, to assess the degree of myocardial fibrosis, cardiac MRI was performed using a Siemens MAGNETOM Skyra (Germany) 6 and 12 months after discharge. This was used to identify changes in myocardial structure associated with COVID-19 exposure.

The study used biochemical methods to analyse blood, including determining the level of inflammatory markers (C-reactive protein), indicators of heart failure (natriuretic peptide), and markers of myocardial damage (troponins I and T). This made it possible to assess the degree of impact of COVID-19 on the cardiovascular system in patients with coronary heart disease. The method of daily monitoring of blood pressure and heart rate was also used to track fluctuations in haemodynamic parameters throughout the day. The monitoring identified episodes of arrhythmias and blood pressure fluctuations that had a critical impact on the condition of patients with CHD.

Several statistical methods were used to process the data. In particular, the Mann-Whitney U test for independent samples was used to compare two groups of patients (those who had COVID-19 and those who did not have the virus). To analyse changes in intracardiac haemodynamics over time, a Student's t-test was used for dependent samples. Correlation analysis was performed to determine the relationship between the level of biochemical markers and changes in cardiac function parameters. The level of statistical significance was set at $p < 0.05$. SPSS version 26 software was used for all calculations. The study was conducted following the requirements of the Declaration of Helsinki [32] and its subsequent amendments, as well as the current legislation on medical research involving human subjects. Before the study, the approval of the Ethics Committee of the Bogomolets National Medical University was obtained (Protocol No. 163 of 07.11.2022). All study participants provided written informed consent to participate after a detailed explanation of the purpose, methods, risks and benefits of the study. All ethical standards related to research involving human subjects were observed, including ensuring the anonymity of participants' data, their voluntary participation and the right to withdraw at any stage of the study without negative consequences for them.

## RESULTS

The study revealed a gradual recovery of cardiac function in most patients, but a significant proportion continued to experience haemodynamic disorders even a year after discharge. This was especially true for LVEF and cardiac output, which remained reduced in patients with severe COVID-19. The analysis showed that the most pronounced changes occurred in the first 6 months after discharge, while in the next 6 months, the patient's condition stabilised.

**Dynamics of the main haemodynamic parameters.** LVEF is one of the key indicators that assesses the contractile function of the heart. In patients with COVID-19, a decrease in this indicator was observed after 3 months of observation, which could be due to damage to the heart muscle due to the virus. The mean LVEF in the main group at month 3 was 47%, which was significantly different from the control group, where it was 53% ($p < 0.05$). At month 6, a partial recovery of LVEF was observed in patients of the main group – up to 50%, but this figure was still lower than in the control group (54%). By month 12, further improvement to 52% was observed, demonstrating a gradual recovery of cardiac function. EDV is another important parameter of intracardiac haemodynamics. In patients with COVID-19, at 3 months after hospitalisation, an increase in EDV to 160 ml was observed, indicating a decrease in ventricular filling efficiency. In the control group, this figure remained stable at 145 ml. After 6 months, the EDV in the main group decreased to 150 ml, and by the 12$^{th}$ month, this indicator stabilised at 147 ml, indicating a gradual improvement in cardiac filling function.

Cardiac output in the main group of patients who had suffered from COVID-19 was reduced at the initial stages of the study. At 3 months after discharge from the hospital, cardiac output was 3.8 l/min, indicating a reduced functional ability of the heart to pump blood. This value was lower compared to the control group, where the cardiac output was 4.2 l/min. The decrease in cardiac output at an

early stage indicates that COVID-19 harmed the efficiency of cardiac function in patients with CHD. At 6 months of follow-up, some improvement was recorded: cardiac output in the main group increased to 4.1 l/min. This indicates a partial recovery of cardiac function, but this figure was still lower than that of the control group. Only at the 12th month after discharge did the cardiac output in the intervention group approach the values of the control group, reaching 4.3 l/min. This result demonstrates a gradual recovery of cardiac function in patients with COVID-19, although it has not yet reached the level of healthy patients with CHD who have not had a viral infection.

**Evaluation of biochemical parameters.** Troponin I and T levels in patients with COVID-19 were significantly elevated at baseline. The average level of troponin I in patients of the main group at month 3 was 0.08 ng/ml, which exceeded normal values and indicated myocardial damage. In the control group, this figure remained at 0.03 ng/ml. After 6 months, a decrease in troponin levels to 0.05 ng/ml was observed in the main group, and by the 12th month, this indicator returned to normal values (0.02 ng/ml). Elevated levels of natriuretic peptide indicate the presence of heart failure. In patients with COVID-19, this indicator was elevated at month 3 and averaged 200 pg/ml, indicating the development of heart failure due to viral infection. By month 6, the level of natriuretic peptide decreased to 150 pg/ml, and by month 12 – to 100 pg/ml, indicating a gradual improvement in heart function. C-reactive protein is a marker of inflammation, and its elevated level indicates inflammatory processes in the body. At month 3, patients with COVID-19 had a high C-reactive protein level of 8 mg/l, which was higher than normal (up to 3 mg/l) and indicated ongoing inflammation in the body. In the control group, this figure was 2.5 mg/l. At 6 months, the level of C-reactive protein decreased to 5 mg/l, and at 12 months – to 3 mg/l, indicating the completion of the inflammatory process.

Cardiac MRI revealed structural changes in the myocardium in 40% of patients in the main group at 3 months after COVID-19. These changes included myocardial fibrosis, which was observed in the form of thickening of the left ventricular walls. In 15% of patients in the control group, minor changes were also detected that could be related to age-related factors. After 6 months, the proportion of patients with signs of fibrosis decreased to 25%, and at 12 months, this figure was only 10%, indicating a possible reverse recovery of myocardial structure. In patients of the main group, an increase in the frequency of arrhythmias and blood pressure fluctuations was observed at month 3. On average, 6 episodes of paroxysmal tachycardia per day were recorded, which was significantly higher than in the control group (2 episodes). After 6 months, the frequency of arrhythmias decreased to 3 episodes per day, and in the 12th month, this indicator reached the values of the control group (2 episodes). Blood pressure fluctuations also decreased, especially in patients with well-controlled hypertension.

**Comparison of results between the intervention and control groups.** According to the study results, patients in the main group (COVID-19 survivors) demonstrated a statistically significant decrease in LVEF compared to patients in the control group ($p < 0.05$). At 3 months after discharge from the hospital, the mean LVEF in patients in the intervention group was 47%, while in the control group, it was 53%. At 12 months, a gradual recovery of this indicator was observed in both groups, but patients in the intervention group still demonstrated slightly lower values (52%) compared to the control group (54%). At month 3 of the study, the EDV in patients of the main group exceeded the corresponding values of the control group (160 ml vs 145 ml). This indicated a decrease in the ability of the heart to effectively fill the ventricles after COVID-19. After 6 months, there was a partial decrease in EDV in both groups, but the difference between the intervention and control groups remained significant (150 ml vs. 140 ml, respectively). By 12 months, the index of EDV in the main group was equal to the control values (147 ml in both groups), indicating a gradual recovery of the functional state of the ventricles in patients of the main group. Cardiac output also differed significantly between the intervention and control groups at baseline. At month 3, the average cardiac output in the intervention group was 3.8 l/min, while in the control group it was 4.2 l/min. By month 12, this figure in patients with COVID-19 approached the values of the control group, but the difference was still statistically significant (4.3 l/min vs. 4.5 l/min, $p < 0.05$) (Table 1).

**Table 1.** Dynamics of changes in key indicators used to assess the state of the cardiovascular system of patients after COVID-19

| Value | Initial value | 3rd month | 6th month | 12th month |
|---|---|---|---|---|
| Troponin I (ng/ml) | Increased | 0.08 (main), 0.03 (control) | N/A | 0.02 (both groups) |
| B-type natriuretic peptide (pg/ml) | Increased | 200 (main), 110 (control) | 150 (main), N/A | 100 (main), N/A (control) |
| C-reactive protein (mg/l) | N/A | 8 (main), 2.5 (control) | N/A | 3 (main), 2 (control) |
| LVEF | N/A | 47% (main), 53% (control) | N/A | 52% (main), 54% (control) |
| EDV, ml | N/A | 160 (main), 145 (control) | 150 (main), 140 (control) | 147 (both groups) |
| Cardiac output (l/min) | N/A | 3.8 (main), 4.2 (control) | N/A | 4.3 (main), 4.5 (control) |

**Source:** compiled by the author

Elevated levels of troponins I and T in patients in the main group at baseline indicated the presence of myocardial damage after COVID-19. At month 3, the mean troponin I level in the intervention group was almost three times higher than in the control group (0.08 ng/ml vs. 0.03 ng/ml, respectively). During 12 months of follow-up, troponin levels in both groups gradually decreased, and at 12 months it was 0.02 ng/ml in both groups, indicating the recovery of myocardial function in patients in the main group. B-type natriuretic peptide, as a marker of heart failure, was significantly elevated in patients in the main group at the initial stages of the study. At month 3, the average level of

natriuretic peptide was 200 pg/ml, while in the control group, this figure was 110 pg/ml. During the 6th and 12th months of follow-up, the level of natriuretic peptide in the main group gradually decreased to 150 pg/ml at month 6 and 100 pg/ml at month 12, indicating a gradual recovery of cardiac function. In patients with COVID-19, the level of C-reactive protein in the 3rd month after hospitalisation averaged 8 mg/l, which was significantly higher than in the control group (2.5 mg/l). This indicated an active inflammatory process in the body after a viral infection. During the study, the level of C-reactive protein gradually decreased, and at month 12, its return to normal values was observed in both groups (3 mg/l in patients of the main group and 2 mg/l in the control group) (Table 2).

**Table 2.** Changes in troponin, natriuretic peptide and C-reactive protein levels during the observation period

| Value | Initial value | 3rd month | 6th month | 12th month |
|---|---|---|---|---|
| Troponin I (ng/ml) | Increased | 0.08 (main), 0.03 (control) | N/A | 0.02 (both groups) |
| B-type natriuretic peptide (pg/ml) | Increased | 200 (main), 110 (control) | 150 (main), N/A (control) | 100 (main), N/A (control) |
| C-reactive protein (mg/l) | N/A | 8 (main), 2.5 (control) | N/A | 3 (main), 2 (control) |

**Source:** compiled by the author

MRI of the heart showed significant changes in the structure of the myocardium in patients in the main group at month 3 of the study. In 40% of patients, signs of myocardial fibrosis were detected, indicating damage to the heart muscle due to COVID-19. In the control group, only 15% of patients had such changes, and they were related to age or concomitant factors. At month 6, the proportion of patients with fibrosis in the main group decreased to 25%, and by month 12 – to 10%. This indicates the possibility of a gradual recovery of myocardial structure in some patients after an infection.

**Results of daily blood pressure and heart rate monitoring.** In patients of the main group, a significant increase in the frequency of arrhythmias, in particular paroxysmal tachycardia, was recorded at month 3 of the study, which occurred on average 6 times per day. In the control group, this figure was significantly lower and amounted to 2 episodes per day. At month 6, the frequency of arrhythmias decreased to 3 episodes per day in the main group, and at month 12, this figure reached the level of the control group (2 episodes per day). Blood pressure fluctuations in the intervention group were also significantly higher at baseline. In the 3rd month of follow-up, the average daily blood pressure in the intervention group was 145/90 mm Hg, while in the control group, it was 135/85 mm Hg. During the following months of follow-up, blood pressure in both groups gradually approached normal values, and in the 12th month, the average blood pressure in the intervention group was 135/85 mm Hg, which was similar to the control values.

The decrease in LVEF in the main group compared with the control group at month 3 was statistically significant ($p < 0.05$). However, at 12 months, this difference decreased significantly, and although the values in the intervention group were still lower, they were no longer statistically significant ($p > 0.05$). Changes in EDV in patients in the intervention group compared with the control group at different stages of the study were statistically significant ($p < 0.05$). The greatest differences were observed in month 3, but by month 12, these values were similar in both groups. Troponin levels: the differences in troponin levels between the intervention and control groups at baseline were significant ($p < 0.001$), indicating severe myocardial damage in patients after COVID-19. However, at 12 months, these parameters normalised, and no statistically significant differences were found ($p > 0.05$). The level of C-reactive protein also showed statistically significant differences at the beginning of the study ($p < 0.01$), but within 12 months there was a decrease in the level to normal, and at the end of the study there was no difference between the groups ($p > 0.05$). The number of patients with myocardial fibrosis was significantly higher in the intervention group at month 3 compared with the control group ($p < 0.05$), but by month 12 the difference became statistically insignificant ($p > 0.05$), indicating a potential regression of fibrotic changes in some patients.

Correlation analysis was performed to determine the relationship between various clinical parameters used to assess the cardiovascular system in patients with COVID-19. The main indicators for the analysis were LVEF, troponin levels, C-reactive protein and natriuretic peptide. The results of the correlation analysis showed a significant positive correlation between troponin levels and LVEF reduction, with a correlation coefficient of $r = 0.58$ and a statistical significance level of $p < 0.01$. This indicates that an increase in troponin levels, which is a marker of myocardial damage, is associated with a significant decrease in LVEF, reflecting a deterioration in cardiac function. This relationship confirms that in patients with COVID-19, there is a close correlation between the presence of myocardial damage and functional cardiac impairment. High levels of troponins indicate damage to the heart muscle, which directly affects its ability to pump blood efficiently, which is reflected in a decrease in ejection fraction. Further analysis also showed that elevated levels of C-reactive protein, as a marker of the inflammatory process, correlated with deterioration in cardiac function, but less strongly than troponins. This suggests that the inflammatory process may have an additional impact on cardiac function, but its role in reducing ejection fraction is less critical compared to direct myocardial damage.

## DISCUSSION

The results of the study of the dynamics of intracardiac haemodynamics in patients with CHD after COVID-19 demonstrate important aspects of the recovery of cardiac function and the impact of the virus on the heart. The gradual improvement in cardiac function, while confirming the general trend towards recovery within 12 months, indicates that cardiac function in many patients may still be reduced even a year after discharge from the hospital. This underscores the importance of long-term monitoring of COVID-19 patients to ensure their full recovery and prevent

potential cardiac complications. LVEF is one of the most important indicators that reflects the ability of the heart to pump blood efficiently, so its decrease indicates significant cardiovascular dysfunction. This study determined that in patients in the main group, LVEF decreased to 47% at 3 months after COVID-19, indicating serious functional changes in the heart caused by the viral infection. This decrease is statistically significant, as in the control group, the LVEF value was 53%, which highlights a significant difference between the two groups ($p < 0.05$). This deterioration in cardiac function is likely to be a consequence of direct myocardial damage due to the inflammatory process caused by the viral infection. The SARS-CoV-2 virus affects the heart muscle through inflammatory mechanisms and the body's immune response, which can cause cardiomyocyte necrosis and myocarditis, which explains this decrease in LVEF.

The results obtained are consistent with other scientific data, in particular the study by A. Rahman *et al.* [33], which also found similar reductions in LVEF in patients after COVID-19. This confirms that the virus can cause serious damage to the heart muscle, which has long-term consequences. LVEF is not only a marker of cardiac performance, but also an indicator of patient survival prognosis, so its decline raises concerns about the long-term recovery of cardiac health. In patients with COVID-19, such functional changes can last for a long time, requiring additional medical intervention to correct the disorders and prevent the development of heart failure. The gradual recovery of LVEF to 52% at 12 months after discharge demonstrates positive dynamics in the restoration of cardiac function, but it should be noted that this figure was still lower than the control values of 54%. This indicates that, despite the progress, the process of cardiac recovery is lengthy and does not always result in a full return to normal functional parameters. Such changes may be due to long-term structural or functional damage to the heart muscle caused by a viral infection, which takes longer to fully recover or may even be irreversible [34].

This tendency for prolonged recovery of cardiac function after COVID-19 is consistent with the results of other studies. For instance, the study by E. Ghantous & Y. Topilsky [35] also determined that the recovery of cardiac function after COVID-19 is slow, and in many patients, LVEF remains below normal values even a year after discharge from the hospital. This underscores the importance of long-term follow-up of such patients, as even minor residual cardiac dysfunction can pose a risk of future complications such as heart failure or arrhythmias. In addition, such a long recovery period can be explained both by the severity of the primary heart damage during the acute phase of the disease and by the individual response to the infection. Some patients may be more susceptible to developing heart damage or recover more slowly due to comorbidities or other risk factors, such as hypertension, diabetes mellitus, or previous cardiovascular disease [36].

EDV is an important indicator reflecting the efficiency of the process of filling the ventricles during diastole. The increase in EF to 160 ml at 3 months after discharge in patients of the main group is a significant violation compared with the control values of 145 ml. Such an increase in volume may indicate a deterioration in diastolic cardiac function, which is typical for patients with COVID-19. In many cases, this increase may be due to fluid accumulation in the ventricles or a decrease in the ability of the heart to adequately relax after contraction, leading to less efficient filling during diastole [37]. A gradual decrease in EDV to 150 ml at month 6 and stabilisation at 147 ml at month 12 indicate an improvement in diastolic cardiac function and gradual normalisation of its function. However, despite the positive dynamics, the achieved indicators are only close to the control values and do not exceed them, which may indicate that some residual functional cardiac disorders may persist for a long time after the main infection [38]. Similar results are present in other studies. For instance, the study by A. Olszanecka *et al.* [39] also observed an increase in EF in patients with COVID-19, which confirms the results and emphasises the importance of assessing diastolic cardiac function in the long term. An increase in EF may be associated with the presence of residual inflammation or damage to the heart muscle, which affects the ability of the heart to effectively perform its pumping function.

Cardiac output, which is one of the key indicators of cardiac performance, was reduced to 3.8 l/min at the initial stages of the study in patients in the main group. This figure is significantly lower than normal and differs significantly from the results of the control group, where the average cardiac output was 4.2 l/min. This decrease indicates severe cardiac dysfunction after COVID-19, which confirms the presence of heart muscle damage or impaired pumping function [40]. During the 12-month follow-up, a gradual improvement in cardiac output was recorded, reaching 4.3 l/min at the final stage of the study. Although this figure was close to the control group's 4.5 l/min, it still did not reach a fully normal level, indicating a long-term recovery of cardiac function. This dynamic is an important indicator of the gradual recovery of cardiac function but also indicates that some patients may require additional therapy to fully restore their cardiovascular system.

The findings are consistent with those of other researchers. For instance, the study by Y. Szekely *et al.* [41] also demonstrated a similar decrease in cardiac output in patients after COVID-19. This study determined that reduced cardiac output may be a consequence of viral myocardial damage, as well as the result of a general systemic inflammatory response that affects cardiac function. Such findings underscore the importance of long-term follow-up of patients who have had COVID-19 to detect residual changes in heart function and correct complications. The level of troponins I and T, which are sensitive and specific biomarkers of myocardial damage, was increased in patients of the main group at 3 months of follow-up. In particular, the troponin level was 0.08 ng/ml, which was significantly higher than in the control group (0.03 ng/ml). This increase in troponin levels indicates the presence of heart muscle damage in patients with COVID-19 and may indicate the development of myocarditis or other complications associated with viral infection.

A gradual decrease in troponin levels to normal values at 12 months of follow-up is a positive sign of cardiac function recovery. This indicates that acute myocardial damage decreases over time, and, accordingly, the functional state of the heart gradually improves. An important point is that the normalisation of troponin levels may reflect not only the cessation of acute injury but also the effectiveness of

treatment measures aimed at supporting the cardiovascular system after COVID-19. The findings are consistent with the results of other studies. For example, the study by D. Zhao *et al*. [42] also showed an increase in troponin levels in patients with COVID-19, which confirms myocardial damage in a significant proportion of patients. This study demonstrated that elevated troponin levels correlate with the severity of COVID-19 and are a predictor of long-term recovery of cardiac function. The similarity of the results underscores the importance of monitoring troponin levels as a diagnostic tool for assessing myocardial health after infection.

A significant increase in the level of natriuretic peptide to 200 pg/ml at month 3 in patients in the main group is an important indicator of the development of heart failure due to COVID-19. A high level of this marker indicates an increased burden on the heart and ventricular dysfunction, which is typical for heart failure [43]. A gradual decrease in natriuretic peptide to 100 pg/ml at month 12 indicates a positive trend in the process of cardiac function recovery, which indicates an improvement in myocardial condition and a decrease in myocardial overload. This is consistent with the results of the study by B. Benhuri *et al*. [44], who also found elevated levels of natriuretic peptide in patients with COVID-19 and its gradual decrease during the recovery period.

Elevated levels of C-reactive protein, which reached 8 mg/l at month 3, indicate the presence of an active inflammatory process in patients in the main group. C-reactive protein is a sensitive marker of inflammation, and its elevation often indicates the presence of a systemic inflammatory response that may be caused by tissue damage, including heart muscle, during or after COVID-19 [45]. The gradual decrease in C-reactive protein levels to normal values over 12 months indicates that the inflammatory process gradually subsided, accompanied by an improvement in the general condition of patients and the restoration of body functions. This downward trend in C-reactive protein is consistent with the results of other studies, including the study by Z. Cui *et al*. [46], which also found similar dynamics in patients after COVID-19. A decrease in C-reactive protein levels indicates positive changes in the body, including a decrease in systemic inflammation, which helps to restore the cardiovascular system and reduce the risk of further complications.

MRI revealed the presence of structural changes in the myocardium in a significant proportion of patients in the main group, in 40% at 3 months of follow-up. One of the most common pathologies was myocardial fibrosis, which is characterised by the replacement of normal muscle tissue with connective tissue, which can adversely affect heart function, contributing to its stiffness and impaired contractility. In the control group, such changes were much less common, which emphasises the severity of myocardial damage in patients with COVID-19. A decrease in the proportion of patients with fibrosis to 10% at 12 months of follow-up indicates that structural changes in the myocardium may be partially reversible and that the recovery process continues. This confirms the possibility of natural or therapeutic remodelling of cardiac tissue after a viral infection. Similar findings were reported by P. Parhizgar *et al*. [47], who also found a decrease in myocardial fibrosis over a long period of follow-up of patients after COVID-19.

A significant increase in the frequency of arrhythmias and blood pressure fluctuations in patients in the main group at month 3 is a clear indicator of the negative impact of COVID-19 on cardiac rhythm and overall haemodynamics. An increase in the frequency of arrhythmias, such as atrial or ventricular extrasystoles, as well as significant fluctuations in blood pressure, indicate that viral infection can disrupt normal heart rhythm function and blood pressure regulation [48]. This may be due to inflammatory processes or the direct effect of the virus on cardiac tissue, leading to dysfunction of the electrical and mechanical processes of the heart. The gradual decrease in the frequency of arrhythmias and blood pressure fluctuations to the level of the control group at month 12 indicates an improvement in blood pressure and heart rhythm control, which may be the result of restoration of heart function and reduction of inflammatory processes. This indicates positive changes in the condition of patients after COVID-19, although functional impairment may persist in the future.

The results of other studies, such as the one by S.H. Kanuri *et al*. [49], also confirm the impact of COVID-19 on heart rhythms and blood pressure. They found an increase in the frequency of arrhythmias and pressure fluctuations in patients with COVID-19, confirming the observations of the virus's long-term effects on cardiac function. Statistical analysis confirms the significance of changes in LVEF, cardiac output and other haemodynamic parameters between the intervention and control groups. The decrease in LVEF and cardiac output at month 3 is statistically significant ($p < 0.05$), which confirms the negative impact of COVID-19 on cardiac function. The gradual recovery of indicators by 12 months indicates positive changes but also demonstrates the need for long-term monitoring.

## CONCLUSIONS

The study aimed to evaluate the impact of COVID-19 on cardiac function in patients with CHD within 12 months after hospitalisation. The results confirmed the objectives. The dynamics of LVEF showed that at month 3, in patients in the main group, this figure decreased to 47%, while in the control group, it was 53%. At 12 months, the ejection fraction partially recovered to 52% in the main group but remained lower than in the control group (54%). As for the EF and cardiac output, at month 3, the EDV in the main group was 160 ml (control group – 145 ml), indicating a volume overload of the left ventricle. By month 12, the EDV was equal in both groups (147 ml), and cardiac output in patients after COVID-19 remained lower but was approaching that of the control group (4.3 l/min vs. 4.5 l/min at month 12). Troponin I and T levels were elevated at baseline but returned to normal after 12 months. B-type natriuretic peptide decreased from 200 pg/ml to 100 pg/ml, and C-reactive protein decreased to 3 mg/l in the main group.

The practical significance of the study is an emphasis on the importance of long-term monitoring of patients after COVID-19. Among the study's limitations is the lack of analysis of other factors, such as comorbidities or the impact of drug therapy, which could have influenced the results. In addition, the study did not include patients with severe COVID-19, which limits the generalisability of the results to the entire patient population. Prospects for

further research include studying the impact of different rehabilitation methods on myocardial function recovery, as well as analysing the long-term effects of COVID-19 in patients with other comorbidities to create more effective treatment programmes.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis. 2020;20(5):533–4. DOI: 10.1016/S1473-3099(20)30120-1
[2] He F, Deng Y, Li W. Coronavirus disease 2019: What we know? J Med Virol. 2020;92(7):719–25. DOI: 10.12/jmv.25766
[3] Adhikari SP, Meng S, Wu YJ, Mao YP, Ye RX, Wang QZ, et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: A scoping review. Infect Dis Poverty. 2020;9:29. DOI: 10.1186/s40249-020-00646-x
[4] Sanche S, Lin YT, Xu C, Romero-Severson E, Hengartner N, Ke R. High contagiousness and rapid spread of severe acute respiratory syndrome coronavirus 2. Emerg Infect Dis. 2020;26(7):1470–7. DOI: 10.3201/eid2607.200282
[5] Lakhani HV, Pillai SS, Zehra M, Sharma I, Sodhi K. Systematic review of clinical insights into novel coronavirus (CoVID-19) pandemic: Persisting challenges in U.S. rural population. Int J Environ Res Public Health. 2020;17(12):4279. DOI: 10.3390/ijerph17124279
[6] Khan MA, Hashim MJ, Mustafa H, Baniyas MY, Al Suwaidi SK, Al Katheeri R, et al. Global epidemiology of ischemic heart disease: Results from the global burden of disease study. Cureus. 2020;12(7):e9349. DOI: 10.7759/cureus.9349
[7] Szarpak L, Mierzejewska M, Jurek J, Kochanowska A, Gasecka A, Truszewski Z, et al. Effect of coronary artery disease on COVID-19 – prognosis and risk assessment: A systematic review and meta-analysis. Biology (Basel). 2022;11(2):221. DOI: 10.3390/biology11020221
[8] Ganatra S, Dani SS, Shah S, Asnani A, Neilan TG, Lenihan D, et al. Management of cardiovascular disease during coronavirus disease (COVID-19) pandemic. Trends Cardiovasc Med. 2020;30(6):315–25. DOI: 10.1016/j.tcm.2020.05.004
[9] Wang S, Zhu R, Zhang C, Guo Y, Lv M, Zhang C, et al. Effects of the pre-existing coronary heart disease on the prognosis of COVID-19 patients: A systematic review and meta-analysis. PloS One. 2023;18(10):e0292021. DOI: 10.1371/journal.pone.0292021
[10] Kaddoura R, Salam AM. Thrombosis management and challenges in COVID-19 patients presenting with acute coronary syndromes. Heart Views. 2020;21(3):195–208. DOI: 10.4103/HEARTVIEWS.HEARTVIEWS_143_20
[11] Basu-Ray I, Almaddah NK, Vaqar S, Soos MP. Cardiac manifestations of coronavirus (COVID-19) [Internet]. Treasure Island: StatPearls Publishing; 2024 [cited 2024 Sep 29]. Available from: https://pubmed.ncbi.nlm.nih.gov/32310612/
[12] Pannucci P, Jefferson SR, Hampshire J, Cooper SL, Hill SJ, Woolard J. COVID-19-induced myocarditis: Pathophysiological roles of ACE2 and toll-like receptors. Int J Mol Sci. 2023;24(6):5374. DOI: 10.3390/ijms24065374
[13] Kounis NG, Gogos C, de Gregorio C, Hung MY, Kounis SN, Tsounis EP, et al. "When," "where," and "how" of SARS-CoV-2 infection affects the human cardiovascular system: A narrative review. Balkan Med J. 2024;41(1):7–22. DOI: 10.4274/balkanmedj.galenos.2023.2023-10-25
[14] Taylor RS, Dalal HM, McDonagh ST. The role of cardiac rehabilitation in improving cardiovascular outcomes. Nat Rev Cardiol. 2022;19(3):180–94. DOI: 10.1038/s41569-021-00611-7
[15] Dalal HM, Taylor RS, Wingham J, Greaves CJ, Jolly K, Lang CC, et al. A facilitated home-based cardiac rehabilitation intervention for people with heart failure and their caregivers: A research programme including the REACH-HF RCT. Programme Grants Appl Res. 2021;9(1). DOI: 10.3310/pgfar09010
[16] Cao J, Dong R, Zhang K, Zhang H. Effects of myocardial viability and left ventricular remodeling on survival of patients with heart failure and reduced ejection fraction after coronary artery bypass grafting. Cardiovasc Diagn Ther. 2020;10(2):183–92. DOI: 10.21037/cdt.2020.01.11
[17] Weinmann K, Werner J, Koenig W, Rottbauer W, Walcher D, Keßler M. Use of cardiac biomarkers for monitoring improvement of left ventricular function by immunoadsorption treatment in dilated cardiomyopathy. Biomolecules. 2019;9(11):654. DOI: 10.3390/biom9110654
[18] Topf A, Mirna M, Bacher N, Paar V, Motloch LJ, Ohnewein B, et al. Analysis of selected cardiovascular biomarkers in Takotsubo cardiomyopathy and the most frequent cardiomyopathies. Front Cardiovasc Med. 2021;8:700169. DOI: 10.3389/fcvm.2021.700169
[19] Atri D, Siddiqi HK, Lang JP, Nauffal V, Morrow DA, Bohula EA. COVID-19 for the cardiologist: Basic virology, epidemiology, cardiac manifestations, and potential therapeutic strategies. JACC Basic Transl Sci. 2020;5(5):518–36. DOI: 10.1016/j.jacbts.2020.04.002
[20] Karagodin I, Singulane CC, Descamps T, Woodward GM, Xie M, Tucay ES, et al. Ventricular changes in patients with acute COVID–19 infection: Follow-up of the World Alliance Societies of Echocardiography (WASE-COVID) study. J Am Soc Echocardiogr. 2022;35(3):295–304. DOI: 10.1016/j.echo.2021.10.015
[21] Castillo EC, Vázquez-Garza E, Yee-Trejo D, García-Rivas G, Torre-Amione G. What is the role of the inflammation in the pathogenesis of heart failure? Curr Cardiol Rep. 2020;22(11):139. DOI: 10.1007/s11886-020-01382-2
[22] Mesquita T, Lin YN, Ibrahim A. Chronic low-grade inflammation in heart failure with preserved ejection fraction. Aging Cell. 2021;20(9):e13453. DOI: 10.1111/acel.13453

[23] Sharma D, Rohila A, Deora S, Garg MK, Misra S. Cardiac assessment of patients during post COVID-19 recovery phase: A prospective observational study. Glob Cardiol Sci Pract. 2022;2022(3):18. DOI: 10.21542/gcsp.2022.18
[24] Alqahtani MS, Abbas M, Alsabaani A, Alqarni A, Almohiy HM, Alsawqaee E, et al. The potential impact of COVID-19 virus on the heart and the circulatory system. Infect Drug Resist. 2022;2022(15):1175–89. DOI: 10.2147/IDR.S351318
[25] Ntchana A, Shrestha S, Pippin M. Cardiovascular complications of COVID-19: A scoping review of evidence. Cureus. 2023;15(11):e48275. DOI: 10.7759/cureus.48275
[26] Polito MV, Silverio A, Bellino M, Iuliano G, Di Maio M, Alfano C, et al. Cardiovascular involvement in COVID-19: What sequelae should we expect? Cardiol Ther. 2021;10(2):377–96. DOI: 10.1007/s40119-021-00232-8
[27] Vazirani R, Feltes G, Hoyo RS, Viana-Llamas MC, Raposeiras-Roubín S, Romero R, et al. Elevated troponins after COVID-19 hospitalization and long-term COVID-19 symptoms: Incidence, prognosis, and clinical outcomes – results from a multi-center international prospective registry (HOPE-2). J Clin Med. 2024;13(9):2596. DOI: 10.3390/jcm13092596
[28] Lionte C, Sorodoc V, Haliga RE, Bologa C, Ceasovschih A, Petris OR, et al. Inflammatory and cardiac biomarkers in relation with post-acute COVID-19 and mortality: What we know after successive pandemic waves. Diagnostics (Basel). 2022;12(6):1373. DOI: 10.3390/diagnostics12061373
[29] Sarzani R, Allevi M, Di Pentima C, Schiavi P, Spannella F, Giulietti F. Role of cardiac natriuretic peptides in heart structure and function. Int J Mol Sci. 2022;23(22):14415. DOI: 10.3390/ijms232214415
[30] Tobler DL, Pruzansky AJ, Naderi S, Ambrosy AP, Slade JJ. Long-term cardiovascular effects of COVID-19: Emerging data relevant to the cardiovascular clinician. Curr Atheroscler Rep. 2022;24(7):563–70. DOI: 10.1007/s11883-022-01032-8
[31] Arévalos V, Ortega-Paz L, Rodríguez-Arias JJ, Calvo López M, Castrillo-Golvano L, Salazar-Rodríguez A, et al. Acute and chronic effects of COVID-19 on the cardiovascular system. J Cardiovasc Dev Dis. 2021;8(10):128. DOI: 10.3390/jcdd8100128
[32] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 Jul 18]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/
[33] Rahman A, Ruge M, Hlepas A, Nair G, Gomez J, du Fay de Lavallaz J, et al. Hyperdynamic left ventricular ejection fraction is associated with higher mortality in COVID-19 patients. Am Heart J Plus. 2022;14:100134. DOI: 10.1016/j.ahjo.2022.100134
[34] Narovlyanskaya O, Winokur EJ. Viral myocarditis. Dimens Crit Care Nurs. 2020;39(2):75–80. DOI: 10.1097/DCC.0000000000000402
[35] Ghantous E, Topilsky Y. Recovery of cardiac function following COVID-19. Eur J Heart Fail. 2021;23(11):1913–5. DOI: 10.1002/ejhf.2364
[36] Ferdinandy P, Andreadou I, Baxter GF, Botker HE, Davidson SM, Dobrev D, et al. Interaction of cardiovascular nonmodifiable risk factors, comorbidities and comedications with ischemia/reperfusion injury and cardioprotection by pharmacological treatments and ischemic conditioning. Pharmacol Rev. 2023;75(1):159–216. DOI: 10.1124/pharmrev.121.000348
[37] Kapoor PM, Bhardwaj V, Sharma A, Kiran U. Global end-diastolic volume an emerging preload marker vis-a-vis other markers – have we reached our goal? Ann Card Anaesth. 2016;19(4):699–704. DOI: 10.4103/0971-9784.191554
[38] Badrinath A, Bhatta S, Kloc A. Persistent viral infections and their role in heart disease. Front Microbiol. 2022;13:1030440. DOI: 10.3389/fmicb.2022.1030440
[39] Olszanecka A, Wojciechowska W, Bednarek A, Kusak P, Wizner B, Terlecki M, et al. Serial echocardiographic evaluation of COVID-19 patients without prior history of structural heart disease: A 1-year follow-up CRACoV-HHS study. Front Cardiovasc Med. 2023;10:1230669. DOI: 10.3389/fcvm.2023.1230669
[40] Nunez-Gil IJ, Feltes G, Viana-Llamas MC, Raposeiras-Roubin S, Romero R, Alfonso-Rodríguez E, et al. Post-COVID-19 symptoms and heart disease: Incidence, prognostic factors, outcomes and vaccination: Results from a multi-center international prospective registry (HOPE 2). J Clin Med. 2023;12(2):706. DOI: 10.3390/jcm12020706
[41] Szekely Y, Lichter Y, Sadon S, Lupu L, Taieb P, Banai A, et al. Cardiorespiratory abnormalities in patients recovering from coronavirus disease 2019. J Am Soc Echocardiogr. 2021;34(12):1273–84. DOI: 10.1016/j.echo.2021.08.022
[42] Zhao D, He Y, Dian Y, Meng Y, Zeng F, Deng G. Elevated troponin levels predict the reduced efficacy of Paxlovid in COVID-19 patients. J Infect. 2023;87(2):148–50. DOI: 10.1016/j.jinf.2023.03.026
[43] Brunner-La Rocca HP, Sanders-van Wijk S. Natriuretic peptides in chronic heart failure. Card Fail Rev. 2019;5(1):44–9. DOI: 10.15420/cfr.2018.26.1
[44] Benhuri B, Aikawa T, Takagi H, Benhuri D, Kuno T. Elevated natriuretic peptides in patients with severe or critical COVID-19: A meta-analysis. Tex Heart Inst J. 2022;49(5):e207404. DOI: 10.14503/THIJ-20-7404
[45] Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018;9:754. DOI: 10.3389/fimmu.2018.00754
[46] Cui Z, Merritt Z, Assa A, Mustehsan H, Chung E, Liu S, et al. Early and significant reduction in C-reactive protein levels after corticosteroid therapy is associated with reduced mortality in patients with COVID-19. J Hosp Med. 2021;16(3):142–8. DOI: 10.12788/jhm.3560
[47] Parhizgar P, Yazdankhah N, Rzepka AM, Chung KY, Ali I, Lai Fat Fur R, et al. Beyond acute COVID-19: A review of long-term cardiovascular outcomes. Can J Cardiol. 2023;39(6):726–40. DOI: 10.1016/j.cjca.2023.01.031

[48] Yajima T. Viral myocarditis: Potential defense mechanisms within the cardiomyocyte against virus infection. Future Microbiol. 2011;6(5):551–66. DOI: 10.2217/fmb.11.40
[49] Kanuri SH, Sirrkay PJ, Ulucay AS. COVID-19 heart unveiling as atrial fibrillation: Pathophysiology, management and future directions for research. Egypt Heart J. 2023;75(1):36. DOI: 10.1186/s43044-023-00359-0

# Ішемічна хвороба серця та динаміка показників внутрішньосерцевої гемодинаміки після госпіталізації через COVID-19: результати 12-місячного спостереження

**Сергій Мостовий**
Кандидат медичних наук
Національний медичний університет імені О.О. Богомольця
01601, бульв. Т. Шевченка, 13, м. Київ, Україна
https://orcid.org/0000-0002-8783-3819

**Анотація.** Метою дослідження було оцінити динаміку показників внутрішньосерцевої гемодинаміки у пацієнтів з ішемічною хворобою серця (ІХС) після перенесеного COVID-19 протягом 12-місячного періоду. Для досягнення цієї мети було проведено спостереження за пацієнтами, які перенесли COVID-19 та мали діагноз ІХС на клінічних базах Національного медичного університету імені О.О. Богомольця і кардіологічного відділення Дорожньої клінічної лікарні № 2, Київської міської клінічної лікарні № 18 та медичного центру «Медбуд». Основними параметрами, що досліджувались, були фракція викиду лівого шлуночка (ФВЛШ), кінцево-діастолічний об'єм (КДО), серцевий викид, рівні тропонінів I та T, натрійуретичного пептиду, а також рівень С-реактивного білка. За результатами дослідження встановлено, що ФВЛШ знизилася до 47 % на 3-му місяці після COVID-19 і частково відновилася до 52 % через 12 місяців, але не досягла контрольних значень. КДО збільшився до 160 мл на 3-му місяці, а потім зменшився до 147 мл на 12-му місяці, що вказує на об'ємне перевантаження лівого шлуночка. Серцевий викид на початку дослідження становив 3,8 л/хв, а на кінець – 4,3 л/хв, що свідчило про позитивну динаміку, але неповне відновлення. Рівні тропонінів та натрійуретичного пептиду спочатку були підвищеними, але поступово знизилися до нормальних показників, що свідчить про відновлення серцевої функції. Підвищений рівень С-реактивного білка, зафіксований на початку дослідження, свідчив про наявність активного запального процесу, який поступово знижувався до нормальних значень протягом 12-місячного періоду. Це є важливим показником зменшення запалення та покращення загального стану пацієнтів після перенесеного COVID-19. Отримані результати дослідження вказали на часткове відновлення функцій серця у пацієнтів, але також продемонстрували, що процес відновлення є тривалим і не завжди завершується повним поверненням до нормальних показників серцевої діяльності

**Ключові слова:** серцева недостатність; запальний процес; відновлення міокарда; функціональні порушення серця; серцеві маркери

Article's History: Received: 11.07.2024; Revised: 05.11.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.34

Journal homepage: https://bmbr.com.ua/en

UDC 616.89-008:355.01

# Correlation between cephalgia and chronic stress progression in wartime

**Pavlo Ometsynskyi***

Medical Intern
Municipal Non-Profit Enterprise "1st City Polyclinic in Lviv"
79000, 20 Ruska Str., Lviv, Ukraine
https://orcid.org/0009-0002-5932-298X

**Abstract.** The aim of this study was to explore the main correlations between chronic stress and the progression of cephalalgia in Ukrainian refugees. A total of 83 Ukrainian refugees participated in the study, with data collected using a questionnaire-based method. The most significant differences in stress levels were observed between the age groups of 14-19 years and 35-39 years ($p = 0.009$). This finding supports the hypothesis that stress influences the development of cephalalgia and highlights that stress levels differ significantly between these age groups, likely due to the distinct life circumstances and stressors characteristic of each group. Using one-way analysis of variance (ANOVA), the F statistic was calculated to compare variations between groups and within each group. For instance, a value of $F = 2.19$ at $p = 0.035$ demonstrated a statistically significant difference in stress levels among different age groups. This result indicated that age had a noticeable effect on stress levels, and consequently, on the progression of cephalalgia. Additionally, the correlation coefficient between participants' age and stress was found to be -0.101 at $p = 0.006$. This revealed a weak but statistically significant inverse relationship between age and the stress indicator. Given that p-value is below 0.05, this correlation can be considered significant. Although the relationship was weak, it suggested a small but clinically relevant connection between chronic stress and cephalalgia as influenced by age

**Keywords:** refugees; headache; fear; anxiety; neurological disorders

## INTRODUCTION

The year 2022 marked a turning point for the world after the COVID-19 pandemic, particularly for Ukrainians. The war brought not only physical destruction but also profound psychological stress, which manifested in a range of physical symptoms, among which cephalalgia (headache) emerged as one of the most prevalent. A key issue is that populations affected by military actions often suffer from chronic cephalalgia, which worsens under constant stress and proves challenging to treat.

