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CONTENTS

ORIGINAL ARTICLES

Inga Samborska, Viktoriia Piliponova, Kostiantyn Ahafonov, Liudmyla Ahafonova, Liudmyla Sokurenko
Correction with Omega-3 fatty acid of lead influence on morpho-functional status of rats' cerebral cortex **2011**

Andrzej Żyluk
Outcomes of treatment of cat-bite injures **2019**

Oksana I. Afanasiuk, Yuliia Y. Masliuk, Valerii P. Ivanov
Association between the severity of anatomical damage to the coronary arteries and clinical indicators and Holter electrocardiogram monitoring indicators **2028**

Alaa H. Fadel, Noor H. Naser
In Silico Study and synthesis of thiazole-based amino coumarin derivatives with promising carbonic anhydrase inhibitory activity **2034**

Oksana O. Kopchak, Kateryna O. Hrynevych, Oleksandr R. Pulyk
Sexual dysfunction in multiple sclerosis: Associations with disability, fatigue, affective symptoms and brainstem lesions **2046**

Dwi Retnoningrum, Nyoman Suci Widyastiti, Meita Hendrianingtyas, Edward Kurnia Setiawan Limijadi, Villa Sekar Cita, Nur Farhanah, Banundari Rachmawati
Inflammatory cytokines as risk factor of septic shock in patients with sepsis **2053**

Lyudmyla P. Sushchenko, Nataliia M. Polishchuk, Yevgen Y. Kachur, Alina I. Kubatko, Vira R. Budzyn, Victoriya I. Romanova, Lidiya A. Zavatska
Adaptive football in physical and sports rehabilitation of individuals with amputations: Impact on health-related quality of life, health status, and cognitive domain **2065**

Karrar Mohammed Abbas AlButhabhak, Sameer abdul Majeed Jasim, Ali Hasan Ismaeel Alshamari
Prevalence of thyroid dysfunction in patients with acute atrial fibrillation attending coronary care unit at Al-Sadder Teaching Hospital **2072**

Nataliia Siranchuk, Sofiia Siranchuk, Lyudmila Lukyanyk, Svitlana Dubovyk, Iryna Sukhopara, Liudmyla Rjmanenko, Nataliia Vyshnivska
Adrenal hormone and histamine levels in children with vasomotor rhinitis **2082**

Asala Riyadh Mohammed, Israa Dheyaa Mohammed, Sundus Baqer Dawood, Adil Ali Hussein
Socioeconomic predictors of maternal coping during pediatric hospitalization **2088**

Ivan I. Shkribliak, Iryna V. Shargorodska, Oleksii V. Kriuchko, Pavla P. Ivaniuta
Clinical and ophthalmological patterns of myopia onset and progression in ukrainian patients of different age groups **2095**

Noor Majeed Albahdly, Ameer Dhahir Hameedi
Ex vivo histological comparison between Er, Cr: YSGG laser and slow-speed hand piece in bone cutting **2102**

Vadym G. Terentyuk, Ruslan S. Tsymbaiyuk, Sergii V. Zemskov, Inna I. Kucherenko, Kateryna O. Bobkovych, Tamara V. Serhiienko, Borys I. Palamar
A study of nurses' readiness to learn about and use telemedicine **2113**

Atheer Kadhim Ibadi, Elaf Ibrahim Ismail
Clinical and sociodemographic predictors of psychological health outcomes in cancer patients undergoing active treatment at the National Teaching Cancer Hospital, Najaf: AI-assisted predictive analysis **2120**

Oleg Hodovanyi, Olesia I. Martovlos, Andrew I. Martovlos, Olha Yu. Klyuchivska, Oksana V. Skybchyk, Ostap O. Myhal, Rostyslav Stoika
Correction of periodontal status and evaluation of disorders in biomarkers of local oxidative metabolism in patients with orthodontic anomalies on the background of generalized periodontitis **2129**

Mariam Suhail, Atheer Kadhim Ibadi
Exploring association between anxiety and infertility characteristics: A combined statistical and machine learning approach **2142**

Maksym Harkavenko, Kostiantyn V. Tarasenko, Oksana Shlykova
The structure of the Poltava region population, determined by the polymorphisms rs3745274 and rs1799971 significant in the kinetics of anaesthetics **2152**

Salim Kadhim, Majeed N. Abdul Al-Shaeer, Maryam K. Alaasam, Aeman Yaakob Almasodi
Vancomycin-resistant Enterococci: Local epidemiology and clinical outcomes **2158**

Viacheslav V. Stepanenko, Lydmyla Y. Petryshyn, Viktoriia I. Stepanenko, Solomiia I. Hnatyshyn, Mariia Ya. Kichula, Vitalii Y. Kulchytsky, Larysa Ya. Fedoniuk
Study of the cognitive aspects of subjective well-being and Mental Health stability among internally displaced schoolchildren **2164**

Ahmed Hasan Kadhim, Nagham Yahya Ghafil
Assessment of physiological changes and levels of KIM-1 and MDA biomarkers resulting from TDM on vancomycin-induced subclinical nephrotoxicity for pediatrics in Al-Najaf city: A case-control study **2173**

Vasylysa G. Suleimanova, Volodymyr F. Kryvetskyi, Volodymyr O. Shaprynskyi, Bohdan O. Mitiuk, Viktor I. Polishchuk, Pavlo S. Lysak, Andrii V. Kovalchuk
Gasless laparoscopy in the treatment of hiatal hernia **2180**

Maryam Alaayedi, Zahraa M. Kadhum, Jamal Ali Ashoor, Ashti M.H. Saeed, Jinan M Al-Mousawy, Hasanin Shakir Mahmmod, Entsar H. Jawad, Mustafa H. ALHamadani
Formulation and characterization of lipophilic chemotherapy as ethosomal gel for cervical cancer **2186**

Maksym A. Chernykh, Olena O. Oshyvalova
Comparative analysis of machine learning models for survival prediction in patients with malignant melanoma **2194**

Vladyslav A. Danylchenko, Roman M. Perkatyi, Valerii O. Kryzhanovskyi, Vadym L. Shvets, Andriy V. Nadutiy, Iryna I. Shaposhikova, Yurii M. Korniichuk
Motor activity as a factor in improving the physical working capacity and somatic health of higher education applicants **2201**

Monya Noori Hassan Almuhmed Agha, Afya Sahib Diab Al-Radha
Radiographic evaluation of the impact of hyaluronic acid and bone graft on peri-implant tissue: A randomized clinical trial **2207**

Ivan M. Leshchyshyn, Leonid Yu. Markulan, Mykola M. Plodienko, Olga I. Okhotska, Pavlo L. Byk
The appendix as a full-thickness morphological surrogate for the histological subtype of the colon in patients with chronic slow-transit constipation **2216**

REVIEW ARTICLES

Ewa Alicja Ogłodek
Increased proteasome and inflammasome activity in response to cellular stress and PTSD-induced damage – a narrative review **2229**

Sofiia M. Maslianchuk, Olena O. Oshyvalova
Quantitative justification of the advantage of 3d bioprinting over conventional skin transplantation in the reconstruction of burn defects **2237**

Ali Jasim Mohammed, Ahmad Fakrurrozi Mohamad, Muhammad Annurdin Sabarudin, Husniyati Roslan
An overview of peri-implant soft tissues dehiscence (PSTD): A narrative review **2245**

Yuliia Lysanets, Olena Bieliaieva, Olena Uvarkina, Mariia Hutsol
The use of eponymic bacterial nomenclature in medical case reports: Etymology and morphological models **2255**

Nasar Khan, Huma Kanwal Atta, Sohima Aznak, Oksana Fishchuk
Exploring the potential and challenges of artificial intelligence (AI) for intervention and prevention of suicidality to achieve SGDs: A thematic review **2265**

Yuliia Savitska, Valerii Ivanov, Andrii Klantsa, Natalia Schepina
Anti-inflammatory therapy in patients with coronary artery disease: a new era in the treatment of atherosclerosis or false hopes? **2273**

Aleksander Krupski, Michal Marusza, Victoria Stielow, Antonina Zatyka
Exercise-related dysglycemia in type 1 diabetes: Endocrine counterregulation, exercise modality, risk modifiers and technology – supported prevention **2278**

ORIGINAL ARTICLE

Correction with Omega-3 fatty acid of lead influence on morpho-functional status of rats' cerebral cortex

Inga Samborska[1], Viktoriia Piliponova[2], Kostiantyn Ahafonov[2], Liudmyla Ahafonova[2], Liudmyla Sokurenko[3]
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ABSTRACT

Aim: Experimental justification for using omega-3 fatty acids to correct the effects of free radical oxidation in the brains of rats with lead poisoning.

Materials and Methods: Experiments were conducted on 24 nonlinear male rats at the age of 3-4 months. Lead intoxication was performed in 16 rats by intragastric administration of plumbum (II) acetate. Part of the animals (n=8) that were given lead intoxication, intragastrically introduced omega-3 fatty acids. Microscopic examination of the cerebral cortex was performed using an Olympus BX-41 light microscope.

Results: Under the condition of lead intoxication, neurocytes of the molecular, outer granular, and pyramid layers had signs of extracellular oedema. The areas of significant haemorrhage in all layers of the cerebral cortex were observed. With correction by omega-3 fatty acids, the microscopic structure of the blood vessel wall was restored. Under the influence of lead, SOD and catalase slowed down the utilisation of hydrogen peroxide, compared to the control group, which mostly normalised with the correction of omega-3 fatty acids.

Conclusions: Thus, studies have shown that under the condition of lead intoxication at a dose of 7.5 mg/ kg body weight for 14 days, histological examination revealed disorders of the cerebral cortex cytoarchitectonics and damage to the structure of the blood vessel walls. With the correction of lead intoxication by omega-3 fatty acids at a dose of 750 mg/kg body weight within 14 days, improvements were noted in the histostructure of the experimental animals' cerebral cortex.

KEYWORDS: lead intoxication; Omega-3 fatty acid; cerebral cortex; neurons, glial cells.

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INTRODUCTION

Chronic lead poisoning is a common problem in both developing and developed countries. The toxic effects of lead on various organs and body systems have been studied for a long time, and the effects of lead intoxication on the hematopoietic system, gastrointestinal tract, liver, kidneys, and reproductive system have been well described [1-3]. Studies have also reported that even small doses (less than 3 μg/dL) of lead compounds (Pb^{2+}) cause central nervous system (CNS) injury and are manifested by a variety of neurological disturbances in the cortex and changes in cortical-subcortical relationships [4, 5]. Exposure to lead intoxication results in the development of acute encephalopathy and peripheral neuropathy, paralysis, toxic coma, reduced cognitive functions and intelligence, and mental disorders [6]. Lead can accumulate in brain tissues by crossing the blood-brain barrier and disrupting the brain's structural components, resulting in injury to glial cells, impaired synapse formation, and disrupted signal transmission [7]. The formation of free radicals has been reported to be associated with increased oxidative stress under lead exposure, which is accompanied by the activation of free radical oxidation processes in cell membranes, lipids and proteins. The lead neurotoxic effect is also released through the displacement of zinc or calcium from compounds enriched with thiol groups, which prevents apoptotic cell death [8, 9].

The morphological changes in the cerebral cortex under lead intoxication, which manifest in the molecular layer nerve cells, include vacuolization, pigmentation, nuclear pyknosis, and dissolution of chromatophilic substance. Foci of haemorrhages, stasis and blood clots characterise severe brain and spinal cord damage forms. There is insufficient information on the lead intoxication pathogenic effect on developing morphological changes in the cerebral cortex ([10]). It may have a protective effect on the central nervous system and serve as a means of preventing various neurological disorders. Confirmation of these facts was the unwinding of the

processes of ω-3 metabolism to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), which have an antioxidant effect: they increase the activity of superoxide dismutase, catalase, reduce the proliferation of neutrophils and the caspase-3 function, increase cellular clearance by macrophages [11-13].

However, the relationship between the high lead concentration in plasma, which damages the nervous system, and the result of correcting the condition with polyunsaturated fatty acids still needs to be studied further. Existing research does not fully disclose cerebral cortex morphology under lead intoxication and the results of its correction.

AIM

The study aimed to provide experimental justification for using omega-3 fatty acids to correct the effects of free radical oxidation in the brains of rats with lead poisoning.

MATERIALS AND METHODS

Experiments were conducted on 24 nonlinear male rats at the age of 3-4 months, weighing 250-280 g. All stages of research are conducted in accordance with international requirements for the humane treatment of animals, as outlined in the "European Convention for the Protection of Vertebrates, which are used for experimental and other scientific purposes" (Strasbourg, 1986).

Lead exposed: 16 rats were intragastrically administered by plumbum (II) acetate in 1% starch solution (in a dose of 7.5 mg/kg body weight) once/day for 14 days. Part of these animals (n=8) were given lead intoxication, intragastrically introduced omega-3 fatty acids (O.N.E. ™ Omega) per 1% starch solution at a dose of 750 mg/kg of body weight once/day for 14 days [14].

The control group consisted of 8 rats that were intragastrically administered an equivalent amount of solvent (1% starch solution) at a rate of 1 ml per 100 g of rat weight over 14 days.

MORPHOLOGICAL RESEARCH

On the 15th day, animals were taken out of the experiment by decapitation under ketamine anaesthesia. The extracted brain pattern was fixed in a 2.5% glutaraldehyde solution at pH 7.2-7.4 in phosphate buffer. It was fixed in an Epon-Araldite mixture, according to the generally accepted method [15].

Demi-thin sections were made from the obtained blocks, and then stained with hematoxylin and eosin. Microscopic examination of the cerebral cortex was performed using an Olympus BX-41 light microscope. We investigated the molecular (plexiform) layer, the external (outer) granular layer and the external (outer) pyramidal layer.

BIOCHEMICAL RESEARCH

On the 15th day, the animals' extracted brains were perfused with a cold 1.15% KCl solution and homogenised at 3000 rpm for 1 minute (Teflon-coated) in a medium of 1.15% potassium chloride (1:3 ratio) for withdrawal. Homogenates were centrifuged for 30 minutes at 600 g, and aliquots of the post-nuclear supernatant were taken into Erpendorf microtubes and stored at -20°C until further processing.

Superoxide dismutase (SOD), catalase, malondialdehyde (MDA), and carbonyl groups of proteins (KGP) levels were determined in brain homogenates, according to the International System of Units recommended for use in clinical laboratory practice.

Statistical processing of measurement results was performed using a software package, including GraphPad Prism 8, Biostat, and MS Excel, and a graph was generated. Statistical analyses were performed using the nonparametric method.

ETHICS

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

AI STATEMENT

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

RESULTS

MORPHOLOGY OF THE EXPOSED RAT'S BRAIN CEREBRAL CORTEX

In the case of lead influence in the molecular layer of the rats' cerebral cortex, the neurocytes exhibited polymorphism phenomena. Their cytoplasm was enlightened, with signs of hydropic dystrophy. Cell nuclei were characterised by hyperchromia. Chromatin in them was usually placed in a lump-shaped region near the nuclear envelope inner membrane. Significant areas of enlightenment were noted around neurocytes, which is a pericellular oedema sign. The glyocytes of the molecular layer under these conditions were also hyperchromic. The latter prevailed, condensed chromatin occupying the eccentric

Table 1. Design groups of experimental animals

Groups	Time, days	Experiment	Animals number
Control	14	intragastric equivalent amount of solvent (1% starch solution at the rate of 1 ml per 100 g of rat weight)	8
Pb	14	intragastric administrated by plumbum (II) acetate in 1% starch solution (in a dose of 7.5 mg/kg body weight 1 time/day for 14 days)	8
Pb+Omega 3	14	intragastric administrated by plumbum (II) acetate in 1% starch solution (in a dose of 7.5 mg/kg body weight 1 time/day for 14 days) + omega-3 fatty acids (O.N.E. ™ Omega) per 1% starch solution at a dose of 750 mg/kg of body weight 1 time/day	8

Source: compiled by the authors of this study

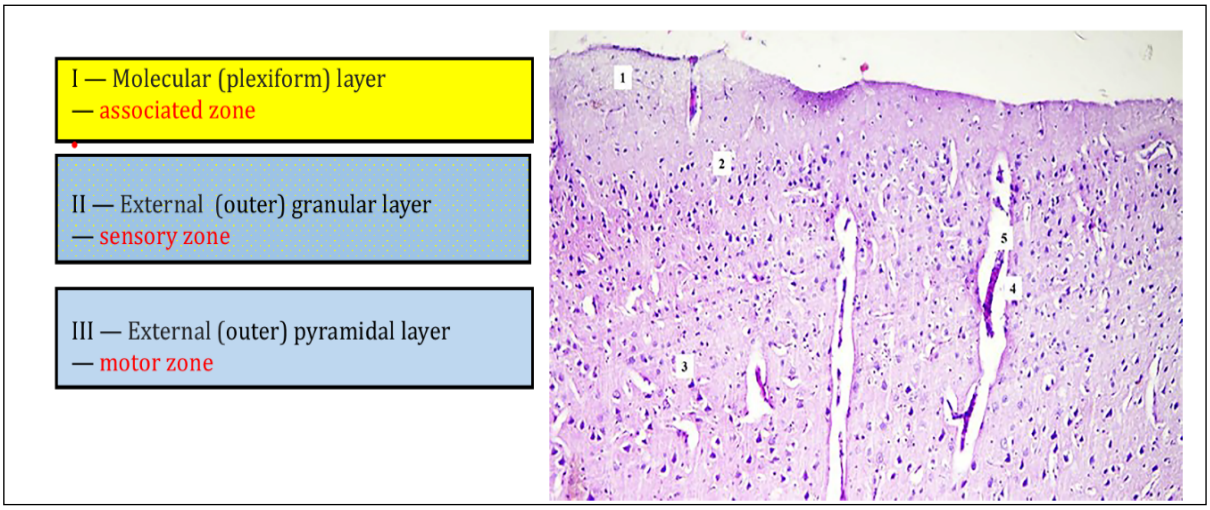


Fig. 1. Histological organization of the cerebral cortex under lead exposed. Molecular layer (1), outer granular layer (2), pyramidal cells layer (3), expansion of the lumen of the blood vessel (4), and blood cells stasis (5). Hematoxylin and eosin x 100
Picture taken by the authors

position. In the molecular and outer granular layers of the experimental animals' cerebral cortex, a significant expansion of the blood vessel lumen was observed in the same experiment. Histological sections revealed blood cell stasis, signs of white blood cell adhesion, and occasionally, the presence of initial blood clots (Fig. 1).

In the outer granular layer of the cerebral cortex, a lesion of the endothelial lining was visualised in the vascular walls. The endothiliocytes were exfoliated from the basal membrane (Fig. 2C). In some fields of view, the fibrin deposition threads on the blood vessels' walls and luminal surface were revealed, and in some places, perivascular oedema manifestations were observed. In the outer granular layer of lead-exposed brain hemispheres, both hyper- and hypochromic cells were noted. Neurocyte nuclei had telocentric localisation, and the presence of binucleated cells was decisive. In the outer granular layer, neurocytes exhibited a significant variation in the nuclear-cytoplasmic index, which may be a possible reaction to the damaging factor's effect. The presence of necrosis areas also characterised this cerebral cortex layer. Various haemorrhage areas were noticeable. In addition, the neurocytes' mitotic activity increased, a compensatory and adaptive response to the influence of plumbum acetate (Fig. 2).

In the experimental group, the animals' cerebral cortex had a pyramid layer with dark cells that predominated. Their shapes and sizes were significantly different. Most pyramidal neurocyte nuclei were hyperchromatic, with a prevailing presence of heterochromatin. Neuronophagia manifestations are often observed, indicating increased functional activity of microglial cells. In some pyramid cells, recesses or niches were formed where glyocytes were localised. It is worth noting that significant changes in the neurocyte nuclei, namely karyopicnosis and karyorrhexis, have been found. A decrease in size, accompanied by hyperchromatosis, was also reported in pyramidal neurocytes. Hromatophilic substance was placed in compact, dark, rod-like formations. Often, pyramid neurocytes were not clearly visualised, as they lost their characteristic shape, their edges were uneven, and the differentiation of the perikaryon, nuclei, and nucleus boundaries was difficult. Ischemic changes in the cerebral cortex's pyramidal layer nerve cells were found.

Microscopic examination of neurocytes revealed the phenomenon of satelitosis, characterised by the

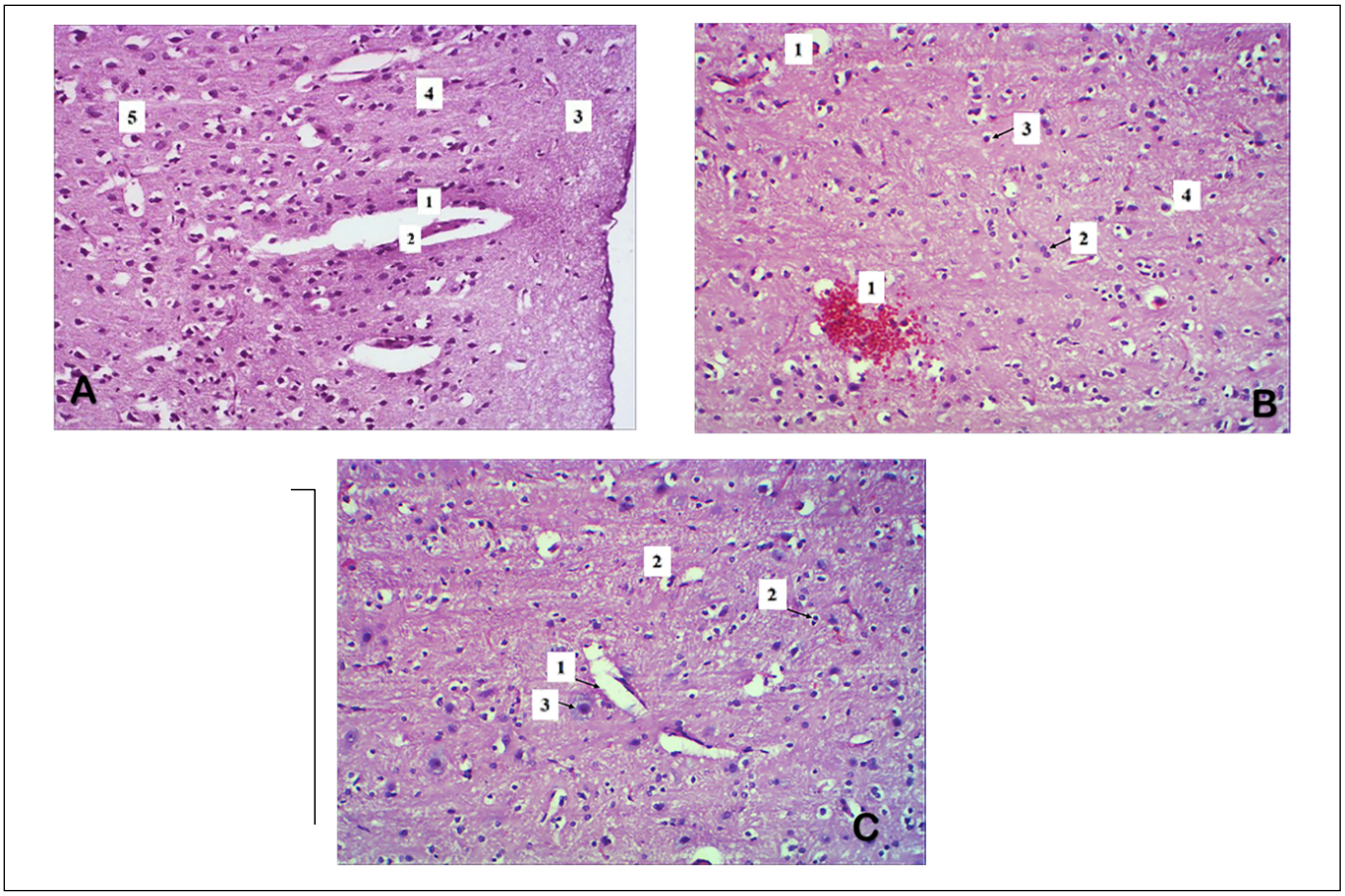


Fig. 2. Microscopic changes of the cerebral rats cortex under lead exposed. A). Perivascular oedema (1), stasis of blood cells (2), molecular layer (3), outer granular layer (4), outer pyramid cells layer (5). B). (1), binucleated neurons (2), vacuolation of the nerve cell cytoplasm and perivascular oedema (3), extracellular oedema (4). C). Endotheliocyte exfoliation of the vessel wall from the basal lamina, fibrin in the lumen of the vessel (1), two-nuclei cells (2), and perinuclear oedema (3). Hematoxylin and eosin. ×100
Picture taken by the authors

presence of glyocytes on the surface of neurocytes. Some pyramid cells also exhibited extracellular oedema signs, as well as cytoplasmic and nuclear hypo- and/or hyperchromasia. Chromatin in such nuclei was diffuse; it was impossible to identify its division into hetero- and euchromatin. The cerebral cortex pyramid layer glial cells showed signs of hyperplasia. Their contours were usually blurred and fuzzy. The cytoplasm of glya cells is enlightened; the nuclei are enlarged and typically contain one nucleolus; chromatin is predominantly diffuse and localised on the nuclear periphery. In addition, the cells with nuclei shifted to the periphery due to hydropic dystrophy of the cytoplasm and perinuclear oedema were detected (Fig. 3).