According to N.M. Malinovska & I.A. Andriychuk [1], emotional trauma resulting from military actions induces severe stress, which may be both acute and chronic, persisting in the victim's life. Prolonged stress impacts all aspects of life and leads to serious mental health consequences, including post-traumatic stress disorder (PTSD), depression, anxiety, sleep disturbances, and other psychosomatic issues. The authors noted that the most detrimental effects on war victims arise from auditory and visual stimuli associated with explosions, as well as the loss of loved ones. These factors are often accompanied by fear, anxiety, and severe headaches. To mitigate such anxiety, the authors suggested engaging in basic psychological self-education to provide foundational support to loved ones prior to seeking professional assistance. S.Y. Romanenko [2] and L. Stovner *et al*. [3] emphasised that children are particularly vulnerable to cephalalgia and chronic stress due to their developing and highly malleable psyches. Their research highlighted that 15% to 50% of visits to paediatric neurologists and psychiatrists are related to complaints of headaches and anxiety. 62% of these cases involve primary cephalalgia, while the remainder are secondary. The study also notes a gender difference in reported cases: 38% of girls report cephalalgia compared to 27% of boys, suggesting that boys may exhibit greater mental resilience to stress.

**Suggested Citation**:
Ometsynskyi P. Correlation between cephalgia and chronic stress progression in wartime. Bull Med Biol Res. 2024;6(4):34–44. DOI: 10.63341/bmbr/4.2024.34

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

Under conditions of constant danger, uncertainty, and anxiety, stress-induced headaches become a frequent physiological response to the stressors of war. The progression of cephalalgia is closely associated with chronic stress, particularly in situations of aggression, where individuals experience significant psychological pressure. Stress in military contexts can be particularly intense due to fear, anxiety, traumatic events, and other factors that contribute to the development or exacerbation of cephalalgia. N.V. Lebedynets & A.S. Negriy [4] described similar phenomena in their work, drawing parallels between anxiety-induced cephalalgia and the processes of accelerated ageing. Cortisol, the primary stress hormone, plays a critical role in many bodily processes. Under conditions of chronic stress, persistently elevated cortisol levels can significantly impact health and expedite ageing. Post-traumatic cephalalgia is often accompanied by fluctuations in cortisol and adrenaline levels. In control groups at rest or without stress, normal cortisol levels range between 6-23 µg/dl (micrograms per decilitre) in the blood. However, during intense stress, cortisol levels can rise to 30-50 µg/dl, depending on the stress's intensity and duration.

Similar changes have been noted by N. Yatsuk *et al*. [5] and S. Freischmidt [6], who highlighted those post-traumatic disorders, particularly those arising from military conflicts, are frequently characterised by cephalalgia, often as a primary symptom. These researchers concluded that creating comfortable and peaceful conditions helps reduce stress levels, thereby minimising cephalalgia. The highest levels of stress and severe headaches were reported among Ukrainian refugees who fled the country before or at the onset of the war and among military personnel. Among refugees, approximately 35-40% are prone to cephalalgia or tension headaches caused by chronic stress. Among military veterans diagnosed with PTSD, the prevalence of headaches reaches 60-65%. In contrast, the lowest levels of anxiety and headaches were observed in individuals who chose to remain in Ukraine despite the ongoing danger. This decision appeared to provide a modest sense of stability, sparing them from the severe symptoms experienced by others.

It is important to note that women of reproductive age were significantly impacted by chronic stress resulting from the war. This stress was primarily manifested in menstrual dysfunction among young girls and hormonal imbalances [7]. Additional complications included difficulties with conception and the development of neoplasms in the uterine cavity and ovaries, which have proven challenging to treat [8]. Clinically, these women often experienced severe migraine attacks, and in some cases, gave birth to anxious newborns. Such conditions require timely diagnosis and appropriate treatment. I. Petukhova *et al.* [9] emphasised the importance of prioritising the prevention and mitigation of stress as a key provocateur of cephalalgia, alongside the timely management of anxiety disorders. However, notable gaps remain in the research, particularly regarding the mechanisms underlying the development of cephalalgia during wartime and its correlation with chronic stress. The aim of this study was to determine the influence of chronic stress on the development and progression of cephalalgia in Ukrainian refugees. The specific objectives were: to describe the characteristics of cephalalgia resulting from chronic stress caused by military conflict; to provide basic recommendations for preventing or alleviating manifestations of stress-related cephalalgia among Ukrainian refugees.

## MATERIALS AND METHODS

To achieve the research objectives, a questionnaire method was employed. The survey was conducted in 2024 at the Municipal Non-Profit Enterprise "1st City Polyclinic in Lviv" in Lviv, Ukraine, among Ukrainians affected by the war who remain in Ukraine, as well as Ukrainian refugees residing in Poland and Spain. Refugees from both countries were combined into a single general group, without division by location. Participants were selected based on key criteria: Ukrainian refugees from areas affected by hostilities (primarily from the eastern regions of Ukraine) and individuals who witnessed or experienced shelling and missile attacks (residents of the northern, southern, and western regions of Ukraine). A total of 83 individuals participated in the study, comprising 52 women and 31 men across various age groups. The participants were recruited six months prior to the full-scale invasion and within six months following 24 February 2022. The study was conducted face-to-face and anonymously, with participants fully informed about the measures taken to ensure the confidentiality of their personal information. They were also made aware of the study's purpose, as well as any potential risks associated with participation. All participants provided informed consent for their involvement in the survey and the collection of their data.

To assess levels of anxiety and sleep quality, in addition to the core questionnaire focusing on headache, participants were asked detailed questions regarding their psycho-emotional state and sleep patterns. The study did not analyse dynamic changes over time; rather, it focused on comparing survey responses before the invasion and after the onset of war. For more detailed analysis, the participants were divided into age categories: children (0-14 years), adolescents (14-19 years), and older adults (35-39 years). It is important to note that individuals aged 19-35 were not surveyed due to the absence of this age category in the study design. This segmentation facilitated the examination of age-related differences in stress perception and the development of cephalalgia, allowing for an assessment of how different age groups responded to war-related stressors. This approach ensured a diverse sample, providing a comprehensive overview of the impact of war on the mental and physical health of individuals across different genders and age groups.

The study revealed a wide range of reactions to stressful events caused by war among different demographic groups. A standardised questionnaire was used for the survey, encompassing key parameters for assessing the presence or absence of cephalalgia (Table 1). This questionnaire facilitated the collection of data on the frequency, intensity, and duration of headaches among participants, as well as the identification of potential relationships between these symptoms and stress induced by war. The standardised approach to data collection ensured the reliability and validity of the findings.

**Table 1.** Sample questionnaire for investigating the presence of cephalgia

| Question | Sex | | Full years | Date of filling |
|---|---|---|---|---|
| | Males | Females | | |
| Please indicate what type of headache you are experiencing? | | | | |
| At what age did you first experience a headache? | | | | |
| Does the pain move around your head? | | | | |
| How long (hours) does the headache last? | | | | |
| What medications are you taking (if any)? | | | | |
| If you take headache medicine, how long (hours) will the headache last? | | | | |
| How would you rate the severity of your headache? (on a scale from 0 – almost no pain, to 10 – very severe) | | | | |
| How many hours do you sleep? | | | | |
| Do you have difficulty falling asleep or staying asleep (yes, no)? | | | | |
| Does a headache wake you up from a sound sleep (yes, no)? | | | | |
| How many cups of coffee, tea, or caffeinated beverages do you drink (per day)? | | | | |
| Has this figure (number of cups) changed after February 2022? | | | | |
| Do you smoke? If so, how long have you been smoking (in years)? | | | | |
| How often do you drink alcohol (per month)? | | | | |
| If you are employed, please indicate the nature of your work (check one or more options):<br>1. Stationary.<br>2. Mobile.<br>3. Physical.<br>4. Working with a computer.<br>5. Working with people.<br>6. Flexible schedule.<br>7. Military. | | | | |
| How concerned are you about your overall health (scale from 0 – I feel completely healthy to 10 – very concerned) | | | | |
| Do you do any form of sports or physical exercise? | | | | |
| How relaxed or tense did you feel until February 2022? (scale from 0 – completely relaxed to 10 – extremely tense) | | | | |
| How relaxed or tense do you feel right now? (scale from 0 – completely relaxed to 10 – extremely tense) | | | | |
| How frequent have your headache attacks (per month) been over the past 2 years? | | | | |
| In your opinion, to what extent did martial law conditions affect the frequency and severity of your headaches?<br>(scale from 0 – did not notice to 10 – very much affected) | | | | |

**Source:** created by the author

To analyse the effects of chronic stress, fear, and anxiety on health, respondents were categorised into three groups: healthy, mildly ill, and seriously ill. This classification considered their lifestyle, including alcohol and tobacco consumption. The healthy group comprised individuals without any underlying pathology, while the mildly ill and seriously ill groups included those with existing chronic diseases or conditions in the acute phase. Chronic conditions in these groups included cardiovascular diseases (e.g., ischaemic heart disease, diffuse cardiosclerosis, prior myocardial infarction, hypertension), chronic inflammatory diseases of the respiratory and digestive systems, and musculoskeletal disorders. This categorisation enabled an investigation of how varying health statuses and behavioural factors, such as alcohol and tobacco use, interact with stress and influence overall health. The results of this analysis provided valuable insights into the role of lifestyle in determining respondents' resilience to stress and identified population groups most vulnerable to the adverse effects of chronic stress.

One-way analysis of variance (ANOVA) was employed to detect statistically significant differences between the means of multiple groups classified based on a single independent variable or factor. This method enabled researchers to evaluate whether an independent variable (such as age, education level, or other characteristics) significantly influenced changes in the dependent variable. One-way ANOVA compared the variation within groups to the variation between groups, allowing determination of whether differences in means are attributable to the independent variable or random fluctuations. This approach provided data on the F statistic, which measured the relative magnitude of variation between groups compared to within groups. In this analysis, the main groups were compared based on age and gender, with the severity of cephalalgia symptoms serving as the primary dependent variable.

A t-test was conducted to assess statistically significant differences in headache frequency between military personnel, refugees affected by war, and pre-war baseline indicators. The primary objective of this test was to evaluate whether the differences in headache frequency or intensity between these groups were statistically significant when compared to pre-war data. If the obtained t-value exceeded the critical t-value, the null hypothesis was

rejected, indicating a statistically significant difference in headache frequency between military personnel, refugees, and pre-war data. The critical t-value was set at 0.01 (1%), establishing a significance level of 0.01. This implied that there was a 1% probability of incorrectly rejecting the null hypothesis. This study adhered to the principles outlined in the Declaration of Helsinki on ethical principles for medical research involving human subjects [10], and informed consent was obtained from all participants.

## RESULTS

### Analysis of stress factors impact on the frequency of cephalgia among different demographic groups

The study included 83 participants, comprising 52 women and 31 men from various age groups. This diverse sample allowed for the collection of comprehensive data, enabling the consideration of gender and age differences in the analysis. The survey results revealed significant variations in stress responses and the occurrence of cephalalgia between men and women. These findings indicate the presence of gender differences in participants' reactions, which may reflect distinct perceptions or experiences of the phenomena under investigation (Table 2). Such differences warrant further analysis to gain a deeper understanding of their nature and to account for them when interpreting the study results. Notably, refugee women exhibited a stronger reaction to life stressors compared to refugee men. This suggests that women may respond more acutely to stressful situations or perceive a higher level of tension in daily life.

**Table 2.** Comparative characteristics of the frequency of cephalgia attacks depending on gender and age

| Characteristics | Refugee men (total number in the group) | Refugee women (total number in the group) | Frequency of attacks of cephalgia in men of this group | Frequency of cephalgia attacks among women of this group |
|---|---|---|---|---|
| Age 0-14 years | 8 people | 10 people | 5 cases (62.5%) | 4 cases (40%) |
| Age 14-19 years | 14 people | 11 people | 9 cases (64.2%) | 8 cases (72.7%) |
| Age 35-39 | 9 people | 31 people | 8 cases (88.8%) | 26 cases (83.8%) |
| Military (out of the total number, those who were discharged from military service) | 2 people | - | 2 cases (100%) | - |
| Other complaints | Complaints of anxiety, depression. Phantom pain – the military | Complaints of anxiety, depression, reproductive system disorders | - | - |
| Comments | - | - | The group of people aged 14-19 perceived any stress factors with vulnerability and the frequency of cephalgia attacks increased | Women had more frequent attacks of cephalalgia than men in the age group 35-39 years |

**Source:** created by the author

These differences may be attributed to both psychological and sociocultural factors that influence the perception and experience of stress across different gender groups. The correlation coefficient between participants' age and the studied indicator was -0.101 at $p = 0.006$. This represents a weak but statistically significant inverse relationship between age and the occurrence of the studied indicator. With p-value of 0.001 (or 0.1%), the results are statistically significant, allowing for the rejection of the null hypothesis. An increase in cephalalgia attacks was observed within the group reporting severe cephalalgia (Table 3). Despite the small correlation coefficient, the findings suggest that as participants' age increases, the studied indicator tends to decrease slightly.

**Table 3.** Dynamics of the main indicators of respondents before the start of the full-scale invasion and after (before February 24, 2022 and after)

| Category of respondents | Increase of cephalgia attacks (per month) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before 24.02.2022 | After 24.02.2022 | % | Average value | | Decreasing the coefficient | | Average value taking into account the coefficient | |
| | | | | In category | Total | | In category | In category | Total |
| Healthy | 2.3 | 3.1 | 34.8 | 56.8% | 62.1% | 1 | 66.7% | 34.8% | 60.4% |
| Mild cephalgia | 3.7 | 6.8 | 83.8 | | | 1.5 | | 125.7% | |
| Severe cephalalgia | 5.2 | 7.9 | 51.9 | | | 2.5 | | 129.8% | |

**Source:** created by the author

Children and adolescents were combined into a single group, referred to as the younger group. The most severe stress levels and, consequently, the most complex cases of cephalalgia in a war context were observed in this younger

group, aged 14-19 years. One-way ANOVA revealed a significant difference in stress levels among the different age groups ($F = 2.19$, $p = 0.035$). The most pronounced differences were identified between the 14-19 and 33-39 age groups ($p = 0.009$). These findings suggest that younger individuals experience war-related stress more acutely than adults, potentially due to lower life resilience or limited experience in managing extreme situations. The study also accounted for lifestyle factors, including smoking and alcohol consumption (Table 4). This approach allowed for an analysis of how varying health behaviours interact with stressful conditions and influence overall health outcomes. The results provided valuable insights into which population groups are most vulnerable to the adverse effects of stress and highlighted lifestyle modifications that could contribute to improved health under conditions of chronic stress.

**Table 4.** Distribution of respondents depending on lifestyle and bad habits

| Category of respondents | Increase of cephalgia attacks (per month) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Before 24.02.2022 | After 24.02.2022 | % | Average value | | Decreasing the coefficient | | Average value taking into account the coefficient | |
| | | | | In category | Total | | In category | In category | Total |
| Lead an active lifestyle | 2.5 | 4.8 | 92 | 79% | 62.1% | 1 | 50% | 92% | 60.4% |
| Lead a sedentary lifestyle | 4.7 | 7.8 | 66 | | | 2 | | 131.9% | |
| Smoke | 4.5 | 7.7 | 71.1 | 64.7% | | 2 | 50% | 142.2% | |
| Do not smoke | 2.4 | 3.8 | 58.3 | | | 1 | | 58.3% | |
| Moderate or no alcohol consumption | 4.6 | 6.1 | 32.6 | 50.6% | | 1.5 | 75% | 48.9% | |
| Often drink alcohol | 3.5 | 5.9 | 68.6 | | | 2 | | 137.1% | |

**Source:** created by the author

The overall level of stress, and consequently the risk of cephalalgia, was closely associated with the number of traumatic factors, including exposure to episodes of military operations. This indicates that the greater the exposure to traumatic situations, the higher the level of perceived stress experienced. This relationship underscores the importance of considering the number and intensity of adverse events in a person's life when assessing their psycho-emotional state and developing appropriate supportive measures. The study revealed that perceived stress levels significantly increased in participants under the following circumstances: direct experience of a difficult event ($p = 0.022$), witnessing the misfortunes of others ($p = 0.004$), traumatic events involving loved ones and friends ($p = 0.015$), and intense fear of potential misfortunes or threats ($p = 0.005$). These findings demonstrate that not only personal experiences of trauma but also empathy for others and fear of possible dangers have a considerable impact on stress levels. A negative correlation was observed between the level of perceived stress and the intensity of positive emotions experienced in the weeks following the onset of the war. This suggests that higher levels of stress are associated with fewer positive emotions [10]. Conversely, perceived stress was positively correlated with the intensity of negative emotions, indicating that greater stress leads to more intense negative emotional experiences. Furthermore, direct and significant correlations were identified between perceived stress levels and symptoms of anxiety and depression ($p = 0.000$). This highlights the importance of assessing perceived stress as a critical indicator of potential psychophysiological disorders. High levels of perceived stress may predict the onset of anxiety and depressive disorders, emphasising the need for early detection and intervention to prevent serious mental health conditions.

**Comprehensive approach to the treatment of cephalgia**

The findings of the study underscore the critical importance of preventing and treating neurological disorders that contribute to cephalalgia. Key measures include psychological and social support, pharmacological treatment, physical activity, and various relaxation techniques. Providing psychological support and counselling to survivors of military conflicts is essential in helping them overcome stress and manage their emotions. Military conflicts often result in profound psychological trauma, frequently accompanied by elevated stress levels [11]. Psychological support plays a vital role in mitigating this stress by teaching relaxation techniques, fostering resilience, and managing anxiety. Following a conflict, individuals may experience a range of negative emotions, including fear, anger, sadness, and despair. Psychological counselling helps stabilise emotional states, build self-control skills, and encourage the constructive expression of feelings. A crucial component of psychological support involves addressing PTSD and other psychological consequences of conflict [12]. Therapy may focus on overcoming traumatic memories, rebuilding self-esteem, and facilitating a return to normal life. In addition to individual counselling, group therapy offers a platform for individuals to share their experiences and provide mutual support. This collective approach helps to alleviate feelings of isolation and alienation. Survivors of military conflicts often encounter challenges in adapting to civilian life. Psychological support aids in developing adaptive skills, addressing new life challenges, and setting positive goals for the future.

The results indicate that treating cephalalgia (headache) often requires an integrated approach, which may include the use of various medications. Depending on the type and intensity of the headache, the following categories of medications may be prescribed: analgesics, anti-inflammatory drugs, psychotropic medications (to alleviate stress and anxiety), and specific treatments for migraines. Headache treatment is tailored to the individual, considering the type of cephalalgia, pain intensity, and accompanying symptoms. In addition to managing acute pain, preventive measures are often recommended, involving the long-term use of antidepressants, antiepileptic drugs, or beta-blockers to reduce the frequency of headache attacks [13-15]. Lifestyle modifications are also advised, including avoiding headache triggers, ensuring regular rest, engaging in physical activity, and maintaining a nutritious diet.

As part of the study, a survey was conducted to assess anxiety and sleep among Ukrainian refugees, particularly those affected by shelling in Ukraine. The impact of these sleep changes on the effectiveness of cephalalgia treatment, especially in terms of the frequency of headache episodes, was also evaluated. The findings showed that a 50% reduction in headache frequency was associated with improved sleep quality and psychosocial factors. This suggests that better sleep not only alleviates the physical symptoms of migraine but also enhances the psycho-emotional well-being of patients, ultimately improving treatment outcomes. Participants were selected based on specific criteria: Ukrainian refugees from areas experiencing active hostilities (primarily in eastern Ukraine) and individuals who had witnessed or been victims of shelling and rocket attacks (residents of northern, southern, and western Ukraine). To assess anxiety and sleep quality, the questionnaire included detailed questions about the psycho-emotional state and sleep patterns in addition to primary questions about cephalalgia. The study did not focus on dynamic changes over time but instead aimed to compare survey results before the invasion with those collected after the start of the war.

**Dependence of cephalgia occurrence on military stressful conditions**

Military conflicts significantly increase the risk of developing or exacerbating cephalalgia (headaches) due to exposure to various stressors, trauma, and adverse conditions. Chronic stress, driven by a persistent sense of danger, anxiety, and uncertainty, is a potent trigger for headaches [16, 17]. Stress activates the sympathetic nervous system, potentially causing muscle tension, vascular changes, and, consequently, cephalalgia. Combat participants often endure both physical and psychological trauma. Traumatic brain injuries are a common cause of post-traumatic headaches, while psychological traumas, such as PTSD, are strongly associated with frequent headaches. Furthermore, the conditions under which military personnel operate often prevent them from achieving regular, high-quality sleep – an essential factor in headache prevention. Sleep deprivation or poor sleep quality can contribute significantly to the development of cephalalgia [18]. In the challenging environment of military conflicts, access to adequate water and food is often limited, leading to dehydration and nutritional deficiencies – both recognised triggers for headaches. Additionally, the constant noise from explosions, gunfire, and other combat activities can provoke cephalalgia, particularly in individuals already suffering from migraines or other headache disorders. Given these contributing factors, military conflicts not only lead to new cases of cephalalgia but also markedly worsen the condition of those with pre-existing headaches [19, 20]. This underscores the critical need for comprehensive medical and psychological support for victims, along with the development of specialised rehabilitation programmes for combatants to mitigate the risk and alleviate the symptoms of cephalalgia.

War conditions, including anxiety, fear, and traumatic experiences, can significantly increase the intensity and frequency of headaches in individuals affected by conflict. The constant psychological pressure and emotional stress associated with military conflicts not only exacerbate existing headaches but also contribute to new cases of cephalalgia, further deteriorating the overall health of those affected. This underscores the urgent need to establish or expand specialised centres for the treatment of headaches and migraines, a critical step towards enhancing the quality of medical care during wartime. Such centres should be staffed with specialists experienced in managing these conditions [21, 22]. The development of facilities focused on the diagnosis and treatment of headaches and migraines – leveraging advanced medical technologies and innovative treatment methods – represents a significant advancement in the prevention and management of cephalalgia. To ensure comprehensive care, it is essential to involve a multidisciplinary team of specialists, including neurologists, psychotherapists, physiotherapists, and dietitians. These integrated efforts can significantly improve the quality of life for patients by providing necessary support and effective treatments, irrespective of their location.

## DISCUSSION

The results of the study indicate significant gender differences in stress responses and the development of headaches. A survey of 83 participants, comprising 52 women and 31 men, revealed distinct variations in responses to stressors, often resulting in differing frequencies of headaches. These findings suggest both biological and psychological differences between the sexes in their perception of stress, as well as the influence of social factors such as gender roles and expectations. Women may exhibit greater susceptibility to stress due to a combination of physiological factors (e.g., hormonal fluctuations) and social pressures (e.g., balancing career and family responsibilities). These factors could make women more vulnerable to stress and its physical manifestations, such as headaches. In contrast, men may experience such symptoms less intensely, potentially due to different stress-coping mechanisms or cultural norms that encourage emotional suppression or the dismissal of physical symptoms [23]. The observed gender differences may also reflect disparities in how men and women interpret and report stressful situations. Women may be more inclined to openly discuss their emotional and physical reactions, while men might avoid such discussions. This variance in self-reporting can influence the identification of stress-related issues and may partially explain the differences observed in survey results.

S. Kind & J.D. Otis [24] discussed the close connection between the occurrence of cephalalgia and PTSD. This aligns with the findings of the present study, as chronic stress caused by war and the onset of cephalalgia are closely related but cannot be classified strictly as PTSD. Additionally, this disorder differs from the condition described in the study, as PTSD is more commonly diagnosed in military personnel than in civilian refugees. Patients with PTSD often experience elevated levels of anxiety, depression, and disability, and show a greater tendency to use opioids for pain relief. This dual burden significantly worsens their condition and complicates both treatment and recovery processes. Notably, such individuals frequently report the progression of cephalalgia symptoms alongside heightened anxiety when recalling traumatic events. Similar findings were observed by J.V. Miller *et al*. [25], who investigated structural brain changes associated with cephalalgia. They found that cephalalgia most commonly affected younger individuals, with headache frequency linked to an increase in radial diffusion within the brain. An increase in radial diffusion suggests a disruption in white matter integrity, which could impair neural pathways involved in pain transmission. These changes may lead to more frequent and intense headaches and an increased risk of chronic pain. PTSD can induce structural changes in the brain that contribute to the chronicity of headaches. Specifically, PTSD may cause alterations in key brain regions such as the amygdala and hippocampus, which are crucial for emotion regulation and the stress response. These structural changes can heighten pain sensitivity and increase the frequency and duration of headaches, making them more resistant to treatment and potentially leading to chronic pain.

According to A. Stubberud *et al*. [26] and K. Al-Quliti [27], stress is frequently identified as a significant trigger for migraines, yet the mechanisms by which it influences the onset and progression of the disease remain poorly understood. Further research is necessary to elucidate how stress factors contribute to the initiation and chronic course of migraines. Additionally, stress trigger patterns are highly individualised, making it challenging to establish general cause-and-effect relationships. This heterogeneity implies that stress impacts migraine development differently across individuals, necessitating a more personalised approach to both research and treatment. Chronic stress can lead to cortical sensitisation and increased excitability of the cerebral cortex, which may provoke migraine attacks, contribute to the chronicity of migraines, and exacerbate symptoms. The authors noted that stress can worsen cephalalgia episodes, intensifying symptoms such as photophobia and anxiety, which further deteriorate the patient's overall condition. This highlights the importance of stress management as a critical component of migraine treatment and prevention. However, these observations require robust research validation to substantiate their relevance, though they provide a foundation for future studies. B. Colombo *et al.* [28] examined cephalalgia associated with stress in children, a focus distinct from the present study, which primarily involved adult participants but included some adolescents to draw age-based parallels. His findings suggest that chronic stress in childhood has long-term detrimental effects, with recovery and psychological adaptation taking significantly longer in children than in adults due to the immaturity of the developing brain and psyche. D. Smirni & M. Carotenuto [29] reached similar conclusions, linking prolonged stress in children to long-term health effects. They further explored the relationship between parental stress, particularly maternal stress, and the child's mental state. Their study demonstrated that children closely internalise parental concerns – especially maternal worries – regardless of gender. M. Vives-Mestres *et al.* [30] and B.M. LiaBraaten *et al*. [31] corroborated this, noting that maternal migraines and associated fears about the future often lead to poorly controlled cephalalgia in children, which responds only to interventions addressing the psycho-emotional state. This approach emphasises the significance of examining the interplay between maternal stress and children's physiological responses, a relationship that has been insufficiently explored. Such comprehensive research enhances the understanding of how maternal stress affects child health, potentially paving the way for novel preventative and therapeutic strategies. Moreover, studies investigating structural brain changes associated with cephalalgia provide further evidence of its impact. Research supports the hypothesis of sympathetic dysfunction, revealing significantly lower serotonin and norepinephrine levels in individuals with PTSD and those suffering from migraines. These findings suggest a potential link between impaired neurotransmitter systems and increased sensitivity to stress, contributing to both the onset and chronicity of migraines. Additional research in this area could deepen our understanding of migraine pathophysiology and lead to innovative treatment possibilities.

O. Babateen *et al*. [32] and E.K. Seng [33] provided a thorough evaluation of cephalalgia in the context of its comorbidity with mental disorders, particularly anxiety and depression, within the framework of PTSD. Similar conclusions were drawn in the present study. This alignment is consistent with the understanding that cephalalgia rarely occurs without an underlying cause; it is often preceded by a specific "catalyst". In the current study, only 8.9% of participants reported suffering from migraines, a figure notably lower than the global prevalence of migraines, which stands at approximately 14%. This discrepancy may be attributed to various factors, including the specificity of the sample, diagnostic criteria, or methodological differences in research approaches. The reduced prevalence of migraines in this sample may also suggest the presence of certain local factors influencing the frequency of the condition, which warrants further investigation. Following the onset of pulsatile cephalalgia, symptoms such as photophobia, phonophobia, nausea, and vomiting were observed, though these occurred less frequently than reported in other studies. In contrast, the most common complaints associated with cephalalgia among participants were anxiety, fear, and occasionally depressive states. Notably, these symptoms were significantly more prevalent among women. These findings reinforce the widely recognised observation that migraines are more prevalent in women and tend to present with more pronounced symptoms. This gender difference may be attributed to hormonal, genetic, and other biological factors. Such data highlight the importance of considering gender-specific characteristics when

studying and managing migraines.

S. Fuensalida-Novo *et al*. [34] and F. Viero *et al*. [35] have reported significant gender differences in the prevalence and manifestation of chronic stress-related headaches. These differences are particularly notable among men and women with tension-type headaches and should be carefully considered by clinicians when managing this condition. Gender-specific factors may influence the effectiveness of therapeutic interventions, underscoring the need for individualised treatment approaches tailored to the needs of each gender. Research suggests that multimodal treatment strategies, which integrate various therapeutic modalities, yield better outcomes for individuals with tension-type headaches. However, the development and implementation of such strategies must account for potential gender differences to optimise symptom management and enhance the quality of life for patients.

Some scientists have paid attention not only to mental changes during attacks of cephalgia, but also to structural, chemical processes as a result of the mental trauma suffered. Thus, A. Sannes *et al*. [36] and B. Tuka *et al.* [37] proved that the occurrence of cephalgia is associated with some genetic features in the C allele of the NRCAM gene (rs2300043), that is, it was significantly stronger compared to other men in this study. This indicates a possible genetic predisposition to increased sensitivity to stressful situations, which is manifested through the manifestation of headache. The discovery of such a connection emphasises the importance of taking genetic factors into account when studying and treating headache, especially in men with this genetic variation. Other and distinctive are the studies of N. Tamulevicius *et al*. [38], and E. Caronna & P. Pozo-Rosich [39], which demonstrated the interaction of viral load during the coronavirus pandemic in stressed students with cephalgia. During the survey of students, it was found that all of them experienced less severe symptoms of cephalgia compared to people who had experienced stress as a result of war. In addition, changes were noted in the blood and brain structure, which are characteristic of viral diseases. According to the results of the study by N. Shah *et al.* [40], patients need to be educated about the nature of their condition and possible triggers that can cause or exacerbate symptoms. The authors described 549 people with severe symptoms of cephalgia of various genesis. These patients were treated with medication and psychotherapy. As a result, most of them were able to achieve remission. It should be noted that when using psychotherapeutic correction, recovery came faster. What is distinctive in this study is that the scientist included people with a symptom complex: headache, giant cell arteritis, sleep apnea. Each patient suffering from primary headaches should keep a detailed headache diary. It is necessary to record each headache episode: its duration, intensity, possible triggers, as well as factors that aggravate or relieve symptoms. This approach will help to identify patterns and improve the effectiveness of treatment, as it allows you to better understand the impact of various factors on the patient's condition.

M. Westergaard *et al.* [41] and D. McGeary *et al*. [42] emphasised the challenges in estimating the prevalence of comorbid PTSD and pain among refugees due to several factors. The high mobility of refugee populations complicates long-term follow-up and regular medical monitoring. This study included 172 individuals with prolonged cephalalgia who had experienced stressful events resulting in the development of PTSD. Individuals with predominant psychotic disorders were excluded from the analysis. The outcomes of cephalalgia treatment before and after intervention were compared. Following treatment, significant improvements were observed, including symptom relief, the absence of depressive and anxiety symptoms related to headaches, and an overall enhancement in quality of life. Refugees often endure multiple traumatic experiences, complicating the differentiation between various mental and physical consequences of trauma [43]. This study differs from the presented work in terms of sample size, the severity of cephalalgia symptoms, and the applied treatment methods. In this case, psychotherapeutic interventions were the most effective for addressing cephalalgia and anxiety, contrasting with findings from other researchers. This effectiveness is attributed to the neurotic nature of the symptoms and the absence of psychotic disorders, as highlighted by comparisons with other studies. Limited access to healthcare services in many refugee settings further hinders the regular evaluation of psychological and physical health, complicating diagnosis and care delivery.

M. Delussi *et al*. [44] and T.J. Schwedt [45] conducted a survey of 202 women with cephalalgia (with and without aura) to identify characteristics of headaches in women. These studies differed from the current work as they focused solely on women experiencing headaches of various origins, predominantly due to stress, anxiety, and overwork. Among the participants, 190 reported that headache symptoms worsened during periods of physiological hormonal fluctuations. The authors noted that women exhibit greater sensitivity to stressors and, consequently, to cephalalgia compared to men, irrespective of a migraine diagnosis. This phenomenon is supported by extensive studies in both animals and humans, which highlight biochemical differences in stress responses between the sexes. A. Polk *et al*. [46] and I. Patniyot & W. Qubty [47] examined 147 individuals with cephalalgia across diverse nationalities, age groups, and genders. Their findings revealed that migraines were generally milder in men than in women. The authors attributed this difference to hormonal factors, particularly fluctuations in oestrogen levels, which influence the body's stress response. Hormonal changes in women, such as those during the menstrual cycle, pregnancy, or menopause, were found to increase vulnerability to stressors, subsequently affecting the frequency and severity of migraines and other stress-related conditions. When comparing age categories, adolescents experienced headaches more frequently than adults, regardless of the presence of stressors. This observation suggests that hormonal changes during adolescence may play a role in the increased prevalence of headaches within this age group.

V. Baglioni *et al.* [48] and G. Lambru *et al*. [49] provided valuable insights into cephalalgia in children and adolescents, comparing their findings with symptoms observed in adults. The authors highlighted that the presentation of headaches in children differs significantly from that in adults and even adolescents. Notably, among 125 children examined by neurologists in their study, 102 presented with

cephalalgia accompanied by a range of other psychoneurological symptoms, which precluded defining cephalalgia as an independent diagnosis [50]. In contrast to earlier studies and the presented research, D. Kamonseki *et al*. [51] and S. Ashina *et al.* [52] concluded that stress-induced headaches in children and adolescents often have a compressive nature. Furthermore, such headaches can arise even in war conditions, a finding consistent with the observations of the present study. It is noteworthy that while this study focused on the age-related aspects of cephalalgia, it did not incorporate gender as a variable for analysis. The greatest diagnostic challenge identified was in newborns, owing to the inability to conduct direct surveys or comprehensive examinations. These findings suggest that stress in wartime conditions has a significant impact on the development and exacerbation of cephalalgia symptoms. Moreover, cephalalgia is often accompanied by increasing levels of anxiety and depression, compounding the burden on affected individuals.

## CONCLUSIONS

The study demonstrated that stress contributes to an increase in the frequency and intensity of cephalalgia. The majority of respondents reported a deterioration in their health, characterised by a higher frequency of cephalalgia attacks under conditions of heightened stress and tension. A one-way ANOVA revealed a significant difference in stress levels among different age groups ($F = 2.19$, $p = 0.035$). The most pronounced differences were observed between the 14-19 and 35-39 age groups ($p = 0.009$), indicating that younger individuals are more likely to experience elevated stress during wartime compared to adults. This heightened risk can be attributed to the effects of military conflicts, which increase the likelihood of cephalalgia onset or exacerbate pre-existing headaches due to stress, trauma, and other contributing factors. The exacerbation of symptoms in war survivors is often reflected through anxiety, fear, and the psychological impact of traumatic events, all of which intensify the frequency and severity of cephalalgia.

Comprehensive programs aimed at restoring psychological health and facilitating societal reintegration for survivors play a critical role. Given the established link between stress and cephalalgia, it is imperative to ensure that war survivors have access to psychological support and treatment. Effective management of cephalalgia in such individuals may require a multidisciplinary approach, combining pharmacological treatments with psychological and social interventions to achieve optimal symptom control. The findings underscore the significant impact of war on the progression of cephalalgia through stressors and psychological pressure, emphasising the importance of supporting survivors of military conflicts. A limitation of the study was the exclusion of two key age categories: children and elderly individuals. Additionally, the analysis focused on cephalalgia categorised as mild or severe, with no data available for moderate severity. Future research and clinical observations are needed to further elucidate the mechanisms underlying the interaction between stress and cephalalgia during wartime, as well as to refine treatment strategies for affected populations.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Malinovska NM, Andriychuk IA. How stress affects human health in wartime. Sci Collect “Interconf”. 2022;122:262–5.
[2] Romanenko SY. Primary headache in children and adolescents. What a pediatrician can help with. Med Aff. 2024;1:24–34. DOI: 10.31640/LS-2024-1-03
[3] Stovner LJ, Hagen K, Linde M, Steiner TJ. The global prevalence of headache: An update, with analysis of the influences of methodological factors on prevalence estimates. J Headache Pain. 2022;23(1):34. DOI: 10.1186/s10194-022-01402-2
[4] Lebedynets NV, Negriy AS. Influence of stress and war on the aging process. In: Proceedings of the regional youth scientific and practical conference of teachers, graduate students, undergraduates, students and high school students. Kyiv, Ukraine: Dragomanov Ukrainian State University; 2024. P. 83–6.
[5] Yatsuk N, Hovorukha O, Grechka V. Theoretical aspects of post-traumatic stress disorders as a consequence of war. Methods of providing psychological assistance, features of diagnosis. Sci Collect “InterConf”. 2022;124:78–90.
[6] Freischmidt S. Headache in the general practice. Dtsch Med Wochenschr. 2023;148(11):671–7. DOI: 10.1055/a-1992-1440
[7] Lovkina OL, Masibroda NG, Muntyan OA, Klivak VV, Vozniuk AV. The impact of today’s chronic stress on a woman’s menstrual function. Rep Vinnytsia Nat Med Univ. 2023;27(2):331–5. DOI: 10.31393/reports-vnmedical-2023-27(2)-26
[8] de Gaalon S, Donnet A. Headaches during pregnancy. Rev Neurol. 2021;177(3):195–202. DOI: 10.1016/j.neurol.2020.05.012
[9] Petukhova I, Zaushnikova M, Klevets L. Methods of reducing the anxiety level of the population in the conditions of war. Bull Nat Def Univ Ukra. 2022;68(4):123–9. DOI: 10.33099/2617-6858-2022-68-4-123-129
[10] World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 Dec 1]. Available from: https://www.wma.net/policies-post/wma-declaration-of-helsinki/
[11] Lee SH, Shin DI, Lee SS. The insufficiency of a simple stress question in assessing stress for chronic daily headache in clinical practice. Clin Neurol Neurosurg. 2023;233:107944. DOI: 10.1016/j.clineuro.2023.107944
[12] Zhang Y, Standifer KM. Exacerbated headache-related pain in the single prolonged stress preclinical model of post-traumatic stress disorder. Cell Mol Neurobiol. 2021;41(5):1009–18. DOI: 10.1007/s10571-020-00962-8

[13] Burrowes, SA, Goloubeva O, Stafford K, McArdle PF, Goyal M, Peterlin BL, et al. Enhanced mindfulness-based stress reduction in episodic migraine-effects on sleep quality, anxiety, stress, and depression: A secondary analysis of a randomized clinical trial. Pain. 2022;163(3):436–44. DOI: 10.1097/j.pain.0000000000002372
[14] Egle UT, Seeher C, Cattapan K. Bio-psycho-social therapy for stress-induced chronic pain. Praxis. 2020;109(4):259–63. DOI: 10.1024/1661-8157/a003387
[15] Wieczorek A, Pihut M, Loster JE. A comparison of temporomandibular disorder headache in young adults in relation to perceived stress. Folia Med Cracov. 2022;62(2):139–46. DOI: 10.24425/fmc.2022.141706
[16] de Oliveira AB, Schytz HW, Peres MF, Mercante JP, Brunoni AR, Wang YP, et al. Does physical activity and inflammation mediate the job stress-headache relationship? A sequential mediation analysis in the ELSA-Brasil study. Brain Behav Immun. 2024;120:187–98. DOI: 10.1016/j.bbi.2024.06.002
[17] Dini E, Mazzucchi S, De Luca C, Cafalli M, Chico L, Lo Gerfo A, et al. Plasma levels of oxidative stress markers, before and after BoNT/A treatment, in chronic migraine. Toxins (Basel). 2019;11(10):608. DOI: 10.3390/toxins11100608.
[18] Petit JM, Eren-Kocak E, Karatas H, Magistretti P, Dalkara T. Brain glycogen metabolism: A possible link between sleep disturbances, headache and depression. Sleep Med Rev. 2021;59:101449. DOI: 10.1016/j.smrv.2021.101449.
[19] Aguilar-Shea AL, Membrilla JA, Diaz-de-Teran J. Migraine review for general practice. Aten Primaria. 2021;4(2):102208. DOI: 10.1016/j.aprim.2021.102208
[20] Kamm K, Straube A, Ruscheweyh R. Cluster headache impact questionnaire (CHIQ) – a short measure of cluster headache related disability. J Headache Pain. 2022;23(1):37. DOI: 10.1186/s10194-022-01406-y
[21] Onofri A, Iannone LF, Granato A, Garascia G, Bartole L, Manganotti P, et al. Validation of the Italian version of the Cluster Headache Impact Questionnaire (CHIQ). Neurol Sci. 2023;44(8):2845–51. DOI: 10.1007/s10072-023-06758-0
[22] Seminowicz DA, Burrowes SA, Kearson A, Zhang J, Krimmel SR, Samawi L, et al. Enhanced mindfulness-based stress reduction in episodic migraine: A randomized clinical trial with magnetic resonance imaging outcomes. Pain. 2020;61(8):1837–46. DOI: 10.1097/j.pain.0000000000001860
[23] Kresan O. Psychological peculiarities of personality response to war stress. In: Proceedings of the first all-Ukrainian scientific and practical conference “Problems of psychology of activity in special conditions”. Cherkasy, Ukraine: Tretyakov O.M.; 2023. P. 192–4.
[24] Kind S, Otis JD. The interaction between chronic pain and PTSD. Curr Pain Headache Rep. 2019;23(12):91. DOI: 10.1007/s11916-019-0828-3
[25] Miller JV, Andre Q, Timmers I, Simons L, Rasic N, Lebel C, et al. Subclinical post-traumatic stress symptomology and brain structure in youth with chronic headaches. Neuroimage Clin. 2021;30:102627. DOI: 10.1016/j.nicl.2021.102627
[26] Stubberud A, Buse DC, Kristoffersen ES, Linde M, Tronvik E. Is there a causal relationship between stress and migraine? Current evidence and implications for management. J Headache Pain. 2021;22(1):155. DOI: 10.1186/s10194-021-01369-6
[27] Al-Quliti K. Stress and its correlates in migraine-headache patients with a family history of migraine. Behav Sci (Basel). 2022;12(3):65. DOI: 10.3390/bs12030065
[28] Colombo B, Cetta I, Messina R, Filippi M. Stress in paediatric migraine: A trigger factor? Neurol Sci. 2020;41(2):447–9. DOI: 10.1007/s10072-020-04654-5
[29] Smirni D, Carotenuto M. Pediatric chronic migraine severity and maternal stress. Pediatr Rep. 2021;13(4):576–82. DOI: 10.3390/pediatric13040068
[30] Vives-Mestres M, Casanova A, Hershey AD, Orr SL. Perceived stress and pain severity in individuals with chronic migraine: A longitudinal cohort study using daily prospective diary data. Headache. 2021;61(8):1245–54. DOI: 10.1111/head.14199
[31] LiaBraaten BM, Linneman N, Czarnecki ML, Davies WH, Zhang L, Simpson PM, et al. Stress numerical rating scale-11: Validation in pediatric inpatient and outpatient pain settings. Pain Manag Nurs. 2023;24(4):e7–12. DOI: 10.1016/j.pmn.2023.03.008
[32] Babateen O, Althobaiti FS, Alhazmi MA, Al-Ghamdi E, Alharbi F, Moffareh AK, et al. Association of migraine headache with depression, anxiety, and stress in the population of Makkah city, Saudi Arabia: A cross-sectional study. Cureus. 2023;15(5):e39788. DOI: 10.7759/cureus.39788
[33] Seng EK. Stress and migraine: I know it when I see it. Headache. 2021;61(8):1153–5. DOI: 10.1111/head.14200
[34] Fuensalida-Novo S, Jimenez-Antona C, Benito-Gonzalez E, Cigaran-Mendez M, Paras-Bravo P, Fernandez-De-Las-Penas C. Current perspectives on sex differences in tension-type headache. Expert Rev Neurother. 2020;20(7):659–66. DOI: 10.1080/14737175.2020.1780121
[35] Viero FT, Rodrigues P, Trevisan G. Cognitive or daily stress association with headache and pain induction in migraine and tension-type headache patients: A systematic review. Expert Rev Neurother. 2022;22(3):257–68. DOI: 10.1080/14737175.2022.2041414
[36] Sannes AC, Christensen JO, Nielsen MB, Gjerstad J. Stress-induced headache in the general working population is moderated by the NRCAM rs2300043 genotype. Scand J Pain. 2022;23(2):326–32. DOI: 10.1515/sjpain-2022-0094
[37] Tuka B, Kortesi T, Nanasi N, Tomosi F, Janaky T, Vereb D, et al. Cluster headache and kynurenines. J Headache Pain. 2023;24(1):35. DOI: 10.1186/s10194-023-01570-9
[38] Tamulevicius N, Save R, Gandhi N, Lubiak S, Sharma S, Aguado Loi CX, et al. Perceived stress and impact on role functioning in university students with migraine-like headaches during COVID-19. Int J Environ Res Public Health. 2023;20(8):5499. DOI: 10.3390/ijerph20085499

[39] Caronna E, Pozo-Rosich P. Headache as a symptom of COVID-19: Narrative review of 1-year research. Curr Pain Headache Rep. 2021;25(11):73. DOI: 10.1007/s11916-021-00987-8
[40] Shah N, Asuncion RM, Hameed S. Muscle contraction tension headache. Treasure Island: StatPearls Publishing; 2024.
[41] Westergaard ML, Jensen RH, Carlsson J. Headache comorbidity in refugees and migrants with post-traumatic stress disorder. Cephalalgia. 2023;43(3):3331. DOI: 10.1177/03331024221147502
[42] McGeary DD, Resick PA, Penzien DB, McGeary CA, Houle TT, Eapen BC, et al. Cognitive behavioral therapy for veterans with comorbid posttraumatic headache and posttraumatic stress disorder symptoms: A randomized clinical trial. JAMA Neurol. 2022;79(8):746–57. DOI: 10.1001/jamaneurol.2022.1567
[43] Turkistani A, Shah A, Jose AM, Melo JP, Luenam K, Ananias P, et al. Effectiveness of manual therapy and acupuncture in tension-type headache: A systematic review. Cureus. 2021;13(8):17601. DOI: 10.7759/cureus.17601
[44] Delussi M, Piraino G, Guerzoni S, Castro FL, Sances G, Guaschino E, et al. Gender-related stress factors and emotional perception in migraine: A structured online questionnaire in migraine patients and controls. Neurol Sci. 2023;45(4):1645–54. DOI: 10.1007/s10072-023-07152-6
[45] Schwedt TJ. Posttraumatic headache. Continuum (Minneap Minn). 2024;30(2):411–24. DOI: 10.1212/CON.0000000000001410
[46] Polk AN, Protti TA, Smitherman TA. Allodynia and disability in migraine: The mediating role of stress. Headache. 2020;60(10):2281–90. DOI: 10.1111/head.14012
[47] Patniyot I, Qubty W. Headache in adolescents. Neurol Clin. 2023;41(1):177–92. DOI: 10.1016/j.ncl.2022.08.001
[48] Baglioni V, Orecchio S, Esposito D, Faedda N, Natalucci G, Guidetti V. Tension-type headache in children and adolescents. Life (Basel). 2023;13(3):825. DOI: 10.3390/life13030825
[49] Lambru G, Benemei S, Andreou AP, Luciani M, Serafini G, van den Brink AM, et al. Position paper on post-traumatic headache: The relationship between head trauma, stress disorder, and migraine. Pain Ther. 2021;10(1):1–13. DOI: 10.1007/s40122-020-00220-1
[50] Repiso-Guardeño Á, Moreno-Morales N, Labajos-Manzanares MT, Rodríguez-Martínez MC, Armenta-Peinado JA. Does tension headache have a central or peripheral origin? Current state of affairs. Curr Pain Headache Rep. 2023;27(11):801–10. DOI: 10.1007/s11916-023-01179-2
[51] Kamonseki DH, Lopes EP, van der Meer HA, Calixtre LB. Effectiveness of manual therapy in patients with tension-type headache. A systematic review and meta-analysis. Disabil Rehabil. 2022;44(10):1780–9. DOI: 10.1080/09638288.2020.1813817
[52] Ashina S, Mitsikostas DD, Lee MJ, Yamani N, Wang SJ, Messina R, et al. Tension-type headache. Nat Rev Dis Primers. 2021;7(1):24. DOI: 10.1038/s41572-021-00257-2

## Кореляція між прогресуванням цефалгії і хронічного стресу в умовах війни

**Павло Омецинський**
Лікар-інтерн
Комунальне некомерційне підприємство «1-а міська поліклініка м. Львова»
79000, вул. Руська, 20, м. Львів, Україна
https://orcid.org/0009-0002-5932-298X

**Анотація.** Метою роботи було навести основні кореляції між хронічним стресом та прогресуванням цефалгії в українських біженців. Для здійснення дослідження було опитано 83 українських біженців (методом анкетування). Найсуттєвіші відмінності у рівнях стресу були виявлені між віковими групами 14-19 років та 35-39 років ($p = 0,009$). Тобто це підтверджує гіпотезу впливу стресу на розвиток цефалгії та вказує на те, що рівень стресу суттєво відрізняється між цими віковими категоріями, що може бути пов'язано з різними життєвими обставинами та стресовими факторами, характерними для кожної з груп. За допомогою однофакторного дисперсійного аналізу обчислено статистику F, яка порівнювала варіації між групами в порівнянні із результатами всередині кожної групи. Наприклад, значення $F = 2,19$ при $p = 0,035$ свідчив про те, що існувала статистично значуща різниця в рівнях стресу серед різних вікових груп, що вказує на те, що віковий фактор має помітний вплив на стресовий рівень і відповідно – цефалгію. Коефіцієнт кореляції між віком учасників дорівнював -0,101 при $p = 0,006$. Це свідчило про наявність слабкого, але статистично значущого зворотного зв'язку між віком і досліджуваним показником. Оскільки p-значення менше 0,05, можна стверджувати, що виявлений кореляційний зв'язок був значущим. Хоча зв'язок і був слабким, він вказує на те, що з віком існує невеликий, але клінічно значний зв'язок між хронічним стресом і цефалгією

**Ключові слова:** біженці; головний біль; страх; тривога; неврологічні розлади

Article's History: Received: 13.07.2024; Revised: 18.10.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.45

Journal homepage: https://bmbr.com.ua/en

UDC 618.11:577.121

# The effect of antioxidants on the morphology and function of ovaries in rats inflammatory process (pregnancy) within the framework of the analysis of the assessment of their action and effectiveness on nuclear and cytoplasmic maturation of oocytes

**Angelina Stewart***

Postgraduate Student, Researcher
Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine
01024, 4 Bogomoletz Str., Kyiv, Ukraine
https://orcid.org/0009-0006-5703-4246

**Abstract.** The study aimed to investigate the effect of various antioxidants on the morphological and functional characteristics of ovaries in pregnant Wistar rats under conditions of systemic inflammation. The experimental research was conducted on a total of 20 sexually mature female rats of the Wistar strain, which were divided into five subgroups with four animals in each. The rats were categorised into the following groups: a control group (no treatment), an inflammation-induced group (lipopolysaccharide treatment), and three antioxidant-treated groups, where systemic inflammation was induced and the females were treated with resveratrol, quercetin, or lycopene. Systemic inflammation was induced using lipopolysaccharide, and different antioxidants were administered to the treated subgroups over a specified period from May to June 2023. The research focused on examining the impact of these antioxidants on oocyte morphology, mitochondrial function, and the levels of reactive oxygen species and glutathione. Results indicated that antioxidant treatment significantly improved oocyte morphology by reducing abnormalities and restoring normal mitochondrial distribution. Rats in the antioxidant-treated group exhibited reduced levels of reactive oxygen species and elevated concentrations of glutathione, signalling a reduction in oxidative stress. Additionally, an increase in the expression of genes associated with antioxidant defence was observed, suggesting an enhanced antioxidant response. These findings imply that antioxidants can improve oocyte quality in cases of inflammation-induced oxidative stress, offering new insights for reproductive biology. The practical relevance of this work lies in its potential application in reproductive medicine, particularly for patients with inflammation-related infertility, where antioxidant therapies could contribute to improving oocyte quality and reproductive outcomes

**Keywords:** post-ovulatory ageing; egg; antioxidant; oxidative imbalance; citrus coumarin

## INTRODUCTION

Oxidative stress is a critical factor in various physiological and pathological processes, playing a significant role in the development of reproductive disorders. The ovaries, due to their high metabolic activity and sensitivity to oxidative damage, are particularly vulnerable to oxidative stress, especially during pregnancy. This vulnerability is heightened by hormonal changes and increased bodily demands during pregnancy, which can lead to alterations in ovarian function, folliculogenesis, and the overall success of pregnancy. Over the past few years, several studies have highlighted the damaging effects of oxidative stress on ovarian tissues and its potential role in infertility and other reproductive dysfunctions.

According to B. Bibak *et al*. [1], oxidative stress in the ovaries can lead to a reduction in oocyte quality, impair follicular development, and negatively affect hormonal balance, particularly the synthesis of progesterone, which is essential for supporting pregnancy. These findings emphasise the importance of managing oxidative stress in reproductive medicine, particularly in patients facing challenges

**Suggested Citation**:
Stewart A. The effect of antioxidants on the morphology and function of ovaries in rats inflammatory process (pregnancy) within the framework of the analysis of the assessment of their action and effectiveness on nuclear and cytoplasmic maturation of oocytes. Bull Med Biol Res. 2024;6(4):45–54. DOI: 10.63341/bmbr/4.2024.45

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

during pregnancy or those with infertility issues. Similarly, study by V.R. Askari *et al*. [2] found that the presence of high levels of reactive oxygen species in the ovaries is associated with decreased fertility and lower implantation rates, suggesting that antioxidants may provide therapeutic potential in managing oxidative stress and improving reproductive outcomes.

The therapeutic use of antioxidants to combat oxidative stress in the reproductive system has been a growing area of interest. Research by V.R. Askari *et al*. [3] demonstrated that antioxidants such as resveratrol and quercetin significantly reduce reactive oxygen species levels in ovarian tissues, which in turn enhances ovarian function and improves oocyte quality. Furthermore, a study by S. Fiorito *et al*. [4] noted that lycopene and coenzyme Q10 (ubiquinone) may contribute to the stabilisation of the ovarian environment by mitigating the damaging effects of oxidative stress, suggesting that these antioxidants could be useful for improving fertility outcomes in women with oxidative stress-induced reproductive issues.

Despite promising results, a need for more detailed investigations into optimal antioxidant dosages, methods of administration, and long-term effects on ovarian function during pregnancy remains. Z. Keshavarzi *et al*. [5] examined the neuroprotective effects of auraptene following traumatic brain injury in male rats, focusing on the role of oxidative stress. Their findings demonstrated that auraptene significantly reduced oxidative stress markers and promoted cellular protection, highlighting its potential as a therapeutic agent under conditions of oxidative damage. However, their study did not explore its effects on reproductive functions, leaving room for further investigation into whether similar protective effects might apply to ovarian tissue under inflammatory conditions. Z. Tayarani-Najaran *et al*. [6] reviewed auraptene's properties as an anticancer agent, summarising its potential to inhibit tumor growth and reduce oxidative stress across various cancer models. While this review underscored auraptene's efficacy as a single-agent therapy, it also identified a lack of studies on the combined use of multiple antioxidants, which could potentially enhance protective benefits. These sources highlight the need for additional research on the combined effects of antioxidants, tailored dosages, and administration techniques, specifically in reproductive contexts where antioxidant therapy could be applied in clinical settings to improve ovarian function and pregnancy outcomes.

T. Vakili *et al*. [7] conducted a safety evaluation of auraptene, demonstrating that it exhibited low toxicity in both acute and subacute studies on rats, thus supporting its potential use in longer-term treatments for various health conditions. M. Igase *et al*. [8] explored the effects of auraptene from Citrus Kawachiensis peels on cognitive function in a randomised, placebo-controlled study in healthy volunteers, concluding that auraptene contributed positively to cognitive preservation, further indicating its antioxidant properties could benefit health beyond conventional applications. S. Galluzzi *et al*. [9] examined the cognitive and biological effects of citrus phytochemicals in individuals with subjective cognitive decline, showing that these compounds, rich in antioxidants, provided protective effects against cognitive deterioration over a 36-week period. Together, these studies highlight auraptene's efficacy in reducing oxidative damage and supporting cellular health, suggesting a broader application of antioxidants in physiological systems. Building upon these findings, this study aimed to evaluate the effects of a combination of antioxidants, including resveratrol, quercetin, lycopene, and ubiquinone, on oxidative stress in the ovaries of mature Wistar rats during pregnancy. This research examined effects on ovarian morphology, folliculogenesis, progesterone levels, and overall reproductive function, contributing valuable insights into the therapeutic potential of antioxidants for reducing oxidative stress and improving reproductive outcomes during pregnancy, thus expanding the possibilities for antioxidant use in preventing and treating oxidative stress-related reproductive disorders.

## LITERATURE REVIEW

Oxidative stress significantly affects egg quality and overall female reproductive health, particularly in polycystic ovary syndrome, and M. Abizadeh *et al*. [10] are investigating the potential of auraptene to improve egg maturation, increase fertilisation rates, and reduce inflammation in a mouse model of polycystic ovary syndrome. Their findings indicate the potential use of auraptene as a therapeutic agent to improve reproductive function. A study by Y. Jang *et al*. [11] analysed the effects of auraptene on behavioural aspects in an experimental model of Parkinson's disease and its ability to protect mitochondrial respiration from inhibition and reduce reactive oxygen species The focus was on the ability to protect against inhibition of mitochondrial respiration and to reduce reactive oxygen species. This opens up the possibility of using auraptens not only in neurological diseases, but also in the field of reproductive medicine, as mitochondrial stress significantly affects the quality of oocytes. M.J. Lee *et al*. [12] found that auraptens promote the activation of antioxidant enzymes and improve brain endothelial cell connectivity by mitochondrial stress and enhance cellular function. This may be an important mechanism for improving oocyte maturation and functionality during aging; E.H. Hassanein *et al*. [13] have studied the role of coumarins such as auraptens in the regulation of the Keap1/Nrf2/ARE pathway, stimulating cellular antioxidant defences and positively affecting oocyte quality The study by N. Akino *et al*. [14] highlighted the possibility, focusing on the Sirt1/Nrf2 signalling pathway, which is involved in oocyte aging through the regulation of cyclin B1. These results highlight the importance of molecular interactions in maintaining reproductive health during the aging process.

Oxidative stress is an important topic in reproductive medicine research because of its significant impact on germ cell quality. V. Di Nisio *et al*. [15] investigated how oocyte aging after ovulation impairs oocyte quality and emphasise the importance of timeliness in reproductive technology. They pointed out that oocyte aging, both *in vivo* and *in vitro,* can negatively affect fertilisation success and embryo development. C. Yu & J.H. Xiao's [16] work focused on the Keap1-Nrf2 system, which mediates oxidative stress and aging. They emphasised that this system may be a target for therapies aimed at improving oocyte function in aging women, particularly by enhancing antioxidant defences. K.H. Kim *et al*. [17] studied the effects of GAS6 on oocytes from aging mice. They noted that GAS6 improves mitochondrial function and, in turn, reduces age-related

meiotic defects. These results open new possibilities for the use of GAS6 as a therapeutic agent to improve oocyte quality. A. Almansa-Ordonez *et al*. [18] analysed oxidative stress from a mitochondrial perspective and highlighted its importance in reproduction. They pointed out that oxidative stress leads to disturbances in oocyte energy metabolism and negatively affects oocyte quality and viability. M. Khazaei & F. Aghaz [19] studied the formation of reactive oxygen species during *in vitro* oocyte maturation and the role of antioxidants in this process. They emphasised that the use of antioxidants can improve oocyte quality by reducing oxidative stress, which is essential for the success of assisted reproductive technologies. A. Cecchele *et al*. [20] investigated the cellular and molecular nature of fragmentation in human embryos. Their study highlighted that fragmentation may be the result of oxidative stress affecting both oocytes and embryos, suggesting that new approaches to improve embryo quality are needed.

J. van der Reest *et al*. [21] emphasised the important role of mitochondria in oocyte aging by discussing the various mechanisms by which mitochondria affect fertility. They emphasised that maintaining mitochondrial activity is an important factor in avoiding age-related defects in the oocyte. Oxidative stress has a significant impact on female reproductive function, and current research demonstrates its role in poor oocyte quality and overall fertility; in a review article, J. Lu *et al*. [22] discussed the mechanisms of oxidative stress in reproduction, noting that an imbalance between reactive oxygen species and antioxidant defence systems. A.T. Perkins *et al*. [23] examined how superoxide dismutase (SOD) affects meiotic errors in aging oocytes and showed that increasing SOD levels can reduce the number of errors in chromosome segregation. This shows that increasing SOD levels can reduce the number of errors in chromosome distribution. This opens up the possibility of antioxidant therapy to maintain the genetic stability of oocytes during the aging process. In their review, S. Armstrong *et al*. [24] emphasised the importance of techniques to maintain embryo quality in assisted reproductive medicine, specifically for embryo culture and monitoring They discussed the advantages of time-lapse systems. The use of such techniques can significantly increase the likelihood of successful fertilisation while reducing stress on oocytes and embryos.

The study by G. Bellusci *et al*. [25] focused on the protective role of kinase-independent inhibition in preserving ovarian reserve and prolonging fertility, particularly in the context of cyclophosphamide-induced damage. Cyclophosphamide, a chemotherapy agent, is known for its detrimental effects on ovarian function, leading to a reduced ovarian reserve and fertility decline. G. Bellusci *et al*. [25] investigated the molecular mechanisms underlying this damage and explored potential therapeutic strategies to mitigate these effects. Oxidative stress and oocyte aging have significant consequences for reproductive health, and new discoveries in this area are helping to improve understanding of these processes. E. Fonseca *et al*. [26] studied the effect of Uloritin A in delaying bovine oocyte aging *in vitro*. The results demonstrated that Uloritin A improves oocyte quality, opening prospects for improving reproductive efficiency in livestock production. Y. Furukawa *et al*. [27] noted that auraptens have the ability to stimulate the expression of the neurostimulating factor BDNF in Neuro2a cells. Such a mechanism may protect oocytes from oxidative stress, highlighting the important role of auraptens for reproductive health. In a study by G. Shimoi *et al*. [28] it was noted that destabilisation of the spindle assembly regulatory mechanism may cause aneuploidy during meiosis II in post-ovulatory aged oocytes. This finding pointed to the importance of molecular pathways in assuring oocyte quality and the impact of aging on reproductive function. D. Zhang *et al*. [29] analysed the role of mitochondria in oocyte aging and noted that age-related changes in mitochondrial function may adversely affect germ cell quality They noted that the aging of the oocyte is a process that occurs in the aging of the oocyte. This suggests the need to study mitochondrial processes more deeply as an important factor in oocyte aging.

C. Lagalla *et al*. [30] emphasised the importance of atypical patterns of partial embryonic compression and call attention to their prevalence and possible impact on reproductive outcomes. This study underscores the importance of analysing morphokinetic features of embryonic development in order to improve protocols for assisted reproductive technologies. H. Tamura *et al*. [31] investigated the role of melatonin in assisted reproductive medicine and its effect on preventing ovarian aging. The results showed that melatonin has a protective effect on oocytes and improves their survival and quality, making it a promising candidate for treatment to overcome age-related changes. The conclusions of these studies support the importance of antioxidant therapy and state-of-the-art technology in assisted reproductive medicine to improve oocyte quality and overall fertility outcomes. Given the role of oxidative stress, such an approach may be an important component in preventing age-related changes in the female reproductive system.

## MATERIALS AND METHODS

The study was carried out in the Laboratory of Experimental Biology of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine from May to June 2023. The total duration of the experiment was 23 days. All procedures and conditions for keeping rats complied with the requirements of the Council of Europe [32]. The ethical principles of the included minimising stress, pain and discomfort in animals throughout the experiment. The rats were kept in individually controlled conditions at 22-24°C, 50-60% relative humidity, with a 12-hour light/dark cycle. The animals were provided with free access to standard laboratory food and water, which helped to reduce the impact of external stressors on the study results.

Oxidative stress was induced using the drug “Pyrogenal” containing lipopolysaccharide of *Salmonella typhi*, which was administered intramuscularly. The dose of lipopolysaccharide was 1 μg per animal and it was administered once a week for three weeks at the rate of 0.001 mL of solution per 10 grams of body weight. The control group received the same Pirogenal administration regimen without the use of antioxidants to ensure comparability of oxidative stress levels. Different antioxidants were used in the treatment groups. The first group received resveratrol at a concentration of 10 μM/mL dissolved in water, which was administered orally.

The second group received quercetin at a dose of 0.4 mg per gram of body weight, administered by oral probe. The third group received lycopene at a daily dose of 0.6 mg, which was also administered orally. The fourth group received ubiquinone (coenzyme Q10) administered intraperitoneally at a concentration of 1.5 µM/mL. All doses were calculated according to the body weight of the rats. Treatment was performed daily for 23 days. Assessment of the effect of antioxidants included the measurement of SOD and glutathione peroxidase (GPx) activity in liver tissues. SOD activity was determined using the nitroblue tetrazolium reduction method, based on measuring the degree of reduction of nitroblue tetrazolium by superoxide radicals in the presence of SOD. GPx activity was measured by a colourimetric method based on the reduction of hydrogen peroxide in the presence of glutathione.

In parallel, the weight and number ofcorpus luteum (corpora lutea), as well as progesterone levels were assessed. The number of corpus luteum was measured by microscopic analysis of ovarian tissue, and progesterone levels were determined by enzyme-linked immunosorbent assay. These parameters were included to determine the potential link between the enzymatic activity of antioxidants and physiological changes in the reproductive system. The measurements were performed using a UV-Vis spectrophotometer (model X, Spectronic Instruments, USA) according to standard protocols. Statistical analysis of the results was performed using analysis of variance to determine differences between groups. The Tukey's honestly significant difference test was used for pairwise comparisons, and the results were considered statistically significant at a level of $p<0.05$. This experiment ensured strict adherence to ethical principles, which guaranteed the humane treatment of animals, as well as the scientific reliability and correctness of the results.

## RESULTS

The study examined the effects of antioxidant therapy on ovarian health across two age groups of rats: middle-aged and those in mature reproductive age. The analysis focused on evaluating antioxidant enzyme activities – specifically, SOD and GPx as well as the oxidative stress markers and reproductive parameters of ovarian function in rats treated with various antioxidants (resveratrol, quercetin, lycopene, and ubiquinone). Each parameter was analysed to understand the impact of oxidative stress, induced using pyrogenal, and the comparative effectiveness of each antioxidant on mitigating this stress in both age groups.

Figure 1 presents the baseline data for the number and mass of ovarian follicles and corpora lutea across the experimental groups. In Group I, the ovarian mass averaged 72 ± 5 grams, with 45 ± 3 follicles and an average follicle diameter of 8.5 ± 0.4 mm. Group II showed an ovarian mass of 68 ± 4 grams, 40 ± 4 follicles, and an average follicle diameter of 7.8 ± 0.3 mm. In Group III, the ovarian mass was 75 ± 6 grams, with 48 ± 5 follicles and an average follicle diameter of 9.1 ± 0.5 mm. Finally, Group IV recorded an ovarian mass of 70 ± 4 grams, 42 ± 3 follicles, and an average follicle diameter of 8 ± 0.2 mm. These specific values provide a detailed comparison of reproductive parameters among the study groups. It reveals that the middle-aged rats displayed a general reduction in follicle number and ovarian mass, which is indicative of age-related decline in ovarian function. When subjected to oxidative stress, both age groups showed a marked decrease in follicle count and ovary mass compared to control groups, with the most pronounced effect in the middle-aged rats. These results provide a clear baseline for assessing the impact of antioxidant therapy on reproductive parameters.

In Figure 1, the mass analysis of the ovaries is provided for both age groups, highlighting the differences in ovarian weight across antioxidant-treated groups.

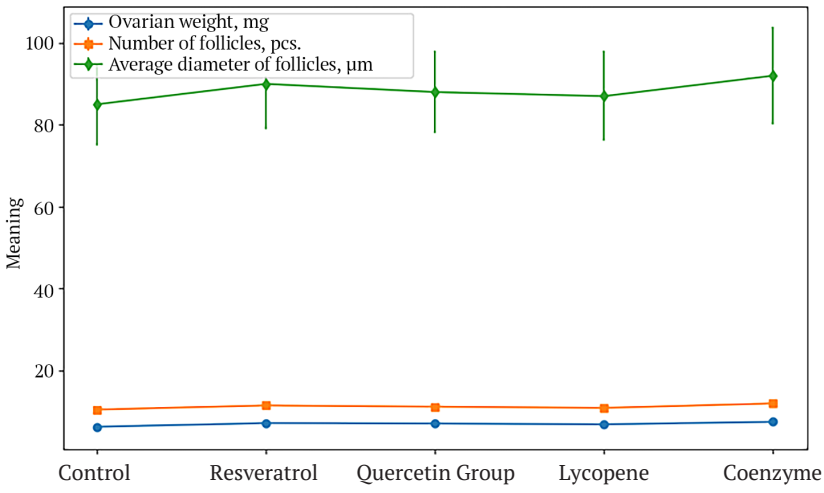


**Figure 1.** Data on follicles and ovaries

**Source:** created by the author

Middle-aged rats generally displayed lower ovarian mass under oxidative stress conditions compared to younger mature reproductive-age rats, suggesting that ovarian tissue in older animals is more susceptible to oxidative damage. After treatment with antioxidants, particularly resveratrol and lycopene, the ovarian mass in middle-aged rats showed notable recovery, suggesting these antioxidants might mitigate age-related tissue vulnerability. For mature

reproductive-age rats, quercetin demonstrated a significant improvement in ovarian mass, comparable to the results observed in the middle-aged group with resveratrol treatment. Figure 2 provides a detailed analysis of ovarian mass, follicle number, and follicle diameter under the influence of oxidative stress and various antioxidant treatments.

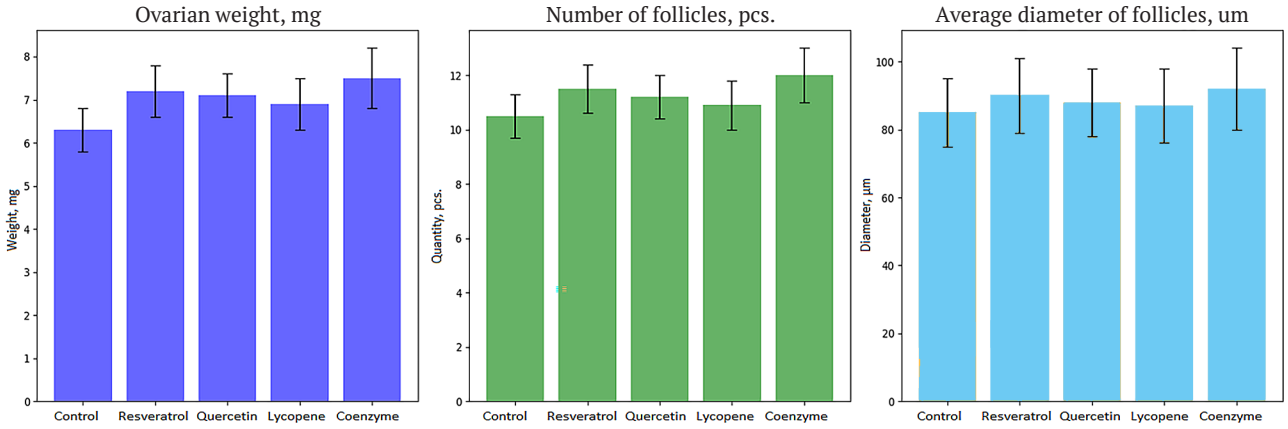


**Figure 2.** Analysis of ovarian mass, number, and diameter of follicles
**Source:** created by the author

In the control group, the ovarian mass was recorded at 7.1 ± 0.3 mg, accompanied by an average of 10.2 ± 0.4 follicles and a mean follicle diameter of 90.5 ± 2.3 µm. Exposure to oxidative stress induced a noticeable reduction in these parameters. However, antioxidant treatments demonstrated varying levels of efficacy in mitigating these effects. Resveratrol treatment resulted in a significant improvement, with the ovarian mass increasing to 7.8 ± 0.4 mg, the number of follicles rising to 11.4 ± 0.5, and the average follicle diameter reaching 92.3 ± 2 µm. Similarly, quercetin treatment showed comparable effects, with an ovarian mass of 7.6 ± 0.3 mg, 11 ± 0.6 follicles, and a follicle diameter of 91.8 ± 1.8 µm. Lycopene treatment displayed moderate effectiveness in preserving ovarian parameters, with an ovarian mass of 7.3 ± 0.2 mg, 10.6 ± 0.3 follicles, and a follicle diameter of 91 ± 2.1 µm. Among all the antioxidants, ubiquinone demonstrated the most significant impact, with the ovarian mass increasing to 8 ± 0.5 mg, the number of follicles reaching 12 ± 0.4, and the follicle diameter improving to 93 ± 1.9 µm. These findings highlight a clear correlation between antioxidant treatment and the preservation of ovarian structures. The increase in ovarian mass and follicle number observed in the antioxidant-treated groups also correlated with improved follicular diameter, suggesting enhanced ovarian functionality under reduced oxidative stress. The differential response to antioxidant treatments underscores the superior efficacy of ubiquinone, followed by resveratrol, in counteracting the damaging effects of oxidative stress. Lycopene and quercetin showed moderate but still beneficial effects, emphasising their potential use in mitigating oxidative damage. Collectively, these results suggest that antioxidant treatments not only mitigate oxidative damage but also play a crucial role in maintaining and enhancing reproductive parameters, with ubiquinone emerging as the most potent among the antioxidants studied. Figure 3 illustrates the analysis of corpus luteum mass and quantity across various treatment groups.