MORPHOLOGY OF THE CEREBRAL RAT'S CORTEX UNDER LEAD EXPOSURE WITH FATTY ACID OMEGA-3 CORRECTION

At the histological examination of the cerebral rat's cortex under lead exposure with fatty acid omega-3 correction, a decrease in the molecular and external granular layers' blood vessel lumina was detected, compared to the experimental group animals without correction. Destruction of the vascular walls' endothelial layer wasn't shown. A small number of blood elements were recorded in the lumen. The perivascular and extracellular oedema manifestations were moderate. There was a significant improvement in the cerebral cortex cytoarchitectonics. Haemorrhage zones were less common. There are still neuronophagia and satellitosis phenomena on the background of the structural cerebral cortex histological restoration. In some fields of view, the outer granular and pyramidal layers of nerve cells showed signs of necrosis. An essential finding under these conditions was an increase in the activity of the histiocytic row cells in the cerebral cortex pyramidal layer, which is probably the launch of protective mechanisms. These layer glyocytes were characterised by a sharp increase in size compared to those without correction. They were located around or within the body of pyramidal neurocytes and had a high nuclear-to-cy-

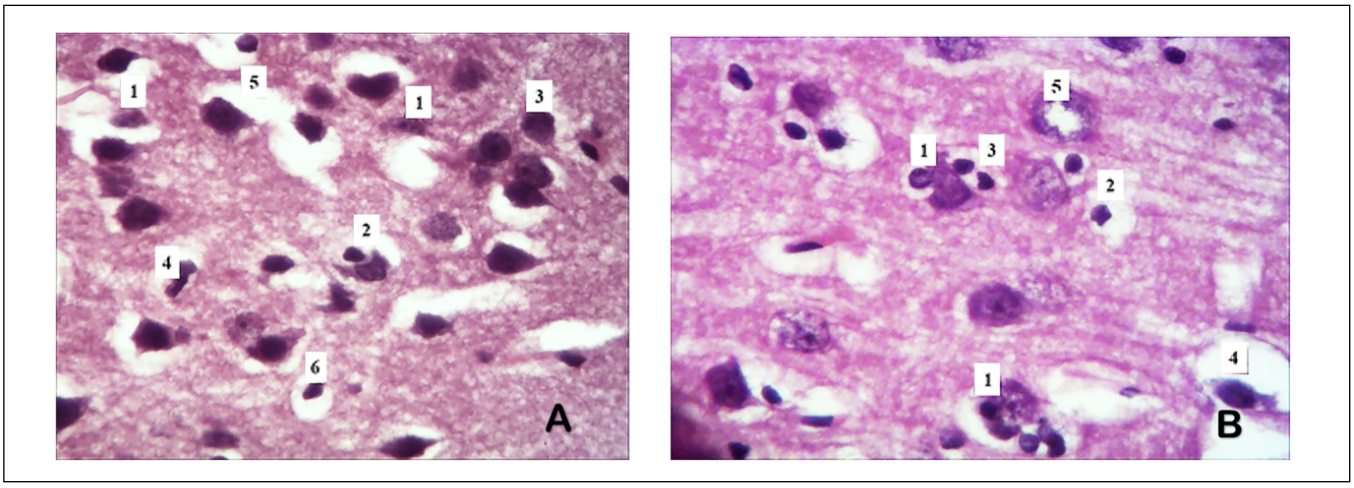


Fig. 3. Histological changes of the cerebral rat cortex under lead exposed. A). Shadow cells (1), neuronophagia (2), karyorrhexis (3), wrinkling pyramidal neurocytes (4), pronounced pericellular oedema (5), karyopyknosis (6). B). Neuronophagia (1), focal neurocyte necrosis (2), satellitosis (3), extracellular oedema (4), karyolysis (5). Hematoxylin and eosin. ×400
Picture taken by the authors

toplasmic ratio. Their nuclei were typically organisation, contained one nucleus, and were surrounded by heterochromatin, which was placed eccentrically (Fig. 4).

BIOCHEMISTRY OF THE CEREBRAL RAT CORTEX UNDER LEAD EXPOSURE AND WITH FATTY ACID OMEGA-3 CORRECTION

Under the influence of lead, SOD (1.44 (1.65; 2.1) conditional units/mg of protein) and catalase (4.16(3.67.4.8) μcat / mg of protein) levels statistically significant decrease compared to the control group ((2.46 (2.11; 2.67) conditional units/mg of protein and 5.92 (5.45; 6.67) μcat / mg of protein, respectively).

The content in the brain of MDA 16.9 (16.3; 17.7) μmol / mg of protein and KGP 6.19 (5.44; 6.55) nmol/mg of protein in this experiment, on the contrary, statistically significantly increases in relation to the control ((11.7 (10.13; 14.48) μmol / mg of protein and 4.19 (4.04; 4.47) nmol/mg of protein, respectively).

The activity of antioxidant enzymes of the rat's lead-exposed brain cerebral cortex is observed during correction by omega-3 fatty acids: SOD level - 2.1 (1.85; 2.27) conditional units/mg of protein, and catalase - 5.34 (4.99; 5.95) μcat / mg of protein. The speed of lipid and protein peroxidation processes slowed down: MDA content was 14.65 (12.8; 1.18) μmol/mg of protein, and KGP was 4.87 (4.68; 5.03) nmol/mg of protein.

DISCUSSION

Under the condition of lead intoxication, neurocytes of the molecular, outer granular, and pyramid layers had signs of extracellular oedema. It can be explained that lead is connected to the endotheliocyte plasmalemma endosurface that damages junctions between endotheliocytes and consequently increases their permeability [16].

Areas of significant haemorrhage in all layers of the cerebral cortex were observed, which may also be associated with damage to the blood-brain barrier [17].

The binucleated neurons were contained in the outer granular layer, which may be an abortive hyperplasia of the nerve cells reacting to unfavourable circumstances [18]. A reduced number of neurons and oligodendrocytes was found in the animal's spinal cord, but an increased number of astrocytes was observed compared with the control [19].

Destruction and, in some cases, nerve cell depletion due to changes in the Nissl substance organisation are observed in the pyramidal layer. The functioning of the chromophilic substance is closely linked to the activity of Nuclear Pore Complex, wich is fundamental for neurons to allow transcription factors to enter into the nucleus [20]. The primary roles of the Nuclear Pore Complex are activation of regulatory elements necessary for adaptation and response to external stimuli, especially those implicated in neuronal plasticity [21, 22].

Neuronophagia, satellitosis, and shadow cells were observed. Shadow cells were described in the case of hematoxylin-eosin staining, like a pale eosinophilic appearance, without nuclear structures, leaving behind bright areas with cytoplasmic silhouettes [23-25]. They were described by several authors (26) as a form of cell death, closely related to apoptosis. It has been proven that lead induces the mitochondrial release of calcium, initiating apoptosis [26].

The glyocytes had perinuclear oedema manifestations. Glia are dynamic cells that sense neuronal meta-

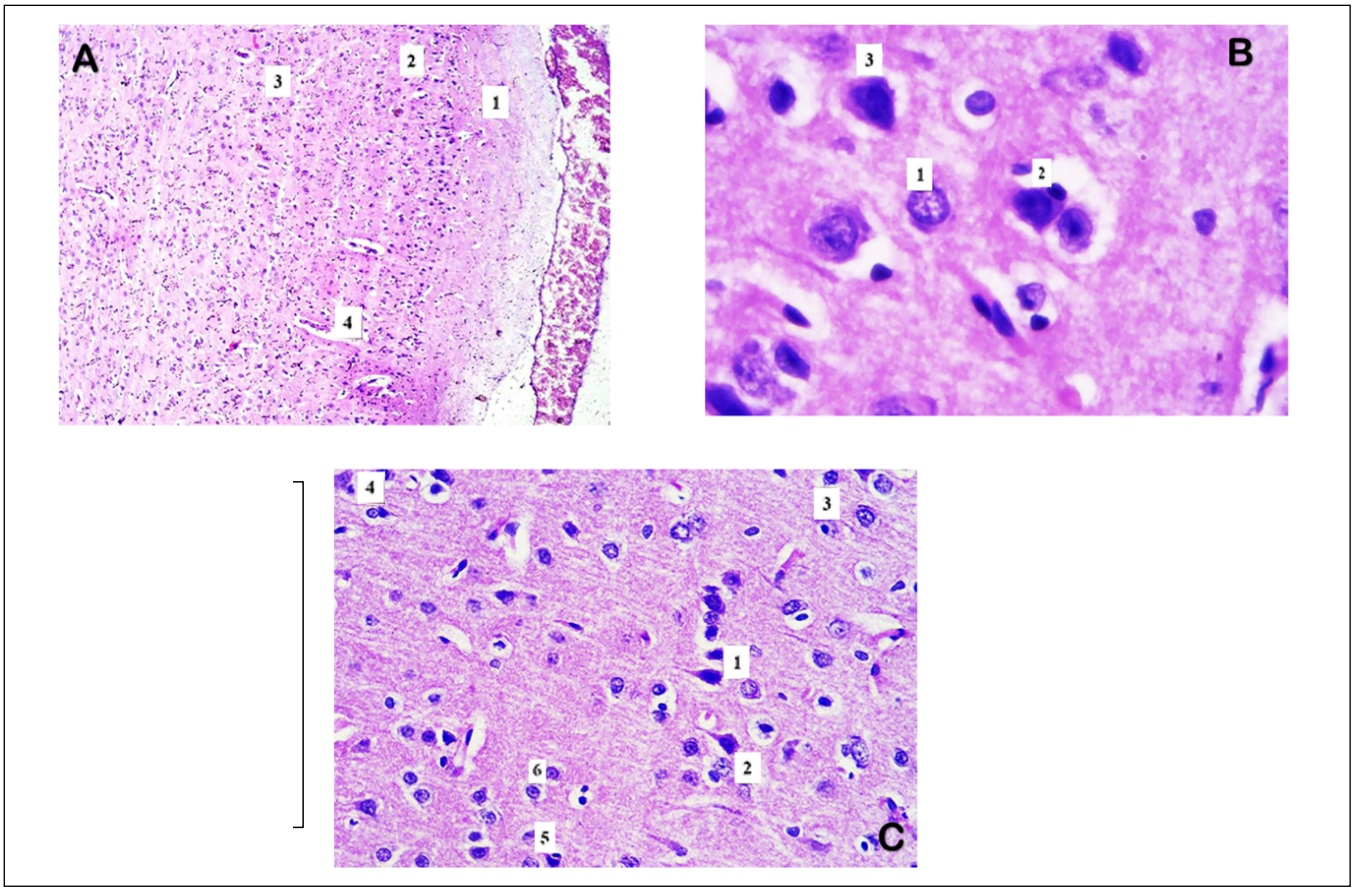


Fig. 4. Histological changes of the cerebral rat cortex under lead exposed with fatty acid omega-3 correction. A). Molecular layer (1), outer granular layer (2), layer of pyramidal cells (3), and lumen of the blood vessel with the molded elements of the blood elements (4). B). Pyramid neurocytes with signs of moderate pericellular edema (1), shadow cells (2), focal necrosis (3), satellitosis (4), neuronophagia (5), microglyocytes (6). C). Glyocytes (1), neuronophagia (2), moderate extracellular neurocyte oedema (3). Hematoxylin and eosin. ×400
Picture taken by the authors

bolic changes and regulate metabolism by transferring metabolites from glia to neurons, such as glucose and lactate, thereby influencing the energy metabolism [27].

All morphological changes depend on different lead-induced neurotoxicity mechanisms, including oxidative stress, alterations in membrane bio-physics, deregulation of cell signalling, and impairment of neurotransmission [28].

Under the influence of lead, SOD slows down the utilisation of hydrogen peroxide with the catalase participation compared to the control group. The peroxidic destruction process increases biomembrane lipids, as evidenced by MDA and KGP, which, conversely, increase in relation to the control. An increase in the intensity of oxidative degradation of cell membrane proteins explains the growth of KGP. The results, confirmed by other authors [29], revealed that lead acetate toxicity induced a significant increase in the levels of BUN, creatinine, and MDA, as well as a significant decrease in SOD and GPx [30]. Lead exposure decreased SOD activities in zebrafish livers due to direct interactions between lead and SOD, resulting in conformational and functional changes of the enzyme [31].

There is no antidote for lead, but it can be removed from the organism with chelation therapy [32]. We can also utilise antioxidants in the alleviation of lead toxicity, including vitamins (Vitamin B6 (pyridoxine), B1 (thiamine), vitamin E (α-tocopherol), and Vitamin C (ascorbic acid)), flavonoids (quercetin and α-lipoic acid), and herbs [33]. Using antioxidant Thiocetam administration, which contains the thiol group, has shown restoration of biochemical and morphological indicators [34].

We use omega-3 fatty acids for correction, and the microscopic structure of the blood vessels' walls was restored, with a reduction in perivascular and periancelular oedema manifestations in the brain hemisphere being characteristic. There were areas of haemorrhage much less often. The phenomena of satellitosis and neuronophagia were stored. There was a significant activation of histiocytic cells, which could be a sign that protective mechanisms are being launched in the cerebral cortex. In the literature, the effectiveness of omega-3 fatty acids in treating and preventing various diseases, as well as their significant

role in combating inflammatory diseases, such as cardiovascular and neurodegenerative diseases [35].

CONCLUSIONS

Thus, studies have shown that under the condition of lead intoxication at a dose of 7.5 mg/ kg body weight for 14 days, histological examination revealed disorders of the cerebral cortex cytoarchitectonics and damage to the walls of the blood vessels. With the correction of lead intoxication by omega-3 fatty acids at a dose of 750 mg/kg body weight within 14 days, improvements were noted in the histostructure of the experimental animals' cerebral cortex.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Outcomes of treatment of cat-bite injures

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ABSTRACT

Aim: The objective of the study was to analyze the results of treatment of hand cat bite wounds in one centre with a hand surgery profile, within one year.

Materials and Methods: Fifteen patients, 9 women and 6 men, aged a mean of 43 years, with hand infections caused by cat bites were treated. All patients were admitted to the hospital, and the treatment consisted of a wide incision in the area of infection. The results were evaluated in 13 patients after an average 1.8 years from the start of treatment.

Results: Most patients did not complain of hand pain at all. Two patients had a slight deficit in finger flexion, and one had incomplete wrist mobility. All patients regained good grip strength, and the dexterity of the hands, and assessment with the qDASH questionnaire revealed almost the same function as that of a healthy hand (mean 8.4 points). One patient developed necrosis of a bitten finger. The most dangerous infections were caused by the *Streptococcus pyogenes* bacterium. The total treatment time was 2.3 months in average. All wounds were sutured after the inflammation had subsided. In 5 patients, treatment was prolonged due to aseptic inflammation after surgical wounds had healed.

Conclusions: Cat bites can be the cause of severe infections and serious complications. Early, wide incision of the area showing fear of infection and intravenous antibiotic therapy allow the disease to be controlled, complications are prevented and a good treatment result is achieved.

KEYWORDS: cat bite; hand phlegmon; surgical treatment; microbiological profile; outcome assessment.

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INTRODUCTION

Hand wounds caused by bites by dogs and cats are relatively common and almost every day bitten patients are seen in emergency departments. The number of dogs kept at home in the author's country is estimated at 8 million, the number of cats at 7 million, with an increasing tendency. In addition, there is an even more unspecified number of stray animals (without an owner). Literature provides estimates on the number of bite wounds inflicted by animals in different countries. In the United States, there are about 1-2 million bites by dogs and about 400 thousands bites by cats per year, which is about 200 bite wounds per 100,000 inhabitants, per year [1]. In Europe, on the other hand, it is estimated that there are 175-740 animal bites per 100,000 inhabitants per year. In Germany, there are about 35,000 bites per year by dogs and cats with an owner (registered) and about 1 million by stray animals [2]. There is no such data in the author's country, however people bitten by dogs or cats present every day to the emergency department of the hospital where the author works (large facility). Of this number, at least 1-2 patients per week require hospitalization due to a deep infection requiring incision and drainage. So this is a relatively serious problem. Dog bites account for about 70-80% of all events, followed by bites and scratches by cats (5-15%), and human bites (3-23%) [3]. The victims are usually dog or cat owners and people who work with animals, such as veterinarians and farmers, on whose farms the animals are very often kept. Hands are the most common bite site in adults and older children, while children < 10 years of age are more often bitten in the head and neck [3]. Bite wounds have been classified (as well as burns) depending on the depth and presence of tissue loss or necrosis [2]. A three-level grading divides bitten wounds into superficial (1°), deep (2°) and complicated (3°) wounds (Table 1). This classification is not as important as in burns and has no major impact on practice, however, it can be useful in record-keeping, research and outcome assessment. Most bite wounds encountered in practice are 1st degree wounds.



Fig. 1a. Hand view 12 hours after being bitten by a cat. The bite site is marked with an arrow
Source: Own materials



Fig. 1b. Swelling and redness of the dorsal side of the metacarpal in the patient from Figure 1a
Source: Own materials



Fig. 1c. Hand view 24 hours after being bitten by a cat. The bite site is marked with an arrow
Source: Own materials



Fig. 2a. Incisions in the patient from Figure 1c. Note pus outflow from the wounds
Source: Own materials



Fig. 2b. Four days after the incision in the patient from Figure 1a
Source: Own materials

Cat bites are considered worse than dog bites. Cats have thin and sharp teeth, causing puncture wounds. Soon after being bitten, the organisms on the teeth and in saliva are introduced relatively deep into the subcutaneous tissue, where they have good conditions for multiplying. A puncture bite is a closed wound where there is no outflow of contents, which additionally promotes the development of infection. Data from the literature indicate a statistically significantly higher risk of infection of the cat bite wounds compared to the dog bite wounds [4-6]. Some wild and farmed animals, such as squirrels, ferrets, chinchillas and foxes, also have teeth similar to cats, but such bites are rare.

For the above reasons, it is recommended to make a primary incision in bite wounds inflicted by cats.

The author's institution deals with a lot of hand bites caused by dogs and cats. Treatment of such injuries is relatively difficult and often long-lasting, which in some cases is the result of bad initial care conducted by emergency and family doctors. Patients with diffuse hand phlegmons are frequently admitted to the hand surgery clinic a few days after the bite, requiring surgical treatment, hospitalization and intravenous antibiotic therapy.

AIM

The aim of this study was to analyze the results of the treatment of cat bites to the hand carried out in dedicated hand surgery centre.

MATERIALS AND METHODS

MATERIALS

In 2023, 15 patients, 9 women and 6 men, aged 43 years (range 27-64) with hand infections caused by cat bites were treated in the author's institution. Table 2 summarises the demographic and clinical data of this patient population. In 12 cases, the bite was caused by a cat's own or known, and in 3 cases by an unknown animal. Of these 3 cases, in one the cat was captured and handed over for observation, and the other 2 cats escaped. In 9 patients, the wound involved the left, and in 6 the right hand. The fingers were the most common bite site - 7 cases, followed by the dorsal metacarpal side - 4, the palmar metacarpal side - 2 and the wrist - 2 (Figs. 1 a-c). The time from the bite to presenting to the hospital was a mean of 14 hours (range 8-76). Two



Fig. 3a. Extensive incisions on the metacarpal and forearm in the patient after biting the index finger. *Streptococcus pyogenes* infection
Source: Own materials



Fig. 3b. Index finger necrosis 3 days after bite; The patient in Figure 3a
Source: Own materials



Fig. 3c. View of the patient's hand from Figures 3 a and b, four days after the amputation of the index. Note full finger flexion and secondary wound closure
Source: Own materials



Fig. 3d. View of the patient's limb from Figure 3a, two days after closure of the wounds
Source: Own materials

patients (13%) reported only redness and swelling in the bite area, and 13 (87%) had signs and symptoms of phlegmon involving the hand and forearm at the time of reporting (Figs. 1 a-c). Two patients had the hand and forearm affected, and one patient had an entire upper limb, up to the middle of the arm.

TREATMENT IN THE SURGICAL WARD

All patients with signs and symptoms of the diffuse infection (phlegmon) were given surgery with extensive incision(s) and were admitted to the hospital. Two patients with superficial inflammation were given an antibiotic and released home, but the next day they came back because of spread of infection. These patients were operated on and admitted to the hospital. Patients were operated on the day of reporting to the hospital, without undue delay. Prior to surgery, they received an intravenous antibiotic: 1.2 g of amoxicillin with clavulonate in 12 cases, and 0.6 g of clindamycin in 3 cases. All patients received tetanus prophylaxis upon admission.

Nine patients were operated on under brachial plexus block anaesthesia and with a tourniquet on the arm, and 6 in WALANT (Wide-Awake Local Anaesthesia With no Tourniquet). In 8 cases of severe phlegmon, the operation was performed in the operating room in the theatre, and in 7 cases of local infections - in the procedure room (in the emergency department or at the surgical ward). The length of the incisions on the fingers was 2-3 cm, and on the metacarpus about 4 cm (Figs. 2 a, b). The incisions on the forearm and arm were the longest, even 10-15 cm. Incisions were made on the palmar and dorsal sides of the fingers and metacarpus, as signs of infection were usually seen on both sides. In four patients, infection involved the hand and forearm, and in 2 it reached the upper arm. Bacterial cultures in these patients revealed streptococcal (*Streptococcus pyogenes*) infection. In these cases, long incisions were made through the entire affected area of the extremity (Fig. 3 a). In 12 cases, inflammatory infiltration of soft tissue was found at the incision, without the presence of a pus, and in 3 cases pus collection was present (Fig. 2 a). In all cases, material was collected for culture. Haemostasis was achieved by compression or by diathermy. In most cases, a rigid drain was inserted into the wound, in the form of a gutter, which facilitated the drainage of the contents from the wound and prevent-



Fig. 4a. View of another patient's wrist and forearm 2 days after wounds closure. *Streptococcal phlegmon*
Source: Own materials



Fig. 4b. Stitched wounds on the forearm and arm. The same patient as in Figure 4 a
Source: Own materials



Fig. 4c. The patient shown in the figures 4 a, b, at follow-up examination at 2 years. The wounds in the forearm are almost invisible, they were marked with a pen
Source: Own materials



Fig. 4d. Full flexion of the patient's fingers from Figure 4c
Source: Own materials



Fig. 4e. Scars after healing wounds on the forearm and arm, in the patient shown on Figure 4b
Source: Own materials

ed it from closing too fast. A moist dressing was applied to the wound. One patient who suffered a bite on the index finger of her right hand developed phlegmon that reached up to the elbow (Fig. 3 a). Bacterial culture showed an infection with *Streptococcus pyogenes*. Despite an extensive incision and antibiotic therapy, a necrosis of the index finger occurred and the finger had to be amputated (Figs. 3 b, c). In another patient, with a primary bite wound on the palmar side of the wrist, severe phlegmon developed involving the metacarpus, forearm and upper arm. Wide incisions were made in all affected areas, which resulted in subsiding infection and a favourable course of treatment. In this patient, the culture also showed infection by *Streptococcus pyogenes*.

Further treatment was continued in the surgical department. Until the result of culturing was obtained, an empirical i.v. antibiotic therapy was continued. The dressings were changed every day, and half an hour of hand baths were also performed in a bowl with antiseptic liquid. Patients were encouraged to make movements with their fingers, possible in the full range of movements, even if there were open wounds on them (Fig. 3 c). On average 4 days after the incision, when the symptoms of infection subsided, a secondary wound suture was performed. Usually 1-3 stitches were placed on the hand, but on the forearm, where the incisions

Table 1. Classification of bite wounds severity

1°	Bruising of the skin by the animal's teeth Skin scratching Superficial, single skin wound A depression in the skin caused by a tooth (interruption of continuity) Tearing of the skin with exposure of part of the subcutaneous tissue
2°	Deep wound, reaching fascia and muscles
3°	Wound with soft tissue loss and necrosis

Source: Compiled by the authors of this study

Table 2. Demographic and clinical data of 15 patients with cat-bite wounds

Variable	Description
Sex	F-9, M-6
Age (mean, range)	43 years (27-64)
Left/Right hand	L - 9, R - 6
Site of bite	Digit - 7, dorsal metacarpal - 4, palmar metacarpal - 2, wrist - 2
Cat known/unknown	Known - 12, unknown - 3
Time from injury to presentation (mean, range)	14 hours (8-76)
Time of hospital stay (mean, range)	4.3 days (4-12)
Post-incision woung suture	All patients
Time form incision to wound suture (mean, range)	4 days (3-8)
Complications	6 patients

Source: Compiled by the authors of this study

Table 3. Types of bacteria grown from 12 wounds after cat-bites

Type of the organism	Number of patients
Pasteurella multocida	9
Streptococcus pyogenes	4
Staphylococcus aureus	3
Neisseria species	3
Bergeyella zoohelcum	2
Negative result	3

Source: Compiled by the authors of this study

were long, even a dozen or so were needed (Fig. 3 d; Figs. 4 a, b). Patients were discharged home at a mean of 4.3 days after admission (range 4-15), and further treatment was carried out in the outpatient clinic.

Rabies prevention. In the case of 3 stray cats, one was captured and handed over for veterinary observation. The bitten patient himself did it, and the result of the observation was negative. In the remaining 2 cases, patients were referred to an infectious diseases clinic after being discharged from the hospital, where they received rabies vaccination and immunoglobulin, according to the post-exposure schedule.

OUTPATIENT TREATMENT

The time of outpatient treatment was 2.3 months (range 1-8) on average. Only in 4 patients did the wounds heal within 2 weeks, without inflammatory reaction. In the remaining 11 patients, the affected finger or hand remained swollen for several weeks, and mobility was limited. Due to the prolonged inflammatory recation (without infection) of the healed wound, 5 patients received 10 days oral steroids course (20 mg of prednisone), which was effective and the symptoms of inflammation slowly resolved. The longest treatment lasted 8 months, in a 45-year-old patient, after her own cat bit the back of her left hand.

METHODS

All patients whose treatment was completed were called for a follow-up examination at a mean of 1.8 years from the injury and admission to the hospital (range 1-3 years). Thirteen patients were available, two could

Table 4. Results of treatment of 13 patients in the study

Variable	Value	
	Median	Range
Pain intensity (NRS)	0	0-3
Finger mobility	Full – 11 Incomplete -2	
Wrist mobility	Full – 12 Incomplete -1	
Grip strength	37 kG	15-54 kG
QuickDASH score (range 0-100)	6	1-28

Source: Compiled by the authors of this study

not be contacted. During the examination, they were asked about pain (in Numeric Rank Scale, NRS), the range of motion of the fingers and wrist was assessed (full- or limited mobility), grip strength was measured with the Jamar dynamometer and the dexterity of the hand was examined using the quickDASH (Disability of Arm, Shoulder and Hand) questionnaire [7].

RESULTS

Bacteriological cultures. The types of bacteria cultured from patients' wounds are summarized in Table 3. The most commonly isolated organism was *Pasteurella multocida*, typical of the normal oral flora of healthy cats. In 4 cases, *Streptococcus pyogenes* was identified and this organism was responsible for the most severe infections (phlegmons). *Staphylococcus aureus* organism was cultured in 3 wounds. In 10 patients, more than one bacterium (mixed flora) was cultured, and in four patients the culture result was negative.

Table 3.

Results of biochemical tests. Blood was taken in all patients to examine inflammation parameters: leukocyte count, CRP (C-reactive protein) and procalcitonin concentrations. The average number of leukocytes in the first blood sample was 9.4 G/L (range 5-24 G/L), CRP concentration was 16.7 mg/dL (range 2-166 mg/dL), and procalcitonin 0.8 ug/dL (range 0.2-12 ug/dL). In 11 patients (73%), inflammatory parameters were within normal limits, while in all 4 cases of streptococcal infection, they were significantly elevated.