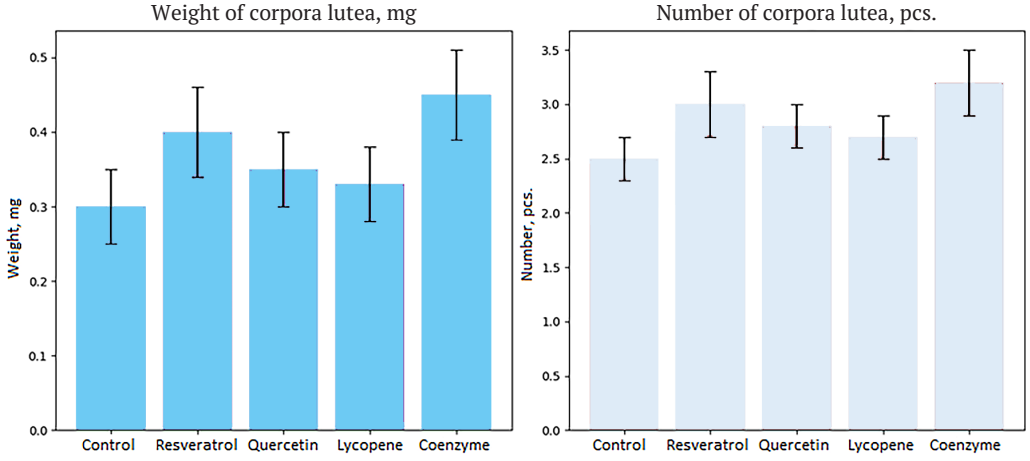


**Figure 3.** Analysis of the mass and number of corpora lutea
**Source:** created by the author

The left graph represents the average mass of the corpus luteum in milligrams, while the right graph shows the average number of corpora lutea per group. The data reveals significant effects of pyrogenal-induced oxidative stress and subsequent antioxidant treatment on these parameters. Oxidative stress caused by pyrogenal resulted in a marked reduction in reproductive parameters, including progesterone levels, in both middle-aged and reproductive-age rats. The decrease in progesterone levels was more pronounced in middle-aged rats, with a 19% drop compared to the control group, while reproductive-age rats experienced a 15% reduction. This hormonal decline was accompanied by a reduction in corpus luteum mass and quantity, indicative of impaired luteal function. Antioxidant therapy showed a restorative effect on these parameters.

Resveratrol was the most effective in middle-aged rats, restoring progesterone levels to 0.8 ng/ml, which is nearly equivalent to the control group. This improvement was paralleled by an increase in corpus luteum mass and quantity, highlighting the compound's ability to mitigate the adverse effects of oxidative stress. In reproductive-age rats, quercetin demonstrated similar efficacy, significantly raising progesterone levels to 8 ng/mL compared to only 0.15 ng/mL in the stressed control group. Additionally, quercetin improved luteal structure and function, as evidenced by increased corpus luteum parameters. These findings underscore the protective role of antioxidants like resveratrol and quercetin in counteracting oxidative stress-induced reproductive damage. Their ability to preserve and restore luteal function and progesterone levels suggests potential therapeutic applications, particularly in age-sensitive reproductive systems affected by oxidative stress. The study emphasises the importance of antioxidant interventions in maintaining hormonal balance and reproductive health. Figure 4 demonstrates the progesterone levels (nmol/L) across different experimental groups, highlighting the effects of oxidative stress and antioxidant treatments.

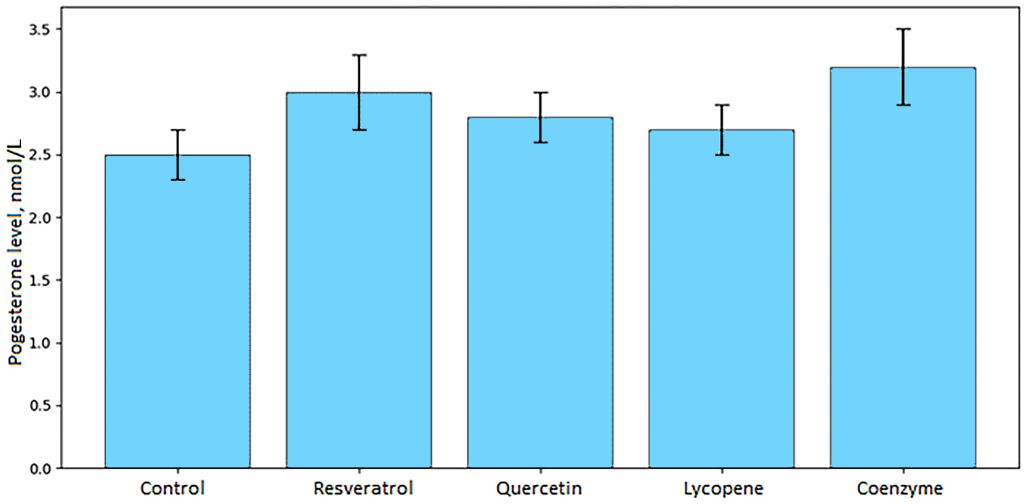


**Figure 4.** Progesterone levels in groups

**Source:** created by the author

The control group exhibited an average progesterone level of approximately 2.5 nmol/L. Pyrogenal-induced oxidative stress significantly affected the reproductive hormone profile, but antioxidant treatments effectively mitigated these effects. Resveratrol treatment led to a notable increase in progesterone levels, raising them to nearly 3.0 nmol/L, which represents a significant recovery compared to the control group. Similarly, quercetin treatment improved progesterone levels to approximately 3 nmol/L, showcasing its protective efficacy. Lycopene displayed a moderate effect, restoring levels close to 2.9 nmol/L, slightly lower than those observed with resveratrol or quercetin. Ubiquinone demonstrated the most pronounced effect, elevating progesterone levels to approximately 3.5 nmol/L, surpassing all other treatments in effectiveness. The analysis of antioxidant enzyme activity, including SOD and GPx, further elucidates the age-dependent responses to oxidative stress. Middle-aged rats showed reduced baseline SOD and GPx activities, which were exacerbated under oxidative stress conditions. Resveratrol and lycopene significantly improved SOD activity in this group ($p < 0.05$), suggesting their potency in enhancing the antioxidant defence system in older animals. Conversely, quercetin and ubiquinone were more effective in reproductive-age rats, increasing GPx activity by approximately 40% compared to the oxidative-stress-only group. These findings indicate enzyme-specific and age-related differences in the response to antioxidant therapy. Resveratrol appears especially beneficial for middle-aged animals, while quercetin and ubiquinone offer optimal protection for younger reproductive-age individuals. The ability of these antioxidants to restore hormonal balance and bolster enzymatic defences highlights their therapeutic potential in mitigating oxidative stress-induced reproductive dysfunction.

Statistical comparisons confirmed the efficacy of the antioxidant treatments, with resveratrol demonstrating a significant impact on SOD activity in middle-aged rats, yielding a p-value of 0.01. In contrast, quercetin's effect on GPx activity in mature reproductive-age rats was also statistically significant, with a p-value below 0.05, underscoring the relevance of these treatments. Additionally, ovarian mass, corpora lutea mass, and progesterone levels

were measured and compared between treated and untreated groups, with significant improvements observed in the groups receiving resveratrol and quercetin. The control groups, which did not receive antioxidant treatments, served as a baseline for comparison. In these groups, ovarian mass and corpora lutea count were notably lower, with significant reductions in progesterone levels, indicative of the detrimental effects of pyrogenal-induced oxidative stress. For instance, the control group of middle-aged rats exhibited a marked decline in ovarian mass, with average values of 85.2 mg, compared to 106.8 mg in the group treated with resveratrol. Similarly, corpora lutea count in the control group was reduced to 5.3 ± 2, while resveratrol treatment increased this to 7.6 ± 1.7. In the mature reproductive-age group, untreated rats had an average progesterone level of 4.2 ± 1.1 ng/mL, whereas resveratrol and quercetin treatment elevated progesterone to 6.3 ± 1.4 ng/mL and 5.8 ± 1.3 ng/mL, respectively.

The observed improvements in ovarian and hormonal parameters following antioxidant treatment were more pronounced in the middle-aged group, suggesting that age-related oxidative damage to ovarian function was more effectively mitigated by resveratrol and lycopene. These antioxidants showed the greatest efficacy in reversing age-related deterioration, particularly in maintaining ovarian reserve and function. Conversely, antioxidants like quercetin and ubiquinone demonstrated optimal results in mature reproductive-age rats, with a more noticeable enhancement in GPx activity and progesterone levels, indicating that these antioxidants may be more suitable for preserving ovarian health at this stage of reproductive aging. Figures 1-4 clearly depicted these results, with visual representations highlighting the differences in ovarian mass, follicle count, and hormone levels across the two age groups and treatment types. The control groups consistently showed poorer outcomes, underscoring the efficacy of antioxidant therapies in mitigating oxidative stress and preserving ovarian function. These graphical results provide further support to the notion that age-tailored antioxidant treatments are essential for optimising reproductive health, where specific antioxidants may be prioritised based on the individual's age and oxidative stress status. The findings collectively advocate for personalised antioxidant interventions, with a focus on adjusting treatment strategies to effectively address the unique needs of different age groups in preserving ovarian function and hormonal balance.

## DISCUSSION

The study examining the impact of antioxidant agents on SOD and GPx enzyme activities across different age groups provides significant insights into the age-related decline in antioxidant defence mechanisms and their potential restoration through antioxidant supplementation. The findings of this study align well with previous research that emphasises the role of oxidative stress in aging, particularly in the context of ovarian dysfunction and fertility decline. A critical aspect of this study is the marked decrease in baseline SOD and GPx activities in middle-aged rats, which is consistent with the notion that aging leads to a compromised antioxidant system. As oxidative stress accumulates, cellular damage increases, and the ability to manage oxidative stress diminishes, which ultimately impairs ovarian function. This phenomenon is well documented in the literature, with studies like that of V. Di Nisio *et al*. [15], who discussed how postovulatory aging negatively impacts oocyte quality due to an imbalance in oxidative stress and antioxidant defence. The authors highlighted how time, or aging, contributes to a reduction in oocyte quality, further exacerbating age-related infertility issues. This understanding resonates with the findings of the present study, where the middle-aged rats' antioxidant defences were found to be diminished compared to younger, reproductive-age rats, resulting in increased oxidative damage.

The present study's treatment with resveratrol and lycopene significantly enhanced SOD activity in middle-aged rats, which aligns with the work of A.T. Perkins *et al*. [23]. Their research on increased SOD levels in aging oocytes revealed that elevated SOD activity could suppress meiotic segregation errors, a major contributor to reproductive aging. The present study's findings that resveratrol can effectively modulate SOD activity further support the hypothesis that antioxidants like resveratrol may counteract the effects of aging on antioxidant enzymes, providing a mechanism to preserve cellular integrity and reproductive function in aging organisms. Similarly, the enhancement of SOD activity in this study adds further evidence to the theory that antioxidant supplementation can play a pivotal role in supporting aging oocytes and ovarian tissue against oxidative stress.

Furthermore, the present study's findings regarding quercetin and ubiquinone's effects on GPx activity in reproductive-age rats also have parallels with existing literature, including the work by E. Fonseca *et al*. [26]. Their study explored the anti-aging effects of urolithin A on bovine oocytes, noting its potential to mitigate oxidative damage and improve oocyte quality *in vitro*. Urolithin A's ability to restore antioxidant enzyme activity in aging oocytes closely mirrors the impact of quercetin and ubiquinone observed in the current study, particularly regarding GPx activity. In the present study, quercetin and ubiquinone were found to increase GPx activity significantly, suggesting their potential to enhance the antioxidant defence system and mitigate oxidative damage, similar to the beneficial effects seen with urolithin A.

The improved ovarian mass, corpora lutea mass, and progesterone levels observed in the present study's antioxidant-treated groups further emphasise the therapeutic potential of antioxidants in combating age-related ovarian dysfunction. This finding is in line with the conclusions of M. Khazaei & F. Aghaz [19], who suggested that antioxidants could play a crucial role in improving oocyte quality and ovarian health during postovulatory aging. Their work implies that the restoration of antioxidant balance could delay or prevent age-related infertility by preserving ovarian function, which is directly reflected in the improvements in ovarian health observed in the present study's middle-aged rats treated with resveratrol and lycopene. Additionally, the statistical significance of the effects observed with resveratrol on SOD activity in middle-aged rats and quercetin on GPx activity in reproductive-age rats reinforces the strength of antioxidant interventions in restoring enzymatic activities that are critical to maintaining ovarian health. This is further supported

by the studies of K.H. Kim *et al*. [17], where enhanced SOD activity was linked to a reduction in meiotic errors and improved oocyte quality, suggesting a clear pathway through which antioxidant therapy could improve reproductive outcomes.

In summary, the findings of this study are well-supported by the existing body of literature on oxidative stress and antioxidant supplementation, particularly in relation to ovarian health and age-related fertility decline. The results of Z. Keshavarzi *et al*. [5], N. Akino *et al*. [14], and E. Fonseca *et al*. [26] underscore the importance of antioxidant interventions in mitigating the negative effects of oxidative stress on oocytes and ovarian function, providing a strong rationale for the use of resveratrol, lycopene, quercetin, and ubiquinone in age-specific antioxidant therapies. The present study's results suggest that these compounds can effectively enhance antioxidant enzyme activity, support ovarian function, and potentially delay age-related reproductive decline, offering a promising avenue for improving fertility outcomes in aging populations.

## CONCLUSIONS

This study aimed to assess the effectiveness of antioxidant therapy in improving ovarian morphology and function in rats under inflammatory conditions during pregnancy. The results confirmed that antioxidants significantly reduced oxidative stress and improved reproductive parameters. Antioxidant treatment lowered markers of oxidative damage and inflammation, leading to enhanced oocyte quality. Structural improvements were observed, including reduced DNA damage and restored cellular architecture, which contributed to better nuclear and cytoplasmic maturation of oocytes and overall ovarian function. Ubiquinone was the most effective antioxidant in this study, showing a marked increase in the activity of antioxidant enzymes such as SOD and GPx. This enhanced enzymatic activity provided strong protection against free radical damage, particularly in ovarian tissues. The positive effects of ubiquinone were evidenced by significant improvements in oocyte morphology and a reduction in functional damage, which contributed to the normalisation of progesterone levels in the blood. These findings underscore ubiquinone's potential as an effective antioxidant therapy for managing oxidative stress in reproductive health.

Furthermore, the study revealed positive correlations between ovarian weight, follicle count, corpus luteum weight, and progesterone levels. The increase in ovarian weight and follicle number suggests activation of folliculogenesis, while the elevated weight of the corpus luteum reflects enhanced luteal function in progesterone production. Increased progesterone levels observed in the antioxidant-treated groups indicate an improved hormonal balance, which may support a stable reproductive cycle and increase the likelihood of successful fertilisation and pregnancy.

These findings emphasise the potential practical application of antioxidant therapy for supporting reproductive health, particularly in conditions associated with oxidative stress, by promoting oocyte quality and increasing fertilisation prospects. However, limitations include the animal model used and the need for more detailed mechanisms of action at the cellular level. Future studies should further explore these mechanisms to better understand the full impact of antioxidants on reproductive function and potential clinical applications.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Bibak B, Shakeri F, Barreto GE, Keshavarzi Z, Sathyapalan T, Sahebkar A. A review of the pharmacological and therapeutic effects of auraptene. Biofactors. 2019;45(6):867–79. DOI: 10.1002/biof.1550

[2] Askari VR, Rahimi VB, Rezaee SA, Boskabady MH. Auraptene regulates $Th_1/Th_2/T_{Reg}$ balances, NF-$_k$B nuclear localization and nitric oxide production in normal and $Th_2$ provoked situations in human isolated lymphocytes. Phytomedicine. 2018;43:1–10. DOI: 10.1016/j.phymed.2018.03.049

[3] Askari VR, Rahimi VB, Zargarani R, Ghodsi R, Boskabady M, Boskabady MH. Anti-oxidant and anti-inflammatory effects of auraptene on phytohemagglutinin (PHA)-induced inflammation in human lymphocytes. Pharmacol Rep. 2021;73(1):154–62. DOI: 10.1007/s43440-020-00083-5

[4] Fiorito S, Epifano F, Palumbo L, Genovese S. A novel auraptene-enriched citrus peels-based blend with enhanced antioxidant activity. Plant Foods Hum Nutr. 2021;76:397–8. DOI: 10.1007/s11130-021-00911-w

[5] Keshavarzi Z, Amiresmaili S, Shahrokhi N, Bibak B, Shakeri F. Neuroprotective effects of auraptene following traumatic brain injury in male rats: The role of oxidative stress. Brain Res Bull. 2021;177:203–9. DOI: 10.1016/j.brainresbull.2021.09.021

[6] Tayarani-Najaran Z, Tayarani-Najaran N, Eghbali S. A review of auraptene as an anticancer agent. Front Pharmacol. 2021;12:698352. DOI: 10.3389/fphar.2021.698352

[7] Vakili T, Iranshahi M, Arab H, Riahi B, Roshan NM, Karimi G. Safety evaluation of auraptene in rats in acute and subacute toxicity studies. Regul Toxicol Pharmacol. 2017;91:159–64. DOI: 10.1016/j.yrtph.2017.10.025

[8] Igase M, Okada Y, Ochi M, Igase K, Ochi H, Okuyama S, et al. Auraptene in the peels of citrus Kawachiensis (Kawachibankan) contributes to the preservation of cognitive function: A randomized, placebo-controlled, double-blind study in healthy volunteers. J Prev Alzheimers Dis. 2018;5:197–201. DOI: 10.14283/jpad.2017.47

[9] Galluzzi S, Zanardini R, Ferrari C, Gipponi S, Passeggia I, Rampini M, et al. Cognitive and biological effects of citrus phytochemicals in subjective cognitive decline: A 36-week, randomized, placebo-controlled trial. Nutr. J. 2022;21:64. DOI: 10.1186/s12937-022-00817-6

[10] Abizadeh M, Novin MG, Amidi F, Ziaei SA, Abdollahifar MA, Nazarian H. Potential of auraptene in improvement of oocyte maturation, fertilization rate, and inflammation in polycystic ovary syndrome mouse model. Reprod Sci. 2020;27(9):1742–51. DOI: 10.1007/s43032-020-00168-9
[11] Jang Y, Choo H, Lee MJ, Han J, Kim SJ, Ju X, et al. Auraptene mitigates Parkinson's disease-like behavior by protecting inhibition of mitochondrial respiration and scavenging reactive oxygen species. Int J Mol Sci. 2019;20(14):3409. DOI: 10.3390/ijms20143409
[12] Lee MJ, Jang Y, Zhu J, Namgung E, Go D, Seo C, et al. Auraptene enhances junction assembly in cerebrovascular endothelial cells by promoting resilience to mitochondrial stress through activation of antioxidant enzymes and mtUPR. Antioxidants. 2021;10(3):475. DOI: 10.3390/antiox10030475
[13] Hassanein EH, Sayed AM, Hussein OE, Mahmoud AM. Coumarins as modulators of the Keap1/Nrf2/ARE signaling pathway. Oxidative Med Cell Longev. 2020;2020:1675957. DOI: 10.1155/2020/1675957
[14] Akino N, Wada-Hiraike O, Isono W, Terao H, Honjo H, Miyamoto Y, et al. Activation of Nrf2/Keap1 pathway by oral Dimethylfumarate administration alleviates oxidative stress and age-associated infertility might be delayed in the mouse ovary. Reprod Biol Endocrinol. 2019;17:23. DOI: 10.1186/s12958-019-0466-y
[15] Di Nisio V, Antonouli S, Damdimopoulou P, Salumets A, Cecconi S. *In vivo* and *in vitro* postovulatory aging: When time works against oocyte quality? J Assist Reprod Genet. 2022;39(4):905–18. DOI: 10.1007/s10815-022-02418-y
[16] Yu C, Xiao JH. The Keap1-Nrf2 system: A mediator between oxidative stress and aging. Oxid Med Cell Longev. 2021;2021:6635460. DOI: 10.1155/2021/6635460
[17] Kim KH, Kim EY, Lee KA. GAS6 ameliorates advanced age-associated meiotic defects in mouse oocytes by modulating mitochondrial function. Aging. 2021;13(14):18018–32. DOI: 10.18632/aging.203328
[18] Almansa-Ordonez A, Bellido R, Vassena R, Barragan M, Zambelli F. Oxidative stress in reproduction: A mitochondrial perspective. Biol. 2020;9(9):269. DOI: 10.3390/biology9090269
[19] Khazaei M, Aghaz F. Reactive oxygen species generation and use of antioxidants during *in vitro* maturation of oocytes. Int J Fertil Steril. 2017;11(2):63–70. doi: DOI: 10.22074/ijfs.2017.4995
[20] Cecchele A, Cermisoni GC, Giacomini E, Pinna M, Vigano P. Cellular and molecular nature of fragmentation of human embryos. Int J Mol Sci. 2022;23(3):1349. DOI: 10.3390/ijms23031349
[21] van der Reest J, Cecchino GN, Haigis MC, Kordowitzki P. Mitochondria: Their relevance during oocyte ageing. Ageing Res Rev. 2021;70:101378. DOI: 10.1016/j.arr.2021.101378
[22] Lu J, Wang Z, Cao J, Chen Y, Dong Y. A novel and compact review on the role of oxidative stress in female reproduction. Reprod Biol Endocrinol. 2018;16:80. DOI: 10.1186/s12958-018-0391-5
[23] Perkins AT, Greig MM, Sontakke AA, Peloquin AS, McPeek MA, Bickel SE. Increased levels of superoxide dismutase suppress meiotic segregation errors in aging oocytes. Chromosoma. 2019;128(3):215–22. DOI: 10.1007/s00412-019-00702-y
[24] Armstrong S, Bhide P, Jordan V, Pacey A, Marjoribanks J, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction. Cochrane Database Syst Rev. 2019;5:CD011320. DOI: 10.1002/14651858.CD011320.pub4
[25] Bellusci G, Mattiello L, Iannizzotto V, Ciccone S, Maiani E, Villani V, et al. Kinase-independent inhibition of cyclophosphamide-induced pathways protects the ovarian reserve and prolongs fertility. Cell Death Dis. 2019;10(10):726. DOI: 10.1038/s41419-019-1961-y
[26] Fonseca É, Marques CC, Pimenta J, Jorge J, Baptista MC, Gonçalves AC, et al. Anti-aging effect of urolithin a on bovine oocytes *in vitro*. Animals. 2021;11(7):2048. DOI: 10.3390/ani11072048
[27] Furukawa Y, Washimi YS, Hara RI, Yamaoka M, Okuyama S, Sawamoto A, et al. Citrus auraptene induces expression of brain-derived neurotrophic factor in Neuro2a cells. Molecules. 2020;25(5):1117. DOI: 10.3390/molecules25051117
[28] Shimoi G, Tomita M, Kataoka M, Kameyama Y. Destabilization of spindle assembly checkpoint causes aneuploidy during meiosis II in murine post-ovulatory aged oocytes. J Reprod Dev. 2019;65(1):57–66. DOI: 10.1262/jrd.2018-056
[29] Zhang D, Keilty D, Zhang ZF, Chian RC. Mitochondria in oocyte aging: Current understanding. Facts Views Vis Obgyn. 2017;9(1):29–38.
[30] Lagalla C, Coticchio G, Sciajno R, Tarozzi N, Zaca C, Borini A. Alternative patterns of partial embryo compaction: Prevalence, morphokinetic history and possible implications. Reprod Biomed Online. 2020;40(3):347–54. DOI: 10.1016/j.rbmo.2019.11.011
[31] Tamura H, Jozaki M, Tanabe M, Shirafuta Y, Mihara Y, Shinagawa M, et al. Importance of melatonin in assisted reproductive technology and ovarian aging. Int J Mol Sci. 2020;21(3):1135. DOI: 10.3390/ijms21031135
[32] Council of Europe. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [Internet]. 1986 [cited 2024 Nov 28]. ETS No. 123. 1986 Mar 18. Available from: https://rm.coe.int/168007a67b

# Вплив антиоксидантів на морфологію та функцію яєчників щурів за умов запального процесу (вагітності) в рамках аналізу оцінки їх дії та ефективності на ядерне та цитоплазматичне дозрівання ооцитів

**Ангеліна Стюарт**

Аспірант, науковий співробітник
Інститут фізіології ім. О. О. Богомольця Національної академії наук України
01024, вул. Богомольця, 4, м. Київ, Україна
https://orcid.org/0009-0006-5703-4246

**Анотація.** Метою роботи було дослідити вплив різних антиоксидантів на морфофункціональні характеристики яєчників вагітних щурів лінії Вістар за умов системного запалення. Експериментальне дослідження проведено на 20 статевозрілих щурах-самках лінії Вістар, які були розподілені на п'ять підгруп по чотири тварини в кожній. Щури були розподілені на такі групи: контрольна група (без лікування), група з індукованим запаленням (лікування ліпополісахаридами) та три групи з антиоксидантним лікуванням, де було індуковано системне запалення, а самиці отримували ресвератрол, кверцетин або лікопін. Системне запалення викликали за допомогою ліпополісахариду, а різні антиоксиданти вводили досліджуваним підгрупам протягом певного періоду з травня по червень 2023 року. Дослідження було зосереджено на вивченні впливу цих антиоксидантів на морфологію ооцитів, функцію мітохондрій, а також на рівні активних форм кисню та глутатіону. Результати показали, що лікування антиоксидантами значно покращило морфологію ооцитів, зменшивши аномалії та відновивши нормальний розподіл мітохондрій. У щурів, які отримували антиоксиданти, знизився рівень активних форм кисню та підвищилася концентрація глутатіону, що свідчить про зменшення окислювального стресу. Крім того, спостерігалося збільшення експресії генів, пов'язаних з антиоксидантним захистом, що свідчить про посилення антиоксидантної відповіді. Ці результати свідчать про те, що антиоксиданти можуть покращувати якість ооцитів у разі оксидативного стресу, спричиненого запаленням, пропонуючи нові можливості для репродуктивної біології. Практичне значення цієї роботи полягає в її потенційному застосуванні в репродуктивній медицині, зокрема для пацієнтів із запальним безпліддям, де антиоксидантна терапія може сприяти покращенню якості ооцитів та репродуктивних результатів

**Ключові слова:** постовуляторне старіння; яйцеклітина; антиоксидант; окислювальний дисбаланс; кумарин цитрусових

Article's History: Received: 02.08.2024; Revised: 25.10.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.55

Journal homepage: https://bmbr.com.ua/en

UDC 616.8:615.82

# General procedures for treating myofascial pain syndrome with therapeutic exercises and massage

**Valerii Yur**

Master
Vinnytsia Social and Economic Institute of the University "Ukraine"
21036, 6 Dmytro Maiboroda Str., Vinnytsia, Ukraine
https://orcid.org/0009-0002-8243-8462

**Oleksandr Lebediev***

Lecturer
Vinnytsia Social and Economic Institute of the University "Ukraine"
21036, 6 Dmytro Maiboroda Str., Vinnytsia, Ukraine
https://orcid.org/0009-0002-9079-1813

**Abstract.** The purpose of the study was to determine the effectiveness of massage and therapeutic exercises for patients with myofascial pain syndrome. The study compared the results of a survey of patients before, during, and after treatment with massage, therapeutic exercises, and a combination of both. Pain characteristics were evaluated on an 11-point scale and functional performance on a 5-point scale. In the course of the study, it was found that the effectiveness of various methods of therapy for myofascial pain syndrome of the trapezius muscle was high. All patients successfully completed the full course of treatment, which demonstrated a significant reduction in pain and improvement in functional activity. The best results were demonstrated by the group that received combined treatment with massage and therapeutic exercises. In patients in this group, positive changes were observed as early as on Day 5 of therapy, while in the group with only massage, relief occurred on Day 6 and Day 7. Patients who had been treated with therapeutic exercises alone began to experience significant improvements towards the end of the course. According to statistics, pain in most patients after treatment decreased to 1-2 points on an 11-point scale. The highest effectiveness was demonstrated by the combined treatment group, in which pain decreased by an average of 5.55 points, and 85% of patients reported only minor pain. In the massage-only group, 75% of patients reported a reduction in pain levels to 1-2 points on an 11-point scale. In the therapeutic exercises group, pain decreased in 65% of patients, but the recovery process was slower. None of the patients reported pain of 7-8 points after the treatment. Overall results showed that a combination of massage and therapeutic exercises is the most effective method for reducing pain and restoring functional activity

**Keywords:** trigger points; physical rehabilitation; manual therapy; musculoskeletal manipulation; muscle relaxation

## INTRODUCTION

Myofascial pain syndrome (MFS) is a common condition that is accompanied by chronic pain in the muscles and fascia. This disorder is a common cause of disability and significantly reduces the quality of life of patients. Given that most treatments have a temporary effect, it is important to find optimal therapeutic approaches that would not only relieve symptoms, but also help to restore muscle function. Therapeutic exercises and massage are among the most common non-drug methods used in the treatment of MPS. Their popularity is conditioned by their availability, minimal health risks, and the possibility of long-term use. The problematic issues of the study were determining the effectiveness of therapeutic exercises, massage, and their combination for the treatment of MPS, and comparing them. The rate of onset of pain relief with the treatment methods under study.

Z. Ostapyak *et al*. [1] compared electroneuromyographic parameters in patients with MPS on the background of multiple sclerosis under the influence of various physical therapy methods. 105 patients were examined with a course of reflexology, muscle stretching exercises, and massage. The results of tests after therapy in patients of the

**Suggested Citation**:
Yur V, Lebediev O. General procedures for treating myofascial pain syndrome with therapeutic exercises and massage. Bull Med Biol Res. 2024;6(4):55–63. DOI: 10.63341/bmbr/4.2024.55

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

experimental group showed a significant improvement in indicators, especially after reflexology with stretching. The use of reflexology in combination with stretching helped reduce muscle spasms and pain in patients with MPS in multiple sclerosis. O. Yaroshevskyi [2] considered the problem of the spread of MPS among young people with cervical-brachial localisation. The researcher evaluated the effectiveness of non-drug methods of therapy. Patients were divided into two groups: manual therapy, a combination of manual therapy with acupuncture. The combination of the two methods showed better results in reducing pain, emotional disorders, and autonomic dysfunctions.

The effectiveness of manual therapy in the treatment of MPS was assessed in a systematic review of L.A. de Melo *et al*. [3]. Of the five selected studies, 156 patients received manual therapy. It was found that this method was better than no treatment, and exceeded counselling. The study by F. Eslamian *et al*. [4] compared the effectiveness of electroacupuncture and biofeedback in the treatment of cervical MPS. The study included 50 patients with chronic neck pain. Specifically, 80% of patients in the electroacupuncture group achieved significant improvement, compared to 40% in the biofeedback group.

The effect of physical therapy on MPS in the neck and shoulder area was assessed in the meta-analysis by C.-M. Kim & J.W. Park [5]. The results showed that combined treatment with physical therapy and exercise showed the highest average degree of effect. The greatest effect was observed in the treatment groups lasting from 16 to 30 days or consisting of 6-10 sessions. Physical therapy and therapeutic exercises significantly reduced the manifestations of MPS. R.D. Gerwin [6] evaluated the treatment of musculoskeletal dysfunctions using evidence-based medicine. The effects of dry acupuncture on trigger points and the use of exercise to reduce myofascial pain were considered. The results confirmed that acupuncture and exercise are effective treatments for MPS.

Objective analysis performed by M.J. Guzmán-Pavón *et al*. [7] investigated the effect of exercise programmes on dysfunctions associated with myofascial trigger points. The effectiveness of the programmes in reducing pain intensity, increasing the pressure pain threshold, and improving range of motion was determined. Exercise has been effective in relieving pain and improving motor function in patients with MPS. Diagnostic criteria for myofascial trigger points used in physical therapy interventions were investigated in a systematic review by L. Li *et al*. [8]. The researchers found that the most common criteria were a combination of "pinpoint pain", "reflected pain", and "local contraction response". The study revealed significant variability in the diagnosis of myofascial trigger points, and a lack of transparency in reports on diagnostic criteria. The study by M. Rahbar *et al*. [9] compared the effectiveness of shock wave therapy and standard treatment for neck and upper back MPS. Both methods significantly reduced pain and disability during the first week of treatment compared to the control group. However, after four weeks, shock wave therapy showed better results in reducing pain intensity. This suggests that shock wave therapy was more effective in the long run.

The main gaps in these studies in the treatment of MPS are the lack of standardisation of diagnostic and therapeutic methods, which makes it difficult to compare the results and limits their applicability. Many studies did not consider the long-term effects of treatment, and the individual characteristics of patients, including psychoemotional state and level of physical activity. In addition, the choice of methods is often limited and possible side effects are not sufficiently analysed, which requires a more comprehensive approach to research and treatment. The purpose of the study was to determine the effectiveness of therapeutic exercises, massage, and their combination in MPS.

## MATERIALS AND METHODS

The study was based on investigating the effectiveness of various treatments for MPS in patients who worked at a computer for at least 6 hours a day. The study involved 60 patients aged 18 to 40 years who had complaints of chronic trapezius muscle pain lasting more than three months (Table 1). Patients were randomly assigned to three groups: the first group (20 patients) received only massage, the second group (20 patients) received therapeutic exercises, and the third group (20 patients) received combined treatment, which included both therapeutic exercises and massage.

**Table 1.** Distribution of patients with MPS by study groups and demographics

| Category | Massage group | Therapeutic exercises group | Therapeutic exercises + massage group | Total |
|---|---|---|---|---|
| Total patients, n | 20 | 20 | 20 | 60 |
| **Age, n** | | | | |
| 18-20 years old | 7 | 5 | 6 | 18 |
| 21-30 years old | 5 | 6 | 7 | 18 |
| 31-40 years old | 8 | 9 | 7 | 24 |
| **Gender, n** | | | | |
| Men | 8 | 10 | 9 | 27 |
| Women | 12 | 10 | 11 | 33 |
| **Nature of pain on an 11-point scale (points)** | | | | |
| 5-6 | 13 | 14 | 12 | 39 |
| 7-8 | 7 | 6 | 8 | 21 |
| Functional performance, average value for all groups (points) | 0.5 | | | - |

**Source:** compiled by the authors

Prior to the start of the study, all participants signed informed consent to participate in accordance with the Declaration of Helsinki of 1964 and its subsequent amendments [10]. All participants were pre-selected based on inclusion criteria. Only patients with active MPS in the trapezoid region (right, left, or both) with symptoms lasting more than three months were allowed to participate in the study. Patients had at least two signs of limited range of motion: flexion, extension, or lateral flexion below normal (45°). Men and women between the ages of 18 and 40 who had computer skills and experienced a pain level of at least 5 on a visual-analogue scale were allowed to participate. In addition, participants were required to have symptoms of muscle tension caused by pain and the appearance of trigger points on the trapezius muscle, and a doctor-confirmed diagnosis of MPS of the trapezius muscle.

The exclusion criteria were patients with a history of hypertension, cardiovascular disease, osteoporosis, cancer, herniated discs, and infectious skin diseases. Patients who had fractures or cracks in the neck or spine, pregnant women, and those who had received any other form of treatment or taken medications (such as diclofenac or steroids) during the previous month were excluded from the study. Patients who did not complete the full course of therapy, or those with worsening symptoms, were referred to conventional treatment and excluded from the study.

All patients were treated in 14 sessions, lasting 60 minutes each. In the massage group, a classic therapeutic massage was performed. In the therapeutic exercises group, exercises were performed for the head, neck, shoulders, and abdomen, which improved blood circulation in the upper body and stretched the muscles of the neck, back, and arms. In the combined treatment group, the session of therapeutic massage and therapeutic exercises was 60 minutes each. Before treatment, on Day 7 (interim survey), and after completing the course of treatment, patients underwent a survey that included pain assessment on an 11-point scale, including the evaluation of improvements in performance and quality of life. Functional performance was evaluated on a 5-point scale, where 0 – low performance and 5 – maximum functionality. Data processing was performed on a computer using a comparative method and a percentage ratio of indicators.

## RESULTS

All patients were able to complete the full course of treatment. The nature of pain and functional performance showed significant results after the completion of treatment. Table 2 shows data that characterise the pain syndrome in patients who have completed a full course of treatment in each group, and functional performance. It should be noted that during the interim survey on Day 7, patients from the combined massage and physical therapy group reported an improvement in their condition as early as Day 5. The group where only massage was used noted a decrease in pain on days 6-7. The group where only therapeutic exercises were used on Day 7 did not yet have significant changes in general condition and pain reduction. However, by the end of the treatment course, these data in the therapeutic exercises group improved significantly.

**Table 2.** Characteristics of pain after physical therapy, massage, or a combination of both

| Category | Massage group | Therapeutic exercises group | Massage+therapeutic exercises group | Total |
|---|---|---|---|---|
| **Nature of pain on an 11-point scale** | | | | |
| 1-2 points | 15 | 13 | 17 | 45 |
| 3-4 points | 4 | 5 | 3 | 12 |
| 5-6 points | 1 | 2 | 0 | 3 |
| Functional performance, average value for each group (points) | 3.5 | 3 | 4.4 | |

**Source:** compiled by the authors

The study found that before starting treatment with 60 patients, 65% of participants described pain of 5-6 points and 35% of patients had severe pain of 7-8 points, which was associated with MPS of the trapezius muscle. After completion of treatment, all patients were divided into 3 groups, of which 75% of patients characterised pain by 1-2 points, 20% described the intensity of symptoms by 3-4 points, and 5% of patients had pain by 5-6 points. Comparing the study of the treatment group, a significant improvement in the condition and a decrease in pain in all three categories can be noted. However, in the group using therapeutic exercises, the indicators were the lowest. Of the 20 participants who rated pain at 5-6 points (70% of patients) and 7-8 points (30% of patients) before therapeutic exercises treatment, pain indicators decreased to 5-6 points in 10% of patients, to 3-4 points in 65%, and to 1-2 points in 65% of patients after treatment.

Massage occupied an average position, although it also showed a separate high effectiveness of the method of treating MPS in the trapezius muscle. Pain characteristics before starting therapy with 20 participants in 65% of patients were rated at 5-6 points and in 35% of patients – was estimated at 7-8 points. After treatment, 75% of participants reported a reduction in pain to 1-2 points, 20% had a reduction in pain to 3-4 points, and 5% of patients maintained pain at 5-6 points.

The most effective treatment for trapezius MPS was a combination of massage and therapeutic exercises. Before the start of the treatment process, 60% of patients had pain syndrome, which was estimated at 5-6 points, in 40% of patients, pain was characterised at 7-8 points. After the end of the therapeutic process, a significant number of patients noted a decrease in pain to 1-2 points, which was 85%, a decrease in pain syndrome to 3-4 points was observed in

15% of patients. After combined treatment with massage and therapeutic exercises, none of the patients reported pain, which was estimated at 5-6 points. This suggests that it was the combined method of treatment that had the most effective result.

Functional performance, as shown in Table 3, generally increased from 0.5 to 3.6 points, which is 3.1 points higher than the value before the start of treatment. In the combined massage+therapeutic exercises group, the average value of functional performance improved by 3.9 points, which is the highest indicator among all therapeutic methods. The second place was taken by the massage group, where performance improved by 3 points. The therapeutic exercises group also had positive results in functional performance, but among other groups showed a minimal indicator with an improvement of 2.5 points.

**Table 3.** Functional performance after treatment

| Category | Massage group | Therapeutic exercises group | Massage+therapeutic exercises group |
|---|---|---|---|
| Functional performance, average value for each group (points) | 3.5 | 3 | 4.4 |

**Source:** compiled by the authors

Figure 1 shows that the most effective treatment tactic was a combination of massage+therapeutic exercises, and the pain syndrome decreased by an average of 5.55 points. The use of massage alone led to a decrease in the indicator by 4.8 points. And therapeutic exercises had the lowest effectiveness, and reduced pain in patients by 4.75 points. This means that the combination of massage+therapeutic exercises is the most effective compared to monotherapy of massage alone or physical therapy only. The effectiveness of trapezius muscle MPS monotherapy did not differ significantly, although it was found that the use of therapeutic exercises was less successful.

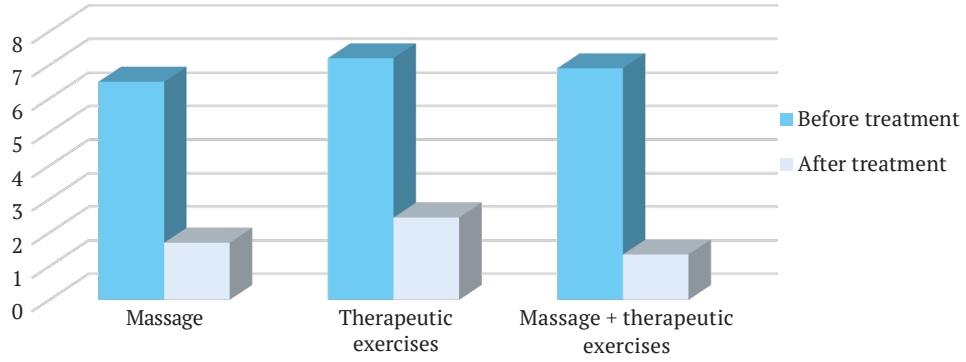


**Figure 1.** Comparative characteristics of pain syndrome by average score
**Source:** compiled by the authors

Thus, all patients noted an improvement in their condition, which indicates a high effectiveness of treatment with therapeutic exercises, massage, and a combination of them to reduce or eliminate MPS of the trapezius muscle. None of the patients after all treatment methods reported severe pain with a score of 7-8 points. Although therapeutic exercises had the lowest rates among all treatments, there was no significant difference between therapeutic exercises and massage. These methods require an individual approach, since long-term severe pain may require longer treatment. Massage shows high effectiveness with short-term use, and therapeutic exercises works for a long time, so the combination of these two methods had the best result. Functional performance was effective, with the maximum indicators for the combined treatment group and the minimum for the group in which only therapeutic exercises were practiced.

MPS is a regional pain disorder that affects all age groups and is characterised by the presence of trigger points in the muscles or fascia [11]. MPS is usually diagnosed during a physical examination, and common diagnostic criteria include the presence of trigger points, pain on palpation, a characteristic pattern of reflected pain, and a local muscle twitch response. The prevalence of MPS among patients who went to medical facilities because of pain ranged from 30% to 93%. This significant change may be conditioned by the lack of clear diagnostic criteria and guidelines for the diagnosis of MPS. Although this syndrome is common, its pathophysiology remains poorly understood. There have been many approaches to treating MPS, including exercise, trigger point injections, medication, and alternative treatments. However, due to the variability of clinical approaches and the lack of uniform diagnostic standards, there is a need for further research to establish unified diagnostic criteria and treatment approaches. Thus, the importance of developing clear protocols for the diagnosis and treatment of MPS remains an urgent public health issue, especially given the significant percentage of patients suffering from this disorder.

The literary review by A. Galasso *et al.* [12] revealed key aspects of MPS, in particular, its prevalence, impact on quality of life, and treatment difficulties. Although MPS can be acute, it often becomes chronic, affecting a significant portion of the population, about 85% of adults. Patients with other chronic pain disorders were more likely to experience MPS, which further complicated treatment. MPS has been shown to reduce physical activity and social

functioning, worsen emotional state, and increase health care costs. The effectiveness of non-pharmacological methods, such as acupuncture and massage, has been noted in some patients.

The complexity of diagnosing and treating MPS was noted in the study by J. Henson [13]. Due to the lack of uniform diagnostic criteria, clinicians relied on physical examination and identification of trigger points that reproduced localised pain or caused pain in remote areas. MPS was associated with prolonged muscle contractions that occurred as a result of insufficient or excessive muscle use, highlighting the importance of behavioural modification in managing this condition. Treatment required a comprehensive approach that included both non-drug and drug methods, including manual therapy and the use of local analgesics. Procedural techniques such as dry acupuncture and shock wave therapy have often been used to reduce symptoms in the early stages of the disease. However, with the chronicity of the process, MPS became less sensitive to treatment, making pain management more difficult. This study emphasised the importance of timely intervention to prevent the transition of MPS to a chronic form and reduce its impact on the quality of life of patients.

The study by Ü. Yalçın [14] compared two treatments for MPS – kinesiotaping, extracorporeal shock wave therapy (ESWT), and both in combination with exercise. Both approaches were found to be effective in reducing pain, raising the pain threshold, and improving patient functionality. However, ESWT showed better results compared to kinesiotaping for all key indicators, including the disability index and pain reduction. An important finding was that ESWT had a higher level of efficacy, making this method a higher priority in the treatment of MPS.

The results of intramuscular electrostimulation (IMES) in the treatment of MPS were considered in the study by M. Hadizadeh *et al*. [15]. Six randomised controlled trials involving 158 patients were evaluated. IMES was compared with placebo, dry acupuncture, or exercise. This helped to establish improvements in indicators such as pain and mobility. IMES was found to be more effective than placebo and other treatments. The research has shown that IMES can reduce pain and improve physical function in patients suffering from chronic pain caused by MPS.

The systematic review by F. Dach & K.S. Ferreira [16] investigated the efficacy of dry acupuncture as a treatment for MPS in patients with low back pain. MPS is a common cause of chronic pain that requires comprehensive treatment, including exercise, trigger point massage, and acupuncture. The study examined the most effective approaches to the use of dry acupuncture based on available scientific evidence. The review was based on meta-analysis and randomised controlled trials, with a total of 509 research papers analysed. They compared dry acupuncture with other treatments such as classical acupuncture, dummy acupuncture, laser therapy, physical therapy, local anaesthetic injections, and ischaemic compression. The results showed that dry acupuncture can significantly reduce pain intensity and improve mobility in patients with both acute and chronic low back pain. Although the effectiveness of the method varied depending on the duration of follow-up and comparable methods, dry acupuncture showed a marked reduction in pain after the intervention. Thus, this method can be considered effective for treating low back pain in patients with MPS.

The study by M.F. Yasar *et al*. [17] compared the effectiveness of kinesiotaping and dry acupuncture for the treatment of MPS of the trapezius muscle. Participants were divided into three groups: a control group, a kinesiotaping group, and a dry acupuncture group. The results showed that both kinesiotaping and dry acupuncture significantly improved pain scores, pressure pain threshold, and neck disability index. In the control group, the improvement was minimal, which indicates the superiority of these methods over simple exercises. The dry acupuncture group showed slightly better results in pain reduction compared to kinesiotaping, which may indicate a more pronounced effect of dry acupuncture on pain reduction and restoration of function. The findings suggest that both methods have a positive effect on the treatment of MPS, but more research is needed to determine the long-term effects.

The results of kinesiotaping treatment for MPS were considered in the paper by F.M. Akpinar *et al*. [18]. The study used two different techniques, which showed that both variants of kinesiotaping significantly reduced the intensity of pain and improved the functional state of patients compared to a home exercise programme. Quality of life, particularly physical function and overall health, improved earlier in the kinesiotaping groups than in the control group. Although the difference between kinesiotaping techniques was minimal, the muscle inhibition technique showed a faster effect on role constraints associated with physical factors.

C.L.A. Ying *et al*. [19] evaluated the effect of Bowen therapy on patients with chronic myofascial neck pain. The results showed that this treatment significantly improved the pressure pain threshold and neck range of motion 12 weeks after starting treatment, and the effects persisted after 24 weeks. In addition to physical improvements, anxiety and depression scores on the General Anxiety Disorder-7 (GAD7) and Patient Health Questionnaire-9 (PHQ9) scales decreased, which had a positive effect on the mental health of patients. Improvements in both physical and mental quality of life indicators have shown the benefits of using this technique in the treatment of myofascial pain. However, given the small sample size, the results may require further confirmation in larger studies.

The study by I. Koukoulithras *et al*. [20] considered a psychosocial model of the MPS treatment approach, where trigger points, which are the main source of pain and discomfort, play a key role. The importance of taking into consideration not only physical but also psychological and social factors that affect patients with MPS was emphasised. Stress and chronic pain have been found to mutually exacerbate each other, worsening patients' condition and reducing their quality of life. Treatment included the use of exercise, posture correction, a balanced diet, and myofascial release and pharmacotherapy. The researchers emphasised the importance of a holistic approach to improving patient health, although the aetiology of MPS remains unclear, which requires further research. Thus, MPS is characterised by damage to all age groups, with the presence of trigger points in the muscles. Diagnosis of MPS in patients is based on physical examination and detection of pain on palpation. Treatment includes medical and non-medical techniques such as massage, exercise, acupuncture, and

shock wave therapy. However, due to the lack of uniform diagnostic criteria, further research is needed to improve the standards of diagnosis and therapy.

## DISCUSSION

MPS therapy with therapeutic exercises and massage is relevant due to its non-invasive nature and minimal side effects, which makes it safe for a wide range of patients. Massage helps to reduce pain and improve blood circulation, while therapeutic exercises help to maintain muscle tone and increase flexibility. Complex application of these methods can improve the quality of life of patients, reduce the frequency of relapses and increase treatment effectiveness.

A comparison of two approaches to the treatment of myofascial pain in the upper trapezius muscle – Thai massage and Thai exercises – was described in the paper by J. Boonruab *et al*. [21]. Both methods significantly reduced pain and improved the volume of movement in the cervical vertebrae in patients. However, Thai massage has been shown to be more effective in reducing the intensity of pain at trigger points compared to exercise. Although both treatments showed similar effects on neck mobility, Thai massage had an advantage in the context of pain relief, making it a more effective method for patients with severe pain symptoms. These results are similar to this study, since the effectiveness of massage also had higher indicators compared to therapeutic exercises. However, the study described here did not investigate combined treatment and used Thai massage rather than therapeutic massage.

The study by S.S. Mansoori *et al*. [22] described how different stretch duration affects patients with chronic MPS. Stretching for 60 seconds was found to negatively affect nerve conduction, while stretching for 30 seconds provided optimal improvement without harming the nervous system. A shorter stretch (15 seconds) had less positive effects on pain and pain pressure threshold. Therefore, the optimal duration of stretching exercises for the treatment of chronic myofascial pain was 30 seconds, since this interval provided the greatest effectiveness with the least side effects. This study differs from the one described in that the details and duration of muscle stretching rights were not considered, however, it confirms the importance of this indicator for reducing pain in MPS.

The relevance of trigger points in the treatment of MPS in primary care settings was highlighted in the study by B. Shipton *et al*. [23]. Methods such as massage, physical therapy, and osteopathy have been found to have an advantage as initial approaches due to their low invasiveness. Injections at trigger points showed some efficacy, but the results were limited due to the small number of participants in the studies, as well as the possible placebo effect. None of the drugs were more effective than others or placebo, which led to recommendations to use injections only in cases where other methods were not successful. This approach emphasised the importance of integrating less invasive techniques before injection, and the need for a comprehensive approach to treating myofascial pain to achieve the best results. The similarity with the current study is to confirm the effectiveness of massage, therapeutic exercises, and combination treatment to reduce MPS.

Two methods of treating chronic mechanical neck pain among students – soft tissue mobilisation with instruments and myofascial release therapy – were compared by F. Shewail *et al*. [24]. Both methods were used for four weeks, but no significant differences in results were found between the groups. Although there was some improvement in pain levels, functional status, and pain threshold after the sessions, the lack of a control group made it difficult to determine the actual effectiveness of the methods. This means that the improvement may have been caused by other factors, such as natural pain reduction or the placebo effect. Further studies with control groups may help to more accurately assess the effectiveness of soft tissue mobilisation using tools and myofascial release therapy. There was a similarity with this study in the use of treatment methods that were similar to massage and led to positive results.

In another study, M. Sadeghnia *et al*. [25] investigated the effectiveness of three different treatments for active trigger points in patients with myofascial pain in the upper trapezius muscle. All three methods – deep transverse friction massage (DTFM), high pain threshold ultrasound, and whole-body vibration (WBV) – showed improvements in pain reduction, increased pressure pain threshold, and extended range of motion for contralateral lateral neck flexion. However, the greatest reduction in pain on the visual analogue scale was observed in the group receiving whole-body vibrations. This showed the potential of WBV as a more effective method for pain relief compared to other methods. The results also showed that although the difference in increasing the pressure pain threshold between the groups was not statistically significant, ultrasound techniques with a high pain threshold and WBV had a better effect on increasing range of motion compared to DTFM. This difference may be due to the fact that the DTFM method requires the therapist to exercise their fingers, which may limit its effectiveness compared to less skill-and effort-dependent methods such as WBV. Therefore, full-body vibration can be considered as a promising option for improving outcomes in patients with active trigger points, especially if resources are limited or less intensive physical intervention is required. In the present study, massage also had a positive effect, although it was also not the leader among all MPS treatments.

The addition of electro-massage to manual therapy in patients with bilateral myofascial pain in the temporomandibular joint was evaluated in the paper by L. Espejo-Antúnez *et al*. [26]. A total of 46 people took part, which were divided into two groups. The first group received only manual therapy, which included mobilisation and work with soft tissues, while the second group additionally underwent a course of electrical stimulation in the cervical region. Both groups underwent a 2-week course of treatment, during which pain intensity, pain sensitivity threshold, and painless mouth opening were assessed. According to the results of the study, the second group, which received electrical stimulation together with manual therapy, showed significantly better results in reducing the intensity of pain, increasing the threshold of pain sensitivity, and improving the mobility of the lower jaw. In addition, participants in this group showed an improvement in the active range of motion of the neck, in addition to rotational movements. Thus, the addition of electro-massage to standard manual therapy significantly improves the results of treatment of

patients with myofascial pain in the temporomandibular joint compared to the use of manual therapy alone. These results indicate similar results to the present study on the prophylactic effectiveness of combination treatment compared to MPS monotherapy.

Ö. Bingölbali *et al*. [27] determined the effect of deep tissue massage on the number of myofascial trigger points, neck mobility, pain, disability, and quality of life in patients with myofascial pain. The study involved 80 patients aged 20 to 57 years, who were divided into two groups: control and experimental. The control group received standard therapy, which included electrical stimulation through the skin of the neuromuscular system, heat packs, and ultrasound therapy. The experimental group underwent an additional 12 deep-tissue massage sessions. The results showed that patients in the experimental group had significantly higher rates of pain reduction (on the neck functional disability scale (NPDS), and quality of life compared to the control group. In addition, patients who received deep-tissue massage showed improvements in neck mobility, in particular, in extension, lateral tilt, and rotation in both directions. Moreover, the difference in flexion rates between the groups was not significant. Therefore, adding deep tissue massage to a standard rehabilitation programme is an effective and safe method to improve neck mobility, reduce pain, and improve the quality of life of patients with MPS. This study confirms the results on the effectiveness of an integrated approach to the treatment of MPS.

The effectiveness of ischaemic compression as a treatment method for MPS was investigated by W. Lu *et al*. [28]. Ischaemic compression was found to significantly improve the pressure pain threshold, demonstrating its positive effect on reducing pain sensitivity. However, when assessing subjective pain perception on a visual analogue scale, no significant differences were found between ischaemic compression and the control group. This may indicate that the method affected the body's physical response, but did not change the patients' sense of pain. The limited number of participants in the studies may influence the overall results, highlighting the need for additional studies with larger samples to more accurately determine the effectiveness of ischaemic compression in the treatment of MPS. However, the use of treatment methods similar to massage and manual therapy confirms their effectiveness.

Studies demonstrated the effectiveness of various treatments for MPS, in particular, massage, stretching, and electrical stimulation, which help to reduce pain and improve the functional state of patients. A combined approach that includes manual therapy with additional methods, such as electric massage or vibration, is more effective. The positive results of various methods indicate the importance of individualising treatment to achieve maximum effectiveness in MPS.

## CONCLUSIONS

The study demonstrated the high effectiveness of various methods of treating MPS of the trapezius muscle. All patients successfully completed the full course of therapy. Significant improvements were recorded both in reducing pain and in improving functional performance.

The combination of massage and therapeutic exercises showed the best results among all the study groups. Patients who received combination treatment reported improvement on Day 5, while in the group where only massage was used, pain relief began on days 6 or 7. In patients who underwent only a course of therapeutic exercises, significant changes were recorded only at the end of the course. Statistics confirm that after treatment, pain in most patients decreased to 1-2 points on an 11-point scale. According to the results obtained, the combined treatment group (massage+therapeutic exercises) showed the highest effectiveness, reducing pain by an average of 5.55 points. In this group, 85% of patients had minimal pain, and none of the patients complained of pain in the range of 5-6 points. This highlights the high efficiency of the combined approach.

The group that used only massage also showed high results. After the course of treatment, 75% of participants reported a reduction in pain to 1-2 points, 20% reported an intensity of 3-4 points, and only 5% continued to experience moderate pain at 5-6 points. Functional performance in this group increased by an average of 3 points, which is quite a high result. Instead, the group that used only therapeutic exercises had the lowest efficacy in the short term. Only 65% of patients reported a reduction in pain of up to 1-2 points, and 10% continued to experience moderate pain of 5-6 points after treatment. However, functional performance in this group increased by 2.5 points, which indicates a positive effect of therapeutic exercises on long-term recovery of physical activity, although this method was inferior to others in terms of symptom relief.

Patients' functional performance also improved significantly after treatment. The highest rates were recorded in the combined treatment group, where the average performance value increased by 3.9 points. This confirms that the combination of massage and therapeutic exercises is the most effective method for both reducing pain and restoring the functional capabilities of patients. The limitations of this study were a small sample of patients and the absence of a control group. Future research should be devoted to the study of methods of treating myofascial pain syndrome that would accelerate the speed of recovery of patients.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Ostapyak Z, Mytckan B, Bejga P, Gerich R, Popel' S. 2020. Effectiveness of physical therapy in myofascial syndrome in patients with multiple sclerosis. Wiad Lek. 2020;73(9):1950–6. DOI: 10.36740/WLek202009210

[2] Yaroshevskyi O. Nonspecific symptoms of pain syndromes of cervicobrachial localization and their dynamics under the influence of non-pharmacological treatment. Wiad Lek. 2016;69(1):10–3.

[3] de Melo LA, de Medeiros AKB, Pinto Campos MF, de Resende CMBM, Barbosa GAS, de Almeida EO. Manual therapy in the treatment of myofascial pain related to temporomandibular disorders: A systematic review. J Oral Facial Pain Headache. 2020;34(2):141–8. DOI: 10.11607/ofph.2530

[4] Eslamian F, Jahanjoo F, Dolatkhah N, Pishgahi A, Pirani A. Relative effectiveness of electroacupuncture and biofeedback in the treatment of neck and upper back myofascial pain: A randomized clinical trial. Arch Phys Med Rehabil. 2020;101(5):770–80. DOI: 10.1016/j.apmr.2019.12.009
[5] Kim C-M, Park JW. Meta-analysis of the effects of physical modality therapy and exercise therapy on neck and shoulder myofascial pain syndrome. Osong Public Health Res Perspect. 2020;11(4):251–8. DOI: 10.24171/j.phrp.2020.11.4.15
[6] Gerwin RD. Evidence-based medicine and low back pain, post-stroke hemiparetic spastic shoulder, and physical exercise as treatment of myofascial pain dysfunction. Pain Med. 2020;21(11):2622–4. DOI: 10.1093/pm/pnaa324
[7] Guzmán-Pavón MJ, Cavero-Redondo I, Martínez-Vizcaíno V, Fernández-Rodríguez R, Reina-Gutierrez S, Álvarez-Bueno C. Effect of physical exercise programs on myofascial trigger points-related dysfunctions: A systematic review and meta-analysis. Pain Med. 2020;21(11):2986–96. DOI: 10.1093/pm/pnaa253
[8] Li L, Stoop R, Clijsen R, Hohenauer E, Fernández-de-Las-Peñas C, Huang Q, et al. Criteria used for the diagnosis of myofascial trigger points in clinical trials on physical therapy: Updated systematic review. Clin J Pain. 2020;36(12):955–67. DOI: 10.1097/AJP.0000000000000875
[9] Rahbar M, Samandarian M, Salekzamani Y, Khamnian Z, Dolatkhah N. Effectiveness of extracorporeal shock wave therapy versus standard care in the treatment of neck and upper back myofascial pain: A single blinded randomised clinical trial. Clin Rehabil. 2021;35(1):102–13. DOI: 10.1177/0269215520947074
[10] The World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects [Internet]. [cited 2024 Nov 10]. Available from: https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/
[11] Urits I, Charipova K, Gress K, Schaaf AL, Gupta S, Kiernan HC, et al. Treatment and management of myofascial pain syndrome. Best Pract Res Clin Anaesthesiol. 2020;34(3):427–48. DOI: 10.1016/j.bpa.2020.08.003
[12] Galasso A, Urits I, An D, Nguyen D, Borchart M, Yazdi C, et al. A comprehensive review of the treatment and management of myofascial pain syndrome. Curr Pain Headache Rep. 2020;24(8):43. DOI: 10.1007/s11916-020-00877-5
[13] Henson J. Chronic pain syndromes: Myofascial pain syndrome. FP Essent. 2023;533:16–20.
[14] Yalçın Ü. Comparison of the effects of extracorporeal shockwave treatment with kinesiological taping treatments added to exercise treatment in myofascial pain syndrome. J Back Musculoskelet Rehabil. 2021;34(4):623–30. DOI: 10.3233/BMR-200234
[15] Hadizadeh M, Rahimi A, Javaherian M, Velayati M, Dommerholt J. The efficacy of intramuscular electrical stimulation in the management of patients with myofascial pain syndrome: A systematic review. Chiropr Man Therap. 2021;29(1):40. DOI: 10.1186/s12998-021-00396-z
[16] Dach F, Ferreira KS. Treating myofascial pain with dry needling: A systematic review for the best evidence-based practices in low back pain. Arq Neuropsiquiatr. 2023;81(12):1169–78. DOI: 10.1055/s-0043-1777731
[17] Yasar MF, Yaksi E, Kurul R, Alisik T, Seker Z. Comparison of dry needling and kinesio taping methods in the treatment of myofascial pain syndrome: A single blinded randomised controlled study. Int J Clin Pract. 2021;75(10):e14561. DOI: 10.1111/ijcp.14561
[18] Akpinar FM, Sindel D, Ketenci A. Investigation of effectiveness of two different kinesiotaping techniques in myofascial pain syndrome: An open-label randomized clinical trial. Pain Phys. 24(6), E721–31.
[19] Ying CLA, Lee TM, Chen PY, Samy W, Au LY, Siu CH. The effect of ISBT-bowen therapy in the treatment of myofascial neck pain – a randomized, single-blinded clinical trial. Int J Ther Massage Bodywork. 2023;16(2):29–38. DOI: 10.3822/ijtmb.v16i2.801
[20] Koukoulithras I, Plexousakis M, Kolokotsios S, Stamouli A, Mavrogiannopoulou C. A biopsychosocial model-based clinical approach in myofascial pain syndrome: A narrative review. Cureus. 2021;13(4):e14737. DOI: 10.7759/cureus.14737
[21] Boonruab J, Poonsuk P, Damjuti W, Supamethangkura W. Myofascial pain syndrome focused on the upper trapezius muscle: A comparative randomized controlled trial of the court-type traditional Thai massage versus the Thai hermit. J Evid Based Integr Med. 2021;26. DOI: 10.1177/2515690X211030852
[22] Mansoori SS, Moustafa IM, Ahbouch A, Harrison DE. Optimal duration of stretching exercise in patients with chronic myofascial pain syndrome: A randomized controlled trial. J Rehabil Med. 2021;53(1):jrm00142. DOI: 10.2340/16501977-2781
[23] Shipton B, Sagar S, Mall JK. Trigger point management. Am Fam Physician. 2023;107(2):159–64.
[24] Shewail F, Abdelmajeed S, Farouk M, Abdelmegeed M. Instrument-assisted soft tissue mobilization versus myofascial release therapy in treatment of chronic neck pain: A randomized clinical trial. BMC Musculoskelet Disord. 2023;24(1):457. DOI: 10.1186/s12891-023-06540-5
[25] Sadeghnia M, Shadmehr A, Mir SM, Hadian Rasanani MR, Jalaei S, Fereydounnia S. The immediate effects of deep transverse friction massage, high-power pain threshold ultrasound, and whole body vibration on active myofascial trigger points. J Bodyw Mov Ther. 2023;36:165–70. DOI: 10.1016/j.jbmt.2023.07.007
[26] Espejo-Antúnez L, Cardero-Durán ML, Heredia-Rizo AM, Casuso-Holgado MJ, Albornoz-Cabello M. Effects of adding electro-massage to manual therapy for the treatment of individuals with myofascial temporomandibular pain: A randomized controlled trial. J Appl Oral Sci. 2024;32:e20240109. DOI: 10.1590/1678-7757-2024-0109
[27] Bingölbali Ö, Taşkaya C, Alkan H, Altındağ Ö. The effectiveness of deep tissue massage on pain, trigger point, disability, range of motion and quality of life in individuals with myofascial pain syndrome. Somatosens Mot Res. 2024;41(1):11–7. DOI: 10.1080/08990220.2023.2165054

[28] Lu W, Li J, Tian Y, Lu X. Effect of ischemic compression on myofascial pain syndrome: A systematic review and meta-analysis. Chiropr Man Therap. 2022;30(1):34. DOI: 10.1186/s12998-022-00441-5

# Основна методика лікування міофасціального больового синдрому лікувальною фізкультурою та масажем

**Валерій Юр**
Магістр
Вінницький соціально-економічний інститут Університету «Україна»
21036, вул. Дмитра Майбороди, 6, м. Вінниця, Україна
https://orcid.org/0009-0002-8243-8462

**Олександр Лебедєв**
Викладач
Вінницький соціально-економічний інститут Університету «Україна»
21036, вул. Дмитра Майбороди, 6, м. Вінниця, Україна
https://orcid.org/0009-0002-9079-1813

**Анотація.** Ціллю дослідження було визначити ефективність масажу і лікувальної фізкультури для пацієнтів з міофасціальним больовим синдромом. В дослідженні було проведено порівняння результатів опитування пацієнтів до, під час та після лікування за допомогою масажу, лікувальної фізкультури та їх комбінації. Оцінювалися характеристика болю за 11-бальною шкалою та функціональна працездатність за 5-бальною шкалою. В ході дослідження було встановлено що ефективність різних методів терапії міофасціальним больовим синдромом трапецієподібного м'яза була високою. Усі пацієнти успішно пройшли повний курс лікування, що дозволило відзначити значне зменшення болю та поліпшення функціональної активності. Найкращі результати продемонструвала група, яка отримувала комбіноване лікування масажем та лікувальною фізкультурою. У пацієнтів цієї групи позитивні зміни спостерігалися вже на 5 день терапії, тоді як у групі з лише масажем полегшення настало на 6-7 день. Пацієнти, які отримували тільки лікувальну фізкультуру, починали відчувати значні поліпшення ближче до завершення курсу. За статистичними даними, біль у більшості пацієнтів після лікування зменшився до 1-2 балів за 11-бальною шкалою. Найвищу ефективність продемонструвала група комбінованого лікування, у якій біль зменшився в середньому на 5,55 балів, причому 85 % пацієнтів відзначали лише незначний біль. У групі пацієнтів, які отримували лише масаж, 75 % учасників відзначили зниження болю до 1-2 балів за 11-бальною шкалою. У групі лікувальної фізкультури біль знизився у 65 % пацієнтів, але процес відновлення був повільнішим. Жоден пацієнт після проведеного лікування не зазначав біль у 7-8 балів. Загальні результати показали, що комбінація масажу та лікувальної фізкультури є найефективнішим методом для зменшення болю та відновлення функціональної активності

**Ключові слова:** тригерні точки; фізична реабілітація; мануальна терапія; маніпуляції з опорно-руховим апаратом; розслаблення м’язів

Article's History: Received: 24.07.2024; Revised: 06.11.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.64

Journal homepage: https://bmbr.com.ua/en

UDC 616.5-006.6: 616.5-007.4

# Optimisation of treatment of chronic wounds in bullous epidermolysis and the influence of microflora: A literature review

**Yevheniia Fedorets***
Postgraduate Student
Bogomolets National Medical University
01601, 13 Taras Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0000-0002-2282-8728

**Olga Golubovska**
Doctor of Medical Sciences, Professor
Bogomolets National Medical University
01601, 13 Taras Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0000-0003-3455-8718

**Leonid Pinsky**
Doctor of Medical Sciences, Professor
Bogomolets National Medical University
01601, 13 Taras Shevchenko Blvd., Kyiv, Ukraine
https://orcid.org/0000-0002-2120-5887

**Abstract.** The study was conducted to find the most effective approaches to the treatment of chronic wounds in bullous epidermolysis, addressing the influence of the wound microbiome on the healing process. The study reviewed relevant scientific sources in dermatology, pharmacology, genetics, immunology, and microbiology, established the role of the wound microbiome in the healing of chronic wounds in bullous epidermolysis and analysed modern treatment strategies to accelerate the healing of these wounds. The results of the study showed that the wound microflora has a significant impact on the healing process. Epidermolysis bullosa provokes constant skin trauma, contributing to the creation of conditions for the growth of pathogenic bacteria in wounds, which leads to the development of dysbiosis and complicates the healing process. The wound microbiome in bullous epidermolysis is significantly affected by the patient's immune status, antibiotic use, location, depth, and duration of the wound. Weakened immunity provokes the development of multidrug-resistant bacteria, an enhanced immune response and autoimmune processes create conditions for the development of microorganisms that can multiply in conditions characteristic of an inflamed wound. The use of antibiotics is a highly effective method of treating infections, but prolonged or irrational antibiotic therapy can provoke the emergence of resistant bacterial strains. Depending on its location, the wound microbiome is influenced by skin characteristics in different parts of the body, such as the level of moisture, the presence of sweat and sebaceous glands, and hair. The influence of the wound depth on the microbiome is realised by creating conditions for the reproduction of the relevant microorganisms at the appropriate depth. The duration of the wound increases dysbiosis in the wound, complicating healing. Modern treatment strategies to accelerate the healing of chronic wounds in bullous epidermolysis include personalised medicine, an integrated approach and the use of modern dressings. The results obtained indicate the need to research and develop new effective methods of eliminating wound dysbiosis to normalise its microbiome and accelerate the healing process

**Keywords:** healing; pathogenic bacteria; dysbiosis; antibiotics; resistance; personalised medicine

**Suggested Citation**:
Fedorets Ye, Golubovska O, Pinsky L. Optimisation of treatment of chronic wounds in bullous epidermolysis and the influence of microflora: A literature review. Bull Med Biol Res. 2024;6(4):64–75. DOI: 10.63341/bmbr/4.2024.64

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

## INTRODUCTION

Epidermolysis bullosa (EB) is a common name for a group of rare complex genetic pathologies that occur as a result of genetic mutations that lead to a disruption in the structure of proteins responsible for the strength of the connection between different layers of the skin. This makes the skin vulnerable to any damage and leads to the formation of chronic wounds, causing pain that leads to restrictions on physical activity and psychological discomfort that can provoke social isolation. Despite significant advances in medicine, the treatment of EB remains a challenge, as there is still no universal and effective method of healing chronic wounds. However, the development of new technologies in the field of healthcare and an increased understanding of the role of microflora in the healing process was used to review existing therapeutic strategies and analyse the possibilities of optimising the treatment of chronic wounds in EB.

Currently, the main challenge facing doctors and scientists involved in the treatment of EB is to find ways to reduce the time it takes for wounds to heal. To address this issue, C. Guttmann-Gruber *et al.* [1] conducted a randomised, double-blind, placebo-controlled trial to evaluate the effect of low-dose calcipotriol ointment on wound healing in patients with dystrophic epidermolysis bullosa. According to the results, the researchers determined that topical treatment with a low dose of calcipotriol (a vitamin D3 analogue) can significantly reduce wound area and itching on the 14$^{th}$ day of therapy without affecting serum calcium levels. Promising treatments for EB were reviewed by P.-C. Hou *et al.* [2]. The authors assessed the high potential of therapeutic areas aimed at primary genetic abnormalities and secondary inflammatory trace of EB, the effectiveness of which is confirmed at various stages of trials of gene, cell and recombinant protein therapy. The efficacy of topical treatments for EB was investigated by M. Pabón-Carrasco *et al.* [3]. Scientists concluded that, according to the criteria of healing speed and reduction of itching, the most effective topical treatment for EB is Oleogel S10 and allantoin, diacerein 1% followed by fibroblasts, and B-VEC gene therapy.

After reviewing new treatments for EB, N.M. Bermudez *et al.* [4] analysed the effectiveness of symptomatic and gene therapy. Investigating the problems of wound healing, scientists determined that the improvement of this process is effectively achieved by a recently approved treatment method using drugs based on natural compounds of birch bark (*Fisulvez*), which have anti-inflammatory, antioxidant and wound healing effects. Among the gene therapy areas, the drug beremagene geperpavec (*Vyjuvek*) demonstrates high performance, a treatment method aimed at correcting the genetic defect underlying EB. In the above works, based on theoretical and practical research, the authors determined the effectiveness of various methods of wound healing in EB, but the analysis of the impact of wound microflora on the process of wound healing was not carried out. An important problem associated with the search for effective treatments for EB is the complications that arise from damage to the skin. Among the common complications of severe EB, researchers identify anaemia. The difficulty in its treatment was noted by C. Liy-Wong *et al.* [5], who studied the problem and presented the first consensus recommendations for the diagnosis and treatment of this complication. In particular, the authors emphasised the importance of an individual approach to therapy, noted the importance of dietary measures as an important part of the treatment process, and suggested the use of iron supplements orally or by infusion, depending on the severity of anaemia.

Based on the results of a multicentre retrospective clinical analysis, E. Raboei *et al.* [6] identified the most common complications in children with EB. Patients with the recurrent-dystrophic subtype of EB (RDEB) most often had complications associated with skin and musculoskeletal diseases. Oesophageal stenosis and pyloric atresia were less common. The prevalence of otological complications in inverse RDEB type was studied by S.J. Robertson *et al.* [7]. Previous reports indicated a low prevalence among patients in this group, but researchers found that 44% of patients with RDEB had otological complications, 90.9% of whom were diagnosed with recurrent otitis externa. These results demonstrate a higher prevalence rate than previously reported and indicate that this group of patients is at high risk of hearing problems.

In a prospective study of 26 patients with EB, Y. Yavuz *et al.* [8] determined complications in patients that included anaemia, growth retardation, gastrointestinal dysfunction, deformity of hands and nails, hair thinning, vision problems, and kidney disease. The most common among them were dental problems, in particular caries, which indicates the importance of preventive and therapeutic measures aimed at improving the condition of the teeth and oral cavity in patients with EB. In the analysed studies, the authors primarily focused on the identification and methods of effective treatment of complications arising from EB, but they did not include an analysis of how these complications can affect wound healing in patients with this disease.

The study aimed to determine the most effective methods of treatment of chronic wounds in EB, addressing modern medical advances. Study tasks included analysis of the role of wound microflora in the wound healing process and assessing the impact of anaemia, vitamin D3 and zinc levels on wound healing in patients with RDEB.

The study was conducted based on the analysis of thematic scientific sources in dermatology, genetics, pharmacology, microbiology, immunology and regenerative medicine published in PubMed, Google Scholar and Scopus databases. The stages of source selection included the formation of an initial sample, screening by titles and abstracts, detailed analysis of the full text, and assessment of the quality of research. The initial sample was formed from keyword searches: “bullous epidermolysis”, “epidermolysis”, “rare skin diseases”, “chronic wounds”, “wound healing”, “vitamin D3”, “zinc”, “anaemia”, “microflora”, “treatment/therapy”. After screening by titles and abstracts, articles that did not correspond to the research topic were excluded.

A detailed analysis of the full text was conducted with sources that met the inclusion criteria, namely containing the results of theoretical and clinical studies written in Ukrainian or English and published from 2016 to 2024. The quality of trials was determined by assessing clinical relevance – the extent to which results can be useful in clinical practice. The methods of processing the sources included thematic analysis, synthesis, identification of general

trends, and data visualisation for a clear presentation of the results (figure, table). A total of 60 relevant publications were selected for review.