The patients were assessed at a mean of 1.8 years after the injury (range 1-3 years). Thirteen patients, 8 women and 5 men, aged at a mean of 43 years were available. The results are summarised in Table 4. Most patients did not complain of pain at all (median NRS 0 points), only 3 reported slight pain in the hand (2-3 NRS points). None of them took painkillers. Four patients reported moderate pain in the hand when exposed to cold. Two patients had a slight deficit in finger flexion, and one had incomplete wrist mobility. All patients regained good grip strength, and the dexterity of the hands: the quickDASH scores were almost the same as in the healthy hand (median 6 points). The patient after the amputation of the index finger also regained good hand function: full flexion of the remaining fingers, grip strength of 83% of the strength of a healthy hand and satisfactory function (14 quickDASH points). Therefore, it can be said that the result of treatment in all patients was good and all of them regained the normal function of their injured hands. Figures 4 a-e illustrate a very good result of treatment of a patient 1.5 years after being bitten by a cat in the metacarpus, who developed streptococcal phlegm of the entire upper limb. The total treatment time was 2.3 months on average (median 1.9 months). One patient bitten by her own cat on the index finger of her left (non-dominant) hand was treated for 8 months due to prolonged swelling of the dorsal side of the hand and limited mobility of the fingers. This patient developed chronic aseptic inflammation of the skin and subcutaneous tissue, which resolved only after 8 months, after the previous two, 10-day prednisone courses. This patient had the worst outcome due to reduced finger mobility, poor strength (15 kG) and hand dexterity (quickDASH 28 points).

COMPLICATIONS

A case in which necrosis and amputation of the finger occurred can be considered a complication. Also, extended, several-week treatment of inflammation after the healing of surgical wounds in 5 patients who received steroid therapy can be considered a complication resulting from the bite.

DISCUSSION

The paper presents the results of treatment of 13 patients with cat-bites to the hand. All patients were treated surgically with incisions in the infected area and antibiotics. A positive cultures were found in 12 cases (80%), which is consistent with the results from other publications (57-86% positive cultures) [4, 5]. *Pasteurella multocida* was the dominant microbe, found

in 9 patients. The results of treatment evaluated almost 2 years after an accident were generally good, because all but one patient regained normal function of their injured hands. The therapeutic algorithm included early surgical intervention: a wide incision not only of the bite site, but of the entire area of visible infection, and empirical antibiotic therapy showed great effectiveness. The most severe infections were caused by *Streptococcus pyogenes* organism, which is not frequent component of normal oral flora of cats. Streptococcal infection spreads rapidly and can involve the entire upper limb (Fig. 3 a; Fig. 4 b). It is characterized by infiltration of the skin and subcutaneous tissue, which is soaked with turbid fluid, which, after the incision, can be squeezed out of the wound like water. Unlike in staphylococcal infection, pus collections are rarely seen. Streptococcal infections quickly cause a systemic inflammatory reaction, which in 4 patients from the study manifested by elevated inflammatory parameters. Eventually, none of them developed sepsis, which can be attributed to rapid surgical intervention and start with antibiotics.

The extent of the incisions in patients in this series is noteworthy: they involved the entire area of visible inflammation (redness of the skin). This is important, because small incisions do not prevent spreading the infection, which results in the need for next operation, usually the subsequent day. Such situations occurred in 3 of our patients. Extensive incisions of the skin and subcutaneous tissue on the forearm, up to 15 cm length, made on the palmar and dorsal sides allow evacuation of infected fluid and limit infection. This is clearly seen when changing the dressing the next day after the procedure, when the originally tight and reddened skin turns pink and begins to wrinkle. After a well-made incision, the improvement occurs almost in the eyes. In all cases of phlegmon after a cat-bites the depth of the incision was limited to the skin and subcutaneous tissue, since the infection did not extend beyond the fascia. Only in 2 cases pus collections were found on the finger and metacarpus (Fig. 2 a). Both patients presented to the hospital more than 2 days after the bite and both were infected with *Pasteurella multocida* and *Staphylococcus aureus* organisms. Deep infection, involving the tendon sheath, joints or bones was seen in none of the patients in this series. A quick and wide incision in the infected area appears to prevent deeper penetration of the bacteria, although with finger wounds, the animal's thin teeth may have reached all the way to tendons and bones. The purulent tendovaginitis, osteomyelitis and joint infections (septic arthritis) are reported in the literature as a complication of cat bites [8, 9]. The relatively quick closure of extensive incisions is an interesting part of our treatment. Wounds were sutured immediately after the signs of inflammation (redness and swelling) had subsided, and there was no wound exudate. Usually the wound was suitable for suturing after 4 days from the original incision. Skin closure was performed in the procedure room of the surgical ward under WALANT anaesthesia. Figures 4 c-e show very good cosmetic result of the treatment, despite such extensive incisions.

In 3 cases of bite wounds, negative cultures were obtained. There are various possible explanations of this. The first is that bite wounds are not always infected, although they are primary contaminated. Signs of infection can develop within a few hours of the bite, but at the time of sampling during surgery, the bacterial multiplication may be not enough to grow on the slide. In addition, the patient's own immune mechanisms and administration of an antibiotic could have already effectively eradicated the bacteria [4]. Another reason may be the difficulty of cultivating *Pasteurella* species: if their number in the sample is too small, the growth in culture may not occur [3, 4, 9].

Another noteworthy observation from this study is the long-term resolution of inflammation in 5 patients, after the wounds have healed. The author noted this phenomenon in several other patients treated conservatively for cat bites, not included in this work. This can be explained by chronic, aseptic inflammation after infection with *Pasteurella multocida*, which smoulders in the subcutaneous tissue, despite the absence of the bacteria themselves. This was observed only in cases where *Pasteurella* species were grown from the wounds, and, for example, wounds after incisions of purulent streptococcus infections healed without reactions. However, this is only a speculation, not supported by evidence. In such cases, a 10-day course of 20 mg/day prednisone was highly effective. In one study the authors used topical steroids for chronic wounds displaying abnormal inflammation [10].

No other similar observations have been found in the literature.

There are single articles in the literature presenting results of the treatment of cat-bite. Below is a summary of a few of them.

Babović et al. (2014) presented the results of treatment of hand cat-bites in 193 patients, over a period of 3 years. Fifty-seven patients (30%) required hospital treatment, which consisted of i.v. antibiotics administration, and in 38 patients (67%) incision of the bite site and wound rinsing. Eight patients (14%) required more than one operation due to local complications. Risk factors for complications were: smoking, immunity defects and the bite over the joint or tendon sheath. However, the time from the bite to the start of treatment

had no effect on the risk of complications and the final outcome of treatment [11].

Kheiran et al. (2019) presented the results of treatment of 20 patients, 18 women and 2 men, aged at a mean of 58 years, after being bitten by cats on their hands. The most common bite site was the index finger in 9 cases. Eleven patients presented to the hospital within 2 days after the bite, and 9 patients after 2 days. All patients underwent surgery by incisions at the sites of infection and received i.v. antibiotics. Seven patients (78%) required repeated operations due to complications: tendon, bone and ligaments involvement, or necrotizing fasciitis. The most common organism isolated from wounds was *Pasteurella multocida.* The mean stay in the hospital was 3.4 days for patients who presented earlier and 9.2 days for those who presented after 2 days ($p < 0.01$). Four patients required amputation of their fingers due to necrosis. The authors conclude that delayed treatment of cat-bite wounds, diabetes and immunocompromised conditions increase the risk of eventful course of infection, the need for multiple surgeries and amputation of fingers. They recommend immediate and wide incision of wounds that show signs of infection [8].

Ng et al. (2025) presented epidemiological data and treatment results of 168 bite wounds to the extremities, registered over a period of 10 years. Hand dog-bites (110 patients, 65%) and hand cat-bites (56 patients, 33%) were predominant. Eighty-eight patients (52%) presented within the first day after the bite, and the rest within 2-6 days. Dog bites were the cause of 38 multi-tissue injuries (tendon, nerve damage, and bone fracture), while cat bites were the cause of infections only. The most common bacterium grown from infected cat wounds was *Pasteurella multocida*. Forty-three of the 56 patients (77%) bitten by cats required hospitalization, and their treatment consisted of incisions, rinses, and i.v. antibiotics. In 7 patients, complications occurred in the form of prolonged healing of wounds and tendinous felons. Necrosis of the finger occurred in one patient, requiring amputation [12].

Some papers suggest the possibility of primary suturing of bite wounds, after thorough rinsing and removal of devitalized tissues. This was especially true for facial bite wounds caused by dog and was motivated by cosmetic reasons [13, 14]. However, wounds inflicted by cats are not suitable for such treatment and, on the contrary, usually require a wide incision.

CONCLUSIONS

The results of our study show that cat-bite wounds be the reason for extensive infections and serious complications. A cat bite causes bacteria on their thin teeth and in their saliva to be introduced relatively deep into the subcutaneous tissue, where they have good conditions for multiplying. Therefore, such wounds that show symptoms of infection require a wide incision, rinsing, admission to the hospital and i.v. antibiotics. Such an intervention prevents the spread of infection, the development of phlegmon and shortens the treatment time. The most dangerous are wounds infected with *Streptococcus pyogenes*, which causes diffuse phlegmons that affect the entire limb. In some cases, a cat bite may cause finger necrosis or prolonged aseptic inflammation that requires treatment with steroids.

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CONFLICT OF INTEREST

The Author declare no conflict of interest

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ORIGINAL ARTICLE

Association between the severity of anatomical damage to the coronary arteries and clinical indicators and Holter electrocardiogram monitoring indicators

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ABSTRACT

Aim: To determine the association between the severity of anatomical damage to the coronary arteries and clinical and Holter electrocardiogram monitoring results.
Materials and Methods: A total of 60 patients were examined; the mean age was 61.67 ± 7.72 years. All patients had stage II hypertension and chronic ischemic heart disease, with or without frequent ventricular premature beats. The nature of the anatomical lesions of the coronary arteries was determined for all patients based on data from coronary ventriculography, which was performed in 3 groups: group 1—all examined patients (n = 60), Group 2 — patients with frequent ventricular extrasystoles (n = 30), Group 3 — patients without frequent ventricular extrasystoles (n = 30) with chronic ischemic heart disease.
Results: In patients with chronic ischemic heart disease, frequent ventricular premature beats (a sign of myocardial electrical instability) are associated with more severe atherosclerotic multivessel disease of the coronary arteries (56.7% of cases), the development of subocclusions and occlusions (100% of cases), as well as with a significantly higher total severity score for anatomical lesions of the coronary arteries based on coronary ventriculography (an average of 5.3 points).
Conclusions: A high total score for the severity of anatomical coronary artery disease and more severe anatomical coronary artery disease in patients with chronic coronary artery disease are associated with male gender, a higher functional class of angina, a longer history of ventricular extrasystoles, the presence of allorhythmia, and an increase in the number of supraventricular and ventricular extrasystoles per day as determined by Holter electrocardiogram monitoring.

KEYWORDS: chronic coronary disease, Holter electrocardiogram monitoring, myocardial electrical instability, premature ventricular contraction

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INTRODUCTION

Coronary heart disease (CHD) is one of the leading causes of death, accounting for 7.4 million deaths worldwide [1]. In approximately half of all patients with CHD, the cause of death is sudden cardiac death (SCD), the main mechanism of which is ventricular arrhythmias (in 84% of cases) [2].

Chronic coronary syndrome, formerly known as stable CHD [3], is associated with stable angina [4]. The severity of atherosclerotic lesions of the coronary arteries (CA) and myocardial changes is an important risk factor for SCD [5].

Ventricular extrasystole (VE) is associated with the risk of SCD [6] and is a marker of electrical instability of the heart [7]. VE are found in 0.7–1% of clinically healthy individuals during standard electrocardiogram (ECG) recording and in 50% during 24-hour Holter ECG monitoring (HM ECG) [8].

Frequent VE can occur in 1–4% of the general population and can provoke life-threatening (malignant) arrhythmias, such as polymorphic ventricular tachycardia or ventricular fibrillation, which are usually associated with structural heart disease [9]. In addition, in people without symptoms and without suspected heart disease, exercise-induced VE has been associated with mortality [10].

Given the above, timely diagnosis of CHD and prevention of the risks of SCD, in particular frequent VE, are important factors in prolonging the life expectancy and improving the quality of life of the population.

AIM

The aim is to determine the association between the severity of anatomical damage to the coronary arteries and clinical and Holter electrocardiogram monitoring results.

MATERIALS AND METHODS

PARTICIPANTS

Sixty individuals with stage II hypertension and concomitant chronic coronary disease (CCD) with and without frequent VE were examined. Among the examined patients, there were 46 (76.7%) men and 14 (23.3%) women. The average age was 61.67±7.72 years.

Inclusion criteria for the study: patients aged 30 to 75 years, stage II hypertension, concomitant coronary artery disease (CAD) limited to a single clinical presentation—exertional angina of functional class II–III (FC), symptomatic frequent premature beats (optional inclusion criterion) (>30 episodes of premature beats during any hour of the study), and the patient's informed consent to participate in the study.

Exclusion criteria: patients younger than 30 or older than 75 years of age, stage I and III hypertension, symptomatic hypertension, acute forms of coronary artery disease (unstable angina, acute myocardial infarction), history of myocardial infarction and stable exertional angina, Class IV according to the New York Heart Association, chronic heart failure Stage D according to the Heart Failure Society of America, left ventricular ejection fraction < 50% as determined by echocardiography, NYHA Class III–IV and Strazhesko-Vasilenko Stages 2–3 (Recommendations of the ESC, 2023 [11] and the All-Ukrainian Association of Cardiologists of Ukraine on the Diagnosis, Treatment, and Prevention of Chronic Heart Failure, 2024 [12]), cardiopathy, acute or previous myocarditis, clinically significant congenital and acquired heart defects, complete bundle branch block, second- or third-degree sinoatrial or atrioventricular block, need for implantation or an implanted pacemaker, diabetes mellitus, severe and clinically significant comorbid conditions with organ dysfunction, mental disorders and alcohol abuse, lack of informed consent, and the patient's unwillingness to participate in the study.

Based on the presence or absence of frequent PE, three groups of patients were identified: Group 1 (overall) – all patients with CHF (n=60); Group 2 – patients with CHF and frequent PE (n=30); Group 3 – patients with CHF without frequent PE (n=30). Male patients predominated (26 men in Group 2 and 20 in Group 3).

Taking into account the presence or absence of frequent VE, three groups of patients were identified: group 1 (general) – all patients with CCD (n=60), group 2 – with CCD and frequent VE (n=30), group 3 – with CCD without frequent VE (n=30). Male patients predominated (26 men in the 2nd group and 20 in the 3rd group).

RESEARCH METHODS

Stage II hypertension was determined in accordance with the recommendations of the European Society of Hypertension 2023 [13] and the clinical protocol for primary and specialised medical care 'Hypertensive disease (arterial hypertension) [14]. Concomitant CCD was verified instrumentally (stress tests) and/or by coronary ventriculography (CVG), which included only exertional angina of functional classes II-III (FC) according to the recommendations of the European Society of Cardiology 2024 [15] and the unified clinical protocol 'Stable ischaemic heart disease' [16]. Cardiac rhythm disturbances were determined using HM ECG [17].

STATISTICAL ANALYSIS

Statistical analysis of the study results was performed using methods of variational statistics with Microsoft Excel (2019) and Statistica 12.0 (Statsoft, USA), in accordance with the recommendations of 'Statistical Analysis in Medical Research' (2019) of the Department of Medical Statistics of the P.L. Shupyk National Medical Academy of Postgraduate Education [18].

Quantitative values were presented as the median and interquartile range (25th and 75th percentiles) due to the abnormal distribution of values in the sample, which was determined by the W-test (Shapiro-Wilk criterion).

The frequency of the trait in the sample was reflected using relative values and presented as percentages (%). The intergroup reliability of the results between quantitative values was calculated using the Kruskal-Wallis ANOVA & median test, and between relative values (%) using the $\chi 2$ criterion for independent samples. Results were considered statistically significant at $p \leq 0.05$.

The relationship between individual parameters was determined using Spearman's nonparametric rank correlation analysis (Spearman Rank Order Correlations, 'Nonparametric Statistics' module of the StatSoft 'Statistica' v. 12.0 package) [19].

ETHICS

The study protocol was developed in accordance with the ethical standards of the Declaration of Helsinki and approved by the local ethics committee of National Pirogov Memorial Medical University, Vinnytsya (protocol No. 8 of 5 October 2017). The patient gave informed consent to participate in the study.

AI STATEMENT

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

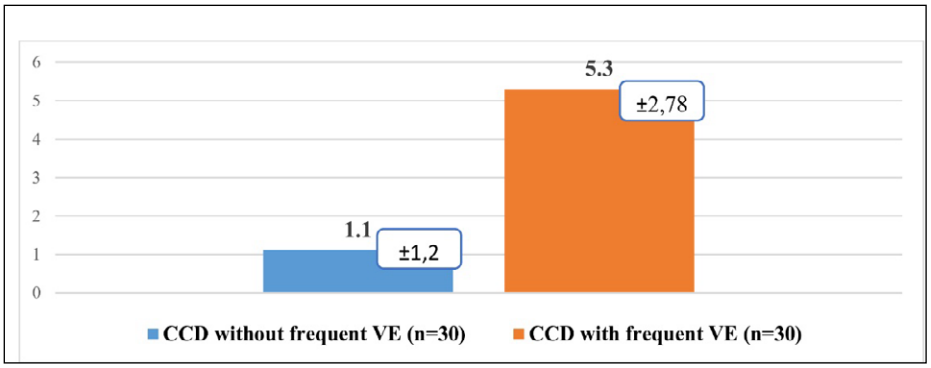


Fig. 1. Total severity score for anatomical lesions of the coronary arteries based on the presence or absence of frequent ST-segment elevation (mean scores ± standard deviation are shown)
Legend: CCD – chronic coronary disease; VE – ventricular extrasystole.
Picture taken by the authors
Picture taken by the authors

RESULTS

In 100% of patients in the 2nd (median value - 1721; interquartile range - 942 and 2435 episodes per day) and 70.0% of the 3rd (median value - 192; interquartile range - 56 and 411) clinical groups, supraventricular extrasystole (SVE) was recorded, which was not a criterion for excluding patients from the study. In the group of patients with frequent VE (group 3), the frequency of SVE per day was significantly higher compared to the group without frequent VE (group 2) (192 vs. 1721, p <0.0001).

In patients in group 2, according to HM ECG data, frequent VE were recorded (> 30 episodes at least during 1 hour of the study within 24 hours). Isolated VE was not a criterion for exclusion from the study and were recorded in 9 (30.0%) patients in group 3. This group was the comparison group in relation to the group with frequent VE (group 2, respectively). Thus, in the 3rd group, a small number of episodes of VE per day were determined (median value - 14; interquartile range - 12 and 18), compared with the 2nd group (median value - 2474; interquartile range - 1663 and 3592). Therefore, the statistical significance of the results obtained was determined (14 vs. 2474 episodes, p<0.0001).

Only 23% (n=7) of patients in group 2 had constant feelings of arrhythmia and used constant antiarrhythmic therapy.

In patients with CCD II and III FC, angina pectoris occurred with equal frequency (41.7% vs. 58.3%, p=0.19). All patients with CCD were diagnosed with the nature of anatomical damage to the CA based on CVG data.

In group 3, compared with group 2 patients, the proportion of cases with atherosclerotic lesions (presence of atherosclerotic plaques of any size) of the main trunk of the left CA was much higher (40.0% vs. 16.7%, p=0.04). We also determined the accuracy of the diagnosis of occlusion (CA stenosis ≥ 90%) of the left CA trunk based on the percentage (10.0% vs. 0%, p = 0.07). In addition, in the second group (with frequent VE), compared with the third group (without frequent VE), we also determined a significantly higher percentage of: 1) occlusions of the middle section of the left anterior descending (interventricular) CA (13.3% vs. 0, p=0.04); 2) atherosclerotic lesions, occlusions and subocclusions (stenosis 60-90%) of the first diagonal branch of the left anterior descending (interventricular) CA (50.0% vs. 20.0%, p=0.01; 16.7% vs. 0, p=0.02 and 20.0% vs. 3.3%, p 0.04, respectively); 3) atherosclerotic lesions of the second diagonal branch of the left anterior descending (interventricular) CA (16.7% vs. 0, p=0.02) and the proximal section of the circumflex branch of the left CA (43.3% vs. 20.0%, p=0.05); 4) atherosclerotic lesions and occlusions of the middle section of the circumflex branch of the left CA (60.0% vs. 33.3%, p=0.03 and 33.3% vs. 3.3%, p=0.003, respectively); 5) atherosclerotic lesions, occlusions and subocclusions of the obtuse edge branch of the left circumflex artery (40.0% vs. 16.7%, p=0.04; 16.7% vs. 0, p=0.02 and 5.0% vs. 0, p=0.07, respectively); 6) occlusions of the proximal section of the right CA (23.3% vs. 0, p=0.005); 7) atherosclerotic lesions of the middle section of the right CA (63.3% vs. 36.7%, p=0.04); 8) atherosclerotic lesions and occlusions of the distal right CA (33.3% vs. 10.0%, p=0.03 and 23.3% vs. 0, p=0.005, respectively).

An analysis was also performed depending on the number of affected CA, which included only patients with haemodynamically significant CA stenoses (CA occlusions and subocclusions). Discrepancies were determined only by the frequency of multivessel disease (lesions of 3 or more vessels) — in the group with CCD and frequent VE, compared with the group without frequent VE, multivessel lesions were determined significantly more often (56.7%, n=17 versus 10.0%, n=3, p=0.0001). This indicates more severe CA lesions in patients with frequent VE and the presence of a certain association between multivessel CA lesions and electrical myocardial instability. A high statistical significance was determined in the percentage difference (p=0.0001) between the groups in terms of the frequency of obstructive CHD (at least one occlusion or subocclusion of the CA): in 100% of cases (n=30) in patients in group 2, this variant of CCD

Table 1. Associations between the severity of anatomical coronary artery disease and clinical parameters and ECG parameters (Spearman's rank correlation)

Indicators associated with the severity of CA damage	R Spirman	P
Male gender (yes -1, no – 0)	0.28	0.03
FC of angina pectoris (FC II -2, FC III – 3)	0.33	0.01
Duration of VE in years	0.71	<0.00001
Presence of persistent VE - bigeminy, trigeminy (Yes -1, No -0)	0.27	0.03
Number of SVE per day	0.54	<0.00001
Number of VE per day	0.63	<0.00001

Legend: CA – coronary arteries; VE – ventricular extrasystole; SVE – supraventricular extrasystole
Source: compiled by the authors of this study

was determined, while in group 3, there were 60.0% (n=18) such cases.

To assess the nature of the anatomical lesion of the CA based on CVG data, we determined the total severity index of the CA lesion, which was calculated as the sum of the lesions of all CA, where subocclusion was 1 point and CA occlusion was 2 points, respectively. In the general group of patients (n=60), a variance analysis of the obtained index was performed, the range of values of which varied from 0 to 11 and averaged 3.22±3.0 points (median index – 3; interquartile range – 1 and 5). Therefore, it was reasonable to assume that half of the patients with CCD had a total score for the severity of anatomical CA lesions ranging from 1 to 5 points. In 25% of patients, it was less than 1 point, i.e. these patients had non-obstructive CCD, and in another 25% it was higher than 5 points, indicating the presence of multivessel (multifocal) coronary artery disease. The data shown in Fig. 1 indicate that in patients with CCD and frequent VE (group 2), compared to the group without frequent VE (group 3), there was a significant increase in the total score of anatomical CA lesions according to CVG data – 5.3 versus 1.1 points, $p<0.0001$ according to the t-test for independent samples (Fig. 1).

After calculating the total score for the severity of anatomical damage to the CA, we performed a non-parametric Spearman's rank correlation analysis to determine the associations between this score and various clinical indicators and HM ECG parameters (Table 1). The table included only those indicators that showed a statistically significant ($p<0.05$) correlation with the initial parameter.

It was determined that the total indicator of the severity of anatomical damage to the CA had a positive correlation with male gender (R=0.28; p=0.03), higher FC of angina pectoris (R=0.33; p=0.01), duration of history of VE in years (R=0. 71; $p<0.00001$), the presence of constant extrasystole - bigeminy, trigeminy (R=0.27; p=0.03) and the number of both SVE (R=0.54; $p<0.00001$) and VE per day (R=0.63; $p<0.00001$).

Thus, a higher total score and, accordingly, more severe anatomical damage to the CA according to CVG should be expected in men with high FC of angina pectoris (> FC II), with a long history of VE (on average 2.1 years with a total score > 3 points), in the presence of arrhythmia and an increased number of SVE (on average 1667 episodes) and VE (2864 episodes with a total score > 3 points) per day according to HM ECG data.

DISCUSSION

We have, for the first time, determined the total coronary artery (CA) lesion score based on coronary angiography (CAG) data in patients with hypertension and concomitant coronary artery disease (CAD), and identified the association between the severity of anatomical CA lesions and clinical parameters and ECG parameters in these patients. Our use of a total score for the severity of coronary artery disease was driven by the need to derive an integrated parameter of the entire coronary bed, taking into account the most problematic segments of the coronary arteries. We considered only subocclusions and occlusions of large vessels in points (1 and 2 points, respectively) and summed them.

V. Maslovskyi et al. [20, 21] proposed a scale for assessing the severity of atherosclerotic coronary artery stenosis in patients with non-ST-segment elevation myocardial infarction (NSTEMI). The authors scored atherosclerotic CA stenosis as follows: 0 points for the absence of atherosclerotic plaques in the CA, 1 point for the presence of hemodynamically insignificant stenosis (up to 50%), 2 – the presence of hemodynamically significant stenosis (50% to 90%), and 3 points – the presence of hemodynamically significant stenosis (90% to complete occlusion). Researchers determined the total severity score of CA disease as the sum of the severity scores for the left CA's diagonal branch/anterior interventricular branch, circumflex branch, and right coronary artery.

Given the above, the timely diagnosis of coronary heart disease and the prevention of risks associated with cardiovascular disease, particularly recurrent myocardial infarction, are crucial for extending life expectancy and improving the quality of life of the population.

CONCLUSIONS

It has been demonstrated that in patients with CHD, frequent premature VE (a sign of myocardial electrical instability) on an HM ECG are associated with more severe anatomical coronary artery lesions as determined by coronary angiography.

It has been shown that signs of myocardial electrical instability in patients with CHD are associated with a significantly higher frequency of multivessel disease and obstructive CHD, as well as a significantly higher total score for the severity of anatomical CAD lesions based on coronary angiography.

It was found that a higher total score of the severity of anatomical CAD lesions, and, accordingly, more severe anatomical CAD lesions in patients with CHD, is associated with male gender, a higher functional class of angina (> Class II), a longer history of angina, the presence of arrhythmias, and an increased number of premature beats and angina episodes per day as determined by Holter ECG monitoring.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

In Silico Study and synthesis of thiazole-based amino coumarin derivatives with promising carbonic anhydrase inhibitory activity

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ABSTRACT

Aim: To develop and assess four newly synthesized compounds designed to target carbonic anhydrases present in solid tumors.

Materials and Methods: The chemical synthesis was done using 6-Aminocoumarin, chloro acetyl chloride, triethylamine, thiourea, glacial acetic acid, and aromatic aldehyde derivatives. To investigate cytotoxic activity, we performed docking simulations with the 2015.10 Molecular Operating Environment program and used the MTT technique.

Results: Compounds IIIc and IIIb showed strong cytotoxicity against tumor cells (IC_{50} = 17.67 and 21.57 μM) and lower toxicity toward normal cells (IC_{50} = 86.63 and 135.01 μM), indicating better selectivity than cisplatin. Docking results revealed higher binding affinity than acetazolamide, due to effective interactions with key amino acids. The substituted thiazole ring also enhanced molecular flexibility and receptor binding.

Conclusions: This study evaluated newly synthesized aminocoumarin–thiazole hybrid compounds as potential anticancer agents against breast cancer. Compounds IIIa–d showed cytotoxic activity with IC_{50} values of 17.67–42.63 μM, with IIIc and IIIb being the most potent. In silico results supported their strong binding potential. These compounds demonstrated lower toxicity in normal cells compared to cisplatin, indicating selective action against cancer cells. Therefore, IIIc and IIIb may serve as promising candidates for breast cancer treatment, although further in vivo studies are required.

KEYWORDS: carbonic anhydrase inhibitors, 6-Aminocoumarin, Antineoplastic, *in silico* study

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INTRODUCTION

Cancer is a complex group of diseases characterized by the unchecked growth and spread of abnormal cells. These cells are capable of producing tumors, invading nearby cells, and spreading to other parts of the body [1, 2]. Numerous new diagnoses are made each year, and it remains a major cause of death worldwide, making it an important global health concern [3, 4]. More than $19.3*10^6$ persons worldwide were diagnosed with cancer in 2020, according to the World Health Organization, and $10*10^6$ of them died from the disease [5, 6]. At the start of the century, effective cancer management appears to be a major issue. This challenge stems from the struggle to discover novel targeted therapies that can inhibit cancer cell multiplication without harming healthy cells [7]. The focus of current scientific efforts is on elucidating the fundamental mechanisms behind cancer development and identifying potential therapeutic targets [8]. Because it has a significant impact on tumor growth, researchers are focusing progressively more on understanding the tumor microenvironment [9]. The presence of hypoxia in the TME around the tumor is induced by the increased oxygen requirements of rapidly reproducing malignant cells [10]. Moreover, highly proliferating cancer cells use anaerobic glycolysis and oxidative phosphorylation of glucose to produce energy. To maintain an alkaline pH and prevent intracellular H' ion accumulation, this metabolic pathway creates considerable volumes of metabolic acid, which include many protein complexes [11]. The cell surface enzymes known as carbonic anhydrases (CAs) are increased in hypoxic environments. These zinc metalloenzymes, which are members of the α-CA family, contribute to the reversible hydration of carbon dioxide into proton and bicarbonate ion [12]. The α-family comprises 15 identified isozymes of human carbonic anhydrases (hCAs). These isoforms differ in their susceptibility to different kinds of modulators, cellular location, protein structure, and catalytic activity [13]. The metabolic and pH-regulating machinery of tumor cells is considerably improved by hCA XII and IX, which are mostly prevalent in hypoxic cancers and stimulate

tumor cell proliferation [14]. As a result, research has concentrated on creating inhibitors that specifically target this enzyme as a means of overcoming medication resistance to chemotherapy [15-17]. The coumarin molecule is one of the most significant inhibitors of CA. Coumarins (2H-chromen-2-one) are a diverse family of heterocyclic compounds that contain a benzopyrone moiety. This moiety is formed by the fusion of an α-pyrone ring with a benzene ring. Coumarins can be derived from both natural and synthetic sources [18]. Coumarin and its derivatives show a fascinating activity of pharmacological properties such as anticancer [19], antifungal [20, 21], antibacterial [22], and antimalarial [23, 24]. The antineoplastic activity of coumarins is due to the ability of these substances to inhibit DNA repair enzymes, induce cell cycle inhibition, induce cell death, and reduce angiogenesis [25, 26]. The biological activities of five-membered heterocyclic compounds, particularly those that contain multiple heteroatoms, are diverse in the field of medicinal chemistry [27]. Thiazole is a heterocyclic molecule with the chemical formula C_3H_3NS, comprising both nitrogen and sulfur. Thiazole rings are aromatic and planar [28]. Its high reactivity, caused by the acidic proton at C-2, makes it a desirable precursor for creating a huge number of novel chemicals. Thiazole derivatives' chemical, physical, and pharmacological features have long fascinated synthetic and biological scientists. Numerous novel compounds with distinct therapeutic potentials have been generated by modifying the thiazole ring at various places, including antioxidants, antineoplastics, antibiotics, diuretics, and anti-inflammatory medications [29]. Because of their diverse pharmacological potential, coumarin–thiazole analogues exhibit a wide range of biological activities, including anti-inflammatory [30]. Anticancer [31]. Antioxidant [32] and carbonic anhydrase inhibitors [33].

AIM

This study focuses on the development and evaluation of four newly synthesized compounds formulated to inhibit carbonic anhydrase isoforms commonly expressed in solid tumor cells. The primary focus is on the objective to develop potent anti-cancer medications through chemical synthesis using 6-aminocoumarin, chloroacetyl chloride, triethylamine, thiourea, glacial acetic acid, and aromatic aldehyde derivatives.

MATERIALS AND METHODS

The anhydrous solvents and reagents were supplied by companies such as Merck, Riedel-de Haën, Sigma-Aldrich, and Hangzhou Hyper Chemicals. The melting temperatures were determined using the Thomas Hover device and capillary tube technique. TLC was used to confirm the reaction procedures; the resulting chemicals were purified, and their retention factors (Rf) were calculated. A combination of 8:2 chloroform and methanol, as well as 3:2 ethyl acetate and hexane, was used as the mobile phase. FT-IR scanning and spectral analysis were performed using a Shimadzu spectrophotometer. KBr discs were created at Ferdowsi University in Iran. Proton NMR spectra were obtained using a Bruker 300 MHz apparatus utilizing DMSO Proton NMR spectra were obtained using a Bruker 300 MHz apparatus utilizing DMSO as a solvent.

GENERAL PROCEDURE

A solution of 6-aminocoumarin in a mixture of benzene and DMF (3:1) was prepared, to which triethylamine (TEA) was introduced. The reaction mixture was chilled in an ice bath, and chloroacetyl chloride, previously diluted with 5ml of benzene, was added dropwise while stirring continuously. The amide derivative was generated upon completion of the reaction. The resulting product was then treated with thiourea in ethanol to create 2-aminothiazole. In the presence of glacial acetic acid, the final primary amine of thiazole reacted with aromatic aldehyde derivatives to form imines. The synthesis of target compounds (IIIa-IIId) and their intermediates is shown in Figure 1.

SYNTHESIS OF COMPOUND I

The 2-chloro-N-(2-oxo-2H-chromen-6-yl) acetamide [compound I] is compiled as follows [34].

(1 g, 0.006 mmol) of 6-aminocoumarin was dissolved in a solution mixture of dimethylformamide and benzene (1:3, 40 mL). The mixture was then treated with TEA (0.85 mL, 0.006 mmol). Chloroacetyl chloride (0.48 mL, 0.006 mol in 5 mL benzene) was added dropwise to the reaction mixture while it was continuously agitated for an hour in an ice bath and then refluxed for 6 hours at 76°C. The product was then precipitated by the addition of excess cold water, filtered, washed with diethyl ether, and recrystallized from ethanol to produce compound (I).

SYNTHESIS OF COMPOUND II

6-((2-((4-chlorobenzylidene)amino)thiazol-4-yl)amino)-2H-chromen-2-one [compound II] has been compiled according to [34].

Thiourea (0.160 g, 0.002 mmol) was introduced to a solution of 0.5 g (0.002 mmol) of Compound I in 40 mL

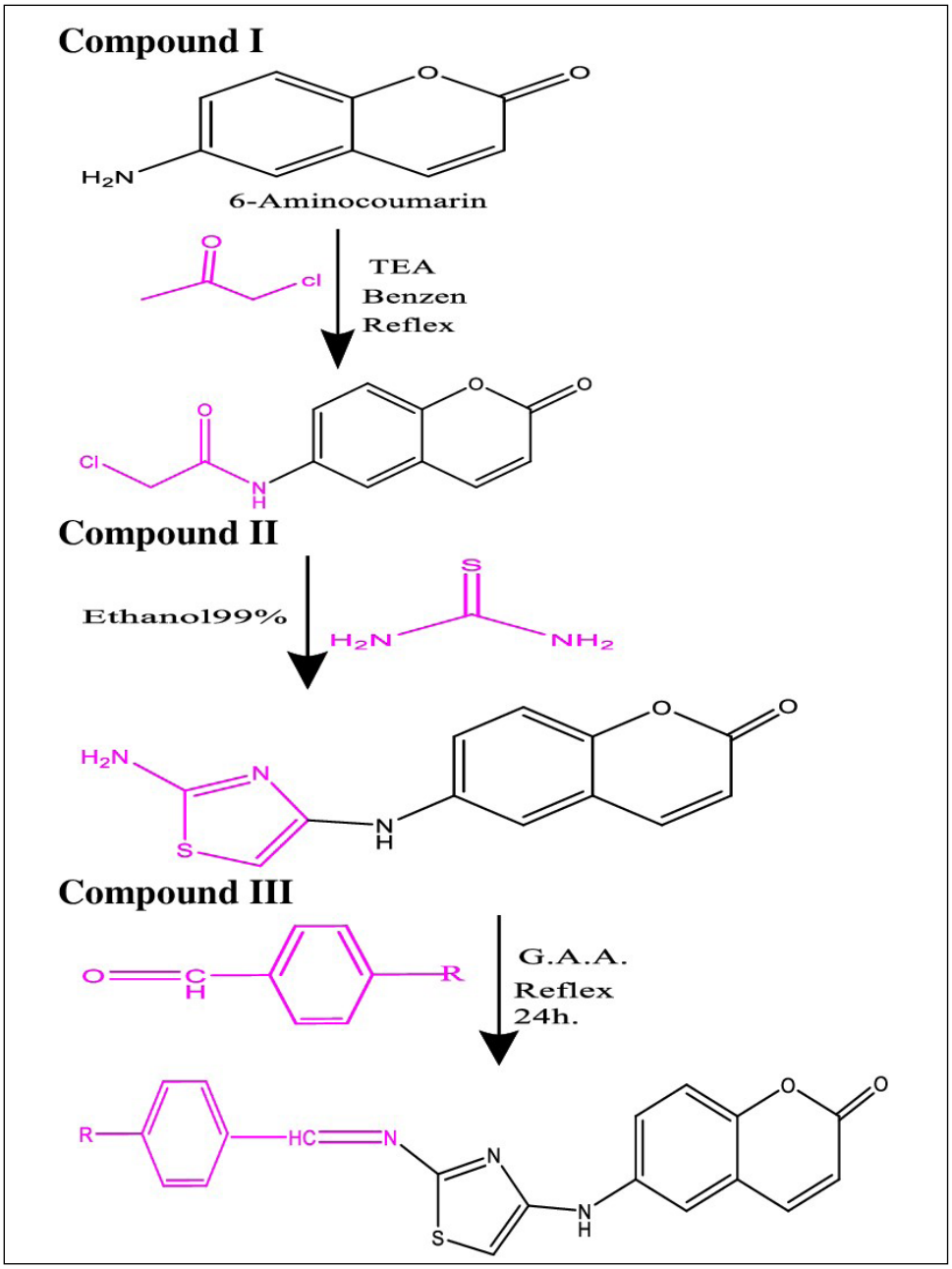


Fig. 1. Synthesis of target compounds (IIIa-IIId) and their intermediates from 6-aminocoumarine. IIIa if R = Br. IIIb if R = Cl, IIIc if R = OH, IIId if R = OCH_3
Source: Own materials

of 99% pure ethanol, and the mixture was constantly agitated until the thiourea dissolved. The reaction mixture was refluxed for 10 hours, the solvent was evaporated, the product was repeatedly washed with diethyl ether, and it was recrystallized from ethanol.

SYNTHESIS OF COMPOUNDS IIIA-D

6-((2-((4-substitutedbenzylidene)amino)thiazol-4-yl) amino)-2H-chromen-2-one has been synthesized according to [35].

A reaction mixture of compound (II) (0.002 mmol, 0.5 g) and the desired aldehyde (0.002 mmol) from Table 1 was mixed with a few drops of glacial acetic acid, all dissolved in ethanol (30 mL). Next, the mixture underwent refluxing for about 24 hours. The generated precipitate was then filtered and washed multiple times with diethyl ether before being recrystallized from ethanol, resulting in compounds (IIIa-d). Table 1 shows type of aldehyde compounds and their amounts were used in the synthesis of compounds IIIa-d.

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism software, version 10, to assess the influence of various factors on the study parameters. Additionally, Microsoft Office Professional Plus 2021-Excel was utilized to determine the significance of differences in the mean and the standard deviation values observed in this study.

Table 1. Type and amount of aldehyde compounds employed in the synthesis of compounds IIIa-d

Symbol of Product	Aldehyde Derivative	Weight (g)
IIIa	4-Bromobenzaldehyde	0.37
IIIb	4-Chlorobenzaldehyde	0.28
IIIc	4-hydroxybenzaldehyde	0.24
IIId	4-methoxybenzaldehyde	0.27

Source: Own materials

Table 2. Cytotoxic activity (IC50, μM) of 6-aminocoumarin derivatives and reference medicines against MCF7 and MCF10a

Compound	IC50(μM) against MCF7	IC50(μM) against MCF10a
Acetazolamide	67.53	53.91
Cisplatin	15.09	13.55
IIIa	42.63	145.52
IIIb	21.57	135.01
IIIc	17.67	86.63
IIId	41.84	135.45

Source: Own materials

Table 3. Molecular docking of the test ligands (IIIa-IIId) in comparison to acetazolamide displays the S-score, RMSD, and primary amino acids that are involved in the ultimate ligand interactions

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

SPECTROSCOPIC DATA

Compound I $C_{11}H_8ClNO_3$: FT.IR (cm^{-1}): 1616 (C=O stretching band of amide), 1703 (ester's C=O stretching band), 3296 (amide's NH stretching band), 3167, 3097 (C-H stretching band of aromatic ring), 1575-1447 (C=C stretching band of lactone and aromatic ring), 684 (C-Cl stretching band).

Compound II $C_{12}H_9N_3O_2S$**:** FT.IR (cm^{-1}): 3437-3305 (asymmetric and symmetric stretching bands of NH_2, respectively), 1633 (C=N (imine of thiazole) stretching band), 1517, 1448 (C=C stretching band of lactone and aromatic ring), 1400 (C-N stretching band of aromatic amines).

Compound IIIa $C_{19}H_{12}BrN_3O_2S$: FT.IR (cm^{-1}): 3192 (N-H stretching band of secondary amine), 1726 (ester's C=O stretching band), 1635, 1568 (C=N stretching bands of thiazole and imine), 1512-1436 (C=C stretching band of lactone and aromatic ring), 644 (C-Br). ^{1}H NMR (ppm): 11.5 (1H, singlet, N-H amine), 10 (1H, singlet, HC=N (imine)), 9.3 (singlet of coumarin) 8.6 - 7.3 (7H, multiplet, of aromatic rings), 6.5 (1H, singlet, vinilic proton).

Compound IIIb $C_{19}H_{12}ClN_3O_2S$**:** FT.IR (cm^{-1})**:** 3157 (N-H secondary amine stretching band), 1722 (ester's C=O stretching band), 1680, 1625 (C=N stretching band of thiazole and band of imine), 1587-1485 (C=C stretching band of lactone and aromatic ring), 675 (C-Cl). ^{1}H NMR (ppm): 10 (1H, singlet, N-H of amine), 9.5 (1H, singlet, HC=N (imine)), 8m6 - 7.3 (7H, multiplet, of aromatic rings).

Compound IIIc $C_{19}H_{13}N_3O_3S$**:** FT.IR (cm^{-1}): 3149 (O–H stretching band), 1670, 1633 (C=N stretching band of thiazole and imine), 1557-1442 (C=C stretching band of lactone and aromatic ring), 1510 (C-N stretching band). ^{1}H NMR (ppm): 10.3 (1H, singlet, N-H of amine), 9.8 (1H, singlet, HC=N (imine)), 8.8–6.8 (7H, multiplet, (of aromatic rings).

Compound IIId $C_{20}H_{15}N_3O_3S$: FT.IR (cm^{-1}): 3188 (N-H secondary amine's stretching band), 1718 (ester's C=O stretching band), 1676, 1633 (C=N thiazole and imine stretching band), 1504, 1439 (C=C stretching band of lactone and aromatic ring). ^{1}H NMR (ppm): 10 (1H, singlet, N-H of amine), 9.4 (1H, singlet, HC=N (imine)), 8.3 – 6.8 (7H, multiplet, of aromatic rings), 3.3–3.8 (3H, singlet, OCH_3 of para methoxyphenyl ring).

DOCKING INVESTIGATION

The MOE program, version 2015.10, was used to conduct molecular research analysis. To accurately illustrate the

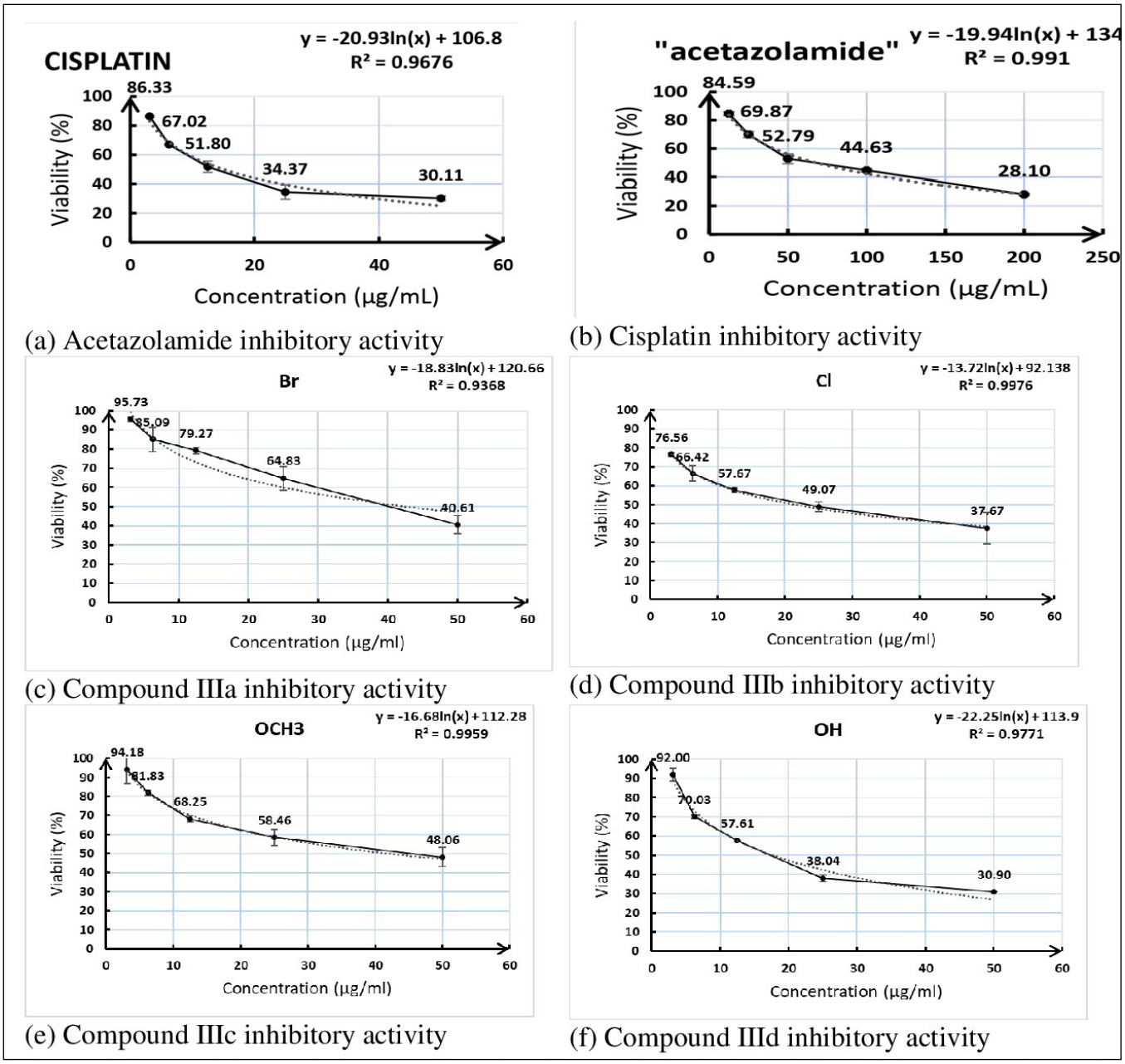


Fig. 2. Cytotoxicity analysis: unveiling the effects of standards and the final compound on MCF7
a - the inhibitory activity of acetazolamide, while; b - the inhibitory activity of cisplatin; c–f - the inhibitory effects of compounds IIIa–IIId
Source: Own materials

ligand formations, the expert program Chem-draw-12.0 was used. Only charges were applied and energy minimization was done after the ligands were protonated in three-dimensional forms inside MOE. Carbonic anhydrase IX's (PDB code: 6fe0) structure was imported into MOE for further analysis after being accessed from the Protein Data Bank. To get the target protein ready, small molecules and non-functional chain fragments were removed. The protein's atomic potentials were changed, and hydrogen bonds were added. The docking process began once the active site was determined and the premade ligands were added to MOE.

CYTOTOXIC STUDY

CELL LINES AND CULTURE

Iran's National Cell Bank (Pasteur Institute) provided the human breast cancer cell line MCF7 and the human normal breast cell line MCF10A. RPMI-1640 medium (Gibco) containing 10% FBS and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) was used to cultivate MCF7 cells. The MCF10A cell line was cultured in DMEM-F12 media with 10% horse serum (Gibco), cholera toxin (100 ng/ml, Sigma), hydrocortisone (0.5 mg/ml, Sigma), EGF (20 ng/ml, Thermofisher), and insulin

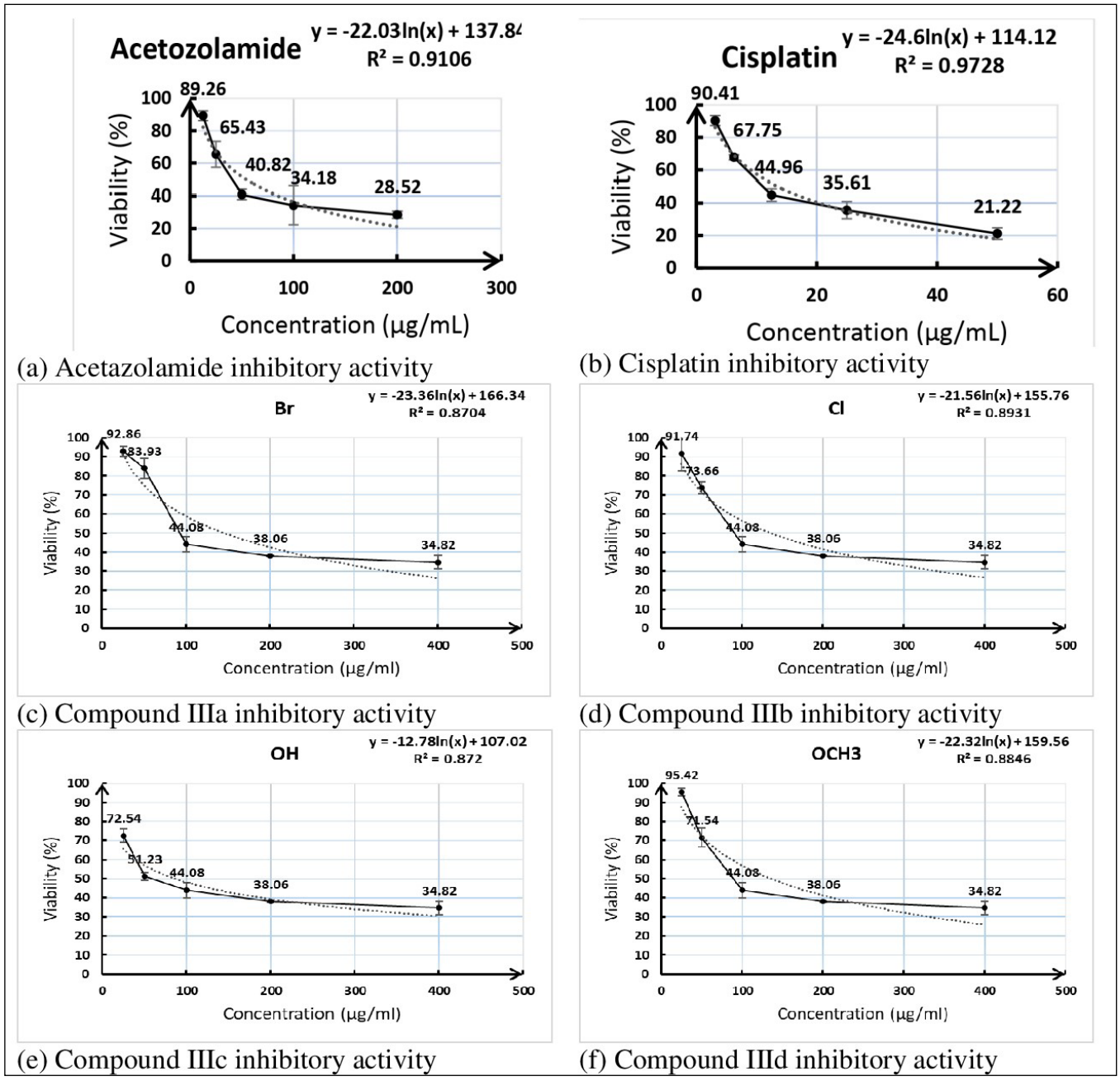


Fig. 3. Cytotoxicity analysis: unveiling the effects of standards and the final compound on MCF10a
a-b - the inhibitory action of acetazolamide and cisplatin, respectively, c–f - the inhibitory effects of compounds IIIa–III
Source: Own materials

(10 µg/ml). Trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution were used to passage the cells, which were grown at 37°C in humidified air with 5% CO_2.