## ROLE OF THE WOUND MICROBIOME IN THE EB HEALING DYNAMICS

Several key factors have contributed to the growing interest in the wound microbiome. The development of DNA sequencing technologies was used to conduct a detailed analysis of the composition of the microbiome, identify new species of microorganisms and study the interaction between them, identify infection markers and detect genes encoding antibiotic resistance factors. The scientific and medical community has grown increasingly aware that the microbiome plays a crucial role in many physiological processes, including immune and metabolic processes, which has further study. An additional impetus for the search for new approaches based on a deep understanding of the mechanisms of wound healing, accounting for the peculiarities of its microbiome, was the decline in the effectiveness of antibiotic therapy caused by the spread of antibiotic-resistant bacterial strains. In 2022, 30% of urinary tract infections and 19% of recurrent wound infections became resistant after antibiotic treatment [9]. This is primarily due to the misuse of these drugs in medicine – up to 50% of all antibiotic prescriptions are clinically unjustified [10]. The development of personalised medicine, which involves an individual approach to patient treatment, was also an impetus to a more detailed study of the microbiome as a tool for developing more accurate diagnostic methods and more effective and safe treatments using its modulation.

The main value of the wound microbiome, which allows it to be used to develop new therapeutic strategies, is its uniqueness, as the wound microflora of a patient with EB differs significantly from that of a healthy person. These differences are due to both the characteristics of the disease itself and the impaired barrier function of the skin. In the wounds of patients with EB, an increased number of pathogenic bacteria (*Staphylococci*, *S. aureus*), fungi (*Candida*) and viruses are often found that can produce toxins that destroy tissue and impede healing [11]. Favourable conditions for their reproduction are provided by the moist environment under the blisters, constant skin trauma that provokes re-infection, impaired immune response and prolonged use of antibiotics, which leads to the development of resistant strains of bacteria. This intensive multiplication of pathogenic microorganisms leads to the displacement of beneficial bacteria, which reduces the diversity of the wound microflora of a patient with EB compared to that of a healthy person.

The imbalance between pathogenic and beneficial bacteria (*dysbiosis*) has a negative impact on the healing process, as it inhibits important functions of beneficial microorganisms, which include the creation of a protective barrier (biofilm formation) aimed at preventing the penetration of pathogens into deeper tissue layers; activation of the immune response; production of substances that promote healing (growth factors, antimicrobial peptides, etc.), stimulating regeneration and regulation of the inflammatory response, which prevents excessive inflammation that can be harmful to healthy tissues [12, 13]. Such functions are performed by certain types of staphylococci, in particular, *Staphylococcus epidermidis*, which are part of the normal skin microflora and can play a protective role by preventing the colonisation of pathogenic microorganisms; certain types of streptococci, which contribute to the production of substances that stimulate regeneration; *Propionibacterium* involved in the production of lactic acid, an important component for inhibiting the growth of pathogenic microorganisms; certain non-pathogenic species of pseudomonas, which produce antimicrobial substances (pseudomonas) with a wide range of antimicrobial activity that inhibit the growth of other bacteria, fungi and some viruses [14, 15]. Dysbiosis caused by genetic features, immune disorders and concomitant diseases characteristic of EB is the main factor in slowing down the healing process and, as a result, chronicity of wounds, so its elimination should be part of the therapeutic strategy for treating this disease. The combination of different wound surface microorganisms and the nature of their interaction depends on many factors, the main ones being the patient's immune status, antibiotic use, location, depth and duration of the wound.

The wound microbiome constantly interacts with the body's immune system, so the immune status of a person significantly affects its composition and functioning, particularly the process of healing chronic wounds in EB. Immunodeficiency provokes a decrease in the body's defence functions, creating favourable conditions for the reproduction of pathogenic microorganisms. This causes an imbalance in the microbiome in favour of pathogens. In this case, opportunistic pathogens are most active – microorganisms that do not usually cause disease in healthy people but can cause infection in people with weakened immune systems. Examples of such pathogens are pneumocystis, candida, aspergillus, etc. In anaerobic conditions, which can occur in deep or poorly drained wounds, anaerobic bacteria, in particular clostridia, actively multiply. Immunodeficiency favours the development of bacterial strains, which makes infection treatment and wound healing much more difficult [16].

An enhanced immune response can provoke chronic inflammation, providing conditions for the growth of bacteria that can survive and multiply in conditions of elevated temperature, low pH, the presence of antimicrobial substances and other adverse factors characteristic of an inflamed wound. In chronic inflammation, an increase in staphylococci, in particular *Staphylococcus aureus* and *Pseudomonas aeruginosa*, is particularly common, as they can form biofilms that protect them from antibiotics and the immune system, facilitating their survival and reproduction [17] (Fig. 1).

Inflammation also increases the number of pseudomonas, which colonise wet wounds and can cause severe infections, *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and other aerobic and facultative anaerobic Gram-negative bacilli [18]. Anaerobic bacteria such as *Clostridium* spp., *Bacteroides* spp. and *Fusobacterium* spp. are commonly found in closed wounds [19].

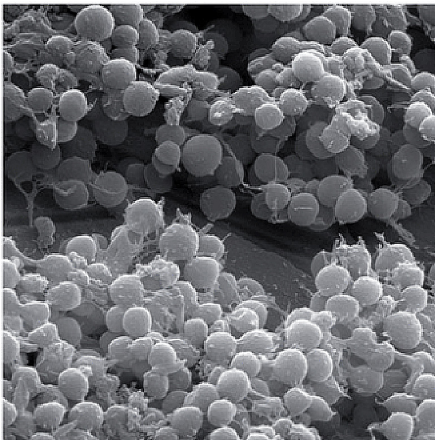

**Figure 1.** An image of *Staphylococcus aureus* biofilms obtained by electron scanning
**Source:** compiled by the authors based on J. Azeredo *et al*. [12]

Although autoimmune diseases and an enhanced immune response are similar in their impact on the wound microbiome, they have significant differences that affect the composition and function of the wound surface microbiota. In autoimmune diseases, inflammation is more specific, while in other causes of an enhanced immune response, it can be more diffuse. The imbalance of the microbiome in the case of activation of autoimmune processes can be more complex and include a greater number of different pathogenic and opportunistic microorganisms, including staphylococci, in particular *Staphylococcus aureus*, streptococci, *Escherichia coli*, pseudomonas, including *Pseudomonas aeruginosa*, anaerobic bacteria and fungi of the *Candida* genus [20]. In addition to the colonisation of the wound by pathogens, wound healing in autoimmune diseases can be complicated by the development of repeated infections and the formation of severe scars.

Identification of the differences between the wound microbiome in immunocompromised, immune-enhanced and autoimmune conditions is an important step in developing effective strategies for the treatment of chronic wounds in EB. Antibiotics are an important tool in the treatment of infections, but their use can have both positive and negative effects on the wound microbiome and the healing process. First, antibiotics effectively destroy pathogenic microorganisms, helping to reduce inflammation, and pain and accelerate healing. The timely use of these drugs helps prevent the spread of infection, the development of sepsis and other complications. At the same time, prolonged or irrational use of antibiotics can cause dysbiosis, promoting the growth of resistant strains of bacteria, fungi and other pathogens and thus disrupting normal wound healing processes, including epithelialisation, angiogenesis and collagen synthesis [21].

Wound localisation is one of the key factors affecting the wound microbiome and the healing process in EB. Different areas of the body have their unique microflora, due to the characteristics of the skin, hair, sweat and sebaceous glands, and the level of hydration. The head and neck areas have a rich microflora, including both resident and transient microorganisms. A breach of skin integrity in these areas can lead to wound colonisation by staphylococci, streptococci and *Candida* fungi [22]. The torso skin usually has a lower number of microorganisms on its surface compared to the skin of the head, neck and extremities. However, in patients with EB, torso wounds can be colonised by both local and exogenous microorganisms (staphylococci, streptococci, pseudomonas, E. coli, anaerobic bacteria, *Candida* fungi) [23]. The skin of the extremities, especially the hands and feet, is exposed to constant mechanical stress and contact with various surfaces. This contributes to the colonisation of wounds by gram-negative bacteria, in particular *Pseudomonas aeruginosa* [24]. Wounds in the skin folds create favourable conditions for the growth of anaerobic bacteria that can cause deep infections [25].

The location of the wounds determines the presence of factors that affect their healing, including the level of hydration, mechanical stress, the presence of hair and the intensity of blood supply. Wet wounds in the skin folds or in areas of constant friction heal more slowly due to maceration and increased risk of infection. These areas include the groin and groin area in general, the popliteal fossae, the interdigital space, especially on the feet, the behind the ears, the folds of the abdominal skin and the area under the breasts. Wounds on body parts subjected to constant mechanical stress (extremities, especially feet and palms, elbows, knees, armpits, places of fixation of medical devices – catheters, drains, plasters, etc. Hair in the wound area complicates its cleaning and creates conditions for the development of infection. Areas of the body with insufficient blood supply (lower extremities, pressure ulcers, areas with large scars, diabetic angiopathy and areas where vascular surgery was performed) heal more slowly due to insufficient oxygen, nutrient deficiencies and slow removal of waste products.

The depth of the wound is a substantial factor that affects its microbiome, as different wound depths create different conditions for the development of microorganisms and affect the speed and quality of tissue regeneration. Superficial wounds are usually colonised by microorganisms that are part of the normal skin microflora, such as staphylococci, streptococci and other gram-positive bacteria, and therefore heal faster and with less risk of complications if they are clean and uninfected [26]. Deep wounds have less access to oxygen, which creates conditions for the growth of anaerobic bacteria (clostridia, bacteroides, etc.) and the development of serious infections [27]. Deep wounds take longer to heal and are sometimes accompanied by complications, the most common of which are infection, bleeding and scarring. The subcutaneous wound microbiome can include aerobic bacteria and fungi. Damage to the subcutaneous tissue is dangerous due to the formation of abscesses and requires surgical intervention.

The duration of a wound has a significant impact on its microbiome and the healing process. The longer the wound exists, the more complicated the processes occurring in it become. In the initial stages, the wound is usually colonised by microorganisms that are part of the normal skin microflora – staphylococci, streptococci and other gram-positive bacteria. Over time, the composition of the microflora changes, and chronic wounds create conditions for the development of more resistant and aggressive microorganisms (pseudomonas, anaerobic bacteria and fungi)

that can form biofilms that protect them from antibiotics and the immune system [28]. Therefore, the healing of chronic wounds takes longer and is accompanied by complications due to impaired regeneration processes and constant tissue irritation. Chronic wounds in EB are characterised by changes in the microenvironment (pH, temperature and oxygen tension), impaired regeneration processes due to the impact on the proliferation and migration of cells necessary for healing, and the formation of scar tissue. In a study conducted by N. Harris *et al.* [29], the average time to closure for chronic wounds was 14.6 weeks compared to recurrent wounds (8 weeks), and spontaneous closure was observed in 26% of chronic wounds, while the same percentage for recurrent wounds was 86.

It is important to note that wound microbiomes may differ depending on the subtypes of EB. Although all forms of EB are characterised by increased skin fragility and a tendency to blistering, certain features affect the composition of the microflora in wounds. For instance, RDEB, the most common subtype of EB, which in some studies reaches 70% of all cases in a study by C.I. Thien *et al.* [30], is usually characterised by deeper and more extensive skin lesions, which can create conditions for the colonisation of a more diverse and potentially pathogenic microflora. In RDEB, colonisation of the wound by fungi, viruses and anaerobic bacteria capable of penetrating deeper layers of the skin is observed, compared to other subtypes of EB, which are mostly superficial infections caused by gram-positive cocci (staphylococci, streptococci) [31]. Due to the prolonged use of antibiotics and the presence of chronic wounds, antibiotic resistance can develop in the microflora of RDEB, while in other types of this pathology, resistance may be less pronounced, as infections are more often acute and treated faster. This is attributed to the fact that the microorganisms of the wound microbiome in RDEB have a higher tendency to form biofilms compared to other subtypes of EB [32]. The difference between the impact on the wound microbiome, depending on the subtypes of BE, is also evident through the association with other diseases. RDEB is often accompanied by atopic dermatitis, which has a negative impact on the wound microbiome, increasing the number of pathogenic microorganisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Candida*) and provoking dysbiosis [33].

In a prospective study, A. Horev *et al.* [34] analysed temporary changes in the skin microbiome of patients with EB after the use of wound dressings. The authors emphasised the importance of determining the characteristics of the skin microbiome of patients with EB to determine the pathophysiology of the processes that occur in it and the factors that affect them. Practical confirmation of this thesis was demonstrated by the results of a study by scientists who found that a decrease in the diversity of bacteria in the wound microbiome due to the potential impact of a topical dressing accelerated the healing process. The authors' findings are notable, and it is worth emphasising the importance of monitoring temporary changes in the microbiome, depending on different treatment methods, to optimise the treatment of chronic wounds in EB.

Based on the results of a case-control study, J. Bar *et al.* [35] provided evidence of skin dysbiosis in patients with dystrophic BE. In addition to the prevalence of staphylococcal species in the affected skin of patients with EB, the results showed that the unaffected skin of patients also had an increased content of staphylococci and significantly different microbial diversity compared to the skin of healthy people. This observation indicates the existence of the influence of EB not only on the wound microflora but also on the microflora of the patient's skin in general. These results indicate the need to expand the impact on the signature of the microbiome associated with dystrophic EB employing specific pathogen-directed therapy. The authors' findings regarding the presence of wound dysbiosis in patients with a dystrophic type of EB are noteworthy, but additional studies should be conducted to confirm the presence of dysbiosis in the intact skin of patients. Understanding the processes occurring in the wound microbiome under the influence of EB and recording the differences inherent in different subtypes of this disease will allow timely identification of the pathogens present, which will help avoid severe complications such as sepsis and select more effective treatment regimens for chronic wounds.

## MODERN THERAPEUTIC STRATEGIES TO ACCELERATE CHRONIC WOUND HEALING IN EB

Current trends in the treatment of chronic wounds in EB include 3 main areas: personalised medicine, an integrated approach and the use of modern dressings. The use of each of them separately in medical practice makes it possible to improve the effectiveness of treatment, and their rational combination allows for optimising the treatment process in general and improving the quality of life of patients with this disease. An additional and important area of research in the field of EB is the study of new therapeutic strategies of gene and mRNA therapy, the use of stem cells and other promising treatments aimed at eliminating the causes (correction or modification of mutated genes) and consequences (repair of damaged skin) of this disease. Difficulties in developing therapeutic strategies for the treatment of chronic wounds in EB are primarily related to the rarity of the disease. A study by German authors found that the overall incidence of EB in the country is 45 per million newborns, of which 15.58 live births occurred in RDEB [36]. Despite its low prevalence, this genetic disease is an important problem for the healthcare industry in general and significantly worsens the quality of life of each patient.

Personalised medicine in the context of EB involves an individual approach to each patient based on knowledge of the subtype of this genetic pathology, the patient's immune status, location, depth of lesions, presence of comorbidities, the effectiveness of previous therapy, including antibiotic therapy, etc. According to this principle, treatment is planned accounting for the individual wound microbiome, genetic characteristics of the patient and the patient's general health. The wound microbiome, as a complex ecosystem of microorganisms that change dynamically depending on many of the above factors, controls both positive (accelerating healing, protecting against pathogens, modulating the immune response) and negative (chronic wound healing, developing infections, sepsis) processes in the wound. Since negative processes occur because of dysbiosis, the main tasks of therapy are to study the microbiome and eliminate dysbiosis, taking into account the factors that cause it.

The development of medical technologies has expanded the range of methods that allow for a detailed study of the composition and interaction of microorganisms in the wound microbiome. Nowadays, in addition to the classical culture method of sowing on nutrient media, which has significant limitations since not all microorganisms can grow on artificial nutrient media, there are more accurate and informative methods for studying the microbiome [37]. Molecular genetic methods include the polymerase chain reaction (PCR) test, which allows the detection of DNA or RNA of specific microorganisms in a sample; next-generation sequencing (NGS), the most informative method that allows determining the full range of microorganisms in a sample without prior cultivation; metagenomics – the study of the totality of genomes of all microorganisms in a sample; metatranscriptomics – the study of the totality of all RNAs, which can be used to assess the activity of microbial genes; metaproteomics – the study of the totality of all proteins, which allows assessing the functional activity of the microbiome [38]. Mass spectrometry is used to identify biomarkers associated with certain types of microbiomes [39]. Different types of microscopies exist depending on the research objectives. Light microscopy is used to assess the morphology of microorganisms, fluorescence microscopy is used to visualise biofilms and the interaction of microorganisms with host cells, and electron microscopy is used to study the ultrastructure of microorganisms in detail [40]. The choice of method depends on the purpose of the study (detection of a specific pathogen, assessment of microbiome diversity, analysis of the functional activity of microorganisms, etc. Molecular genetic methods currently demonstrate the best performance. They have high sensitivity, which allows the detection of even a small number of microorganisms and are characterised by informative and fast analysis [41].

There is an increasing number of studies investigating the potential of exudates in personalised wound care [42]. P. Doerfler *et al.* [43] studied more than 800 exudates from healing and non-healing wounds and found a strong correlation (76%-90%) between wound chronicity and the inhibitory effect of individual exudates on fibroblast proliferation. The validity of the scientists' observation was confirmed by testing wound healing products, the results of which were consistent with clinical experience. In the context of a personalised approach to the treatment of chronic wounds in EB, the use of autologous keratinocyte transplants with gene correction (EB-101) demonstrates efficacy and safety. The results of the second phase of the randomised controlled trial are currently available and the results of the third phase are expected [44]. The main advantage of autologous keratinocyte transplants is their long-term efficacy and the absence of serious side effects during 5.9 years of follow-up.

Depending on the level of wound dysbiosis in EB, local therapy, systemic therapy, physiotherapy, surgery, vacuum therapy, plasmolifting, or an integrated approach combining these methods can be used to eliminate it. Topical therapy uses antiseptic solutions, antibiotic ointments and gels, antifungal drugs, and wet and honey dressings [45]. Phage therapy is a promising area in the fight against bacterial infections, especially antibiotic-resistant ones. It is based on the use of bacteriophages – viruses that infect and destroy individual bacteria [46]. The advantages of phage therapy are its high specificity (each phage infects a specific type of bacteria, which allows targeted treatment of the pathogen without damaging the beneficial microflora) and permeability (they can penetrate biofilms and destroy bacteria from the inside). Bacteriophages can be used in the form of ointments, gels, solutions for washing and as part of dressings. To restore the balance of the microbiome, systemic therapy involves the use of antibiotics prescribed for severe infections that cannot be treated locally, immunomodulators aimed at correcting the immune response, and probiotics to help restore normal intestinal and skin microflora [47]. Among the physiotherapeutic methods that can be used to eliminate dysbiosis, the most effective are laser therapy, which has anti-inflammatory and bactericidal effects, and ultrasound therapy, which aims to soften necrotic tissue and improve blood circulation [48]. Surgical treatment is used when necessary to remove necrotic tissue to create a clean bed for healing and skin grafting to repair visible defects [49]. Vacuum therapy can be used to ensure the outflow of exudate and stimulate healing, and autoplasma injection (plasmolifting) can be used to stimulate tissue regeneration [50]. Each of these methods has its advantages and disadvantages (Table 1), which can be used to optimise the treatment of chronic wounds in EB.

**Table 1.** Advantages and disadvantages of the main methods of treatment of chronic wounds in EB

| Treatment method | Advantages | Disadvantages | Side effects | Price |
|---|---|---|---|---|
| Local therapy | Availability, ease of use | Insufficiently effective in severe EB, possibility of allergic reactions | Redness, itching, dryness of the skin | Relatively low |
| Systemic therapy | Effective in many forms of EB | Serious side effects (immunodeficiency, osteoporosis), high risk of infections | Immunosuppression, increased susceptibility to infections, growth retardation in children | High |
| Physiotherapy methods | An additional method of treatment promotes wound healing, reduces pain | Not a standalone treatment, effectiveness may be limited | Burns, redness of the skin | Average |
| Surgical treatment | An effective method for removing large blisters, restoring the skin | Invasiveness, risk of complications (infection, bleeding), long rehabilitation period | Pain, scarring, risk of transplant rejection | High |

*Continued Table 1.*

| Treatment method | Advantages | Disadvantages | Side effects | Price |
|---|---|---|---|---|
| Vacuum therapy | Promotes wound healing, stimulates blood circulation | Can be painful, requires special equipment | Skin redness, bleeding | Average |
| Plasmolifting | Stimulates tissue regeneration, reduces inflammation | Efficacy is not sufficiently studied, high cost, possible allergic reactions | Pain at the injection site, haematomas | High |

**Notes:** the effectiveness is assessed by the speed of wound healing, pain relief, and improvement of quality of life; the most common side effects are listed; the relative cost of treatment is indicated for comparison (the exact cost may vary significantly depending on the country, clinic, and treatment method chosen)
**Source:** compiled by the author based on M. Titeux *et al.* [45], G.A. Suh *et al.* [46] and J. Riedl *et al.* [47]

A personalised approach to the treatment of chronic wounds in EB, accounting for the patient's genetic characteristics, can be used to determine the causes of the disease more accurately, select the optimal treatment methods and predict its effectiveness. Determining the patient's genetic characteristics defines the causes of EB, as each mutation provokes different phenotypes of the disease, which requires different treatment approaches. Genetic factors can also determine a patient's response to various types of therapy, allowing for the planning of an optimal treatment strategy in advance, avoiding complications (infections, scarring, malignant tumours), and predicting its outcome. Currently, the main areas of genetic research used in personalised medicine for the treatment of EB are pharmacogenetics – the study of the influence of genetic factors on the effectiveness of drugs, the development of gene therapy methods to correct mutations that cause the disease, and regenerative medicine based on the use of stem cells to repair damaged tissues [48]. The effectiveness of medicines in the treatment of EB can vary significantly depending on the genetic characteristics of each patient. The influence of these characteristics can be realised through changes in the metabolic rate due to variations in the genes encoding the enzymes involved in this process, the tendency to develop comorbidities and individual sensitivity to therapy. Immunosuppressants (corticosteroids, azathioprine, mycophenolate mofetil, cyclosporine) and biological drugs (tumour necrosis factor α – TNF-α inhibitors, interleukin-1 inhibitors, integrin inhibitors) have the highest variability in effectiveness [49].

Planning the treatment of chronic wounds in EB, addressing the patient's genetic characteristics, involves several stages. The first stage involves genetic testing to determine the type of mutation. On its basis, an individual treatment strategy is developed, which includes the selection of optimal methods of local therapy, selection of systemic therapy, determination of the need for surgery and development of a rehabilitation programme. Regular monitoring of the patient's condition and, if necessary, adjustments to the treatment plan are mandatory throughout the treatment period [50]. Currently, the development of drugs based on the principles of gene therapy is ongoing. One of the most famous is Vyjuvek (B-Vec) for the treatment of dystrophic E. It contains a copy of a healthy gene encoding the protein collagen type XVII, an important component of the skin, the absence of which leads to the development of dystrophic E. To deliver the gene to cells, an adenoviral vector is used, i.e. a weakened virus that does not cause the disease but effectively delivers genetic material to cells. Vyjuvek (B-Vec) is designed as a topical medication and is applied directly to the affected skin. The introduced gene begins to produce type XVII collagen, which helps to strengthen the skin and reduce the formation of blisters [51].

An important part of planning the treatment of chronic wounds in EB based on the principle of personalised medicine is the assessment of the patient's general health and, depending on it, the correction of treatment. The course of EB can be affected by concomitant diseases and vitamin and mineral deficiencies. Due to permanent skin damage and impaired barrier function, patients with EB most often face deficiencies of vitamin D3, iron and zinc. The main factor behind this deficiency is impaired absorption of nutrients in the intestine due to the large surface area of the wound on the body, which increases the body's need for vitamins and minerals to ensure an active healing process.

Vitamin D3 is central in many physiological processes in the body, including the immune response, cell proliferation and angiogenesis, which is why its deficiency is often observed in patients with chronic skin diseases. The effect of this vitamin on wound healing is realised in several ways [52]. It activates receptors that stimulate epithelial cell division (proliferation), thereby accelerating the healing process. Additionally, it has an anti-inflammatory effect by regulating the activity of immune cells, which helps to avoid scarring during healing. The vitamin also possesses antimicrobial properties, which help to prevent wound infection, and it stimulates the formation of new blood vessels (angiogenesis), improving blood circulation in the wound and providing it with the necessary nutrients.

Vitamin D3 deficiency is often observed in patients with RDEB, as most of them try to avoid sunlight, which is the main source of this vitamin in the body, due to skin sensitivity. At the same time, as a result of constant damage and chronic inflammation, vitamin D3 is synthesised less efficiently and excreted faster. The situation is further complicated by the comorbidities that patients in this group have (e.g., celiac disease), which can further disrupt the absorption of this vitamin [53]. Elimination or correction of vitamin D3 deficiency in patients with BE is carried out by including foods rich in vitamin D in their diet (fatty fish, egg yolk, dairy products, etc.), sunbathing under medical supervision, and prescription of vitamin D3 supplements in an individually selected dosage.

Iron deficiency in EB occurs due to the depletion of iron stores in the body due to constant skin damage chronic bleeding and impaired absorption caused by

inflammatory processes in the intestine. A decrease in iron levels slows down the production of haemoglobin, leading to a decrease in the number of red blood cells and contributing to the development of anaemia. Its effect on wound healing is to reduce tissue oxygenation, which provokes a slowdown in energy processes, weakening of the immune system, delayed cell proliferation, and disruption of collagen synthesis, the main connective tissue protein that provides strength and elasticity to the skin [54]. Treatment of anaemia should begin with determining its type (iron-deficiency, B12-deficiency, folic acid-deficiency). Accordingly, iron therapy or vitamin therapy is prescribed, and a diet is developed that includes iron-rich foods (low-fat red meat such as beef, lamb, pork, dark poultry such as turkey, chicken, rabbit meat, beef and chicken liver, legumes, green leafy vegetables), with vitamin C supplemented to improve iron absorption from food. In severe cases, blood transfusion may be used. Similarly to vitamin D3 deficiency, anaemia is particularly common and acute in patients with RDEB. It is influenced by a combination of factors, including chronic bleeding, comorbidities, including inflammatory bowel disease, and impaired absorption of vitamin B12 and folic acid.

Like most trace elements in the human body, zinc is central to many physiological processes, including wound healing. It stimulates the proliferation of cells, in particular keratinocytes, which are involved in skin repair, enhances collagen synthesis, strengthens the immune system, helps maintain the integrity of cell membranes to preserve cellular functionality, and has antioxidant properties that help protect cells from free radical damage [55]. Depending on the severity of the type of BE, zinc deficiency is also more acute, so patients with RDEB are the most sensitive to a decrease in zinc levels. The process of eliminating its deficiency has the same strategy as for most other trace elements – developing a diet with the inclusion of foods rich in zinc (seafood, beef, lamb, pork, dark poultry meat – chicken, turkey, nuts, legumes), prescribing zinc preparations in the form of tablets or capsules, taking into account the required dosage, and monitoring its blood level to assess the effectiveness of treatment and timely dosage adjustment. Considering the wound microbiome, the patient's genetic characteristics and general health allows to increase the effectiveness of treatment of chronic wounds in EB, using an integrated approach, for example, combining local and systemic therapy or surgical methods and physiotherapy procedures.

Modern therapeutic strategies to improve the effectiveness of local treatment of chronic wounds in EB are aimed at the development and use of dressings that provide optimal conditions for healing. The main requirements for such materials are softness and elasticity, high hygroscopicity, air permeability, and antibacterial properties, ensuring painless dressing changes [56]. Following these requirements, modern dressings have been developed that are effectively used in the treatment of EB. Hydrocollagen dressings create a moist environment, promote the detachment of necrotic tissue and stimulate healing. Hydrogel dressings contain a large amount of water, which helps to moisten the wound and reduce pain during healing. Silver-containing dressings have antibacterial properties and prevent wound infection. Polyurethane foam dressings absorb a large amount of exudate, ensuring long-term use. Alginate dressings made from algae also effectively absorb exudate and create a moist environment that is optimal for healing. Thin, transparent dressing films, called "films" because of the material properties that allow for observation of the healing process, protect the wound from external influences and allow for visual assessment of tissue repair dynamics [57, 58]. When choosing a particular type of material, the stage of wound healing (acute, subacute, chronic), wound depth (superficial, deep), the presence of infection (absent, mild, moderate, severe) and the amount of exudate (dry wound, moderately moist, very moist) should be addressed.

In the context of the study of dressings, the study by M. Nita *et al.* [59], in which scientists reviewed the latest treatments for EB, including the use of the BIOOPA dressing, is noteworthy. The authors analysed the achievements in the field of tissue engineering, which was used to create a material that mimics the structure and natural healing process and can be effectively used to treat chronic wounds, especially in EB. Among the advantages of the modern BIOOPA dressing system, the scientists pointed out the possibility of creating an optimal moist environment for healing, providing protection against infections, reducing pain, resistance to maceration, and ease of application and removal. The authors' conclusions regarding the effectiveness of using the BIOOPA dressing for the local treatment of chronic wounds are notable, as they meet the requirements for dressings specified in this study.

Integrated treatment strategies for EB were investigated by H. Sait *et al.* [60]. The authors' study and the present study have a common problem related to the difficulty in identifying a single approach to wound care in EB. The results of both studies coincide with the importance of comprehensive therapy, and their differences are related to the view of the prospects of the direction of treatment. H. Sait *et al.* [60] expect promising approaches of gene, mRNA and cell therapy aimed at the main cause of the disease, while this paper, in addition to the importance of researching ways to eliminate or correct genetic mutations that lead to severe skin diseases, emphasises the need for improved treatments to alleviate the symptoms and consequences associated with chronic wounds (pain, infection, scarring). The wide range of scientific interests and the increasing technical and information potential of the medical industry make it possible to develop both areas with equal intensity. This will help improve the quality of life of patients with different types of EB now and avoid severe consequences of this disease in the future.

## CONCLUSIONS

The diversity and interaction of microorganisms in the wound microbiome allow it to be used in the development of therapeutic strategies for the treatment of chronic wounds in EB. The moist environment under the blisters and constant skin trauma creates favourable conditions for the growth of pathogenic bacteria in the wounds of patients with EB, provoking dysbiosis, which slows down the healing process.

The main factors affecting the wound microbiome in EB are the patient's immune status, antibiotic use, location, depth and duration of the wound. A weakened immune system leads to the development of multidrug-resistant bacteria, making it difficult to treat infections and slowing down the healing process. An enhanced immune response and

the presence of autoimmune diseases provoke chronic inflammation, promoting the growth of staphylococci, pseudomonas, *Escherichia coli*, *Klebsiella*, *Proteus*, *Clostridium*, bacteroides, fusobacteria, *Candida*, etc. Antibiotic therapy is a traditional method of treating infections, but its irrational use can lead to the development of resistant strains of pathogens, complicating the wound-healing process. The microbiome differs in different parts of the body due to the characteristics of the skin, sweat and sebaceous glands, its moisture level and the presence of hair. Depending on the depth of the wound, appropriate conditions are created for the growth of certain microorganisms, so superficial and deep wounds have significant differences in their microbiome. The composition of the wound microflora is also influenced by the duration of its existence – an increase in duration increases dysbiosis, complicating the healing process.

The main directions in the treatment of chronic wounds in EB are personalised medicine, an integrated approach and the use of modern dressings. The area of personalised medicine is based on the study of the individual wound microbiome, understanding of genetic characteristics and assessment of the overall health of each patient. An integrated approach to therapy allows combining different methods of treating EB to increase their effectiveness. Modern dressing materials include hydrocolloid, hydrogel, silver-containing, polyurethane foam, alginate dressings and thin transparent films (films). Based on the results of the study, the methods of personalised medicine, an integrated approach and the use of modern dressings can be recommended for involvement in the clinical practice of a dermatologist to work with patients with EB.

The limitation of this review was the difficulty of assessing the long-term effects of chronic wound treatment in EB due to the short duration of follow-up in most studies on this disease. Given the difficulty of healing chronic wounds in EB, it is worth continuing to research methods of treating this disease in two directions: correcting mutated genes to eliminate the causes of increased skin vulnerability and finding methods to eliminate wound dysbiosis based on the study of its microbiota.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] Guttmann-Gruber C, Hofbauer JP, Tockner B, Reichl V, Klausegger A, Hofbauer P, et al. Impact of low-dose calcipotriol ointment on wound healing, pruritus and pain in patients with dystrophic epidermolysis bullosa: A randomized, double-blind, placebo-controlled trial. Orphanet J Rare Dis. 2021;16:473. DOI: 10.1186/s13023-021-02062-2
[2] Hou PC, Wang HT, Abhee S, Tu WT, McGrath JA, Hsu CK. Investigational treatments for epidermolysis bullosa. Am J Clin Dermatol. 2021;22:801–17. DOI: 10.1007/s40257-021-00626-3
[3] Pabón-Carrasco M, Caceres-Matos R, Roche-Campos M, Hurtado-Guapo MA, Ortiz-Romero M, Gordillo-Fernández LM, et al. Management of skin lesions in patients with epidermolysis bullosa by topical treatment: Systematic review and meta-analysis. Healthcare. 2024;12(2):261. DOI: 10.3390/healthcare12020261
[4] Bermudez NM, Warp PV, Hargis A, Yaghi M, Schachner L. Epidermolysis bullosa: A review of wound care and emerging treatments. Cur Dermatol Rep. 2024;13:123–31. DOI: 10.1007/s13671-024-00437-9
[5] Liy-Wong C, Tarango C, Pope E, Coates T, Bruckner AL, Feinstein JA, et al. Consensus guidelines for diagnosis and management of anemia in epidermolysis bullosa. Orphanet J Rare Dis. 2023;18:38. DOI: 10.1186/s13023-022-02448-w
[6] Raboei E, Alabdali A, Owiwi Y, Yousef Y, Alsaggaf A, Bustanji N, et al. Overview of complications associated with epidermolysis bullosa: A multicenter retrospective clinical analysis of 152 cases. J Pediatr Surg. 2021;56(12):2392–8. DOI: 10.1016/j.jpedsurg.2021.05.023
[7] Robertson SJ, Prodinger C, Liu L, Skilbeck C, Petrof G, Martinez AE, et al. Otological complications in inversa type recessive dystrophic epidermolysis bullosa. Clin Exp Dermatol. 2022;47(4):717–23. DOI: 10.1111/ced.15029
[8] Yavuz Y, An I, Yazmaci B, Akkus Z, Ortac H. Evaluation of clinical and oral findings in patients with epidermolysis bullosa. Medicina. 2023;59(7):1185. DOI: 10.3390/medicina59071185
[9] Stracy M, Snitser O, Yelin I, Amer Y, Parizade M, Katz R, et al. Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. Science. 2022;375(6583):889–94. DOI: 10.1126/science.abg9868
[10] Bassetti S, Tschudin-Sutter S, Egli A, Osthoff M. Optimizing antibiotic therapies to reduce the risk of bacterial resistance. Eur J Intern Med. 2022;99:7–12. DOI: 10.1016/j.ejim.2022.01.029
[11] Reimer-Taschenbrecker A, Künstner A, Hirose M, Hübner S, Gewert S, Ibrahim S, et al. Predominance of *Staphylococcus* correlates with wound burden and disease activity in dystrophic epidermolysis bullosa: A prospective case-control study. J Investig Dermatol. 2022;142(8):2117–27. DOI: 10.1016/j.jid.2022.01.020
[12] Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T, Costa AR, et al. Critical review on biofilm methods. Critic Rev Microbiol. 2016;43(3):313–51. DOI: 10.1080/1040841X.2016.1208146
[13] Marvasi M, Monici M, Pantalone D, Cavalieri D. Exploitation of skin microbiota in wound healing: Perspectives during space missions. Front Bioeng Biotechnol. 2022;10:873384. DOI: 10.3389/fbioe.2022.873384
[14] White EK, Grice EA. The wound microbiome. Cold Spring Harbor Perspect Biol. 2023;15:a041218. DOI: 10.1101/cshperspect.a041218
[15] Żółkiewicz J, Marzec A, Ruszczyński M, Feleszko W. Postbiotics – a step beyond pre- and probiotics. Nutrients. 2020;12(8):2189. DOI: 10.3390/nu12082189
[16] Rajer F, Sandegren L. The role of antibiotic resistance genes in the fitness cost of multiresistance plasmids. mBio. 2022;13(1):e03552-21. DOI: 10.1128/mbio.03552-21