MTT CELL VIABILITY ASSAY

The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test (Sigma-Aldrich) was used to measure cell growth and viability. To summarize, dcells were digested with trypsin, collected, adjusted to a density of 1.4 × 104 cells/well, and seeded onto 96-well plates filled with 200 µl fresh media in each well for 24 hours. After forming a monolayer, cancer cells were treated with 50-3.125 µg/ml and normal cells with 400-25 µg/ml of the chemical for 24 hours at 37°C and 5% CO_2. At the final stage of the treatment (24 hours), the monolayer culture was maintained in the original plate, but the supernatant was removed and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added. After that, the plate was incubated at 37°C for a further four hours. MTT solution: 100 µl of dimethyl sulfoxide was added to each well after the cell supernatant was extracted. Until the crystals were totally dissolved, cells were shaken at 37°C. An enzyme-linked immunosorbent

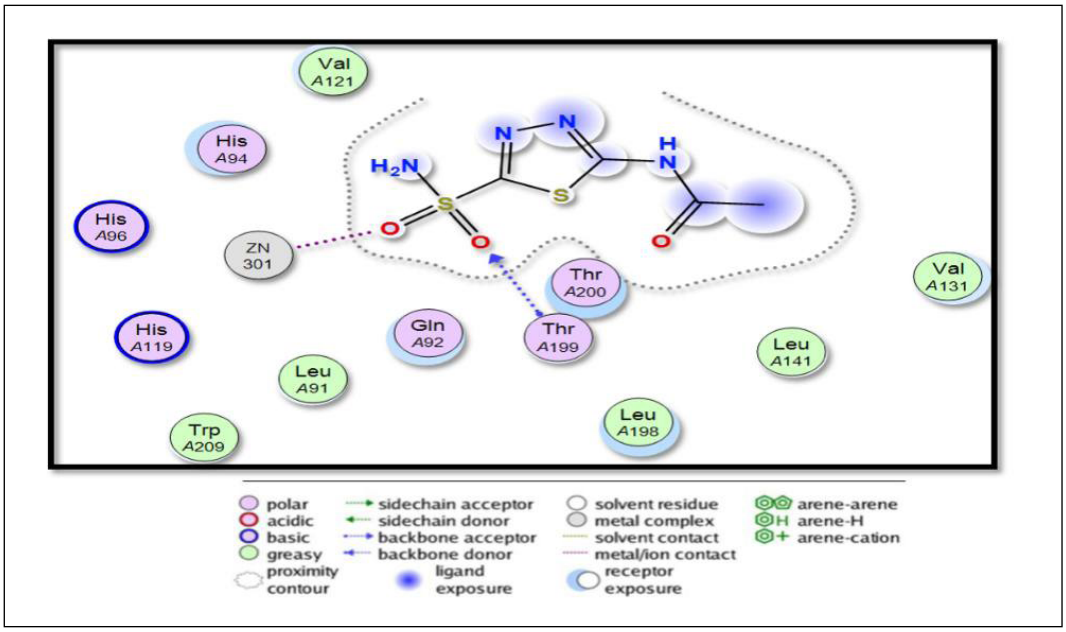


Fig. 4. Docking simulation of reference ligand (acetazolamide) with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

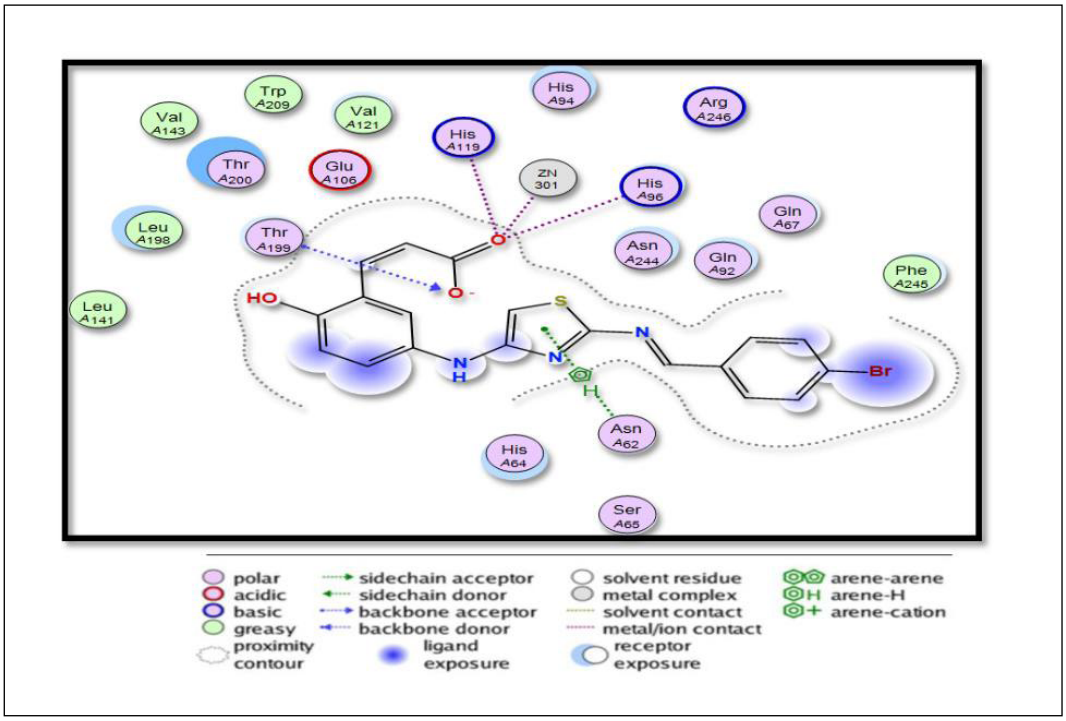


Fig. 5. Docking simulation of compound IIIa with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

assay reader (Model wave xs2, BioTek, USA) was used to measure absorbance at 570 nm to quantify cell viability. The corresponding dose-response curves were used to calculate the concentration of the chemicals that caused 50% of cell death (IC50).

RESULTS AND DISCUSSION

CHEMICAL REACTION

The IR spectra support the electron pair's nucleophilic attack on the primary amine in 6-aminocoumarin towards the carbonyl group of chloroacetyl chloride. The sharp band at 1616 cm^{-1} indicates the amide carbonyl (C=O) bond in the 2-chloroacetamide derivative. Chloroacetyl chloride exhibits selectivity at the α-carbon atom due to higher nucleophilic reactivity toward acid chlorides. This selectivity is due to differences in the electrophilia of the two carbon atoms in chloroacetyl chloride. Steric and electrical elements also have a role in the selecting process as a whole [36]. Compound I is the end product of this reaction. The reaction of the 2-chloroacetamide derivative with thiourea is what creates the thiazole ring. The acetyl chloride's electrophilicity is increased by the hydrogen

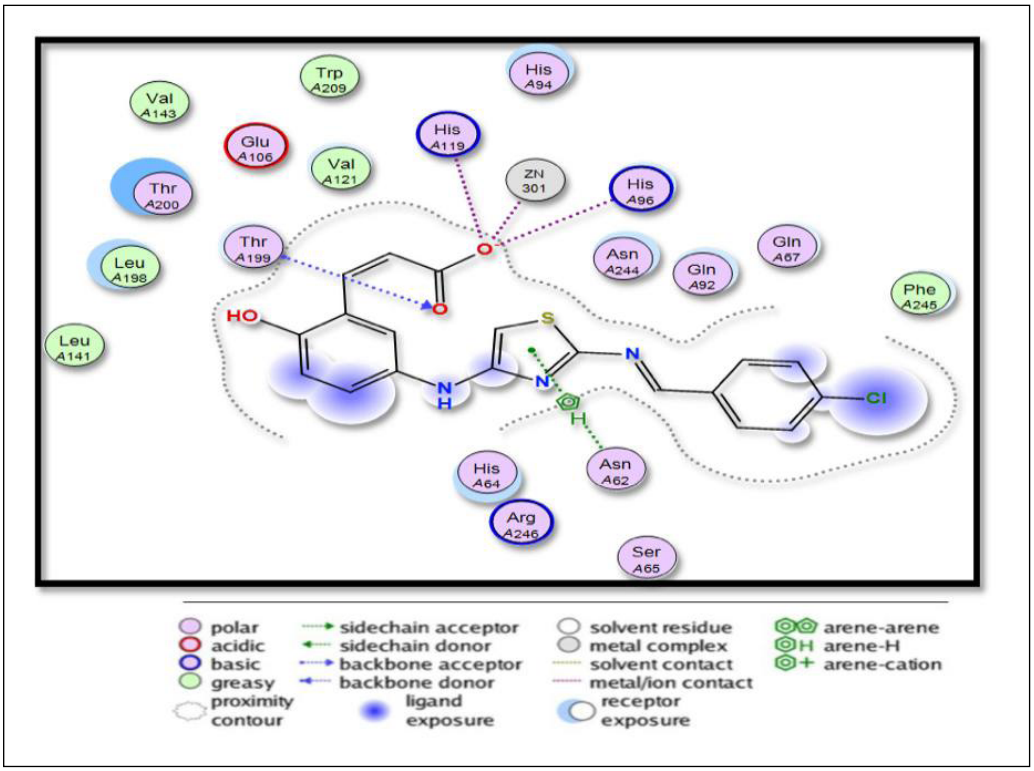


Fig. 6. Docking simulation of compound IIIb with carbonic anhydrase IX (PDB code: 6fe0) *Source: Own materials*

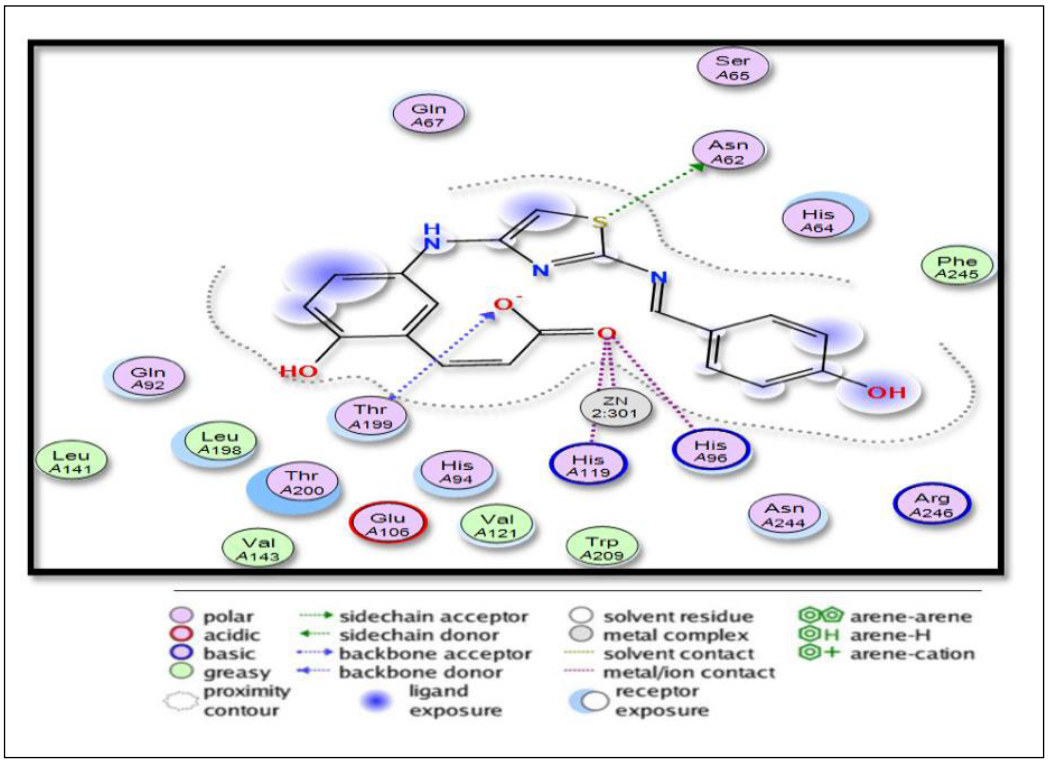


Fig. 7. Docking simulation of compound IIIc with carbonic anhydrase IX (PDB code: 6fe0) *Source: Own materials*

bond that exists between its carbonyl oxygen, making it simpler for the thiourea's amino nitrogen and the sulfur of the chloromethyl carbon to attack and generate a thiazole ring. After that, the removal of an HCl molecule occurs. The creation of the heterocycle is confirmed by the presence of a peak at 1633 cm^{-1}. With the use of glacial acetic acid (GAA) as a catalyst, compounds (IIIa-d) have been produced by Schiff base development from the interacting compound (II) with the corresponding aldehydes. FT-IR spectroscopy confirmed the production of imine bonds, with absorption in the 1568 cm^{-1} region. The mechanism was further supported by the removal of the aldehyde C-H stretch in the 2750–2850 cm^{-1} region and the lack of the aldehyde carbonyl peak in the 1720–1740 cm^{-1} area.

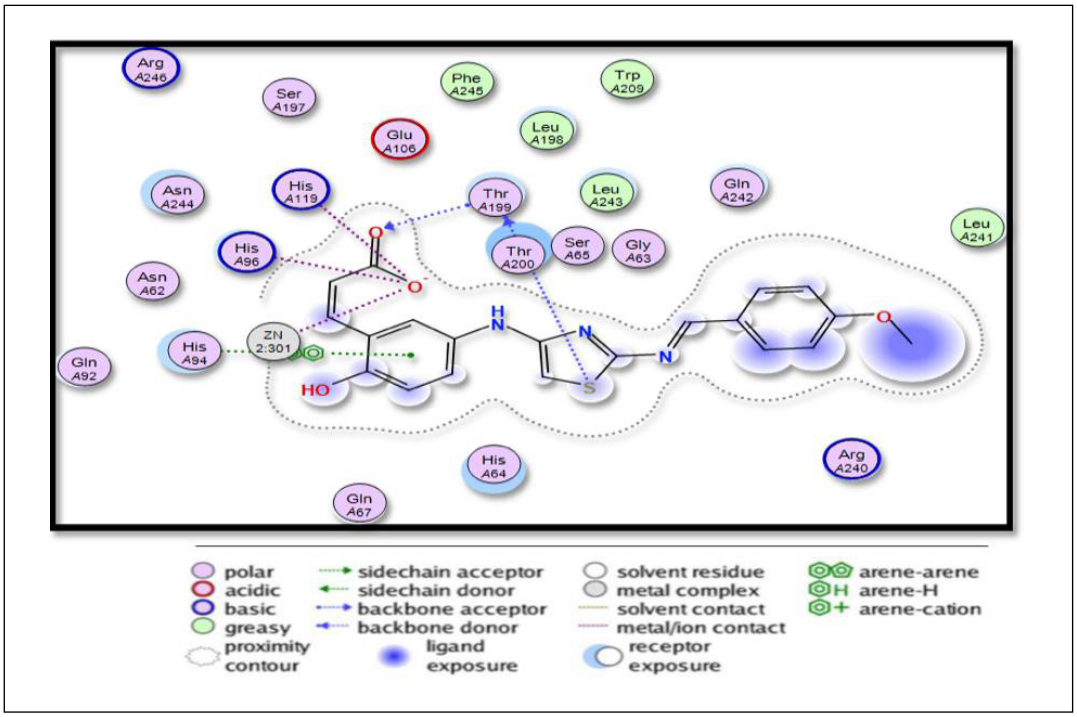


Fig. 8. Docking simulation of compound IIId with carbonic anhydrase IX (PDB code: 6fe0)
Source: Own materials

THE DESCRIPTION AND PHYSICAL CHARACTERISTICS OF THE COMPILED COMPOUNDS AND THEIR INTERMEDIATES

1. 6-aminocoumarin ($C_9H_7NO_2$) as starting material, M. wt = 161.05, green crystals, melting point = 166-168°C, and Rf value = 0.73.
2. Compound I ($C_{11}H_8ClNO_3$), M. wt = 237.64, light yellow crystals, yield = 80%, melting point = 195-198°C, and Rf value = 0.85.
3. Compound II ($C_{12}H_9N_3O_2S$), M. wt = 259.04, yellowish to green powder, yield = 60%, melting point = 221-223 °C, and Rf value = 0.57.
4. Compound IIIa ($C_{19}H_{12}BrN_3O_2S$), M. wt = 426.29, yellow powder, yield = 68%, melting point = 240-242 °C, and Rf value is 0.67.
5. Compound IIIb ($C_{19}H_{12}ClN_3O_2S$), M. wt = 381.83, yellowish to orange powder, yield = 70%, melting point = 230-232 °C, and Rf value is 0.66.
6. Compound IIIc ($C_{19}H_{13}N_3O_3S$), M. wt = 363.39, brown powder, yield = 73%, melting point = 233-235°C, and Rf value = 0.71.
7. Compound IIId ($C_{20}H_{15}N_3O_3S$), M. wt = 377.42, yellow crystals, yield = 75%, melting point = 215-217 °C, and Rf value = 0.60.

CYTOTOXIC EVALUATION

MTT assays were used to evaluate the cytotoxicity of compounds (IIIa, IIIb, IIIc, and IIId) in two cell lines: normal cells (MCF10A) and cancer cells (MCF7). The IC50 values for each medication were determined, and their inhibitory effects were compared to those of acetazolamide. The cytotoxic efficacy of these compounds was compared to that of cisplatin. Compounds IIIa-IIId showed significantly higher cytotoxicity against tumor cells (IC50 = 42.63 µM, 21.57 µM, 17.67 µM, and 41.84 µM, respectively) in comparison with acetazolamide. They also revealed a considerable drop in healthy cell viability (IC50 = 145.52 µM, 135.01 µM, 86.63 µM, and 135.45 µM, respectively) (Table 2).

The results show that each synthesized chemical exhibited excellent inhibitory efficacy and has the potential to be developed into an effective antineoplastic medication. The compounds had IC50 values ranging from 17.67 to 42.63 µM for MCF7 cells and 86.63 to 142.52 µM for MCF10a cells, respectively.

Compounds IIIc and IIIb showed significantly higher cytotoxicity against tumor cells (IC50 = 17.67 µM and 21.57 µM, respectively) and decreased normal cell viability (IC50 = 86.63 µM and 135.01 µM, respectively) compared to cisplatin, indicating higher levels of selectivity and fewer side effects.

These findings show that the synthesized chemicals are effectively selective inhibitors of tumor cells, while causing little harm to healthy cells.

Figure 2 displays the cytotoxicity analysis of several compounds against MCF7 cells, indicated by the mean ± SD of triplicate measurements. Figure 2a shows the inhibitory activity of acetazolamide, while Figure 2b shows the inhibitory activity of cisplatin. Figure 2c–f shows the inhibitory effects of compounds IIIa–IIId.

Figure 3 displays the cytotoxicity analysis of several compounds against MCF10a cells, indicated by the

mean ± SD of triplicate measurements. Figures 3a and 3b indicate the inhibitory action of acetazolamide and cisplatin, respectively. Figure 3(c–f) shows the inhibitory effects of compounds IIIa–IIId.

DOCKING INVESTIGATION

Docking computer simulations demonstrated various binding mechanisms for ligands within the 6fe0 protein's interacting zone. These modes of binding were characterized by specific ligand-protein interactions, including electrostatic interactions and hydrogen bonding. The results showed varied binding strengths for the compounds that have been synthesized, with stronger interactions noted with reference to the target protein compared to acetazolamide. The inhibitory effects of the compounds were clearly identical; all of them bound to the necessary zinc ion for their inhibitory activity, creating hydrogen bonds with the Thr199 residue in the active site of the enzyme. The thiazole moiety was also necessary for attachment to the catalytic region of the enzyme because it interacted with important amino acids that were involved in substrate binding. Table 3 shows for every compound the S. score and RMSD values of the docking study. The S. score values are -9.98, -9.95, -10.12, and -10.6, while the RMSD values are 1.8, 1.8, 1.5, and 1.7, respectively, as listed in Table 3.

Fig. 4 shows the docking simulation of acetazolamide with HCA IX (PDB code 6fe0). Figures 5-8 show the docking simulations of compounds IIIa-IIId with HCA IX (PDB code 6fe0).

CONCLUSIONS

This study sought to explore the efficacy of recently developed aminocoumarin-thiazole hybrid compounds as possible anticancer medicines, with a particular focus on breast cancer. Compounds IIIa-d were meticulously constructed, chemically produced, and simulated to determine their antitumor efficacy. The compounds were tested for cytotoxicity on a cell line and showed IC50 values ranging from 17.67 to 42.63 µM. IIIc and IIIb had the maximum cytotoxicity against breast cancer cell lines (IC50 values of 17.67 and 21.57 µM, respectively). Furthermore, in silico investigations revealed that these compounds had superior S. scores of -10.12 and -9.95, indicating that they could be chemotherapeutic agents. Nonetheless, they showed lesser toxicity in normal cells than cisplatin, meaning that they may do less harm to healthy tissues. The findings show the ability of the developed aminocoumarin-thiazole compounds to selectively target tumor cells while preserving normal cells. The results reveal that compounds IIIc and IIIb may be potential therapy agents for breast cancer; more study is needed to analyze their in vivo anticancer activity more carefully.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

Sexual dysfunction in multiple sclerosis: Associations with disability, fatigue, affective symptoms and brainstem lesions

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ABSTRACT

Aim: To assess the prevalence and severity of sexual dysfunction in patients with multiple sclerosis and its associations with clinical, neuropsychological, and MRI-related factors, considering the primary, secondary, and tertiary components of sexual dysfunction.

Materials and Methods: This cross-sectional observational study included 109 patients with multiple sclerosis (80 women, 73.4%; 29 men, 26.6%). Sexual dysfunction was assessed using the Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 (MSISQ-19), including total and subscale scores. Cognitive function, fatigue, depression, and anxiety were assessed using the Symbol Digit Modalities Test (SDMT), Modified Fatigue Impact Scale (MFIS), Beck Depression Inventory (BDI), and Hamilton Anxiety Rating Scale (HAM-A), respectively. Spearman's correlation analysis and the Mann–Whitney U test were used; $p < 0.05$ was considered statistically significant.

Results: Clinically relevant sexual dysfunction was identified in 60 patients (55.1%). The total MSISQ-19 score correlated positively with age ($\rho = 0.26$; $p = 0.016$), EDSS ($\rho = 0.40$; $p < 0.001$), BDI ($\rho = 0.36$; $p = 0.004$), HAM-A ($\rho = 0.57$; $p < 0.001$), and MFIS ($\rho = 0.64$; $p < 0.001$) score. Brainstem lesions on MRI were associated with higher primary sexual dysfunction subscale score (median 11.0 vs. 8.0; $p = 0.013$).

Conclusions: sexual dysfunction was common in patients with multiple sclerosis and was significantly associated with age, disability level, fatigue, depression, anxiety and brainstem lesions on MRI, supporting its multifactorial nature and the need for routine clinical assessment.

KEYWORDS: multiple sclerosis, sexual dysfunction, fatigue, anxiety, depression, brainstem lesions

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INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system that leads to progressive neurological deficits and most commonly affects young people [1]. In addition to obvious physical symptoms, patients with multiple sclerosis may experience so-called "hidden symptoms," one of which is sexual dysfunction (SD) [2]. SD is one of the most common, often underestimated manifestations of the disease, that can be diagnosed at any stage of the disease, quite often at the early stages [3,5]. According to recent meta-analyses, the combined prevalence of this symptom is approximately 61% in women and 63% in men [4,5].

Sexual dysfunction in MS comprises three components: primary, secondary, and tertiary [6]. Primary dysfunction is associated with direct damage to the structures and neural pathways of the central nervous system involved in the regulation of sexual desire, sexual arousal, genital sensitivity, and orgasm. The secondary component is caused by the physical and functional consequences of the disease, which indirectly affect the patient's sex life. These include fatigue, weakness, spasticity, muscle spasms, impaired coordination and mobility, cognitive impairment, pain or sensory symptoms, adverse treatment effects, and pelvic organ dysfunction. The tertiary component of sexual dysfunction is associated with the psycho-emotional and sociocultural aspects of the condition. Its most common manifestations include low self-esteem, difficulties with emotional regulation, a negative body image, fear of rejection, and communication difficulties with a partner [7].

Despite growing interest in MS sexual dysfunction, this issue needs further investigation. Most studies have focused on assessing its prevalence, risk factors, and impact on quality of life, while associations with clinical disease characteristics, comorbid symptoms, neuroimaging data, and specific components of sexual dysfunction have been analyzed less frequently. Assessment of these aspects is important for a more complete understanding of the nature and clinical significance of sexual dysfunction in MS patients.

AIM

The aim of this study was to assess the prevalence and severity of sexual dysfunction in MS patients and to analyze its associations with age, sex, disease duration, neurological disability, cognitive function, fatigue, affective symptoms, and brainstem lesions on magnetic resonance imaging (MRI), with consideration of the primary, secondary, and tertiary components of sexual dysfunction.

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANTS

A cross-sectional observational study was conducted. The study included patients with multiple sclerosis diagnosed according to the McDonald (2017) diagnostic criteria [8]. Demographic, clinical, and questionnaire-based data were obtained during routine outpatient consultations with a neurologist at the State Scientific Institution "Center for Innovative Medical Technologies of the National Academy of Sciences of Ukraine" (Kyiv, Ukraine) from September 2024 to December 2025. The study included adults aged from 18 years or older who provided written informed consent and completed clinical and questionnaire-based data required for the analysis. Exclusion criteria were pregnancy, severe mental disorders, and clinically significant somatic comorbidities that could affect the assessed outcomes.

109 patients with MS were enrolled into the study including 80 (73.4%) women and 29 (26.6%) men. According to the presence of clinically relevant sexual dysfunction, patients were divided into two groups: the sexual dysfunction group (n = 60; 55.1%) and the comparison group of patients without sexual dysfunction (n = 49; 44.9%). The mean age of the patients at the time of study enrollment was 36.5 ± 10.2 years, and the mean duration of the disease was 5.5 ± 5.4 years. Based on the clinical course of the disease, 96 (88.1%) patients had relapsing-remitting MS. Progressive forms of multiple sclerosis (primary- and secondary-progressive) were identified in 13 (11.9%) patients. The level of disability, assessed using the EDSS scale, was characterized by a median value of 2.5 points (IQR 1.5–4.0).

CLINICAL AND PSYCHOMETRIC ASSESSMENT

The level of neurological deficit was assessed using the Expanded Disability Status Scale (EDSS) [9]. Sexual dysfunction was evaluated using the Multiple Sclerosis Intimacy and Sexuality Questionnaire-19 (MSISQ-19) [6] with analysis of the total score and the scores of the primary, secondary, and tertiary sexual dysfunction subscales. Cognitive function was determined using the Symbol Digit Modalities Test (SDMT) [10]. The level of depressive symptoms was assessed using the Beck Depression Inventory (BDI) [11], the level of anxiety was measured with the Hamilton Anxiety Rating Scale (HAM-A) [12]. The severity of fatigue was evaluated using the Modified Fatigue Impact Scale (MFIS) [13]. Fixed cutoff scores were used to interpret the results of the clinical and psychometric scales. Cognitive impairment was diagnosed when the SDMT score was < 40 points [10]. Sexual dysfunction was defined as a total MSISQ-19 score >30, in line with a previously used cut-off in MS research [14]. Clinically significant fatigue was defined as a total MFIS score ≥38 [13]. Clinically relevant depressive symptoms were defined as BDI score ≥10 [11], and clinically relevant anxiety symptoms as HAM-A score >15 [12].

STATISTICAL ANALYSIS

Statistical analysis was performed using nonparametric statistical methods. Quantitative variables were described as the median and interquartile range [Q1; Q3], while categorical variables were described as absolute numbers and percentages. Spearman's correlation analysis was used to assess the relationships between indicators. The Mann–Whitney U test was used to compare two independent groups. Data analysis was performed using GraphPad Prism 8 software.

ETHICS

The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients were informed of the study's objectives and conditions and provided written informed consent to participate. Participants' personal data were anonymized and used exclusively for scientific purposes.

AI STATEMENT

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

RESULTS

Clinically relevant sexual dysfunction was present in 60 (55.1%) of MS patients. Among these patients, the median total MSISQ-19 score was 40.0 [35.0; 46.0]. Using the tertile-based approach, high levels of manifestations were observed in 30 patients (33.3%) for the primary, 31 patients (34.1%) for the secondary, and 30 patients (33.3%) for the tertiary MSISQ-19 subscales. Clinically significant sexual dysfunction was observed in 45 women (56.3%) and 15 men (51.7%). The total MSISQ-19

Table 1. Comparison of the main characteristics of patients with and without clinically significant sexual dysfunction

Variable	Patients with SD (n=60)	Patients without SD (n=49)	p-value
Age, years	36.0 [29.0; 42.5]	32.5 [26.75; 38.5]	0.124
EDSS score, points	3.0 [2.0; 4.1]	2.0 [1.5; 3.0]	0.0026
Disease duration, years	4.0 [2.0; 10.0]	3.0 [1.0; 5.5]	0.081
SDMT score, points	47,5 [35,0; 57,0]	51.5 [45.5; 60.25]	0.077
MFIS score, points	38,0 [26,5; 47,5]	18.0 [10.0; 25.75]	<0.0001
BDI score, points	13.0 [7.0; 17.25]	6.0 [3.0; 10.5]	0.0002
HAM-A score, points	16.0 [10.0; 25.0]	8.0 [6.0; 15.25]	<0.0001

Source: compiled by the authors of this study

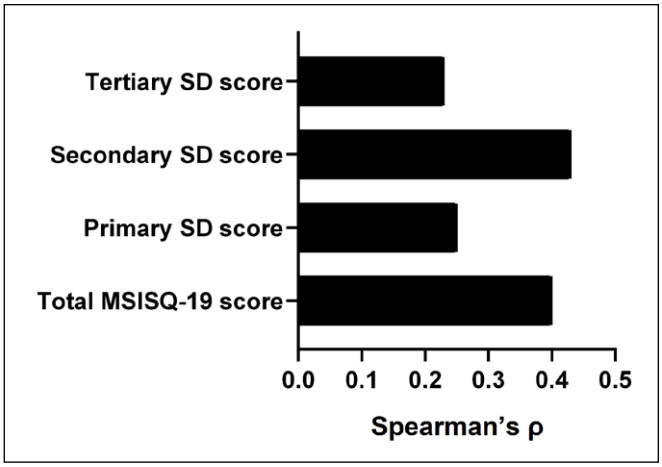


Fig. 1. Correlations between EDSS score and total and subscale MSISQ-19 score
Picture taken by the authors

score did not differ significantly between women and men (p = 0.335). No significant sex-related differences were found for the primary or secondary MSISQ-19 subscales (p = 0.137 and p = 0.958, respectively). At the same time, tertiary subscale scores were significantly higher in women than in men (median 8.0 vs. 6.5; p = 0.032). Disease duration was not significantly associated with sexual dysfunction measures. Specifically, multiple sclerosis duration did not correlate with the total MSISQ-19 score (ρ = 0.11; p = 0.308), as well as with the primary (ρ = 0.01; p = 0.901), secondary (ρ = 0.12; p = 0.257), and tertiary subscale scores (ρ = 0.07; p = 0.512). Moderate positive correlation between patient`s age and the total MSISQ-19 score (ρ = 0.26, p = 0.016) was found. Analysis of the MSISQ-19 subscales revealed that the strongest association was observed between patient`s age and secondary SD scores (ρ = 0.37, p < 0.001). A weak but statistically significant correlation was also found between patient`s age and the level of tertiary SD (ρ = 0.24, p = 0.029). At the same time, the association between age and primary sexual dysfunction was weak and statistically insignificant (ρ = 0.04, p = 0.69). Correlation analysis revealed a statistically significant positive association between the level of disability according to the EDSS score and the total MSISQ-19 score (ρ = 0.40, p < 0.001). Analysis of individual MSISQ-19 subscales revealed that the strongest correlation was found between the EDSS score and the secondary sexual dysfunction subscale score (ρ = 0.43, p < 0.001). Less pronounced but statistically significant correlations were found between the EDSS score and the scores of the primary (ρ = 0.25, p = 0.023) and tertiary (ρ = 0.23, p = 0.034) subscales (Fig. 1). A comparative analysis between patients with relapsing-remitting MS and those with progressive forms of the disease revealed no statistically significant differences in the total MSISQ-19 score (median 34.0 vs. 38.0; p = 0.211). Similarly, scores on the primary (p = 0.399) and tertiary subscales (p = 0.968) did not differ between groups. At the same time, for the secondary subscale of sexual dysfunction, there was a trend toward higher scores in patients with progressive forms of multiple sclerosis (median 20.0 vs. 15.0), which did not reach statistical significance (p = 0.080). Correlation between the SDMT score and the total MSISQ-19 score did not reach statistical significance (ρ = –0.20, p = 0.072). At the same time, a weak inverse correlation was found between SDMT scores and the parameters of the MSISQ-19 subscales. Thus, lower SDMT scores were associated with higher scores on the subscales assessing the severity of primary (ρ = –0.22, p = 0.046) and secondary sexual dysfunction (ρ = –0.22, p = 0.047). The association between SDMT scores and the parameters of the tertiary subscale was weak and statistically insignificant (ρ = –0.13, p = 0.237). Depressive and anxiety symptoms were notably associated with the severity of sexual dysfunction in patients with MS. In particular, the BDI score had a moderate positive correlation with the total MSISQ-19 score (ρ = 0.36, p = 0.004) (Fig.2). An analysis of the MSISQ-19 subscales revealed a statistically significant correlation between BDI score and on all three subscales score: tertiary (ρ = 0.39, p = 0.002), primary (ρ = 0.28, p = 0.031), and secondary (ρ = 0.34, p = 0.008). A strong positive correlation was found between the HAM-A score and the total MSISQ-19 score (ρ = 0.57, p < 0.001). Analysis

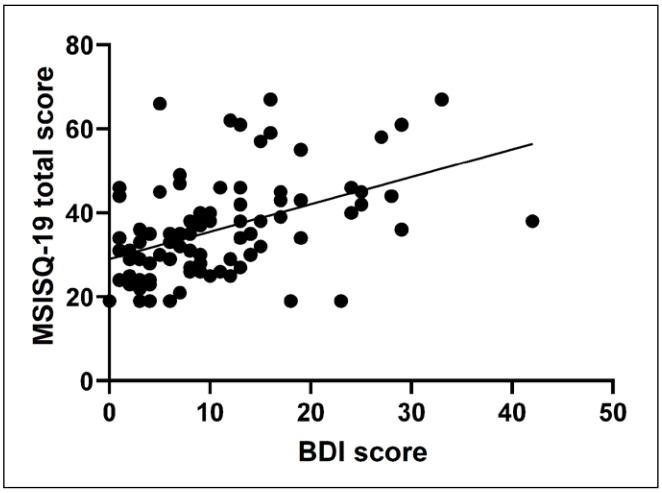


Fig. 2. Association between BDI score and total MSISQ-19 score
Picture taken by the authors

of the subscales also revealed statistically significant positive correlations between HAM-A score and the subscale score characterizing primary ($\rho = 0.46$, $p < 0.001$), secondary ($\rho = 0.42$, $p < 0.001$), and tertiary SD ($\rho = 0.42$, $p < 0.001$). Strong positive correlations were found between fatigue severity and parameteres of sexual dysfunction. In particular, the total MFIS score had a strong, statistically significant association with the total MSISQ-19 score ($\rho = 0.64$, $p < 0.001$). Analysis of individual MSISQ-19 subscales revealed that MFIS scores were significantly correlated with all the components of sexual dysfunction. The strongest correlation was observed for secondary sexual dysfunction ($\rho = 0.52$, $p < 0.001$), whereas for primary ($\rho = 0.46$, $p < 0.001$) and tertiary ($\rho = 0.47$, $p < 0.001$) dysfunction, the correlations were moderate but also statistically significant.

Our study also included a comparative analysis of the main characteristics between both groups of patients with sexual dysfunction (n=60) and those without it (n=49). Patients with sexual dysfunction had significantly higher EDSS, MFIS, BDI, and HAM-A scores than those in the comparison group (Table 1, Fig. 3). No significant difference was observed in age, disease duration, or SDMT score between both groups of patients (Table 1).

To assess the probable association between brainstem lesions and presence of sexual dysfunction, MSISQ-19 scores were compared between patients with and without brainstem lesions on MRI. Patients with brainstem lesions had significantly higher primary sexual dysfunction subscale scores than those without such lesions (median 11.0 vs. 8.0; $p = 0.013$). In contorary, between-group differences in the total MSISQ-19 score (median 35.0 vs. 31.0; $p = 0.072$), as well as secondary (median 15.5 vs. 15.0; $p = 0.522$) or tertiary MSISQ-19 subscale scores (median 8.0 vs. 7.0; $p = 0.068$) did not reach statistical significance.

DISCUSSION

Clinically significant sexual dysfunction was common in our cohort of patients with multiple sclerosis. These findings are consistent with recent studies showing that sexual dysfunction remains highly prevalent in MS. Thus, in a study by Dehghaniathar R. et al. [15], sexual dysfunction was reported in 40–80% of women and 50–90% of men with multiple sclerosis, depending on the age group and the assessment tool used. In a large cohort study by Totuk O. et al. [16], sexual dysfunction was found in 69.0% of patients, specifically in 73.9% of women and 57.6% of men. Previous studies have reported heterogeneous findings regarding sex-related differences in sexual dysfunction among patients with multiple sclerosis [15,16]. In our cohort, no statistically significant differences between men and women were found in the total MSISQ-19 score or in the primary and secondary sexual dysfunction subscales. However, sex-related differences were more apparent in the tertiary MSISQ-19 subscale, with higher scores observed in women than in men. In contrast, a study by Celik D. et al. [17], in which patients were also classified according to the

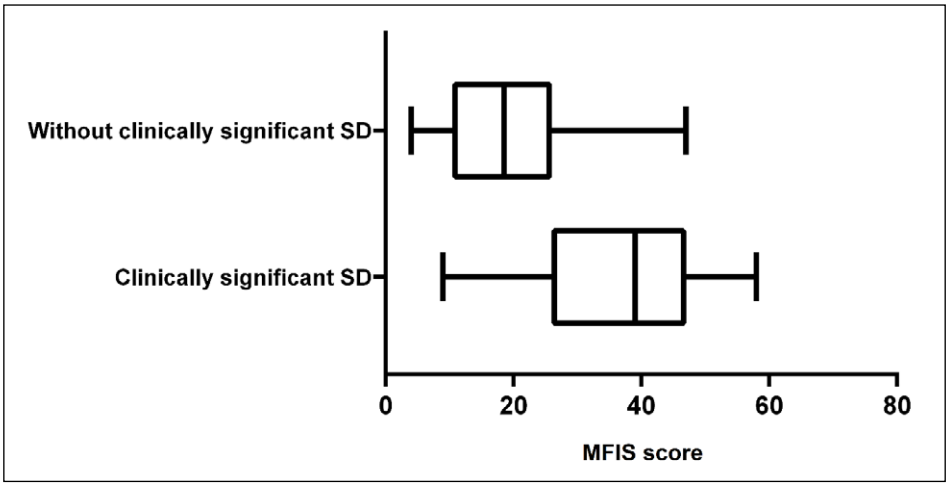


Fig. 3. Comparison of MFIS score between patients with and without clinically significant sexual dysfunction ($p < 0.0001$)
Picture taken by the authors

three subscales of the MSISQ-19, showed that women had higher total MSISQ-19 scores and reported more severe symptoms of secondary sexual dysfunction compared to men. At the same time, the study by Matusik E et al. [18] showed that male gender was associated with indicators of sexual dysfunction; however, this association pertained only to the primary component of the MSISQ-19. According to the results of our study, the duration of the disease was not significantly associated with sexual dysfunction. The available data on this issue are inconsistent. In contrast to our findings, a systematic review and meta-analysis by Yazdan Panah M. et al. [19] found an association between disease duration and sexual dysfunction in multiple sclerosis. Similar results were obtained in a study by Yeni K. et al. [20] among women with MS. Meanwhile, the results of a number of studies indicate that there is no independent association between disease duration and sexual dysfunction, which is consistent with the findings of our study. In particular, a large cohort study by Totuk O. et al. [16] found no statistically significant association between the presence of sexual dysfunction and disease duration. Similar results were obtained in the multicenter study by Petracca M. et al. [14] and the study by Altmann P. et al. [21], where the duration of MS was not associated with sexual dysfunction. In addition, these scientific publications identified patient age as one of the factors associated with sexual dysfunction. A similar pattern was observed in our cohort, where older age was positively correlated with increased severity of sexual dysfunction. This finding is also consistent with the study by Matusik E. et al. [18].

Our study found a statistically significant moderate positive correlation between the level of disability on the EDSS scale and the total MSISQ-19 score, as well as all of its subscales. The strongest association was observed for secondary sexual dysfunction. Similar results were obtained in the study by Mohammadi K. et al. [22], which also demonstrated significant associations between EDSS and both the total MSISQ-19 score and individual subscales, with the strongest correlation noted specifically for the secondary component. The results obtained are also consistent with the studies mentioned above [14, 16, 19–21], in which the level of disability as measured by the EDSS scale is considered one of the key factors associated with the severity of sexual dysfunction in multiple sclerosis.

In our study, the severity of sexual dysfunction was not associated with the clinical course of multiple sclerosis. In particular, no statistically significant differences in MSISQ-19 scores were found between patients with different clinical types of the disease, that is compatiable with data from recent studies [14, 16].

No statistically significant association was found between the total MSISQ-19 score and the SDMT score depicting cognitive functions state, that was consistent with other scientific data and pointed out that cognitive impairment was not among the leading factors contributing to sexual dysfunction in multiple sclerosis [14]. At the same time, our study revealed weak correlations between SDMT and primary and secondary subscales score. However, the available data were limited on a detailed analysis of the relationship between cognitive functions and the MSISQ-19 subscales. In this context, in the study by Marinetto S. et al. [23] executive functions were associated with greater severity of sexual dysfunction, mainly due to the tertiary component.

Taking into consideration obtained results, positive correlations between MSISQ-19 scores and levels of depression and anxiety measured by the BDI and HAM-A scales were observed. The most pronounced associations were found between parameters of BDI and HAM-A scales and MSISQ-19 secondary and tertiary SD subscales, which is partially consistent with the conceptual structure of tertiary SD subscale that mainly reflects psychoemotional and social aspects related to sexual function [6]. The association with the secondary subscale score and the BDI and HAM-A scales score identified in our study may reflect the role of the physical manifestations of the disease, which are potentially linked to the development of psychoemotional disorders. The results obtained in our study are partially consistent with those reported by Domingo S. et al. [24], who showed that patients with sexual dysfunction had higher depression severity; however, no statistically significant difference was found between level of depression and all the sexual dysfunction subscales score. Similar results were highlited in the study by Sari Y. et al. [25], in which higher depression and anxiety score were associated with more pronaunced sexual dysfunction severity. However, the current clinical data are inconsistent, in particular Altmann P. et al. [21] found no association between depression and anxiety and sexual dysfunction in patients with MS.

Our study identified moderate positive correlations between fatigue severity scale score and sexual dysfunction level according to the total and MSISQ-19 subscales score. This is consistent with the current studies, which indicated fatigue as one of the key factors associated with sexual dysfunction in MS. Systematic review and meta-analysis by Yazdan Panah M. et al. [19] showed that in women with MS, decreased sexual function was associated with higher fatigue severity. According to findings reported by Dourou P. et al. [26], the level of fatigue severity in women with MS notably correlated with both the total MSISQ-19 score and the subscale scores describing primary, secondary, and tertiary sexual dysfunction. Similar associations were found in the studis of both sex patient cohorts with MS; specifically, the international study by Marck C. et al. [27] showed that sexual dysfunction and dissatisfaction

with sexual function were associated with presence of fatigue, along with other clinical and psychoemotional factors. Additionally, in a 6-year prospective study by Kisic-Tepavcevic D. et al. [28], fatigue was identified as an independent prognostic factor for the deterioration of sexual function.

According to the our data, the presence of brainstem lesions on MRI was associated with higher primary sexual dysfunction MSISQ-19 scale score. This findings can be explained by the brainstem role in the regulation of the autonomic, sensory, and motor mechanisms involved in sexual function [29]. The available data regarding associations of the MRI lesions and SD in MS remain inconsistent. Thus, our results are partially coherent with the findings of previous studies. Ramezani M. et al. [30], in their systematic review, suggested that the localization of demyelinating lesions can be relevant to sexual dysfunction, particularly in cases involving the brainstem. They also noted a possible link between orgasmic dysfunction and pons involvement. In contrast, Seyman E. et al. [31] found no statistically significant association between quantitative MRI measures of the brain and spinal cord and the severity of sexual dysfunction.

CONCLUSIONS

Sexual dysfunction in MS patients is a common symptom, and is mainly associated with fatigue, affective symptoms, and the level of neurological disability. The strongest associations were observed for the secondary component of sexual dysfunction, whereas the tertiary component was more closely related to affective manifestations. Cognitive impairment did not show a statistically significant association with the overall level of sexual dysfunction; however, it was weakly associated with its individual components. The presence of brainstem lesions on MRI was associated with greater severity of primary sexual dysfunction. Our results support multifactorial nature of SD in MS and emphysize the need for its routine clinical assessment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Inflammatory cytokines as risk factor of septic shock in patients with sepsis

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ABSTRACT

Aim: To prove pro-inflammatory and anti-inflammatory cytokines as risk factors of septic shock in patients with sepsis.

Materials and Methods: This study used a prospective observational study. The target population were sepsis patients who were admitted to the emergency room of Dr Kariadi Hospital. TNF-α, IL-6, IL-10, and IL-4 levels were examined using the ELISA method. Bivariate and multivariate logistic regression analyses as well as receiver operating characteristic curve analysis were used to predict septic shock.

Results: Patients with septic shock had higher levels of IL-6, TNF-α, and IL-10 compared to those with sepsis. Multivariate analysis results showed that IL-6 and IL-10 were independent risk factors for septic shock. IL-6 level ≥11.4 ng/ml had an OR of 7.02, CI 95% 1.78-27.70 (p = 0.005), and IL-10 level had an OR of 3.89, CI 95% 1.13-13.45 (p = 0.031), as risk factors for septic shock.shock.

Conclusions: IL-6 and IL-10 are risk factors of septic shock in sepsis. Meanwhile, TNF-α and IL-4 levels are not associated with shock in sepsis.

KEYWORDS: sepsis, interleukin, tumor necrosis factor, septic shock

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INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by an inappropriate body response to infection [1]. An estimated 49 million cases of sepsis and 11 million sepsis-related deaths occurred worldwide in 2017, accounting for approximately 20% of all causes of death globally. The incidence and mortality of sepsis vary greatly across regions, with the highest incidence in sub-Saharan Africa, Oceania, South Asia, East Asia, and Southeast Asia [2, 3]. A systematic review and meta-analysis by Fleischmann et al. in 2020 reported that from 51 studies, the pooled incidence of sepsis was 189 cases per 100,000 population per year, with an estimated mortality rate of 26.7%. The estimated incidence of sepsis in the intensive care unit (ICU) was 58 per 100,000 people per year, with an estimated mortality rate of 41.9% [4]. The prevalence of sepsis in Asia was reported by Li et al. (2022) from 386 ICUs in 22 Asian countries, with a prevalence of sepsis of 22.4% and a 90-day mortality rate of 36.6% [5]. Research by Purba et al. in Indonesia found that the mortality rate of sepsis patients was 58.3% [6]. The study by Valles et al. reported that 55.5% of deaths from sepsis occurred in the ICU within 72 hours of treatment [7].

Septic shock is a more severe condition than sepsis and is a critical condition involving acute circulatory dysfunction that requires vasopressors to correct hypotension, decreased urine output, and changes in the patient's level of consciousness. Septic shock is defined as sepsis accompanied by persistent hypotension after the administration of 30 ml/kg of intravenous (IV) crystalloid fluid within 1 hour of sepsis identification, requiring vasopressors to maintain a mean arterial pressure (MAP) of ≥ 65 mmHg and serum lactate levels > 2 mmol/L (18 mg/dL). Using this criterion, the mortality rate for septic shock increases to 40% [8, 9].

In sepsis, the balance between pro-inflammatory and anti-inflammatory cytokines is critical. Pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, stimulate systemic inflammation. Anti-inflammatory cytokines such as IL-10 and IL-4 work to restore immunological balance. Sepsis can cause tissue and organ damage if pro-inflammatory cytokines are produced

in excess. Levels of pro- and anti-inflammatory cytokines increase significantly in patients with sepsis [10]. Several studies have indicated that the most important pro-inflammatory cytokine that plays a significant role in the pathogenesis of acute inflammatory responses is tumor necrosis factor-alpha (TNF-α). TNF-α is a pro-inflammatory cytokine that affects antimicrobial and anti-inflammatory responses by activating leukocytes, increasing cell proliferation, differentiation, and lymphocyte apoptosis. According to Deng et al., TNF-α is an inflammatory cytokine associated with sepsis-induced cardiomyopathy [11]. Research by Zhai et al. in patients with abdominal injuries found that TNF-α levels were higher in sepsis than in non-sepsis ($p < 0.001$). TNF-α plays a central role in the systemic inflammatory response due to its ability to release other cytokines in the early stages of infection and its direct effect on septic shock, and plasma TNF-α levels are associated with mortality from sepsis [12, 13].

The hyperinflammatory state in sepsis is also characterized by increased levels of IL-6 as a proinflammatory cytokine. Interleukin 10 (IL-10) is a pleomorphic cytokine with various phenotypic roles released by granulocytes, dendritic cells, macrophages, B cells, and T cell subsets. IL-10 is a major anti-inflammatory cytokine with immunomodulatory effects that suppress the production of pro-inflammatory cytokines [14]. The study by Zhang et al. reported differences in IL-10 levels between sepsis and non-sepsis infections. In sepsis patients, IL-10 correlated with leukocyte count and IL-6 and IL-8 levels [15].

Interleukin-4 (IL-4) is an anti-inflammatory cytokine that plays an important role in modulating the immune response during sepsis. IL-4 functions by inhibiting the production of pro-inflammatory cytokines such as TNF-α and IL-1β, as well as reducing the expression of adhesion molecules on endothelial cells, which collectively help control the excessive inflammatory response that occurs in sepsis. However, the role of IL-4 in sepsis remains controversial. Some studies suggest that IL-4 may have a protective effect by suppressing excessive inflammatory responses, while other studies indicate that IL-4 may contribute to excessive immunosuppression, increasing susceptibility to secondary infections [16]. Research by Schrijver et al. reports that interleukin-4 (IL-4) has the ability to suppress acute inflammation while inducing trained immunity in monocytes. To take advantage of this paradoxical property of IL-4 in the body, they developed a fusion protein between apolipoprotein A1 (apoA1) and IL-4, which was then integrated into lipid nanoparticles. Therapy using IL-4 in the form of these nanoparticles successfully overcame immunoparalysis in mice with hyperinflammation induced by lipopolysaccharides [17].

AIM

To prove pro-inflammatory and anti-inflammatory cytokines as risk factors of septic shock in patients with sepsis.

MATERIALS AND METHODS

This study was a prospective observational study. The study was conducted from March to October 2025. Physical examinations, medical history, and informed consent for research subject screening were conducted at the emergency room of Dr. Kariadi Hospital in Semarang. Complete blood tests and laboratory examinations were performed at the hospital laboratory. Tests for IL-6, TNF-α, IL-10, and IL-4 levels were performed at the GAKI Laboratory, Faculty of Medicine, Universitas Diponegoro, Semarang.

The inclusion criteria were a sepsis diagnosis according to SEPSIS-3 criteria, enrollment within 24 hours of admission to the emergency department, age >18 years, and being free of radiotherapy or chemotherapy. Septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and a serum lactate level more than 2 mmol/L (>18 mg/dL). Exclusion criteria were patients with immunosuppressive therapy, a history of autoimmune disease, and malignancy. IL-6, TNF-α, IL-10, and IL-4 were examined using the ELISA (Enzyme Linked Immunosorbent Assay) kit, Elabscience Biotechnology Inc., United States.

STATISTICAL ANALYSIS

Categorical data were presented as a percentage, and comparisons were made using the chi-square test. Bivariate and multivariate logistic regression analyses were used to investigate the independent risk factors associated with septic shock. Variables with $p < 0.25$ in bivariate analysis were included in multivariate analysis. The odds ratio (OR) and confidence interval (CI) were then computed. The Mann-Whitney test was used to test the difference in IL-6, TNF-α, IL-10, and IL-4 levels between shock and sepsis. The cut-off levels for the variables were determined based on the Youden index from the Receiver Operating Characteristic (ROC) curve or from the literature. The study received ethical clearance from the Health Research Ethics Committee of Dr Kariadi General Hospital, Semarang, No. 16447/EC/KEPK-RSDK/2025.

AI STATEMENT

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

FRAMEWORK
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RESULTS

This study involved 82 subjects consisting of 44 males (53.7%) and 38 females (46.3%). The subjects in this study ranged in age from 18 to 77 years. Most subjects required ICU care, with a mortality rate of 54.9%. Diabetes mellitus was the most prevalent comorbidity among patients, with cardiovascular diseases and hypertension coming in second. Patients had an average SOFA score of 7.7 and a maximum score of 14. The average hemoglobin level of the research participants was 10.03 g/dL, which suggested mild anemia. While the median platelet count stayed within the normal range at $248 \times 10^3/\mu L$, leukocyte counts tended to be higher at $14.28 \times 10^3/\mu L$. With median values of 83.5 mg/dL and 1.9 mg/dL, respectively, the majority of patients had increased urea and creatinine levels. The NLR, which measures systemic inflammation, and serum lactate levels were both high, with median values of 3.7 mmol/L and 9.1, respectively. The basic characteristics of the research population are shown in Table 1.