[17] Seebach E, Kraus FV, Elschner T, Kubatzky KF. Staphylococci planktonic and biofilm environments differentially affect osteoclast formation. Inflammat Res. 2023;72:1465–84. DOI: 10.1007/s00011-023-01745-9
[18] Liu C, Ponsero AJ, Armstrong DG, Lipsky BA, Hurwitz BL. The dynamic wound microbiome. BMC Med. 2020;18:358. DOI: 10.1186/s12916-020-01820-6
[19] Tipton CD, Wolcott RD, Sanford NE, Miller C, Pathak G, Silzer TK, et al. Patient genetics is linked to chronic wound microbiome composition and healing. PLoS Pathog. 2020;16(6):e1008511. DOI: 10.1371/journal.ppat.1008511
[20] Suárez LJ, Garzón H, Arboleda S, Rodríguez A. Oral dysbiosis and autoimmunity: From local periodontal responses to an imbalanced systemic immunity. A review. Front Immunol. 2020;11:591255. DOI: 10.3389/fimmu.2020.591255
[21] Singh A, Amod A, Pandey P, Bose P, Pingali MS, Shivalkar S, et al. Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. Biomed Mat. 2022;17(2):022003. DOI: 10.1088/1748-605x/ac50f6
[22] Kunimitsu M, Nakagami G, Kitamura A, Minematsu T, Koudounas S, Ogai K, et al. Relationship between healing status and microbial dissimilarity in wound and peri-wound skin in pressure injuries. J Tissue Viability. 2022;32(1):144–50. DOI: 10.1016/j.jtv.2022.10.006
[23] Tang Q, Xue N, Ding X, Tsai KH, Hew JJ, Jiang R, et al. Role of wound microbiome, strategies of microbiota delivery system and clinical management. Adv Drug Del Rev. 2023;192:114671. DOI: 10.1016/j.addr.2022.114671
[24] Campbell A, Bae J, Hein M, Hillis SL, Rebeck ON, Rakel BA, et al. The heterogeneous wound microbiome varies with wound care pain, dressing type, and inflammatory gene expression. Wound Repair and Regen;2024;32(6):811–25. DOI: 10.1111/wrr.13184
[25] Durand BA, Pouget C, Magnan C, Molle V, Lavigne JP, Dunyach-Remy C. Bacterial interactions in the context of chronic wound biofilm: A review. Microorganisms. 2022;10(8):1500. DOI: 10.3390/microorganisms10081500
[26] Yang Y, Huang J, Zeng A, Long X, Yu N, Wang X. The role of the skin microbiome in wound healing. Burns Trauma. 2024;12:tkad059. DOI: 10.1093/burnst/tkad059
[27] Shibata K, Ogai K, Ogura K, Urai T, Aoki M, Arisandi, D, et al. Skin physiology and its microbiome as factors associated with the recurrence of pressure injuries. Biol Res Nurs. 2021;23(1):75–81. DOI: 10.1177/1099800420941100
[28] Thaarup IC, Iversen AKS, Lichtenberg M, Bjarnsholt T, Jakobsen TH. Biofilm survival strategies in chronic wounds. Microorganisms. 2022;10(4):775. DOI: 10.3390/microorganisms10040775
[29] Harris N, Fulchand S, Nazaroff J, Li S, Tang JY. Natural history of spontaneous wound healing in recessive dystrophic epidermolysis bullosa wound types using a mobile photography application. J Investig Dermatol. 2023;143(5):S260. DOI: 10.1016/j.jid.2023.03.1534
[30] Thien CI, Bessa VR, Miotto IZ, Samorano LP, Rivitti-Machado MC, de Oliveira ZNP. Hereditary epidermolysis bullosa: Clinical-epidemiological profile of 278 patients at a tertiary hospital in São Paulo, Brazil. Braz Ann Dermatol. 2024;99(3):380–90. DOI: 10.1016/j.abd.2023.06.009
[31] Nyström A, Bruckner-Tuderman L, Kiritsi D. Dystrophic epidermolysis bullosa: Secondary disease mechanisms and disease modifiers. Front Gen. 2021;12:737272. DOI: 10.3389/fgene.2021.737272
[32] Kondo M, Takashima S, Goto H, Habe K, Natsuga K, Yamanaka K. Dominance of methicillin-resistant *Staphylococcus aureus* in a Japanese infant with recessive dystrophic epidermolysis bullosa. Case Rep Dermatol. 2021;13(2):278–81. DOI: 10.1159/000516354
[33] Chua C, Sethi R, Ong J, Low JH, Yew YW, Tay A, et al. Late inflammatory monocytes define circulatory immune dysregulation observed in skin microbiome-stratified atopic dermatitis. J Dermatol. 2023;112(3):158–61. DOI: 10.1016/j.jdermsci.2023.10.006
[34] Horev A, Brandwein M, Vaknin A, Motro Y, Moran-Gilad J. Temporal changes in the skin microbiome of epidermolysis bullosa patients following the application of wound dressings. J Clin Med. 2023;12(20):6435. DOI: 10.3390/jcm12206435
[35] Bar J, Sarig O, Lotan-Pompan M, Dassa B, Miodovnik M, Weinberger A, et al. Evidence for cutaneous dysbiosis in dystrophic epidermolysis bullosa. Clin Exp Dermatol. 2021;46(7):1223–9. DOI: 10.1111/ced.14592
[36] Has C, Hachem ME, Bučková H, Fischer P, Friedová M, Greco C, et al. Practical management of epidermolysis bullosa: Consensus clinical position statement from the European Reference Network for Rare Skin Diseases. J Eur Acad Dermatol Venereol. 2021;35(12):2349–60. DOI: 10.1111/jdv.17629
[37] Forss R, Hugman Z, Ridlington K, Radley M, Henry-Toledo E, O'Neill B. Does the application of a semiocclusive dressing alter the microflora of healthy intact skin on the foot? J Am Podiatr Med Associat. 2021;111(1):e01. DOI: 10.7547/18-141
[38] Mudrik-Zohar H, Carasso S, Gefen T, Zalmanovich A, Katzir M, Cohen Y, et al. Microbiome characterization of infected diabetic foot ulcers in association with clinical outcomes: Traditional cultures versus molecular sequencing methods. Front Cell Infect Microbiol. 2022;12:836699. DOI: 10.3389/fcimb.2022.836699
[39] Bauermeister A, Mannochio-Russo H, Costa-Lotufo LV, Jarmusch AK, Dorrestein PC. Mass spectrometry-based metabolomics in microbiome investigations. Nature Rev Microbiol. 2022;20:143–60. DOI: 10.1038/s41579-021-00621-9
[40] Hatzenpichler R, Krukenberg V, Spietz RL, Jay ZJ. Next-generation physiology approaches to study microbiome function at single cell level. Nature Rev Microbiol. 2020;18:241–56. DOI: 10.1038/s41579-020-0323-1
[41] Schmidt BM. Emerging diabetic foot ulcer microbiome analysis using cutting edge technologies. J Diabet Sci Tech. 2022;16(2):353–63. DOI: 10.1177/1932296821990097

[42] Jozic I, Tomic-Canic M. Flipping the script: Are cellular assays and wound fluids the next frontier in personalized wound care? J Investig Dermatol. 2024. DOI: 10.1016/j.jid.2024.08.007
[43] Doerfler P, Schoefmann N, Cabral G, Bauer W, Berli MC, Binder B, et al. Development of a cellular assay as a personalized model for testing chronic wound therapeutics. J Investig Dermatol. 2024. DOI: 10.1016/j.jid.2024.05.029
[44] So JY, Nazaroff J, Iwummadu CV, Harris N, Gorell ES, Fulchand S, et al. Long-term safety and efficacy of gene-corrected autologous keratinocyte grafts for recessive dystrophic epidermolysis bullosa. Orphanet J Rare Dis. 2022;17:377. DOI: 10.1186/s13023-022-02546-9
[45] Titeux M, Bonnet des Claustres M, Izmiryan A, Ragot H, Hovnanian A. Emerging drugs for the treatment of epidermolysis bullosa. Expert Opin Emerg Drug. 2020;25(4):467–89. DOI: 10.1080/14728214.2020.1839049
[46] Suh GA, Lodise TP, Tamma PD, Knisely JM, Alexander J, Aslam S, et al. Considerations for the use of phage therapy in clinical practice. Antimicr Agen Chemother. 2022;66:e02071–21. DOI: 10.1128/aac.02071-21
[47] Riedl J, Popp C, Eide C, Ebens C, Tolar J. Mesenchymal stromal cells in wound healing applications: Role of the secretome, targeted delivery and impact on recessive dystrophic epidermolysis bullosa treatment. Cytotherapy. 2021;23(11):961–73. DOI: 10.1016/j.jcyt.2021.06.004
[48] Pânzaru M, Caba L, Florea L, Braha EE, Gorduza EV. Epidermolysis bullosa – a different genetic approach in correlation with genetic heterogeneity. Diagnostics. 2022;12(6):1325. DOI: 10.3390/diagnostics12061325
[49] Oliver J, Mason W. Gene variation: The key to understanding pharmacogenomics and drug response variability. Fusion Multidiscipl Res. 2020;1(2):97-109.
[50] Kolimi P, Narala S, Nyavanandi D, Youssef AAA, Dudhipala N. Innovative treatment strategies to accelerate wound healing: Trajectory and recent advancements. Cells. 2022;11(15):2439. DOI: 10.3390/cells11152439
[51] Khan A, Riaz R, Ashraf S, Akilimali A. Revolutionary breakthrough: FDA approves Vyjuvek, the first topical gene therapy for dystrophic epidermolysis bullosa. Ann Med Surg. 2023;85(12):6298–301. DOI: 10.1097/ms9.0000000000001422
[52] Yerlett N, Loizou A, Bageta M, Petrof G, Martinez AE. Establishing an appropriate level of vitamin D supplementation in paediatric patients with recessive dystrophic epidermolysis bullosa. Clin Exp Dermatol. 2022;47(7):1307–13. DOI: 10.1111/ced.15156
[53] Hou PC, del Agua N, Lwin SM, Hsu CK, McGrath JA. Innovations in the treatment of dystrophic epidermolysis bullosa (DEB): Current landscape and prospects. Therapeut Clin Risk Manag. 2023;19:455–73. DOI: 10.2147/TCRM.S386923
[54] Reimer A, Hess M, Schwieger-Briel A, Kiritsi D, Schauer F, Schumann H, et al. Natural history of growth and anaemia in children with epidermolysis bullosa: A retrospective cohort study. Brit J Dermatol. 2020;182(6):1437–48. DOI: 10.1111/bjd.18475
[55] Santos MLD, Monteiro AC, Nascimento A, Barbosa S, Delgado A, Zamberlan P. Nutritional profile and topic management for wound healing in children with epidermolysis bullosa: What is the evidence? A systematic review. Rec Progr Nutrit. 2024;4(3). DOI: 10.21926/rpn.2403010
[56] Konop M, Rybka M, Drapała A. Keratin biomaterials in skin wound healing, an old player in modern medicine: A mini review. Pharmaceut. 2021;13(12):2029. DOI: 10.3390/pharmaceutics13122029
[57] Nguyen HM, Le TTN, Nguyen AT, Le HNT, Pham TT. Biomedical materials for wound dressing: Recent advances and applications. RSC Adv. 2023;13:5509–28. DOI: 10.1039/D2RA07673J
[58] Mirhaj M, Labbaf S, Tavakoli M, Seifalian AM. Emerging treatment strategies in wound care. Int Wound J. 2022;19(7):1934–54. DOI: 10.1111/iwj.13786
[59] Nita M, Pliszczyński J, Kosieradzki M, Fiedor P. Review of the latest methods of epidermolysis bullosa and other chronic wounds treatment including BIOOPA dressing. Dermatol Therap. 2021;11:1469–80. DOI: 10.1007/s13555-021-00578-w
[60] Sait H, Srivastava S, Saxena D. Integrated management strategies for epidermolysis bullosa: Current insights. Int J Gen Med. 2022;15:5133–44. DOI: 10.2147/IJGM.S342740

# Оптимізація лікування хронічних ран при бульозному епідермолізі та вплив мікрофлори: огляд літератури

**Євгенія Федорець**
Аспірант
Національний медичний університет імені О. О. Богомольця
01601, бульв. Тараса Шевченка, 13, м. Київ, Україна
https://orcid.org/0000-0002-2282-8728

**Ольга Голубовська**
Доктор медичних наук, професор
Національний медичний університет імені О. О. Богомольця
01601, бульв. Тараса Шевченка, 13, м. Київ, Україна
https://orcid.org/0000-0003-3455-8718

**Леонід Пінський**
Доктор медичних наук, професор
Національний медичний університет імені О. О. Богомольця
01601, бульв. Тараса Шевченка, 13, м. Київ, Україна
https://orcid.org/0000-0002-2120-5887

**Анотація.** Дослідження було проведене з метою пошуку найефективніших підходів до лікування хронічних ран при бульозному епідермолізі з врахуванням впливу мікробіому рани на процес її загоєння. Під час дослідження проведено огляд актуальних наукових джерел з дерматології, фармакології, генетики, імунології, мікробіології, встановлено роль мікробіому рани в загоєнні хронічних ран при бульозному епідермолізі та проаналізовано сучасні стратегії лікування для прискорення загоєння даних ран. Результати дослідження виявили, що мікрофлора рани має значний вплив на процес їх загоєння. Бульозний епідермоліз провокує постійне травмування шкіри, сприяючи створенню умов для розмноження в ранах патогенних бактерій, що призводить до розвитку дисбіозу та ускладнює процес загоєння. Значний вплив на мікробіом ран при бульозному епідермолізі має імунний статус пацієнта, застосування антибіотиків, розташування, глибина та тривалість рани. Ослаблений імунітет провокує розвиток мультирезистентних бактерій, посилена імунна відповідь та аутоімунні процеси створюють умови для розвитку мікроорганізмів, здатних розмножуватися в умовах характерних для запаленої рани. Застосування антибіотиків є високоефективним методом лікування інфекцій, проте тривала або нераціональна антибіотикотерапія може спровокувати виникнення резистентних штамів бактерій. На формування мікробіому рани, залежно від її локалізації, впливають особливості шкіри на різних ділянках тіла – рівень зволоженості, наявність потових та сальних залоз, волосяний покрив. Вплив глибини рани на мікробіом реалізується створенням умов для розмноження відповідних мікроорганізмів на відповідній глибині. Тривалість існування рани посилює в ній дисбіоз, ускладнюючи загоєння. Сучасні стратегії лікування для прискорення загоєння хронічних ран при бульозному епідермолізі включають напрямок персоніфікованої медицини, комплексного підходу та застосування сучасних перев'язочних матеріалів. Отримані результати вказують на необхідність дослідження та розробки нових ефективних методів усунення дисбіозу рани для нормалізації її мікробіому та прискорення процесу загоєння

**Ключові слова:** загоєння; патогенні бактерії; дисбіоз; антибіотики; резистентність; персоніфікована медицина

Article's History: Received: 08.07.2024; Revised: 01.11.2024; Accepted: 26.11.2024

Bulletin of Medical and Biological Research

Vol. 6, No. 4
2024

DOI: 10.63341/bmbr/4.2024.76

Journal homepage: https://bmbr.com.ua/en

UDC 616.31:613.84

# Health effects of nicotine products on oral tissues: Prevention approaches

**Bohdan Kravchenko***

Postgraduate Student
Shupyk National Healthcare University of Ukraine
04112, 9 Dorohozhytska Str., Kyiv, Ukraine
https://orcid.org/0009-0003-4511-1994

**Kostiantyn Lykhota**

Doctor of Medical Sciences, Associate Professor
Shupyk National Healthcare University of Ukraine
04112, 9 Dorohozhytska Str., Kyiv, Ukraine
https://orcid.org/0000-0003-0912-6470

**Abstract.** The study aimed to analyse the impact of smoking and modern nicotine release systems on the state of the oral cavity, addressing the stratification of risk factors for the development of dental pathologies, determining the possibility of complications and introducing preventive programmes for dentists to minimise the harmful effects of this habit. PubMed, Scopus, Web of Science, and Google Scholar sources were analysed to conduct the research. E-cigarettes, traditional cigarettes and tobacco heating systems were determined to have a negative impact on the oral cavity, causing risks of leukoplakia, gingivitis and periodontal disease. Smoking traditional cigarettes causes significant destructive changes, including deep periodontal pockets (68%), loss of tissue attachment (42%) and gingival leukoplakia (85.1%). E-cigarettes, although less harmful, cause mucosal irritation, and dysbiosis and increase the risk of periodontal disease by 1.43 times compared to non-smokers. Tobacco heating systems show lower toxicity, but the risk of inflammation and tissue destruction remains. The results show that smoking cessation has a positive impact on dental health. A year after smoking cessation, there was a 1.5 mm reduction in the depth of periodontal pockets and a 1.2 mm improvement in tissue attachment. This confirms the importance of combating addiction for the prevention of dental diseases. Recommendations for physicians include history analysis, recording nicotine use patterns, applying the 5A principle of a patient's tobacco use history, providing cessation information, assessing their readiness to quit, assisting with the cessation process, and providing follow-up support through regular contact. Informing patients about the risks associated with all forms of tobacco and promoting complete cessation should be prioritised. However, if it is not possible to quit smoking, it is necessary to visit the dentist regularly, stop smoking during periodontal disease treatment or dental implants, carefully follow the rules of oral hygiene with the use of rinses and abrasive pastes, and seek professional help in a timely manner. Such measures help reduce the risk of dental pathologies and contribute to the long-term maintenance of oral health

**Keywords:** periodontitis; squamous cell carcinoma; leukoplakia; caries; smoking; electronic cigarettes

## INTRODUCTION

Tobacco smoking remains one of the most popular negative habits. Smoking traditional cigarettes, e-cigarettes and tobacco heating systems negatively impact oral health, which can cause cancer, tooth decay, periodontitis, as well as microbiome and salivary disorders. The spread of alternative ways of consuming nicotine is a cause for concern due to insufficient research on their health effects and potential harm to young people. The research relevance is determined by the need for effective control over new types of tobacco products and raising public awareness of their risks.

According to the World Health Organisation (WHO) [1], tobacco use causes more than 8 million deaths each year, of

**Suggested Citation**:
Kravchenko B, Lykhota K. Health effects of nicotine products on oral tissues: Prevention approaches. Bull Med Biol Res. 2024;6(4):76–87. DOI: 10.63341/bmbr/4.2024.76

*Corresponding author

Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/)

which 1.3 million were attributed to second-hand smoke. Approximately 80% of tobacco users lived in low- and middle-income countries, where the tobacco epidemic was most severe. In 2003, WHO member states adopted the Framework Convention on Tobacco Control, which has become a key instrument in tackling this global problem. In the spring of 2023, the WHO, together with the Ministry of Health of Ukraine and the International Institute of Sociology in Kyiv, conducted a new survey aimed at studying tobacco consumption habits among the adult population of Ukraine [2]. The survey found that the prevalence of smoking and the use of new tobacco products among adults remained almost unchanged. In particular, 27.4% of respondents (44% of men and 13.7% of women) reported use of any tobacco or nicotine products. Of these, 23% are daily users (37.5% of men and 11.1% of women).

A survey conducted by Kyiv International Institute of Sociology [3] revealed a downward trend in tobacco consumption in Ukraine from 32% in 2022 to 27% in 2024. The highest level of consumption is observed among young people aged 18-29. The popularity of heated tobacco products is growing: 45% of young people use them. Most respondents supported anti-tobacco measures, including bans on advertising, visible packaging and flavours, and increased excise taxes, which help to reduce the popularity of traditional cigarettes. The findings confirmed that smoking remains an important issue, especially due to the growing popularity of tobacco heating systems.

The first barrier for tobacco combustion products to reach the lungs is the oral mucosa (OME). According to the American Lung Association, cigarette smoking produces more than 7,000 chemical compounds, 69 of which are classified as carcinogens [4]. These substances are associated with the development of oral diseases, including cancer, which is the eighth leading cause of cancer-related deaths in the world. According to the Centres for Disease Control and Prevention [5], the use of tobacco products, including cigarettes and smokeless tobacco, significantly increases the risk of developing oral diseases. Smoking is associated with higher levels of tooth decay: 40% of smokers aged 20-64 have untreated tooth decay, compared to 20% of those who have never smoked. In the older age group (65 years and older), these figures are even worse: 43% of smokers have completely lost their teeth. Smoking is also a key risk factor for oral and upper respiratory cancers, which underscores the need for medical intervention to combat this problem.

Tobacco combustion products, including aerosols, contain carcinogenic substances that can initiate oxidative stress and damage to epithelial cells, potentially leading to malignant transformation. Abuse of tobacco-containing products is associated with mucosal diseases (MD), such as leukoplakia, keratosis, erythroplakia and nicotinic stomatitis, especially in areas of contact with chewing tobacco or snus. According to G.D.G. Da Silva & T.N. Pinheiro [6], 3-6% of leukoplakias have a high risk of malignancy, which highlights the importance of timely detection of these conditions in smokers or tobacco users.

The association between smoking and periodontal disease was confirmed by numerous clinical and epidemiological studies by G.V. Popa *et al*. [7], which show that tobacco combustion products can affect the clinical manifestations and progression of the disease. The study established that cigarettes are one of the key risk factors for the development and severity of periodontal disease. In the United States, the prevalence of periodontitis is about 40%, and among smokers, the disease is at least 50% more common than among non-smokers. Studies have also shown that smokers show less pronounced improvement in symptoms after non-surgical treatment of periodontitis compared to non-smokers.

The results of the study by A.C. Devlin & P.A. Fee [8] demonstrated that the risk of dental implant failure is significantly increased in patients who smoke. In the group that consumed less than 10 cigarettes per day, the failure rate was higher than in non-smokers ($p = 0.046$; 95% CI: 1-1.64). At the same time, patients who smoked more than 10 cigarettes per day showed an even higher failure rate compared to those who consumed lower doses ($p < 0.001$; 95% CI: 1.31-2.17). These data confirm that an increase in the number of cigarettes smoked directly correlates with an increase in the rate of implant failure. E. Jonas *et al*. [9] found that tobacco smoking is associated with sialolithiasis and changes in the rheological characteristics of saliva. Factors such as the presence of *Streptococcus mutans*, poor oral hygiene and a diet rich in carbohydrates are central to the development of caries. According to a study by M.A. Khan *et al*. [10], smoking is associated with an increase in the number of cariogenic microorganisms, including *S. mutans*. Nicotine disrupts the balance of the oral microflora, creating conditions for colonisation by this pathogen, which, together with a reduced saliva buffering capacity, contributes to caries.

Smokers are at an increased risk of oral diseases. Given the popularity of tobacco heating systems, vapes and tobacco pouches in Ukraine, their impact on dental health needs to be assessed, as they are often considered less harmful, even among dentists. Objective of the study: to assess the impact of smoking on oral health, identify the risks of complications and justify the implementation of anti-smoking programmes to reduce the negative impact of smoking on oral tissues. Tasks of the study: to compare the impact of smoking classic cigarettes and modern tobacco heating systems on the state of the oral cavity; to develop recommendations for dentists to avoid oral diseases among smokers.

## MATERIALS AND METHODS

The study was conducted using a theoretical literature review. The comparison of the incidence of oral diseases, such as gingivitis, stomatitis, and leukoplakia, among users of classic cigarettes and modern nicotine systems, was emphasised. The analysis was based on scientific articles, clinical trials, meta-analyses and retrospective reviews available in international databases such as PubMed, Scopus, Web of Science and Google Scholar. The following keywords were used in the literature search: oral diseases, tobacco impact on oral health, electronic cigarettes, tobacco heating systems, keratosis, leukoplakia, caries, periodontitis, periodontal disease, oral cancer, smoking, and smoking prevention.

Following the research topic, 103 scientific papers were selected that reflected the relevance of the problem. The following inclusion criteria were used to ensure the relevance and scientific reliability of the data: by type of study, the list of references included original articles containing

data from clinical, epidemiological or laboratory studies, systematic reviews and meta-analyses, and retrospective and prospective studies. Clear criteria were established for including articles in the review that met the standards of systematic reviews. The period of publication was prioritised: papers published between 2021 and 2024 were included in the analysis. This ensured that the materials were up-to-date and in line with modern scientific approaches. However, articles published before 2020 were included in the analysis if they were of high scientific value if they contained unique data, fundamental findings, or the results of large-scale research that are still relevant.

In addition, the level of evidence was an important criterion: preference was given to randomised controlled trials, meta-analyses and systematic reviews, as well as clinical trials with a high degree of confidence. The type of study population was also considered. The review included studies that analysed the health status of adults or adolescents who used tobacco products or modern nicotine release systems. The criterion of data completeness was also important: only those publications that contained detailed research results, a formed abstract, and were relevant to the research topic were considered. Another important factor was language accessibility. The analysis included papers written in English and Ukrainian, which were used to address relevant and accessible materials for analysis. The relevance of the study was also assessed following the topic: only those publications that covered oral diseases in smokers or users of modern nicotine systems were considered.

The study examined the impact of different ways of consuming nicotine, including classic cigarettes and modern tobacco release systems, on the condition of periodontal tissues, oral mucosa, saliva pH and the intensity of plaque formation. The carcinogenic status of smokers, including the number of carcinogenic microorganisms and changes in the acid-base balance of saliva, which affect the risk of caries and periodontal disease, was studied. Particular attention was devoted to the prediction of the development of re-implantation in patients who consume nicotine in various forms. The effectiveness of individual oral hygiene products, their adaptation to the needs of smokers, and the frequency of informing patients by dentists about the risks of nicotine consumption for oral health and prevention measures were also studied.

## RESULTS AND DISCUSSION

### The impact of tobacco smoking and heating systems on the occurrence of salivary gland disorders and inflammatory disorders of the oral cavity

Smoking habit is a significant risk factor for periodontal diseases, caries, re-implantation and other pathologies that significantly reduce the quality of life of patients. Smoking not only deteriorates hygiene but also affects the acidity of saliva (pH), changes the microflora and promotes the formation of plaque, which creates the preconditions for the progression of inflammatory processes. Therefore, the study of changes in oral tissues caused by tobacco products is important for the development of effective preventive and therapeutic measures.

Research data confirm the significant impact of smoking on the clinical condition of the oral mucosa and hygiene indicators. For instance, in the group of smokers with more than five years of experience, the mean value of bacterial plaque was 35.68 ± 12.45, which was twice as high as in non-smokers (16.32 ± 6.61) [11]. Plaque in smokers had a mean value of 2.24 ± 1.02, while in the control group, it was 0.94 ± 0.68. The gingival bleeding index was also significantly higher in smokers (42.86 ± 14.93) compared to non-smokers (19.54 ± 7.89) (Table 1). Smoking contributed to the progression of gingivitis and plaque accumulation, creating conditions for the development of periodontitis. Gingivitis, which is an initial reversible inflammation of the gums, became chronic in smokers and was complicated by poor oral hygiene. Thus, monitoring of hygiene among smokers is a mandatory measure to prevent the progression of diseases.

**Table 1.** Descriptive characteristics for groups of non-smokers and smokers by age categories

| Age category (years) | Bacterial plaque (NS, %) | Bacterial plaque (S, %) | Gingival bleeding during probing (NS, %) | Gingival bleeding during probing (S, %) |
|---|---|---|---|---|
| 18-25 | 8.66 | 12.02 | 14.16 | 16.42 |
| 26-35 | 19.75 | 14.41 | 17.35 | 21 |
| 36-45 | 25.25 | 21.03 | 25.09 | 21 |
| 46-55 | 31.26 | 29.24 | 30 | 37.5 |
| 56-65 | 36.78 | 36.56 | 44.16 | 43.93 |
| >65 | 43.45 | 52.14 | 53.14 | 52.14 |

**Notes:** NS – non-smokers; S – smokers
**Source:** compiled by the authors based on [11]

Other studies confirm the link between smoking duration and its harmful effects. In adolescents who smoked regularly, there was a high prevalence of inflammatory changes in the periodontium, which correlated with the duration and intensity of the habit ($r = 0.79$ and $r = 0.75$, respectively) [12]. Poor oral hygiene ($r = 0.71$) increased the risk of developing pathologies. Smoking led to a decrease in the elasticity of the mucous membranes, changes in pH and microcirculation, which intensified the destructive processes. A study of the impact of new tobacco devices that heat but do not burn (HNB) on patients' oral cavities revealed a decrease in the impact of biomarkers associated with tobacco harm. Compared to conventional cigarettes, the use of HNB devices reduced levels of 12 major BoEs, including nicotine, by an average of 30-50% ($p < 0.05$). Despite the negative effects, modern alternatives such as HNBs show reduced toxicity, but the risk of inflammatory reactions remains.

Despite the reduction in overall toxic exposure, BoE analysis showed that some biomarkers associated with the

risk of inflammatory reactions in oral tissues remained elevated [13]. Compared to abstinence, HNB 4 users had higher BoEs, which may indicate a risk of chronic mucosal irritation. The results highlighted the need for further independent research to assess the long-term effects of HNB on oral tissues, as the involvement of the tobacco industry in most existing studies limits their objectivity. Comparing HNB to traditional smoking, there is some harm reduction, but abstinence remains the best way to stay healthy.

E-cigarettes also affect the oral cavity. An analysis of the oral cavity of patients who used e-cigarettes revealed changes in the microbiome compared to non-vapers [14]. E-cigarette users showed an increased content of unclassified *Veillonella* species and an increase in alpha diversity among dual users (vaping and regular smoking). Beta diversity also varied depending on the way nicotine products were used. A link was found between the dual use of tobacco products and the presence of pathogenic microbes that may contribute to the progression of periodontal and oral mucosal diseases. Thus, the use of e-cigarettes is not safe and requires careful analysis.

An analysis of the oral health of female smokers revealed significant changes in salivary calcium levels and gingival bleeding index (GBI) compared to non-smokers [15]. In a study of 26 smokers and 37 non-smokers, the mean salivary calcium levels of female smokers were significantly lower than those of non-smokers. At the same time, the average BOP in the smoking group was significantly higher ($p < 0.05$). There was a positive correlation between salivary calcium and BOP among smokers. The longer the duration of smoking, the more the calcium levels decrease and the more the gingival bleeding increases. The overall dietary calcium intake was not statistically different between the groups, but the low salivary calcium levels may have been caused by nicotine, which reduces its concentration. These findings highlighted the importance of preventing smoking among women and educating them about its negative effects on the oral cavity. Comparing these findings with subsequent studies of the effects of alternative nicotine delivery methods, similarities in impairment can be noted, but the mechanisms of change are different.

Significant changes in oral health were observed in young smokers of traditional cigarettes, e-cigarettes and heated tobacco products [16]. The concentration of sphingolipids and ceramides in saliva was reduced in all groups of smokers compared to non-smokers, indicating a lipid imbalance. The levels of malondialdehyde and 4-hydroxynonenal were highest in traditional smokers, indicating increased oxidative stress. E-cigarette and HNB users showed lower levels of these indicators in stimulated saliva. Thus, although the level of oxidative stress is lower in e-users, lipid imbalance and possible oral health consequences remain a common concern for all forms of nicotine consumption.

The study of the impact of heat-treated tobacco products on oral health requires attention due to the growing popularity of these products as an alternative to conventional cigarettes [17]. At the same time, their benefits compared to traditional cigarettes remain controversial due to the lack of long-term studies. A study [17] showed that smokers are still at risk of developing various oral diseases when using HNB products such as IQOS, albeit to a lesser extent compared to traditional smoking. However, research indicates a growing interest in these products among young people and non-smokers, which may help attract new smokers. At the same time, the study observed that IQOS attempts were more popular among younger smokers (25.1%) compared to traditional cigarettes (19.3%) and less so among e-cigarettes (29.1%).

The clinical condition of the oral cavity after exposure to e-cigarettes was characterised by changes that included dry mouth, mucosal irritation and an increased risk of gum disease [18]. There were also changes in the microbiome, which demonstrated a potentially pathogenic orientation compared to the microbiome of non-smokers. *In vitro* studies demonstrated that the effect of e-cigarette components on cells was less pronounced than tobacco smoke but had the potential for long-term tissue changes. Epidemiological data indicated the need for further clinical trials to assess long-term effects. In summary, despite some benefits, e-cigarettes and HNB products share a common risk to oral health that requires preventive measures. These findings are consistent with previous studies of e-cigarettes that also show significant oral health effects, but the difference in mechanisms and potential consequences between different nicotine delivery methods is of interest for further comparative analysis. Prolonged exposure to tobacco led to a significant deterioration in the condition of the oral cavity, manifested in the form of pathologies such as periodontitis, leukoplakia and caries. In patients with long-term tobacco use, an increase in the depth of periodontal pockets was detected in 68% of cases, loss of clinical gingival attachment in 42%, and mucosal leukoplakia in 34% [19]. These pathological changes tended to be localised in the cheeks, gums and tongue, which is consistent with general clinical observations.

An analysis of the state of periodontal tissues of patients after tobacco exposure revealed a significant deterioration in their functional state. Tobacco smoking contributed to the progression of periodontitis, loss of tissue attachment and increased risk of tooth loss. The analysis determined that smoking cessation was accompanied by a gradual decrease in the negative impact on periodontal tissues [20]. After several years of tobacco cessation, the risk of periodontitis decreased, and the effectiveness of non-surgical treatment increased. Positive changes were observed in patients who stopped smoking and received individualised recommendations for prevention and treatment. This confirms the need to focus on smoking cessation programmes as an effective method of reducing the risk of periodontal disease.

Accordingly, the results of smoking cessation programmes indicate a significant potential for improving oral health, even in patients with a history of long-term smoking. The state of the patient's oral cavity after tobacco exposure indicated the progression of periodontal disease, in particular, an increase in the depth of periodontal pockets (PD) and loss of tissue attachment (CAL). Participants in a smoking cessation programme [21] showed a marked improvement in these parameters after 12 months: a 1.5 mm reduction in PD depth and a 1.2 mm improvement in CAL. A reduction in plaque and gingival inflammation was observed, indicating a positive impact of smoking cessation on periodontal health. This emphasises the importance

of smoking cessation for maintaining periodontal tissue health and supporting overall oral health [21].

After using electronic nicotine delivery systems (ENDS), including e-cigarettes and vapes, patients experienced a proliferation of symptoms such as dry mouth, mucosal irritation, ulcers, inflammation, and dysbiosis [22]. Exposure to ENDS was associated with oxidative stress and DNA damage in oral epithelial cells, which was confirmed by *in vitro* studies. Some components of ENDS liquids, including formaldehyde and acetaldehyde, were found to be carcinogenic, increasing the risk of developing oral malignancies. In addition, prolonged use of these devices contributed to changes in the oral microbiome, which could lead to periodontal disease. Cases of oral cancer have been reported in ENDS users without other traditional risk factors, such as tobacco smoking. Despite the absence of traditional risk factors, such as smoking, ENDS use appears to be potentially carcinogenic, suggesting that it is necessary to investigate the long-term effects of ENDS use on oral health.

Thus, tobacco smoking has a significant negative impact on oral health, including bacterial plaque levels and gum bleeding. Smokers have an average plaque level twice as high and a gum bleeding index 20% higher than non-smokers. Statistical analysis confirms that 68% of smokers experience an increase in the depth of periodontal pockets, and 42% have a loss of tissue attachment, which is accompanied by significant risks of gum disease. The use of alternative tobacco products, such as tobacco heating devices, reduces the toxic effects on the oral cavity, but the risk of inflammation remains, which requires additional research and preventive measures. Compared to classic smoking, e-cigarettes cause less tissue irritation and have a less pronounced effect on the depth of periodontal pockets. However, both types of nicotine products contribute to pathogenic changes in the oral microbiome. In addition, the cessation of both classic cigarettes and electronic products demonstrates a significant improvement in oral health parameters: a 1.5 mm reduction in periodontal pocket depth and a 1.2 mm improvement in tissue attachment one year after quitting. This underscores the importance of preventive measures and active anti-addiction efforts to reduce the negative impact of tobacco products.

**The impact of tobacco on the occurrence of leukoplakia and oral cancer**

An analysis of the clinical condition of the oral mucosa among tobacco users showed a high incidence of potentially malignant diseases [23]. A study involving 4,500 people found that submucosal fibrosis (OSMF), mucositis from chewing tobacco and squamous cell carcinoma (OSCC) were the most observed conditions among people in Group A (chewing tobacco) and Group C (combined chewing and smoking). Group B (smoking only) was dominated by smoker's palate lesions. The prevalence of OSCC and OSMF increased significantly with increasing frequency and duration of tobacco use. Lesions were more common in men, as smoking was not recorded in women in this study. A statistically significant correlation was found between increased tobacco use and the risk of lesions ($p < 0.05$). The findings emphasised the need to inform the population about the harmful effects of tobacco use to prevent mucosal pathologies.

Among the population of Hazaribagh, the impact of tobacco on oral tissues was characterised by a high frequency of mucosal lesions [24]. Among the tobacco users, the most common lesion was tobacco pouch keratosis, followed by OSMF, lichenoid reaction, nicotinic stomatitis or melanosis, leukoplakia, erythroplakia and oral cancer (Table 2). According to the results, most lesions were associated with smokeless tobacco use (74.7%), compared to smoking (25.2%). The findings may provide a basis for preventive measures aimed at reducing the prevalence of tobacco-related oral lesions. These results are consistent with the above data on the significant impact of smokeless tobacco on the mucous membrane, which indicates the need to inform the population about the possible risks.

**Table 2.** Types of mucosal lesions associated with tobacco habits

| Type of lesion | Percentage |
|---|---|
| Erythroplakia | 50% |
| OSMF | 17% |
| Lichenoid reaction | 15% |
| Proliferative ulcerative changes (carcinoma) | 3% |
| Tobacco keratosis | 2% |
| Hyperkeratosis / smoker's palate | 13% |

**Source:** compiled by the authors based on [24]

The clinical condition of the oral cavity after tobacco exposure was manifested in a significant proportion of patients as leukoplakia, which was confirmed histopathologically [25]. Among 5,720 examined patients with oral mucosal diseases in Northern Poland, 416 were diagnosed with leukoplakia. The highest incidence of the disease was observed in the 41-60 age group (46.6%), where 85.1% of patients were active smokers, of whom 86.2% were men. Among women, the highest prevalence of smoking (86.8%) was found in the 21-40 age group. The homogeneous form of leukoplakia was the most common (95%) and was mostly localised on the buccal mucosa. Lesions on the gums, alveolar rims and lower part of the tongue were less common. A statistically significant correlation between tobacco smoking and leukoplakia was confirmed. The results emphasised the importance of early diagnosis of leukoplakia by dentists and the need to eliminate smoking as a key risk factor.

Patients exposed to tobacco had a high incidence of squamous cell carcinoma, including gingival cancer [26]. In 46.9% of patients included in the retrospective study, gingival cancer was diagnosed, while other sites were less commonly affected. The average age of patients with

gingival cancer was 74.2 years, which was significantly higher than the average age of patients with tumours in other sites (63.9 years). Most gingival lesions were observed among men (60%), while the gender ratio in patients with other forms of cancer was more even. Patients who combined tobacco and alcohol use accounted for 26.7% of those with gum cancer, all of whom were men. The data confirmed a significant association between tobacco use and an increased risk of squamous cell carcinoma. The findings emphasised the need for timely diagnosis and implementation of preventive measures for patients with risk factors.

Clinical analysis of the oral tissues of patients exposed to tobacco showed significant changes [27]. In most cases, OSCC was observed, with a higher incidence among men with a history of smoking and among women who used chewing tobacco products. The most common lesions were located on the tongue in women, while in men, cancer was detected in the retromolar region and on the tonsils. The age of diagnosis in women was significantly younger (46 years) than in men (57 years). This indicates a difference in cancer development patterns depending on gender and type of tobacco used, which allows for detailed prevention strategies for different groups of patients.

A clinical analysis by L. Amato *et al*. [28] of the oral cavity of patients who used tobacco products showed significant changes in the tissues. E-cigarette smokers developed localised mucosal lesions, the development of a villous black tongue, and an increased frequency of dry mouth. Allergic reactions, carcinogen formation and an increased risk of developing oral cancer were also observed. Cough, irritation and headache were common complaints. In patients with a long history of smoking, bone resorption increased, and chronic inflammatory processes developed. These observations support the findings of other studies that indicate the need for further research on the long-term effects of e-cigarettes. In summary, the impact of tobacco on the development of leukaemia and oral cancer is significant, particularly in patients who are regular tobacco users. Studies have shown that out of 5,720 patients, 416 were diagnosed with leukoplakia, including 85.1% of smokers, which indicates a close link between smoking and the onset of pathologies. In the group of patients with gum cancer, which was detected in 46.9% of cases, smokers accounted for 60%, which confirms the importance of timely diagnosis and preventive measures for people at high risk.

**Periodontal diseases are caused by tobacco or electronic heating systems**

A study by R. Nazaryan *et al*. [29] of the clinical state of the oral cavity in patients who used tobacco revealed significant changes in dental health indices. In adolescents with 1-3 years of smoking experience, an increase in the level of inflammatory processes in periodontal tissues was observed according to the papillary-marginal-alveolar index, which reached 36 ± 2.04%. The simplified index of oral hygiene exceeded the value of the control group, indicating a deterioration in the hygienic state. The biophysical properties of oral fluid, such as elasticity and pH, demonstrated a negative effect of tobacco smoke, which contributed to the development of periodontal disease. This suggests that smoking is a major risk factor for gum health, as it changes the oral microenvironment, creating conditions for disease progression.