Table 2 showed bivariate analysis of risk factors associated with septic shock. The bivariate analysis revealed that males are at higher risk of developing septic shock (OR 2.60, p=0.037, CI 1.05–6.43). Among vital signs, systolic blood pressure and an increased heart rate were associated with a higher risk of septic shock (OR 5.01, p = 0.001, and CI 1.95–12.92 and OR 5.67, p = 0.001, and CI 2.18–14.74, respectively). Based on the source of infection, this study found that intra-abdominal infections carry a higher risk of septic shock compared to other infections (OR = 9.00, p = 0.04, CI 1.03–78.57).

The results of the bivariate laboratory analysis revealed that elevated levels of bilirubin and serum lactate, as well as a high NLR, were risk factors for septic shock compared to other laboratory findings (OR = 2.54, p = 0.047, CI 1.00–6.41; OR = 5.00, p = 0.001, CI 1.93–12.96; OR = 3.10, p = 0.016, CI 1.22–7.86, respectively). Meanwhile, a bivariate analysis of inflammatory cytokines revealed that higher levels of IL-6, TNF-α, and IL-10 were risk factors for septic shock (OR = 8.06, p = 0.001, CI 2.66–24.46; OR = 3.8, p = 0.008, CI 1.39–10.41; OR = 3.59, p = 0.005, CI 1.43–9.00, respectively).

Table 3 showed the differences in pro-inflammatory and anti-inflammatory cytokines between sepsis and septic shock. The Mann-Whitney U test results showed a difference in IL-6, TNF-α, and IL-10 levels between sepsis and septic shock (p = 0.001, p = 0.019, and p = 0.008), while no difference was found in IL-4 levels between sepsis and septic shock (p = 0.352). The level of IL-6, TNF-α, and IL-10 was higher in septic shock than in sepsis.

Based on Table 4, the results of the bivariate analysis with a p-value < 0.25 were included in the multivariate analysis. Thus, the variables gender, WBC, total bilirubin, sodium, lactate, NLR, intra-abdominal infection, IL-6, TNF-α, and IL-10 were included in the multivariate analysis. Table 4 indicated a multivariate analysis of inflammatory cytokines and other risk factor-related septic shocks. The risk factors of septic shock were as followed: lactate serum (OR=6.02; 95% CI, 1.6-22.65, p=0.008), intraabdomen infections (OR=16.76, 95% CI, 1.04-269.44, p=0.047, IL-6 (OR=7.02, 95% CI, 1.78-27.70, p=0.005) and IL-10 (OR=3.89, 95% CI, 1.13-13.45, p=0.031).

Table 5 showed the cut-off value of inflammatory cytokines and other risk factors with area under the curve, sensitivity and specificity. The area under the curve (AUC) values from the ROC curve for IL-6, IL-10, intra-abdomen infection, lactate and the incidence of septic shock were 0.709, 0.673, 0.428 and 0.696. IL-6 has the best AUC value (Fig. 1).

Fig. 1 showed the Receiver Operating Characteristic (ROC) curves comparing the diagnostic capabilities of IL-6, IL-10, lactate levels, and the presence of intra-abdominal infection in predicting the study outcome. As shown in the graphical representation, for most of the 1-specificity range, the IL-6 curve is farther above the reference line than the other biomarkers, demonstrating the best discriminatory ability. The curves for lactate and IL-10 also showed a fairly satisfactory discriminatory capacity, as they are above the reference line, but their performance is still inferior to IL-6. On the other hand, the intra-abdominal infection variable has the curve closest to the reference line, which indicates low predictive ability.

DISCUSSION

The results of this study indicate that being 60 years or older was not associated with septic shock, but males were found to have a higher risk of developing septic shock than females. In general, males are more susceptible to sepsis than females. Several studies have reported a higher incidence of sepsis in males and varying mortality rates between the sexes. Females tend to have a better immune response, which is thought to be due to the role of the hormone estrogen in enhancing the adaptive immune system and higher levels of anti-inflammatory mediators in females, while males often show a weaker cellular immune

Table 1. Characteristics of Research Subjects

Variable (n=82)	n (%)	Mean ±SD	Median (Min-Max)	p-value
Gender				
Male	44 (53.7)			
Female	38 (46.3)			
Age (yr)		54.67±14.12	58.5 (18-77)	0.001*
Sepsis state				
Sepsis	46 (56.1)			
Septic shock	36 (43.9)			
Outcome				
Survive	37 (45.1)			
Non-survive	45 (54.9)			
Ward				
ICU	45 (54.9)			
Non-ICU	37 (45.1)			
Blood pressure				
Systole (mmHg)		108.89±26.62	100 (60-180)	0.001*
Diastole (mmHg)		69.04±16.35	68 (39-107)	0.047*
Heart rate (bpm)		100.30±23.90	98 (60-172)	0.073
Temp (°C)		36.74±0.69	36.7 (35.6-39.1)	0.001*
Respiratory rate (bpm)		23.88±3.33	24 (18-36)	0.001*
Comorbidity				
DM	34 (41.5)			
Hypertension	27 (32.9)			
CKD	3 (3.7)			
Cardiovascular disorder	19 (23.2)			
SOFA score		7.70±2.95	8 (2-14)	0.099
Hemoglobin (g/dL)		10.03±2.53	9.5 (4.9-18.1	0.039
WBC (x10^3/µL)		16.6±10.45	14.28 (3.0-63.3)	0.001*
Platelet (x10^3/ µL)		273.78±166.54	248 (9-785)	0.002*
Blood glucose (mg/dL)		154.46±104.9	122 (34-631)	0.001*
Urea (mg/dL)		97.60±60.76	83.5 (20-262)	0.005*
Creatinine (mg/dL)		2.67±2.67	1.9 (0.3-14.0)	0.001*
Total bilirubin (mg/dL)		2.08±3.8	0.71 (0.15-25.54)	0.001*
Sodium (mmol/L)		133±8.03	134 (107-162)	0.017*
Potassium (mmol/L)		4.09±1.09	4.0 (1.5-7.0)	0.005*
Lactate (mmol/L)		4.88±3.09	3.7 (0.7-15.5)	0.001*
NLR		11.11±7.93	9.10 (0.10-30.01)	0.001*
IL-6 (ng/mL)		20.55±17.99	15.04 (1.31-98.01)	0.001*
TNF-α (ng/mL)		28.29±28.37	17.88 (12.58-161.2)	0.001*
IL-10 (ng/mL)		29.66±28.98	15.73 (1.13-99.19)	0.001*
IL-4 (ng/mL)		49.69±38.89	39.03 (0.84-154.4)	0.001*

p: Kolmogorov-Smirnov, *p<0.05: abnormal distribution of variable, ICU: intensive care unit,
DM: diabetes mellitus, CKD: chronic kidney disease, SOFA: Sequential Organ Failure Assessment, WBC: white blood cells, NLR: neutrophil lymphocyte ratio, IL: interleukin, TNF: tumour necrosis factor.
Source: compiled by the authors of this study

response, possibly due to the suppressive effects of testosterone [18, 19]. Aa complex network of cytokine, immune, and endothelial cell interactions occur and disturbances in the microcirculation cause organ dysfunction or even failure leading to high mortality in those patients. In this respect, numerous experimental and clinical studies indicate sex-specific differences in infectious diseases and sepsis. Female gender has been demonstrated to be protective under such conditions, whereas male gender may be deleterious

Table 2. Bivariate analysis of risk factors associated with septic shock

Variables	Cut-off	Total subject (n=82)		Sepsis		Septic shock		p-value	OR	95% CI (Lower-Upper)
		n	%[a]	n	%[b]	n	%[b]			
Demography										
Age (yr)	≥ 60	37	45.1	20	54.1	17	45.9	0.735	1.16	0.48-2.79
	< 60	45	54.9	26	56.6	19	42.2			
Gender	Males	44	53.7	20	45.5	24	54.5	0.037*	2.60	1.05-6.43
	Females	38	46.3	26	68.4	12	31.6			
Ward	ICU	45	54.9	22	48.9	23	51.1	0.147**	1.90	0.79-4.71
	Non-ICU	37	45.1	24	64.9	13	35.1			
Vital Sign										
Systole (mmHg)	<100	36	43.9	12	33.3	24	66.7	0.001*	5.01	1.95-12.92
	≥100	46	56.1	34	73.9	12	26.1			
Heart rate (bpm)	≥102	39	47.6	15	38.5	24	61.5	0.001*	5.67	2.18-14.74
	< 102	43	52.4	31	72.1	12	27.9			
Temperature (°C)	≥37.5	26	31.7	17	65.4	9	34.6	0.248	0.569	0.22-1.49
	<37.5	56	68.3	29	51.8	27	48.2			
Respiratory rate (bpm)	≥ 22	43	52.4	41	95.3	26	60.5	0.049*	0.317	0.10-1.03
	< 22	39	47.6	5	12.8	10	25.6			
Comorbidities										
Diabetes mellitus	Yes	34	41.5	21	61.8	13	38.2	0.384	0.67	0.28-1.65
	No	48	58.5	23	47.9	23	47.9			
Hypertension	Yes	27	32.9	19	70.4	8	29.6	0.068**	0.41	0.15-1.08
	No	55	67.1	27	49.1	28	50.9			
CKD	Yes	3	3.7	2	66.7	1	33.3	0.592	0.75	0.15-3.80
	No	79	96.3	44	55.7	35	44.3			
Cardiovascular	Yes	8	9.8	5	62.5	3	37.5	0.502	0.75	0.17-3.35
	No	74	90.2	41	55.4	33	44.6			
Type of infection										
Pneumonia	Yes	66	80.5	36	54.5	30	45.5	0.565	1.39	0.45-4.27
	No	16	19.5	10	62.5	6	37.5			
Urinary tract infection	Yes	19	23.2	12	63.2	7	36.8	0.479	0.68	0.24-1.97
	No	63	76.8	34	54.0	29	46.0			
Skin and soft tissue infection	Yes	6	7.3	4	66.7	2	33.3	0.461	0.62	0.11-3.58
	No	76	92.7	42	55.3	34	44.7			
Leptospirosis	Yes	2	2.4	1	50.0	1	50.0	0.688	1.14	0.28-4.67
	No	80	97.6	45	56.3	35	43.8			
Intra-abdominal infection	Yes	7	8.5	1	14.3	6	85.7	0.040*¥	9.00	1.03-78.57
	No	75	91.5	45	60.0	30	40.0			
Laboratory values										
Hemoglobin (g/dL)	<10.4	50	61.0	30	60.0	20	40.0	0.373	0.67	0.27-1.63
	≥10.4	32	39.0	16	50.0	16	50.0			
WBC (x10^3/µL)	≥15.6	34	41.5	16	47.1	18	52.9	0.165**	1.88	0.77-4.57
	<15.6	48	58.5	30	62.5	18	37.5			
Platelet (x10^3/µL)	<150	21	25.6	12	57.1	9	42.9	0.911	0.94	0.35-2.57
	≥150	61	74.4	34	55.7	27	44.3			
Blood glucose (mg/dL)	≥ 122	41	50.0	25	61.0	16	39.0	0.373	0.67	0.28-1.61
	<122	41	50.0	21	51.2	20	48.8			

Table 2. Cont.

Urea (mg/dL)	≥85	40	48.8	21	52.5	19	47.5	0.522	1.33	0.56-3.19
	<85	42	51.2	25	59.5	17	40.5			
Creatinine (mg/dL)	≥1.2	55	67.1	32	58.2	23	41.8	0.587	0.77	0.31-1.95
	<1.2	27	32.9	14	51.9	13	48.1			
Total bilirubin (mg/dL)	≥1.2	29	35.4	12	41.4	17	58.6	0.047*	2.54	1.00-6.41
	<1.2	53	64.6	34	64.2	19	35.8			
Sodium (mmol/L)	≥143.5	5	6.1	1	20.0	4	80.0	0.093**¥	5.63	0.60-52.72
	<143.5	77	93.9	45	58.4	32	41.6			
Potassium (mmol/L)	<4	39	47.6	22	56.4	17	43.6	0.957	0.98	0.41-2.34
	≥4	43	52.4	24	55.8	19	44.2			
Lactate (mmol/L)	≥4.46	33	40.2	11	33.3	22	66.7	0.001*	5.00	1.93-12.96
	<4.46	49	59.8	35	71.4	14	28.6			
NLR	≥7.15	36	43.9	10	27.8	26	72.2	0.016*	3.10	1.22-7.86
	<7.15	46	56.1	25	54.3	21	45.7			
Inflammatory cytokine										
IL-6 (ng/ml)	≥11.4	51	62.2	20	39.2	31	60.8	0.001*	8.06	2.66-24.46
	<11.4	31	37.8	26	83.9	5	16.1			
TNF-α (ng/ml)	≥15.9	53	64.6	24	45.3	29	54.7	0.008*	3.80	1.39-10.41
	<15.9	29	35.4	22	75.9	7	24.1			
IL-10 (ng/ml)	≥21.6	36	43.9	14	38.9	22	61.1	0.005*	3.59	1.43-9.00
	<21.6	46	56.1	32	69.6	14	30.4			
IL-4 (ng/ml)	≥48.9	32	39.0	17	53.1	15	46.9	0.664	1.22	0.50-2.98
	<48.9	50	61.0	29	58.0	21	42.0			

Chi-Square test; ¥: Fisher exact test, significant if *p < 0.05; ** p<0.25; [a]: calculate percentage in total subjects of recruitment (n = 82), [b]: calculate percentages in a row.
Source: compiled by the authors of this study

Table 3. Comparison of inflammatory cytokines between sepsis and septic shock

Variable	Sepsis Median (min-max)	Septic shock Median (min-max)	p
IL-6	10.08 (1.31-52.30)	18.45 (4.84-98.01)	0.001*
TNF-α	16.34 (12,58-119,5)	20.43 (12.65-161.2)	0.019*
IL-10	13.15 (1.13-97.85)	29.19 (3.06-99.19)	0.008*
IL-4	40.38 (3.27-154.4)	33.54 (0.84-152.2)	0.352

p= Mann-Whitney U test; * p<0.05
Source: compiled by the authors of this study

due to a diminished cell-mediated immune response and cardiovascular functions. Male sex hormones, i.e., androgens, have been shown to be suppressive on cell-mediated immune responses. In contrast, female sex hormones exhibit protective effects which may contribute to the natural advantages of females under septic conditions. Thus, the hormonal status has to be considered when treating septic patients. Therefore, potential therapies could be derived from this knowledge. In this respect, administration of female sex hormones (estrogens and their precursors

The mortality rate of sepsis in this study was quite high, with more than 50% of patients dying within 28 days of treatment. These results are consistent with a study by Purba et al., which found a mortality rate of 58.3% among sepsis patients in Indonesia, with the national burden of sepsis estimated at US$130 million per 100,000 patients. The cost of ICU care is much higher, with an average of US$1,618 per patient [6]. The study by Valles et al. reported that 55.5% of deaths from sepsis occurred in the ICU within 72 hours of treatment, with an average mortality rate related

Table 4. Multivariate analysis of risk factors and inflammatory cytokine of septic shock

Variable	Cut-off	p values	OR	95% CI (Lower-Upper)
Gender	-	0.082	0.32	0.09-1.16
WBC (x10^3/µL)	15.6	0.080	3.16	0.87-11.42
Total bilirubin (mg/dL)	1.2	0.980	1.02	0.26-4.03
Sodium (mmol/L)	143.5	0.094	20.68	0.60-717.35
Lactate (mmol/L)	4.46	0.008*	6.02	1.60-22.65
NLR	7.15	0.194	2.44	0.63-9.40
Intra-abdominal infection	-	0.047*	16.76	1.04-269.44
IL-6 (ng/ml)	11.4	0.005*	7.02	1.78-27.70
TNF-α (ng/ml)	15.9	0.943	1.05	0.25-4.14
IL-10 (ng/ml)	21.6	0.031*	3.89	1.13-13.45

*p<0.05

Source: compiled by the authors of this study

Table 5. Area under the curve, sensitivity and specificity biomarker and inflammatory marker as risk factor of septic shock

Variable	Cut-off	AUC	Sensitivity	Specificity
WBC (x10^3/µL)	15.6	0.526	50.0 %	62.2%
Total bilirubin (mg/dL)	1.2	0.692	47.2%	73.9%
Sodium (mmol/L)	143.5	0.455	11.1%	97.8%
Lactate (mmol/L)	4.46	0.696	61.1%	76.1%
NLR	7.15	0.668	72.2%	54.3%
Intra-abdominal infection	-	0.428	16.7%	97.8%
IL-6 (ng/ml)	11.4	0.709	86.1%	56.5%
TNF-α (ng/ml)	15.9	0.652	80.6%	47.8%
IL-10 (ng/ml)	21.6	0.673	61.1%	69.6%

Source: compiled by the authors of this study

to septic shock of 25.8% in the ICU and 29.6% in the hospital [7].

The most common comorbidities in the subjects of this study were diabetes mellitus and hypertension. This is consistent with studies by Xia et al. and Sun et al., in which diabetes mellitus and hypertension were the highest comorbidities causing sepsis mortality [20, 21]. Diabetes, as a metabolic disorder characterized by chronic hyperglycemia, has widespread effects on various physiological systems, including immune function, endothelial integrity, and inflammatory response, all of which are critical in the development and outcome of sepsis. Diabetes mellitus is associated with several immune dysfunctions, such as impaired neutrophil function, decreased phagocytic activity, and altered cytokine production, which can increase susceptibility to infection. Hypertension is associated with chronic inflammation that can exacerbate the inflammatory response to sepsis, thereby increasing the risk of mortality. Research indicates that the use of ACE inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) to manage hypertension in sepsis patients who have not yet experienced septic shock can reduce in-hospital mortality rates [21-23].

The most common source of infection in this study was pneumonia, although pneumonia is not statistically a risk factor for septic shock. This is consistent with previous studies, which found that the most common source of infection causing sepsis was pneumonia [24]. This study found that intra-abdominal infection sources are a risk factor for septic shock. Intra-abdominal infections can cause shock in sepsis through an uncontrolled systemic inflammatory cascade, starting with the translocation of bacteria and endotoxins into the bloodstream. An excessive immune response triggers a cytokine storm that damages the vascular endothelium, causing massive vasodilation, capillary leakage, and refractory hypotension. This leads to tissue hypoperfusion, myocardial depression, and multiple organ failure [25-27].

Lactate is a metabolic product produced primarily by anaerobic metabolism when oxygen to tissues is insufficient for aerobic respiration. In septic shock, there is an increase in serum lactate levels indicating

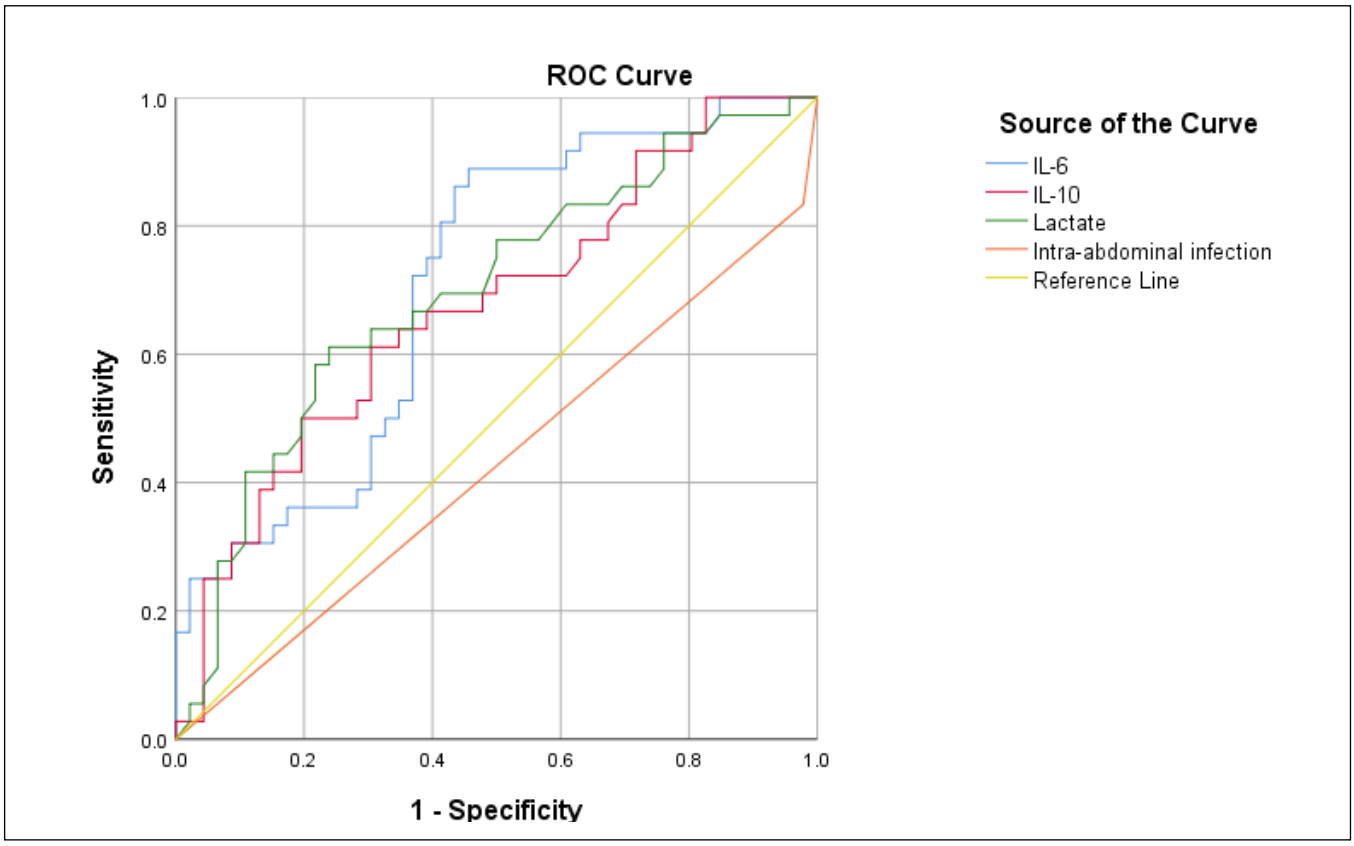


Fig 1. ROC multivariate analysis of single risk factor septic shock of IL-6, IL-10, lactate and intra-abdomen infection with incidence of shock septic
Picture taken by the authors

tissue hypoperfusion and cellular hypoxia, reflecting an imbalance between oxygen supply and demand. This accumulation is caused by increased anaerobic glycolysis and impaired clearance by the liver and kidneys. Elevated lactate is used clinically as a biomarker to assess the severity of sepsis, guide resuscitation efforts, and predict patient outcomes [28, 29]. This study found a mean lactate value of 4.88 mmol/L with a median of 3.7 mmol/L. Multivariate analysis results showed that lactate levels above 4.46 were a risk factor for septic shock. Song et al. reported lactate levels as predictors of sepsis mortality on days 7 and 28. Lactate levels ≥4 mmol/L had odds ratios (OR) of 1.286 and 1.346 in patients with septic shock who received appropriate treatment in the emergency department [30]. A study by Putri et al. found that lactate levels were positively correlated with SOFA scores. This is consistent with the finding that higher lactate levels indicate relatively greater organ dysfunction [31].

The analysis of differences in pro-inflammatory and anti-inflammatory cytokines between patients with sepsis and septic shock revealed a difference in IL-6, TNF-α, and IL-10 levels. Meanwhile, no differences were found in IL-4 levels. After conducting a multivariate analysis of other factors that could be risk factors for septic shock, only IL-6 and IL-10 levels were found to be statistically independent risk factors for septic shock. IL-6 levels above 11.4 ng/mL had a 7.02 times higher risk of septic shock compared to patients with lower IL-6 levels. This observation is consistent with a study by Vivas et al. (2021), which showed significant differences in plasma IL-6 levels between patients with sepsis and septic shock during the first 48 hours after admission to the ICU. In patients with pure sepsis, this study found a significant decrease in serum IL-6 levels, with the average value decreasing from 60.00 pg/mL at admission to 7.00 pg/mL 48 hours later ($p = 0.002$). In contrast, in patients who progressed to septic shock, IL-6 levels remained high and stable during the same period, from 70.00 pg/mL at admission to 50.00 pg/mL at 48 hours ($p = 0.001$). This difference indicates that the persistence of high IL-6 levels is a marker of clinical deterioration toward septic shock, while the progressive decrease in IL-6 reflects a controlled inflammatory response in sepsis without shock [32]. Research by Smok et al. shows that serum levels of IL-6, procalcitonin, and C-reactive protein at hospital admission are statistically significantly higher in sepsis compared to the control group (21.4 pg/ml, 0.6 ng/ml, and 24.9 mg/l vs. 3.48 pg/ml, 0.0 ng/ml, and 1.0 mg/l [33].

Interleukin-6 is synthesized by mononuclear phagocytes, endothelial cells, fibroblasts, and some activated

T cells in response to pathogen-associated molecular patterns (PAMPs), IL-1, and TNF-α. IL-6 stimulates acute-phase protein synthesis by the liver, production of neutrophils from the bone marrow, and differentiation of TH17 T helper cell subsets. In severe sepsis that progresses to septic shock, high IL-6 concentrations are associated with a poor prognosis, and high serum IL-6 levels are closely related to increased inflammatory severity and the development of organ dysfunction. The occurrence of septic shock is primarily mediated by TNF-α, which stimulates the release of other cytokines, including IL-6, thereby causing the amplification of the excessive inflammatory response characteristic of septic shock [32, 34].

In this study, TNF-α levels in sepsis differed from those in septic shock; however, after multivariate analysis, no results were found to indicate that TNF-α is a risk factor for septic shock. A prospective multicenter study of 163 adult patients in the ICU showed that plasma TNF-α levels were significantly higher in the septic shock subgroup (median 57.3 pg/mL) compared to sepsis without shock (median 42.9 pg/mL, $p = 0.0007$). [13] Sepsis has diverse etiologies (Gram-negative vs. Gram-positive, abdominal vs. non-abdominal), causing variable TNF-α responses. Patients with comorbidities or different stages can show inconsistent TNF-α levels, making them non-independent in multivariate analysis. Meta-analyses highlight the divergent effects of TNF-α, with some studies showing adaptive rather than maladaptive effects of TNF-α in experimental studies [35].

The results of the study found that IL-10 levels above 21.6 ng/ml had a 3.89 times higher risk of shock. Research by Fabri et al. (2021) showed that in patients with septic shock, IL-10 production by monocytes and CD4 T cells increased, which was associated with a decrease in HLA-DR expression and a decrease in TNF-α production by monocytes. This data indicates the development of an immunosuppressive profile with increased IL-10, which may contribute to immune dysfunction in septic shock [36]. Research by Mazer et al. demonstrates the dual effect of IL-10 on the immune system of sepsis patients, whereby IL-10 suppresses TNF-α production and HLA-DR expression in monocytes but actually increases IFN-γ production in T cells, indicating the complex modulatory role of IL-10 in post-sepsis immune balance. This indicates that IL-10 acts as an anti-inflammatory cytokine and has the potential to play a stimulatory role in some aspects of the adaptive immune response during sepsis [37]. Research by Harviendo et al. showed that there was a moderate positive correlation between IL-10 levels and APACHE II scores, with a correlation coefficient of 0.430 and a p-value of 0.010. Interleukin-10 is an anti-inflammatory cytokine that functions to stop inflammation in sepsis. High levels are usually associated with a state of homeostatic immunological tolerance or as a counter-response (negative feedback) to increased pro-inflammatory cytokines [38].