The effect of tobacco smoke on the health of patients' gums was studied by B. Liu *et al*. [30]. The authors noted that the risk of developing periodontitis was significantly increased. In particular, the incidence of this disease among people exposed to tobacco smoke reached 56.57%. The likelihood of developing periodontitis was almost twice as high in patients exposed to tobacco smoke compared to those who were not (OR 1.96; 95% CI, 1.67-2.31). At the same time, a protective role of adequate zinc intake was found to reduce the risk of periodontitis (OR 0.86; 95% CI, 0.76-0.98). Exposure to tobacco smoke interacted with zinc deficiency, contributing to the progression of the pathology. The antagonistic effect of this interaction emphasised the importance of maintaining optimal zinc levels in the diet to prevent periodontal disease. Optimising the diet to ensure adequate zinc levels and reducing tobacco smoke exposure were considered effective preventive measures to prevent disorders in the oral cavity. These results highlight the importance of maintaining optimal micronutrient levels in the diet to minimise the effects of smoking on periodontal health.

A study by S. Al Kawas *et al*. [31] on the effect of different types of tobacco on the subgingival microbiome and periodontal health showed significant changes in the microflora in smokers. Compared to nonsmokers, cigarette, Medwakh and hookah smokers showed a significant increase in the relative abundance of pathogens such as *Prevotella denticola*, *Treponema*, *Streptococcus mutans*, *Veillonella dispar*, *Streptococcus sanguinis* and *Tannerella forsythia*. This was associated with an increased risk of developing periodontal disease, even in the absence of clinical manifestations or in mild disease. Among 40 patients in a dental clinic in the United Arab Emirates, subgingival plaques were analysed using Oxford Nanopore sequencing technology. The results indicated a pronounced microbiome dysbiosis that contributes to the progression of periodontitis. The detected microbiological changes can be used as a prognostic marker to assess the risk of developing severe periodontal disease among smokers. This emphasises the importance of further studying the effects of smoking cessation to stabilise the microbiome and prevent periodontal disease. Changes in the microbiota show how smoking, even without severe symptoms, can lead to oral pathologies.

The clinical condition of the oral cavity of patients after tobacco exposure was characterised by significant changes in tissues due to the toxic effects of tobacco smoke components, when using tobacco heating systems such as IQOS [32]. Patients showed signs of periodontal inflammation, deterioration of the mucous membrane and reduced elasticity, as well as changes in saliva pH, which created favourable conditions for the progression of inflammatory processes. Studies have shown that IQOS extracts increased the viability of fibroblast and keratinocyte cells and stimulated their migration, which may indicate the potential for increased proliferation. At the same time, the expression of genes responsible for apoptosis and cell cycle regulation changed a decrease in p53 protein expression and an increase in Bcl2 protein in keratinocytes were observed. Such changes at the cellular level indicate potential mechanisms for the development of mucosal pathologies when using tobacco heating systems.

In a study by O. Uehara *et al*. [33] on the Ploom TECH+ heating system, the condition of the oral cavity in patients

was characterised by significant changes at the cellular level. Prolonged stimulation of gingival epithelial cells with heated tobacco extracts led to disruptions in the processes of differentiation and keratinisation. RNA ribonucleic acid analysis showed a twofold increase in the expression of 284 genes and a decrease in the expression of 145 genes. In particular, the activation of genes related to root formation and keratinisation indicated an increase in the level of pathological changes in the tissues. CpG island methylation showed a significant increase in 158 genes and a decrease in 171 genes, which may indicate epigenetic changes under the influence of Ploom TECH+ products. An increase in the expression of the S100A7 protein, which is associated with inflammatory processes and impaired barrier functions of mucous membranes, was also recorded. The data indicate that regular use of the Ploom TECH+ system is a risk factor for the occurrence of oral mucosal pathologies, including inflammatory changes and hyperkeratosis.

According to T. Yoshioka & T. Tabuchi [34], users of HNB, combustible cigarettes, and their combination showed an increased incidence of periodontal disease. HNB use was associated with periodontal disease prevalence (PR 1.43; 95% CI 1.03-1.62), whereas combustible cigarette users had a PR of 1.29 (95% CI 1.03-1.62). Ex-users (PR 1.56; 95% CI 1.35-1.80) and combined users (PR 1.55; 95% CI 1.20-1.99) also showed significantly higher risk compared to those who had never used tobacco products. These results indicate a negative impact of both traditional and alternative tobacco products on periodontal tissue health, which highlights the need to develop preventive measures.

In a systematic review by C.A. Figueredo *et al*. [35], e-cigarette users showed significant increases in plaque, periodontal pocket depth, clinical attachment loss, and marginal bone loss (MBL) compared to a nontobacco control group. A total of 1,659 studies were analysed, of which eight case-control studies were selected. The authors determined that vaping caused increased pathological changes, including loss of bone support for the teeth and deeper penetration of the probe into periodontal pockets. The results indicate a potentially negative impact of vaping on periodontal tissue, which may contribute to the progression of periodontitis. Despite the limitations of the data, this indicates serious risks associated with vaping for gum health.

However, in a comparative study of the effects of classic tobacco and heating systems on oral health, the results were somewhat different. In a randomised trial by S. Pouly *et al*. [36] of 172 participants, 86 people who continued to smoke cigarettes were compared with 86 participants who switched to HNBs. The results showed that patients using HNB had significantly better rates of PD depth reduction in areas with an initial depth of ⩾4 mm. Among the secondary outcomes, there was an improvement in clinical tooth attachment and a reduction in gingival inflammation in the HNB group. Differences in disease evolution were most pronounced after mechanical therapy, indicating the potential of HNB to reduce the risk of periodontal disease progression. The study confirms the harm reduction by alternative methods of tobacco use.

The state of periodontal tissues in patients who used tobacco was investigated by assessing the main periodontal indices – PD depth and CAL [37]. The study involved 66 people aged 26 to 56 years (median age 38 years), 64% of whom were women. Three groups of patients were divided by the type of tobacco product use: classic cigarettes, HNB, and non-smokers. Mean PD and CAL scores differed significantly between groups ($p \leqslant 0.002$). The highest values were recorded in cigarette smokers, and the lowest in non-smokers. HNB users had significantly lower CAL values than smokers ($p = 0.011$). However, PD and CAL in HNB users remained higher than in non-smokers, but without a statistically significant difference. Cigarette smoking was the main predictor of periodontitis (mean CAL ⩾ 4 mm), increasing the risk by 4.7 times (95% CI 1.2-18.3; $p = 0.027$).

Studies show that smokers of classic cigarettes have higher periodontal pocket depth and CAL scores, indicating a higher risk of developing periodontitis. Patients who use HNB show improvement compared to cigarette smokers, although their results are still inferior to those of non-smokers. At the same time, HNBs can reduce harm compared to traditional smoking, although they are not without negative effects. Thus, in patients who used tobacco products, the risk of developing periodontitis was significantly increased – up to 56.57% among people exposed to tobacco smoke and up to 1.43 times in users of e-cigarettes and tobacco heating systems compared to non-smokers. The depth of PD and CAL were significantly higher than in the control group, especially in smokers of classic cigarettes, who had a 4.7-fold increased risk of periodontitis progression (95% CI 1.2-18.3; $p = 0.027$). Optimisation of preventive measures, such as smoking cessation, mechanical periodontal therapy and ensuring adequate levels of trace elements (e.g., zinc), can reduce the risk of periodontal disease and stabilise the condition of oral tissues.

**The effect of tobacco and heating systems on dental implants**

The study of the clinical condition of the oral cavity after tobacco exposure revealed a significant correlation between traditional smoking and an increased risk of dental implant failure. Data from H.L. Stiller *et al*. [38] study, which included 29,519 implants in 18,301 patients, showed that smoking had a negative impact on both the early and late stages of osseointegration. In 25 of these studies, a dose-response relationship was found: an increase in the number of cigarettes smoked per day increased the risk of implant failure. In particular, the results highlight the need to control the number of cigarettes to reduce risks, as smokers performed significantly worse than non-smokers, especially in the case of uncontrolled tobacco use. Tobacco exposure limited tissue regeneration and interfered with implant stability, requiring more resources for treatment and prevention in these patients. The findings highlight the need for smoking cessation programmes, patient education and individualised care approaches in dental implantology. Future research should consider the impact of not only smoking but also alternative tobacco products such as e-cigarettes on the increased risk of dental implant failure.

A. Tanik & F. Demirci [39] demonstrated a significant effect of smoking on MBL in patients with dental implants. The study included 419 implants placed in 188 patients aged 23 to 76 years. It was found that smokers, especially those who used unfiltered cigarettes, demonstrated significantly greater bone loss compared to non-smokers ($p < 0.05$). This aspect adds to the understanding of the

negative effects of tobacco, as unfiltered cigarettes appear to be more harmful than filtered cigarettes. MBL levels on the mesial and distal surfaces of heavy smokers of unfiltered cigarettes (>20 cigarettes per day) were significantly higher than those of filter smokers ($p = 0.013$). The effect of smoking also varied depending on the location and length of the implant. These data showed a high dependence of bone loss on smoking intensity and cigarette type, especially without a filter. Overall, the study emphasises the importance of smoking cessation as an effective method of preventing and maintaining implant stability. In addition, the authors demonstrate the difference in harm between types of tobacco products, which requires further research to optimise clinical recommendations.

Consideration of the impact of tobacco smoking on the condition of oral tissues revealed a significant increase in the risk of periodontal disease and complications of implant therapy [40]. Smoking caused changes in the microbial composition of subgingival biofilms, including an increase in pathogenic microorganisms, and suppressed inflammatory and immune responses. In contrast to previous findings, the focus here is on the impact of smoking on the microbiological level, which explains the clinical manifestations. This led to a disturbance in the homeostasis of periodontal tissues and a decrease in the healing potential. Early studies and modern methods such as deep sequencing have confirmed a significant deterioration in clinical parameters, including bleeding indices and loss of tissue attachment. Smokers have also been found to have lower success rates in periodontal treatment and dental implant stability. In addition, passive smoking was found to be potentially harmful to oral tissues. The results highlighted the importance of smoking cessation as a preventive measure. Recommendations for dentists should include informing patients about the negative effects of smoking on periodontal tissues and implant prognosis. Thus, the study of D.A. Apatzidou [40] adds another important aspect to the overall picture, indicating a significant effect of smoking on the microflora and immune response, which enhances the clinical significance of the previously obtained data.

In addition, smoking affects the state of the patient's oral cavity, worsening the processes of osseointegration of dental implants [41]. Smokers had an increased risk of implant failure, which reached 140.2% compared to non-smokers. Significant changes were recorded in both the upper (OR 2.910) and lower jaw (OR 2.866). Smoking contributed to MBL (MD 0.580 mm). Compared to other studies, this analysis focuses more on specific anatomical areas and their association with tobacco exposure. These complications reduced the effectiveness of implant treatment, especially with prolonged exposure to tobacco smoke. These findings highlight the need for tobacco prevention to improve dental health.

A study by M. Alqahtani [42] determined that in smokers, the mean PD probing depth was $4.2 \pm 0.5$ mm, while in non-smokers it was $3.1 \pm 0.4$ mm. The bone crest resorption in smokers was $1.5 \pm 0.3$ mm, which was significantly higher than in non-smokers – $0.9 \pm 0.2$ mm ($p < 0.05$). A statistically significant higher bleeding rate during probing was observed among smokers in 62% of cases, compared to 42% in non-smokers. The data confirm that smoking contributes to peri-implant inflammation and bone loss. This confirms the findings of other studies on the association between smoking and peri-implant inflammation, adding new evidence of a statistically significant difference between the groups. O. Vámos *et al*. [43] studied the condition of peri-implant tissues in patients who used various nicotine-containing products showed a significant deterioration in parameters compared to non-smokers. In conventional cigarette smokers, the mean MBL was 1.34 mm (CrI: 0.85-1.79), in hookah smokers (WP) – 1.58 mm (CrI: 0.84-2.35), and in smokeless tobacco (ST) users – 2.53 mm (CrI: 1.20-3.87). For e-cigarette (EC) users, MBL was not significantly different from NS (MD: 0.52 CrI: -0.3-1.36). Nonsmokers also had the best PD probing depth, plaque index, and proinflammatory cytokine levels (TNF-α, IL-1β). Despite being considered less harmful, e-cigarettes also showed lower tissue scores compared to NS, although the difference was not always statistically significant. These findings highlight the importance of close monitoring of oral health in all users of nicotine-containing products and the implementation of preventive measures.

A comparison of the oral cavity after exposure to e-cigarettes and traditional cigarettes showed that the use of EC may reduce or not affect peri-implant disease indicators such as bleeding on probing, PD probing depth, and MBL [44]. However, the study demonstrated that EC, similarly to ST, contains nicotine, which negatively affects tissue healing and implant integration. Comparing the effect of different types of nicotine-containing products on the condition of oral tissues, it is evident that traditional and alternative cigarettes negatively affect peri-implant parameters, although the level of influence varies. For example, peri-implant bone loss in EC users may be slightly higher than in non-smokers, and the response to treatment in these patients was less predictable. These findings suggest the need for additional studies to better assess the impact of EC on clinical outcomes of implant treatment and the prevention of oral disease. The results of these studies highlight the need for further study and patient education about the risks of using various nicotine-containing products, as well as the development of effective preventive measures. In conclusion, tobacco products, including traditional cigarettes and HNB, have a negative impact on peri-implant tissue, but their effects differ significantly. Traditional cigarette smoking is associated with significant bone loss (MBL 1.34-2.53 mm) and worse clinical parameters such as pocket depth and plaque index compared to EC users, who have less severe changes [43, 44]. At the same time, although EC are considered less harmful, their effect on healing and implant stability also remains negative due to the presence of nicotine, which highlights the need for preventive measures regardless of the type of product.

As for the recommendations for doctors, in the context of combating the effects of the use of newer nicotine products, they are based on current research on their impact on the oral cavity. According to the World Dental Federation [45], e-cigarettes and tobacco heating systems cause chronic irritation of the mucous membrane, which increases the risk of leukoplakia, gingivitis and periodontal disease. Accordingly, a detailed medical history and recording of nicotine consumption patterns are recommended to optimise prevention and treatment. The key elements of preventive work are informing patients about the dangers

of aerosols, as well as the use of the 5A principle [46]. This principle is based on clarifying smoking status, advising on risks, assessing the patient's readiness to quit, offering support, and organising follow-up. Basic behavioural therapy and pharmacotherapy, including nicotine replacement therapy, are recommended to combat nicotine dependence. Regular monitoring of the oral cavity in patients with addiction allows early detection of pathological changes, such as hyperplasia or mucosal erosion, and referral to dentists or periodontists. A focus on education, including information about complete nicotine cessation, helps to increase the effectiveness of preventive measures. Thus, the implementation of these recommendations will help improve the quality of prevention and treatment of oral diseases caused using new nicotine products and reduce the risks to patient's health.

**Risks of complications and prevention of oral diseases in smokers**

Based on the analysis, the main risks of complications caused by the impact of smoking (traditional, e-cigarettes and tobacco heating systems) on the oral cavity included aspects such as the risks associated with traditional smoking included an increase in the depth of periodontal pockets and loss of gingival attachment, which was accompanied by the development of chronic periodontitis. Pathological changes in the form of leukoplakia and progression of gingivitis, increased plaque formation, which contributed to the development of caries, were often observed. There was a disturbance in the oral microflora, an increase in pathogenic bacteria, oxidative stress and an increase in the level of biomarkers of cellular damage. Although the toxicity of tobacco heating systems was 30-50% lower, the risk of chronic mucosal irritation and partial maintenance of inflammatory processes in tissues remained. The spread of these systems among young people created additional risks of attracting new tobacco users.

The use of e-cigarettes contributed to the disruption of the bacterial balance, increased the number of pathogens such as *Veillonella*, caused inflammation, and irritation of the mucous membrane, and increased the risk of carcinogenic changes due to the impact of toxic compounds. Common risks for all forms of smoking include lipid metabolism disorders and reduced calcium concentrations in saliva, which contribute to the development of periodontal disease, dysbiosis, bleeding gums and tooth loss. Smoking had a negative impact on bone regeneration, osseointegration of dental implants and tissue healing.

Studies highlighted the importance of prevention programmes and early diagnosis to reduce the impact of tobacco products on oral health. K. Kawamura *et al*. [47] determined that smokers sought medical care when their symptoms became serious. Therefore, dentists are recommended to encourage people who smoke any type of tobacco products to visit dentists more often for early detection and timely treatment of oral diseases. B.W. Chaffee *et al*. [48] emphasised that quitting smoking tobacco products is the most effective model for the prevention of all oral diseases and their complications. However, if the patient is unable to completely quit smoking, the optimal approach is to stop smoking for the period of periodontal disease treatment and dental implantation. If the patient was unwilling to abandon tobacco, dentists had to weigh the risks and benefits by discussing the potential consequences with the patient. Increased follow-up, frequent check-ups (every 6 months or more), and risk communication were recommended.

According to the National Institute on Aging [49], several basic rules are recommended for maintaining oral health. Teeth should be brushed regularly with fluoride toothpaste twice a day, and dental floss should be used to clean the interdental spaces. A dentist should be visited every six months for a preventive examination and professional cleaning. A balanced diet, avoiding sweets and sour foods, is also essential. Water is necessary to prevent mouth dryness. Avoid tobacco and limit alcohol consumption, which significantly increases the risk of oral diseases. If bleeding gums or any changes in the oral cavity occur, it is recommended to visit a dentist. In addition, it is also recommended that smokers use deodorising rinses to neutralise odour [50]. To combat "smoker's plaque", toothpaste with a high abrasive index from the "antitaro" series should be used. Regular dental check-ups every 3-4 months. Less harmful alternatives, such as e-cigarettes or tobacco heating systems, should be considered to reduce the harm from smoking.

## CONCLUSIONS

HNBs and traditional cigarettes both have a negative impact on oral health, but their effects differ in intensity. Smoking traditional cigarettes significantly increases the risk of periodontal disease, and caries and changes the oral microbiome. Deep periodontal pockets are found in 68% of smokers, and the incidence of periodontitis among smokers reaches 56.57%, with gingival leukoplakia observed in 85.1% of cases. Long-term cigarettes use also causes bone loss (MBL 1.34-2.53 mm) and worse clinical parameters such as PD probing depth and gingival bleeding, which reaches 62%.

E-cigarettes, which are 30-50% less harmful than traditional cigarettes, still change the microbiome of the oral cavity, contributing to the development of pathogenic processes. E-cigarette users have a 1.43-fold increased risk of developing periodontal disease compared to non-smokers. E-cigarettes also cause mucosal irritation, and dysbiosis, and increase the risk of malignant tumours due to carcinogens such as formaldehyde. The incidence of peri-implant inflammation among HNB users is lower than among cigarette smokers, but implant stability is also reduced. The long-term safety of HNBs remains questionable and requires additional research. HNBs reduce toxicity compared to traditional cigarettes, but the risk of inflammation and tissue damage remains. In smokeless tobacco users, the incidence of keratosis reaches 46.1% and OSMF – 16.1%. HNB use is associated with a reduction in the depth of PD and an improvement in CAL, but the negative effects cannot be eliminated.

Smoking cessation has a positive effect on the state of the oral cavity. A year after smoking cessation, there is a 1.5 mm decrease in the depth of periodontal pockets and a 1.2 mm improvement in tissue attachment. This confirms the importance of combating addiction for the prevention of periodontal disease and maintaining implant health. Optimisation of oral hygiene, and dietary correction, in particular zinc levels, help reduce the risk of pathologies. To improve the oral health of patients who use nicotine

products, it is recommended to conduct a detailed identification of their forms of use through a thorough history and registration of data in the medical record. Regular information should be provided about the risks of chronic mucosal irritation, leukoplakia, gingivitis and periodontitis, including the harmful effects of alternative products such as e-cigarettes and tobacco heating systems. Using the 5As principle can improve the effectiveness of prevention and treatment by covering all aspects of patient monitoring and support. Particular attention should be devoted to the early diagnosis of mucosal changes, the use of nicotine replacement therapy and individual behavioural support aimed at complete nicotine cessation.

In summary, traditional e-cigarettes have a negative impact on the oral cavity, although the extent of their harm differs. Smoking traditional cigarettes causes significant destructive changes, while the impact of HNBs is less pronounced, but carries long-term risks. The importance of preventing the use of and quitting all types of tobacco products remains key to maintaining dental health. However, if it is not possible to quit smoking, it is recommended to visit the dentist more often, temporarily stop smoking for interventions related to periodontal disease and dental implants, observe hygienic oral care using rinses, and abrasive pastes and seek timely medical care from specialists. A limitation of the study was the lack of clinical trials on the long-term effects of modern tobacco and e-cigarette heating systems on dental health. Future research directions include studying the mechanisms of oral tissue recovery after smoking cessation.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] World Health Organization. Tobacco [Internet]. 2023 [cited 2024 June 17]. Available from: https://www.who.int/news-room/fact-sheets/detail/tobacco
[2] World Health Organization. World No Tobacco Day 2023: Ukraine survey results highlight challenges [Internet]. 2023 [cited 2024 June 17]. Available from: https://www.who.int/europe/news-room/31-05-2023-world-no-tobacco-day-2023--ukraine-survey-results-highlight-challenges
[3] Kyiv International Institute of Sociology. Consumption of tobacco and nicotine products and attitude towards anti-tobacco measures: October 2024 [Internet]. 2024 [cited 2024 Oct 25]. Available from: https://center-life.org/wp-content/uploads/2024/11/Analytical-report-Omnibus-2024.pdf
[4] American Lung Association. Health effects of smoking [Internet]. 2024 [cited 2024 June 17]. Available from: https://www.lung.org/quit-smoking/smoking-facts/health-effects/smoking
[5] Tobacco use and oral health facts [Internet]. 2024 [cited 2024 June 17]. Available from: https://www.cdc.gov/oral-health/data-research/facts-stats/fast-facts-tobacco-use-and-oral-health.html
[6] Da Silva GDG, Pinheiro TN. Histomorphometric comparative analysis between the oral mucosa of fibrous inflammatory hyperplasia and oral leukoplakia. Transl Cancer Res. 2020;9(4):3101–6. DOI: 10.21037/tcr.2019.12.23
[7] Popa GV, Costache A, Badea O, Olimpia Cojocaru M, Mitroi G, Lazăr AC, et al. Histopathological and immunohistochemical study of periodontal changes in chronic smokers. Rom J Morphol Embryol. 2021;62(1):209–17. DOI: 10.47162/rjme.62.1.20
[8] Devlin AC, Fee PA. How do different levels of smoking affect dental implants? Evid Based Dent. 2021;22(1):28–9. DOI: 10.1038/s41432-021-0155-5
[9] Jonas E, Muchnik D, Rabinovich I, Masri D, Chaushu G, Avishai G. Characteristics of sialolithiasis in Israel, a big-data retrospective study of 5100 cases. Oral Dis. DOI: 10.1111/odi.15095
[10] Khan MA, Vichayanrat T, Ngoenwiwatkul Y. The association between smoking and smokeless tobacco use with dental caries among Pakistani patients. BMC Oral Health. 2024;24(1):723. DOI: 10.1186/s12903-024-04508-y
[11] Popa GV, Mogoantă L, Badea O. Macroscopic changes in oral mucosa and hygiene indicators in smokers. Curr Health Sci J. 2021;47(3):383–92. DOI: 10.12865/chsj.47.03.08
[12] Moroka R, Povaliaiev V, Tkachenko I, Fomenko Y, Babai O, Mikulinska-Rudich Y, et al. The relationship between the condition of the oral cavity and the use of tobacco products in different age groups. Georg Med New. 2024;350(5):25–30.
[13] Drovandi A, Salem S, Barker D, Booth D, Kairuz T. Human biomarker exposure from cigarettes versus novel heat-not-burn devices: A systematic review and meta-analysis. Nicotine Tob Res. 2020;22(7):1077–85. DOI: 10.1093/ntr/ntz200
[14] Yang I, Rodriguez J, Young Wright C, Hu YJ. Oral microbiome of electronic cigarette users: A cross-sectional exploration. Oral Dis. 2023;29(4):1875–84. DOI: 10.1111/odi.14186
[15] Tjahajawati S, Rafisa A, Lestari EA. The effect of smoking on salivary calcium levels, calcium intake, and bleeding on probing in female. Int J Dent. DOI: 10.1155/2021/2221112
[16] Majid OW. Salivary lipid changes in young adult tobacco smokers and e-cigarette users: A hidden risk to oral health? Evid Based Dent. 2024;25(2):67–8. DOI: 10.1038/s41432-024-00998-5
[17] Ratajczak A, Jankowski P, Strus P, Feleszko W. Heat not burn tobacco product – a new global trend: Impact of heat-not-burn tobacco products on public health, a systematic review. Int J Environ Res Public Health. 2020;17(2):409. DOI: 10.3390/ijerph17020409
[18] Holliday R, Chaffee BW, Jakubovics NS, Kist R, Preshaw PM. Electronic cigarettes and oral health. J Dent Res. 2021;100(9):906–13. DOI: 10.1177/00220345211002116

[19] Taheri N, Fattahi P, Saeedi E, Sayyari M, Abdi S, Khaki M, et al. A decade of tobacco control efforts: Implications for tobacco smoking prevalence in Eastern Mediterranean countries. PLoS One. 2024;19(2):e0297045. DOI: 10.1371/journal.pone.0297045
[20] Duarte PM, Nogueira CFP, Silva SM, Pannuti CM, Schey KC, Miranda TS. Impact of smoking cessation on periodontal tissues. Int Dent J. 2022;72(1):31–6. DOI: 10.1016/j.identj.2021.01.016
[21] Dixit A, Dhanalakshmi P, Rameshchandra PT, Chachlani KS, Dithi C, Dash KC, et al. Effect of smoking cessation programs on periodontal disease progression. J Pharm Bioallied Sci. 2024;16(Suppl 1):771–3. DOI: 10.4103/jpbs.jpbs_1004_23
[22] Raj AT, Sujatha G, Muruganandhan J, Kumar SS, Bharkavi SI, Varadarajan S, et al. Reviewing the oral carcinogenic potential of e-cigarettes using the Bradford Hill criteria of causation. Transl Cancer Res. 2020;9(4):3142–52. DOI: 10.21037/tcr.2020.01.23
[23] Ramasamy J, Sivapathasundharam B. A study on oral mucosal changes among tobacco users. J Oral Maxillofac Pathol. 2021;25(3):470–7. DOI: 10.4103/jomfp.jomfp_105_21
[24] Choudhary A, Kesarwani P, Chakrabarty S, Yadav VK, Srivastava P. Prevalence of tobacco-associated oral mucosal lesion in Hazaribagh population: A cross-sectional study. J Family Med Prim Care. 2022;11(8):4705–10. DOI: 10.4103/jfmpc.jfmpc_1990_21
[25] Kusiak A, Maj A, Cichońska D, Kochańska B, Cydejko A, Świetlik D. The analysis of the frequency of leukoplakia in reference of tobacco smoking among northern Polish population. Int J Environ Res Public Health. 2020;17(18):6919. DOI: 10.3390/ijerph17186919
[26] Smail Y, Troizier-Cheyne M, Lutz CM, Ejeil AL. Clinico-pathological specificities of gingival carcinoma among 32 patients with oral cancer: A cross sectional retrospective and observational study. BMC Oral Health. 2024;24(1):1317. DOI: 10.1186/s12903-024-05078-9
[27] Bhattacharjee T, Kerketa M, Babu NA. Differences of oral cancer in men and women of West Bengal, India. J Oral Maxillofac Pathol. 2021;25(1):200. DOI: 10.4103/jomfp.jomfp_476_20
[28] Amato L, Cruciani F, Solimini R, Barca A, Pacifici R, Davoli M. Effects of electronic cigarettes on health: A systematic review of the available evidence. Recenti Prog Med. 2020;111(1):30–43. DOI: 10.1701/3294.32651
[29] Nazaryan R, Kryvenko L, Zakut Y, Karnaukh O, Gargin V. Application of estimated oral health indices in adolescents with tobacco addiction. Pol Merkur Lekarski. 2020;48(287):327–30.
[30] Liu B, Yang L, Liu X. Interaction between tobacco smoke exposure and zinc intake and its effect on periodontitis: Evidence from NHANES. Int Dent J. 2024;74(5):978–86. DOI: 10.1016/j.identj.2024.04.007
[31] Al Kawas S, Al-Marzooq F, Rahman B, Shearston JA, Saad H, Benzina D, et al. The impact of smoking different tobacco types on the subgingival microbiome and periodontal health: A pilot study. Sci Rep. 2021;11(1):1113. DOI: 10.1038/s41598-020-80937-3
[32] Pagano S, Negri P, Coniglio M, Bruscoli S, Di Michele A, Marchetti MC, et al. Heat-not-burn tobacco (IQOS), oral fibroblasts and keratinocytes: Cytotoxicity, morphological analysis, apoptosis and cellular cycle. An *in vitro* study. J Periodontal Res. 2021;56(5):917–28. DOI: 10.1111/jre.12888
[33] Uehara O, Nakamoto N, Hiraki D, Paudel D, Sugiyama N, Morikawa T, et al. Effects of prolonged stimulation with heated tobacco products (Ploom TECH+) on gingival epithelial cells. J Periodontal Res. 2023;58(3):553–63. DOI: 10.1111/jre.13123
[34] Yoshioka T, Tabuchi T. Combustible cigarettes, heated tobacco products, combined product use, and periodontal disease: A cross-sectional JASTIS study. PLoS One. 2021;16(3):e0248989. DOI: 10.1371/journal.pone.0248989
[35] Figueredo CA, Abdelhay N, Figueredo CM, Catunda R, Gibson MP. The impact of vaping on periodontitis: A systematic review. Clin Exp Dent Res. 2021;7(3):376–84. DOI: 10.1002/cre2.360
[36] Pouly S, Ng WT, Benzimra M, Soulan A, Blanc N, Zanetti F, et al. Effect of switching to the tobacco heating system versus continued cigarette smoking on chronic generalized periodontitis treatment outcome: Protocol for a randomized controlled multicenter study. JMIR Res Protoc. 2021;10(1):e15350. DOI: 10.2196/15350
[37] Mišković I, Kuiš D, Špalj S, Pupovac A, Prpić J. Periodontal health status in adults exposed to tobacco heating system aerosol and cigarette smoke vs. non-smokers: A cross-sectional study. Dent J (Basel). 2024;12(2):26. DOI: 10.3390/dj12020026
[38] Stiller HL, Ionfrida J, Kämmerer PW, Walter C. The effects of smoking on dental implant failure: A current literature update. Dent J (Basel). 2024;12(10):311. DOI: 10.3390/dj12100311
[39] Tanik A, Demirci F. Effect of unfiltered cigarettes on marginal bone loss of dental implants: A single center 4-year retrospective clinical study. Am J Dent. 2022;35(5):255–62.
[40] Apatzidou DA. The role of cigarette smoking in periodontal disease and treatment outcomes of dental implant therapy. Periodontol 2000. 2022;90(1):45–61. DOI: 10.1111/prd.12449
[41] Mustapha AD, Salame Z, Chrcanovic BR. Smoking and dental implants: A systematic review and meta-analysis. Medicina (Kaunas). 2021;58(1):39. DOI: 10.3390/medicina58010039
[42] Alqahtani M. Influence of moderate cigarette smoking on the peri-implant clinicoradiographic inflammatory parameters around cement- and screw-retained dental implants. J Oral Implantol. 2021;47(6):473–7. DOI: 10.1563/aaid-joi-d-19-00352
[43] Vámos O, Komora P, Gede N, Hegyi P, Kelemen K, Varga G, et al. The effect of nicotine-containing products on peri-implant tissues: A systematic review and network meta-analysis. Nicotine Tob Res. 2024;26(10):1276–85. DOI: 10.1093/ntr/ntae085

[44] Fathi A, Salehi S, Sadeghi S, Atash R, Monirifard R, Farahmand S. Electronic cigarettes and peri-implantitis: An umbrella review. J Oral Implantol. 2024;50(6):653–8. DOI: 10.1563/aaid-joi-D-24-00157
[45] FDI World Dental Federation. The role of oral health practitioners in tobacco cessation [Internet]. 2021 [cited 2024 June 17]. Available from: https://www.fdiworlddental.org/role-oral-health-practitioners-tobacco-cessation
[46] Kaleta D, Świątkowska B, Szulc M, Wojtysiak P, Jankowski M. Anti-smoking intervention, e-cigarettes, and heated tobacco products – what to remember? Recommendations for doctors who have direct contact with nicotine-addicted patients regarding anti-smoking intervention. Gen Med Health Sci. 2024;30(2):81–6. DOI: 10.26444/monz/189601
[47] Kawamura K, Doi T, Kano K, Matsui M, Hattori Y, Onishi F, et al. Association between smoking habits and dental care utilization and cost using administrative claims database and specific medical check-up data. BMC Oral Health. 2022;22(1):372. DOI: 10.1186/s12903-022-02397-7
[48] Chaffee BW, Couch ET, Vora MV, Holliday RS. Oral and periodontal implications of tobacco and nicotine products. Periodontol 2000. 2021;87(1):241–53. DOI: 10.1111/prd.12395
[49] National Institute of Aging. Taking care of your teeth and mouth [Internet]. 2024 [cited 2024 June 17]. Available from: https://www.nia.nih.gov/health/teeth-and-mouth/taking-care-your-teeth-and-mouth
[50] Osadchiy AI. Oral health: Prevention and hygiene. Recommendations of a dentist [Internet]. 2020 [cited 2024 June 17]. Available from: https://umj.com.ua/uk/publikatsia-175350-zdorov-ya-porozhnini-rota-profilaktika-i-gigiyena-rekomendatsiyi-likarya-stomatologa

## Наслідки вживання нікотинових виробів для тканин ротової порожнини: підходи до профілактики

**Богдан Кравченко**
Аспірант
Національний медичний університет імені П.Л. Шупика
04112, вул. Дорогожицька, 9, м. Київ, Україна
https://orcid.org/0009-0003-4511-1994
**Костянтин Лихота**
Доктор медичних наук, доцент
Національний медичний університет імені П.Л. Шупика
04112, вул. Дорогожицька, 9, м. Київ, Україна
https://orcid.org/0000-0003-0912-6470

**Анотація.** Метою дослідження було здійснити аналіз впливу куріння та сучасних систем вивільнення нікотину на стан ротової порожнини, враховуючи стратифікацію факторів ризику розвитку стоматологічних патологій, визначити можливість виникнення ускладнень та впровадження профілактичних програм в роботу лікарів-стоматологів для мінімізації шкідливого впливу цієї звички. Для проведення дослідження було здійснено пошук досліджень у базах даних PubMed, Scopus, Web of Science та Google Scholar. Було встановлено, що електронні сигарети, традиційні сигарети та системи нагрівання тютюну негативно впливають на ротову порожнину, викликаючи ризики лейкоплакії, гінгівіту та пародонтальних захворювань. Куріння традиційних сигарет спричиняє суттєві деструктивні зміни, зокрема глибокі пародонтальні кишені (68 %), втрату тканинного прикріплення (42 %) та лейкоплакію ясен (85,1 %). Електронні сигарети, хоча менш шкідливі, викликають подразнення слизової оболонки, дисбактеріоз і підвищують ризик пародонтозу у 1,43 рази порівняно з некурцями. Системи нагрівання тютюну демонструють нижчу токсичність, проте ризик запалень і тканинної деструкції зберігається. Результати свідчать, що відмова від куріння позитивно впливає на стоматологічне здоров'я. Через рік після припинення куріння спостерігається зменшення глибини пародонтальних кишень на 1,5 мм і покращення тканинного прикріплення на 1,2 мм. Це підтверджує важливість боротьби із залежністю для профілактики стоматологічних захворювань. Рекомендації для лікарів включають ретельний збір анамнезу, реєстрацію форм вживання нікотину, застосування принципу "5А" про досвід вживання тютюну пацієнтом, надати інформацію про відмову від куріння, оцінити їхню готовність до відмови, допомогти в процесі позбавлення залежності та забезпечити подальшу підтримку через регулярний контакт. Особливу увагу слід приділяти інформуванню пацієнтів про ризики, пов'язані з усіма формами тютюну, та сприяти повній відмові від їх використання. Однак, якщо відмовитися від куріння неможливо, важливо регулярно відвідувати стоматолога, призупинити куріння під час лікування пародонтозу чи проведення імплантації зубів, ретельно дотримуватись правил гігієни ротової порожнини із застосуванням ополіскувачів й абразивних паст, а також своєчасно звертатися до спеціалістів за професійною допомогою. Такі заходи дозволяють зменшити ризик стоматологічних патологій і сприяють довгостроковому збереженню здоров'я ротової порожнини

**Ключові слова:** парадонтит; плоскоклітинний рак; лейкоплакія; карієс; куріння; електронні сигарети

**ВІСНИК МЕДИЧНИХ І БІОЛОГІЧНИХ ДОСЛІДЖЕНЬ**
**науково-практичний журнал**

**Том 6, № 4**
**2024**

**Відповідальний редактор:**
Т. П'ятковський

**Редагування бібліографічних списків**:
Т. П'ятковський

**Редагування англомовних текстів**:
К. Касьянов

**Комп'ютерна верстка**:
О. Глінченко

Підписано до друку 26.11.2024
Формат 60*84/8
Ум. друк. арк. 10,4
Наклад 100 прим.

Видавництво:
Тернопільський національний медичний університет імені І. Я. Горбачевського
46001, майдан Волі, 1, м. Тернопіль, Україна
Тел.: +380 352 524492
E-mail: info@bmbr.com.ua
https://bmbr.com.ua/uk

**BULLETIN OF MEDICAL AND BIOLOGICAL RESEARCH**
**Scientific-Practical Journal**

**Volume 6, No. 4**
**2024**

**Managing Editor:**
T. Pyatkovskyy

**Editing bibliographic lists:**
T. Pyatkovskyy

**Editing English-Language Texts**:
K. Kasianov

**Desktop publishing:**
O. Glinchenko

Signed to the print 26.11.2024
Format 60*84/8
Conventional Printed Sheet 10.4
Circulation 100 copies

Publisher:
I. Horbachevsky Ternopil National Medical University
46001, 1 Maidan Voli, Ternopil, Ukraine
Tel.: +380 352 524492
E-mail: info@bmbr.com.ua
https://bmbr.com.ua/en