In the present study we demonstrated that both IL-6 and IL-10 were significant predictors of poor clinical outcomes in sepsis. These results are consistent with a recent systematic review showing that IL-6 is one of the most sensitive early inflammatory response biomarkers. IL-10, on the other hand, is a compensatory anti-inflammatory response and development of sepsis-associated immunosuppression. However, the current evidence seems to indicate that the concomitant increases in IL-6 and IL-10 are indicative of the presence of hyperinflammation and immune dysregulation, which are now considered defining characteristics of the pathophysiology of sepsis, rather than being in opposition to biological pathways. Moreover, recent extensive reviews highlight that no single inflammatory biomarker is adequate for prognostic assessment and propose the use of complementary panels of biomarkers to enhance risk stratification. Therefore, the co-predictive value of IL-6 and IL-10 in this study demonstrates that a combination of biomarkers reflecting both pro- and anti-inflammatory pathways could be a better predictor of disease severity and prognosis than each cytokine alone [39, 40].

Although IL-6 and IL-10 are not yet part of routine sepsis management protocols, both may be useful as additional biomarkers for early risk stratification because they reflect complementary biological mechanisms of the host response. IL-6 is a pro-inflammatory mediator associated with the early hyperinflammatory phase, while IL-10 reflects compensatory anti-inflammatory and immunoregulatory activity. Current sepsis guidelines remain dependent on clinical assessment of the patient and on markers already in routine use. These guidelines recommend blood lactate measurement in suspected sepsis, use of periodic lactate measurements to guide resuscitation and use of procalcitonin in conjunction with clinical evaluation to assist in deciding when to cease antimicrobial therapy. In this context, one should think of IL-6 and IL-10 as investigative, not decision-making, tools by themselves. Both may be useful, in combination with lactate and procalcitonin, to improve early prognosis and identify patients at higher risk of worsening or death. Hence, their complementary roles are particularly precious at the time of patient presentation or early in ICU admission when the balance of inflammatory activation, tissue hypoperfusion, and

immune suppression may not be fully reflected by a single biomarker [41].

IL-4 levels in this study were not found to be associated with septic shock. This is consistent with the study by Wu et al., in which plasma IL-4 levels did not differ significantly between patients who survived and those who died from sepsis [42]. In other infections such as COVID-19, research by Ivanov et al., also reported that IL-4 was not associated with severity [43]. Interleukin 4 (IL-4) functions as an anti-inflammatory cytokine that regulates the immune response during sepsis and septic shock. IL-4 serves to inhibit the activation of proinflammatory immune cells, suppress T and B lymphocyte activity, and assist in regulating the equilibrium between proinflammatory and anti-inflammatory responses during sepsis. However, some studies suggest that IL-4 mRNA expression may be associated with the survival rate of patients with severe sepsis. Studies analyzing the IL-4delta2/IL-4 ratio suggest that this ratio may serve as an immunological indicator, but IL-4 expression does not have a direct relationship with the occurrence of sepsis or septic shock in general [42].

The study has limitations that should be considered when interpreting the results. The study was performed at a single center and thus, the results may not be generalizable to other health care settings and patient populations with different demographic and clinical characteristics. Second, the sample size was rather small, which would have reduced the statistical power to detect small associations and the precision of the estimated predictive performance of the biomarkers. Third, this study did not perform a complete evaluation of potential confounding factors and pre-existing comorbidities such as obesity, diabetes mellitus and other underlying diseases that impact the course of sepsis and the development of septic shock. As a result, a comprehensive assessment of the potential contribution of these factors to the observed relationships was not possible. However, in spite of these limitations, the validation of the considerable predictive value of IL-6 and IL-10 supports the validity of the present findings.

CONCLUSIONS

In this study, IL-6 and IL-10 are independent risk factors for septic shock, whereas TNF-α and IL-4 are not independent risk factors for septic shock in patients with sepsis. Additional multicenter studies in larger and more heterogeneous cohorts, with appropriate adjustment for relevant comorbidities and other potential confounders, are needed to confirm our findings and to further define the therapeutic utility of these biomarkers for risk stratification in sepsis patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Adaptive football in physical and sports rehabilitation of individuals with amputations: Impact on health-related quality of life, health status, and cognitive domain

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ABSTRACT

Aim: To investigate the impact of adaptive football training sessions on the indicators of health-related quality of life, health status, and the cognitive domain of individuals with amputations.

Materials and Methods: The research included 32 male service members and law enforcement officers who had suffered limb amputations as a result of blast and shrapnel injuries sustained during the war in Ukraine (2022-2025). Twenty-six participants had lower-limb amputations, and six had upper-limb amputations. Indicators of health-related quality of life and health status were assessed via the "SF-36 Health Survey". Cognitive domain status was evaluated using the indicators of "Attention Distribution and Span" and "Attention Concentration and Sustainability".

Results: It was found that adaptive football training sessions contributed to a statistically significant improvement in the physical and mental components of health among individuals with amputations. The greatest positive changes were observed in social functioning (12.28 %), vitality (9.99 %), and physical functioning (9.77 %) indicators. A statistically significant improvement was also observed in cognitive indicators: attention distribution and span (15.03 %) and attention concentration and sustainability (3.26 %). At the same time, changes in pain intensity did not reach statistical significance.

Conclusions: Adaptive football is an effective means of physical and sports rehabilitation for individuals with amputations, providing a comprehensive positive impact on indicators of health status, health-related quality of life, and cognitive domain. Regular training sessions help increase physical activity, improve psychological and emotional well-being, promote social adaptation, confirming the value of incorporating adaptive football into rehabilitation programs for people with amputations.

KEYWORDS: adaptive sports, amputee football, health, individuals with amputations, physical and sports rehabilitation, quality of life

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INTRODUCTION

The full-scale war in Ukraine has been accompanied by a significant increase in the number of people who have suffered severe injuries resulting in limb amputations. According to estimates by international organizations, during the first two years of the Russian-Ukrainian war alone, more than 100,000 people lost an upper or lower limb [1]. Such a high number of amputations significantly highlights the challenge not only of the medical recovery of those affected but also of ensuring their long-term physical, psychological, and social rehabilitation.

Limb amputation is a complex biopsychosocial problem, the consequences of which are not limited to the loss of a specific anatomical structure but encompass a wide range of functional, psychological, and social changes [2]. People with amputations experience reduced motor activity, impaired movement biomechanics, limited functional capabilities, changes in their usual lifestyles, and the need to adapt to new living conditions [3]. In addition to physical limitations, a significant proportion of these individuals experience psycho-emotional difficulties, the most common of which include post-traumatic stress disorder, anxiety

and depression, low self-esteem, social isolation, and a reduced quality of life [4]. Common somatic consequences include phantom pain, postural abnormalities, muscle imbalances, and functional limitations caused by changes in movement patterns [5].

Under these conditions, comprehensive rehabilitation for individuals after amputation becomes particularly important; it must combine medical, physical, psychological, and social components of recovery. One of the promising areas of modern rehabilitation practice is the use of adaptive physical education and adaptive sports, which contribute to the restoration of the body's functional capabilities, increased levels of physical activity, the development of motivation for an active lifestyle, and successful social integration.

The analysis of current scientific sources indicates that rehabilitation for individuals following amputation is comprehensive in nature and must encompass the physical, psychological, and social components of recovery [6]. Researchers emphasize that, alongside medical interventions, psychological support is a key factor in successful rehabilitation; this support aims to overcome the consequences of a traumatic event, adapt to changes in physical condition, and foster readiness for active participation in social life [7]. It has been established that the effectiveness of psychological adaptation is largely determined by the level of motivation for physical activity, social interaction, and professional self-fulfillment among individuals with amputations [8].

After the acute postoperative period ends, the main goals of rehabilitation are preparation for prosthetics, restoration of functional independence, improvement of physical performance, and ensuring the individual's social integration [9]. In this regard, significant attention should be paid to developing strength, endurance, coordination, and balance, which help compensate for existing functional limitations and increase independence in daily activities [10].

Adaptive sports play a significant role in modern rehabilitation programs, and their effectiveness has been confirmed by numerous scientific studies [11]. It has been established that regular participation in adaptive sports positively affects the body's functional state, promotes the development of strength, endurance, and coordination, and improves balance and spatial orientation [12]. In addition, participation in sports activities helps improve physical health, expand social contacts, and foster a positive attitude toward one's own capabilities.

In recent years, team adaptive sports – which combine physical exertion with the need for constant interaction among participants – have attracted particular interest from researchers. It has been proven that participation in such activities helps reduce anxiety and depressive symptoms, boost self-esteem, increase self-confidence, and enhance motivation for further rehabilitation [13]. At the same time, the team-based training format creates favorable conditions for rebuilding social connections, developing communication skills, and successfully integrating people with amputations into society [14].

Among adaptive team sports, adaptive football (amputee football) is a promising tool for physical and sports rehabilitation. The nature of the game involves performing complex coordination tasks, moving quickly across the field, constantly monitoring the game situation, and making quick decisions. In this regard, adaptive football training sessions have the potential to influence not only participants' physical condition but also their cognitive domains, including attention, information-processing speed, spatial perception, and executive control [15, 16]. At the same time, the analysis of the scientific literature indicates that most studies focus on specific aspects of rehabilitation for individuals with amputations or on the impact of adaptive sports on physical fitness and psycho-emotional well-being. In contrast, the issue of a comprehensive assessment of the impact of adaptive football training sessions on indicators of health status, health-related quality of life, and cognitive domain in people with amputations has not been sufficiently addressed. This issue is particularly relevant in the current context of a growing number of people who have undergone amputations as a result of the Russian-Ukrainian war and who require effective means of long-term recovery and social adaptation. This highlights the need to study the impact of adaptive football training sessions on indicators of health-related quality of life, health status, and cognitive domain among individuals with amputations.

AIM

The aim is to investigate the impact of adaptive football training sessions on the indicators of health-related quality of life, health status, and the cognitive domain of individuals with amputations.

MATERIALS AND METHODS

PARTICIPANTS

The research, conducted between 2025 and 2026, included 32 service members and law enforcement officers who had suffered limb amputations as a result of blast and shrapnel injuries sustained during the war in Ukraine (2022-2025). All participants were male. Twen-

ty-six participants had lower-limb amputations, and six had upper-limb amputations. All research participants underwent rehabilitation at rehabilitation facilities in Kyiv. Inclusion criteria: the research included individuals with amputations who voluntarily expressed a desire to play amputee football to accelerate the restoration of physiological functions, improve their psychological well-being, and facilitate social adaptation. In addition, a mandatory condition for inclusion in the study group was approval from rehabilitation specialists and physicians, as well as the participants' clearance to participate in amputee football. The participants' age, education, occupation, and marital status were not taken into account. The volume of other motor activity types and the types of treatments participants received during rehabilitation were also not monitored. Exclusion criteria included a participant's desire to stop playing amputee football or a doctor's prohibition on continuing participation due to a deterioration in the participant's health.

The duration of the experiment was 6 months. The initial phase began in September 2025, and the final phase concluded in February 2026. Amputee football training sessions were held three times a week under the guidance of coaches and under the supervision of physical education and sports rehabilitation specialists at Dragomanov Ukrainian State University (Kyiv, Ukraine). Data processing and evaluation of the research results were carried out at the Department of Fitness and Adaptive Sports of Dragomanov Ukrainian State University.

MATERIALS AND METHODS

To achieve the set aim, a set of general scientific theoretical research methods was used. The analysis, systematization, generalization, and interpretation of scientific publications on the rehabilitation of people with amputations, adaptive physical education, and adaptive sports were conducted. The information base for the research consisted of scientific articles found in the international scientometric databases Scopus, Web of Science, and PubMed. The research process involved the bibliographic method, content analysis of scientific sources, comparative analysis, and the generalization of research results.

The research also involved empirical methods and mathematical statistics. Dynamics in health-related quality of life, health status, and cognitive domain indicators were assessed using the "SF-36 Health Survey" sociopsychological questionnaire, the "Number Searching" test, and the Bourdon-Anfimov cancellation test.

According to the method for assessing health-related quality of life and health status indicators, participants in the rehabilitation program were required to answer 36 questions on the "SF-36 Health Survey" questionnaire. The assessment was conducted by comparing the results obtained before and after the course of training sessions using adaptive football. The method does not specify normative or critical values. For each item, several response options are provided and scored proportionally on a scale from 0 to 100 points. A score of 0 points indicates the poorest health-related quality of life, while 100 points indicates the highest health-related quality of life. The results are presented across 8 scales, grouped into two categories that define physical and mental health components. The Physical Health (PH) component includes the following indicators: Physical Functioning (PF); Role-Physical Functioning (RP); Bodily Pain (BP); and General Health (GH). The Mental Health (MH) component includes the following indicators: Vitality (VT); Social Functioning (SF); Role-Emotional (RE); and Mental Health (MH) [17].

To assess the cognitive functioning of the research participants, the indicators "Attention Distribution and Span" and "Attention Concentration and Sustainability" were measured.

The "Attention Distribution and Span" indicator was assessed using the "Number Searching" test [18]. According to the test instructions, participants had to find and cross out on the answer sheet, as quickly as possible, the numbers missing from the main test sheet. The level of development of this aspect of attention was assessed using the number of correctly identified and crossed-out numbers on a 9-point scale. Depending on the result obtained, the following levels were identified: high – 8-9 points, sufficient – 6-7 points, satisfactory – 3-5 points, low – 1-2 points.

To determine the "Attention Concentration and Sustainability" indicator, the Bourdon-Anfimov cancellation test [19] was used. Scores were expressed as a percentage of the maximum possible result. Based on the obtained values, attention concentration and sustainability levels were interpreted as follows: low – less than 80 %, below average – 80-84 %, average – 85-89 %, above average – 90-94 %, and high – 95 % and above.

METHODS OF MATHEMATICAL STATISTICS

The statistical method was used to process the data obtained. The reliability of the difference was determined using the Student's t-test. The results were presented as Mean ± m, where Mean is the arithmetic mean, m is the standard error. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using STATISTICA 13.3.1 software package (2018), adapted for medical and biological research.

Table 1. Dynamics in health-related quality of life, health status, and cognitive domain indicators among individuals with amputations (n = 32)

Indicators	Research phases	Mean±m	t	p
Physical health, points				
Physical Functioning – PF	Initial phase	50.25±1.41	2.49	p≤0.05
	Final phase	55.16±1.38		
Role-Physical Functioning – RP	Initial phase	54.05±1.33	2.29	p≤0.05
	Final phase	58.15±1.20		
Bodily pain – BP	Initial phase	71.41±2.08	1.01	p≥0.05
	Final phase	74.30±1.95		
General Health – GH	Initial phase	64.69±1.05	2.35	p≤0.05
	Final phase	68.28±1.11		
Overall physical health component	Initial phase	60.10±1.38	2.33	p≤0.05
	Final phase	63.97±0.92		
Mental Health, points				
Vitality – VT	Initial phase	66.80±1.23	4.58	p≤0.001
	Final phase	73.47±0.78		
Social Functioning – SF	Initial phase	73.06±1.60	4.61	p≤0.01
	Final phase	82.03±1.11		
Role-Emotional – RE	Initial phase	72.05±1.30	2.97	p≤0.05
	Final phase	77.21±1.15		
Mental Health – MH	Initial phase	71.25±1.13	3.80	p≤0.01
	Final phase	75.94±0.50		
Overall mental health component	Initial phase	70.79±0.62	7.81	p≤0.001
	Final phase	77.16±0.53		
Cognitive domain indicators				
Attention Distribution and Span, points	Initial phase	5.92±0.24	2.79	p≤0.01
	Final phase	6.81±0.21		
Attention Concentration and Sustainability, %	Initial phase	87.68±0.82	2.56	p≤0.05
	Final phase	90.54±0.76		

Legend: Mean – arithmetic mean; m – error of arithmetic mean; n – number of female students; t – Student's test value; p – reliability value
Source: compiled by the authors of this study

COMPLIANCE WITH ETHICAL STANDARDS

The participants were informed of the research aim and voluntarily agreed to participate. The research results were reviewed and approved by the Academic Integrity Committee of the Dragomanov Ukrainian State University (Minutes No. 5 dated April 20, 2026).

FRAMEWORK

This study was carried according to the research plan of the Faculty of Physical Education, Sports and Health of the Dragomanov Ukrainian State University in 2023-2027 in accordance with the theme "Monitoring, control and evaluation of learning results in physical culture, the basics of healthy lifestyle" (state registration number 0113U009185).

AI STATEMENT

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

RESULTS

The results of the study on dynamics in health-related quality of life, health status, and cognitive domain indicators among individuals with amputations before and after a six-month course of adaptive football training sessions are presented in Table 1. The analysis of the SF-36 questionnaire results revealed positive changes in most of the indicators studied. In particular, a statistically significant increase in the overall physical health component indicator was observed, from 60.10 ± 1.38 to 63.97 ± 0.92 points ($p \leq 0.05$), corresponding to a 6.44 % increase.

Statistically significant positive changes were also observed in the physical functioning indicator. Its mean value increased from 50.25 ± 1.41 to 55.16 ± 1.38 points ($p \leq 0.05$), representing a 9.77 % increase. A similar trend was observed for role-physical functioning: the indicator increased from 54.05 ± 1.33 to 58.15 ± 1.20 points ($p \leq 0.05$), representing a 7.59% increase. Positive dynamics were also observed on the general health scale. The indicator rose from 64.69 ± 1.05 to 68.28 ± 1.11 points ($p \leq 0.05$), corresponding to an increase of 5.55 %. At the same time, the pain intensity indicator showed a trend toward improvement but did not reach statistical significance ($p \geq 0.05$).

The most significant changes were observed in the structure of the mental health component. Its composite indicator increased from 70.79 ± 0.62 to 77.16 ± 0.53 points ($p \leq 0.001$), representing an 8.71 % increase. The largest increase among all SF-36 indicators was observed on the social functioning scale. The indicator rose from 73.06 ± 1.60 to 82.03 ± 1.11 points ($p \leq 0.001$), corresponding to a 12.28 % increase. A high positive effect was also observed for the vitality indicator, which increased from 66.80 ± 1.23 to 73.47 ± 0.78 points ($p \leq 0.001$), representing a 9.99% increase. Self-assessed mental health improved significantly from 71.25 ± 1.13 to 75.94 ± 0.50 points ($p \leq 0.001$), representing a 6.58 % increase. The role-emotional functioning indicator, increased from 72.05 ± 1.30 to 77.21 ± 1.15 points ($p \leq 0.01$), corresponding to an increase of 7.16%.

The results of the cognitive domain assessment also showed positive changes. According to the "Number Searching" test, the "Attention Distribution and Span" indicator significantly improved from 5.92 ± 0.24 to 6.81 ± 0.21 points ($p \leq 0.01$). The relative increase was 15.03%, the highest among the cognitive indicators studied. At the same time, the level of development of this aspect of attention changed from satisfactory to sufficient.

According to the results of the Bourdon-Anfimov cancellation test, a statistically significant increase in the "Attention Concentration and Sustainability" indicator was observed, from 87.68 ± 0.82 % to 90.54 ± 0.76 % ($p \leq 0.05$). The relative increase was 3.26 %, accompanied by a shift from an average level of attention development to an above-average level.

Thus, six months of adaptive football training sessions resulted in statistically significant improvements in most physical and mental health indicators, as well as in cognitive aspects of attention, among individuals with amputations. The most significant positive changes were observed in the indicators of attention distribution and span (15.03 %), social functioning (12.28 %), vitality (9.99 %), and physical functioning (9.77 %), indicating the comprehensive impact of adaptive football on various components of the participants' health-related quality of life.

DISCUSSION

The results indicate a positive impact of six months of adaptive football training sessions on health-related quality of life, health status, and cognitive domain indicators among individuals with amputations. The most significant changes were observed in the mental components of health, which attests to the considerable rehabilitative potential of amputee football not only as a means of physical recovery but also as a tool for psychosocial adaptation.

Improvements in the overall indicator of physical health component, physical functioning, and role-physical functioning may be linked to the specific nature of amputee football training. Systematic practice of crutch-assisted locomotion, changing direction, accelerating, stopping, kicking the ball, and performing coordination exercises contributes to the development of muscle strength in the upper limbs, shoulder girdle, trunk, and the intact lower limb; improves the functional state of the cardiorespiratory system; and increases the level of motor independence [20]. It is precisely this improvement in the body's functional capabilities that likely underpinned the research participants' improved self-assessment of their health.

At the same time, the absence of statistically significant changes in pain intensity may be explained by the fact that post-amputation pain largely depends on individual characteristics of the injury, the presence of phantom pain, the condition of the residual limb, and the effectiveness of prosthetics. Thus, even amid positive changes in physical condition, the short-term impact of sports activities on pain sensations may be less pronounced.

Significant positive dynamics were observed in the social functioning indicator. This result is to be expected, as amputee football is a team sport in which performance depends largely on interaction among players, mutual support, and a sense of belonging to the team. Participation in training sessions and competitions helps expand social contacts, restore communication skills, and overcome the social isolation that often arises after limb loss [21].

Improvements in levels of vitality, mental health, and role-emotional functioning are likely linked to the comprehensive psychophysiological effects of training sessions. Regular motor activity helps normalize nervous system function, reduce psychological and emotional stress, and enhance subjective well-being. An additional factor is the achievement of athletic goals, increased confidence in one's own abilities, and the development of a positive outlook on life, which is particularly important for individuals who have experienced severe trauma and amputation [22, 23].

Positive changes in the cognitive domain are evidenced by significant improvements in indicators of attention distribution, span, concentration, and sustained attention. These results may be attributed to the high cognitive demands of amputee football. During the game, athletes must simultaneously monitor the ball's position, the movements of teammates and opponents, assess the game situation, and make quick decisions under time pressure. Such conditions stimulate the development of selective attention mechanisms, improve the ability to shift attention, and sustain it on relevant objects for extended periods. In addition, moderate, varied physical exercise positively affects cerebral blood flow, neurometabolic processes, and central nervous system function, which may also contribute to improved cognitive performance [24].

Overall, the research's results confirm that adaptive football has a comprehensive impact on the physical, mental, and cognitive domains of individuals with amputations. Its advantage lies in the combination of physical activity, elements of social interaction, and sustained cognitive engagement, which creates favorable conditions for improving participants' health-related quality of life and facilitating their successful social adaptation.

CONCLUSIONS

It was found that six months of adaptive football training sessions contributed to a statistically significant improvement in the physical and mental components of health among individuals with amputations. Positive dynamics were observed in indicators of physical functioning, role-physical functioning, and self-assessment of general health. The most significant positive changes were observed in the mental component of health, particularly in social functioning, vitality, and mental health indicators. At the same time, changes in the pain intensity indicator showed a positive trend but did not differ significantly.

The results of the cognitive domain assessment also demonstrated a positive impact of adaptive football training sessions, as evidenced by a statistically significant improvement in indicators of attention distribution and span, as well as concentration and sustained attention. The results obtained provide grounds for concluding that adaptive football has a comprehensive effect on the physical, psycho-emotional, and cognitive state of individuals with amputations and confirm the feasibility of using it as an effective means of physical and sports rehabilitation aimed at improving the health-related quality of life and functional capabilities of this group of individuals.

PROSPECTS FOR FURTHER RESEARCH

We see horizons for future research in the development and experimental testing of physical education and sports rehabilitation programs using adaptive football for individuals with amputations.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Prevalence of thyroid dysfunction in patients with acute atrial fibrillation attending coronary care unit at Al-Sadder Teaching Hospital

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ABSTRACT

Aim: Assessment of prevalence of thyroid dysfunction in patients with acute atrial fibrillation.

Materials and Methods: A total of 110 patients participated in this cross-sectional study, admitted to the coronary care unit with AF not more than 48 hours from symptom onset and investigated by TSH, T3, and T4 to show how prevalent thyroid dysfunction in patients with acute AF, a study done at Al-Sadr Medical City – Najaf Al-Ashraf city, Iraq.

Results: This study showed Prevalence of thyroid dysfunction was 21.7% which represents 24 patients that include (one patient with subclinical hypothyroidism 0.9%, 4 patients with subclinical hyperthyroidism 3.6%, 5 patients with hypothyroidism 4.5%, 14 patients with hyperthyroidism 12.7% and patients with euthyroid represent (N=86, 78.3%) of patients.

Conclusions: For patients who admitted to the coronary care unit with acute atrial fibrillation both hypothyroidism and hyperthyroidism were frequent with the latest exceeding the prevalence in the general population necessitating screening for hidden thyroid dysfunction for early diagnosis and management to reduce morbidity and mortality.

KEY WORDS: atrial fibrillation, cardiac arrhythmia, hyperthyroidism, acute atrial fibrillationx

Wiad Lek. 2026;79(9):2072-2081. doi: 10.36740/WLek/226172 DOI

INTRODUCTION

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia worldwide, affecting over 30 million people. Its prevalence and incidence continue to rise. AF markedly increases morbidity and mortality by elevating the risks of stroke, heart failure, and hospitalization [1-2]. Notably, AF raises stroke risk by approximately fivefold, and AF-related strokes tend to be more severe and deadly than strokes in patients without AF. Clinically, AF is characterized by an irregularly irregular ventricular rhythm and absent distinct P waves on ECG. The condition is categorized by episode duration and frequency, as outlined in the 2014 AHA/ACC/HRS guidelines on AF management [3]. Paroxysmal AF (self-terminating or intermittent): AF that stops on its own or with treatment within 7 days of onset; episodes may recur. Persistent AF: AF that does not terminate spontaneously within 7 days and typically requires pharmacologic or electrical cardioversion to restore sinus rhythm. Patients with persistent AF may later have paroxysmal episodes, reflecting disease progression.

Long-standing persistent AF: AF that has been continuous for more than 12 months.

Permanent AF: Persistent AF for which the patient and clinician have agreed not to pursue further rhythm-control attempts.

Acceptance of persistent AF may shift as symptoms, treatment options, and patient–clinician preferences change. Although AF often progresses from paroxysmal to persistent forms, individuals may experience both types at different times. Despite a slightly higher prevalence in men, AF-related complications tend to be more severe in women [4]. Numerous conditions predispose to AF, including but not limited to hypertension, obesity, metabolic syndrome, diabetes, obstructive sleep apnea, ischemic heart disease, heart failure, and thyroid disorders. The thyroid is a highly vascular, brownishred gland located anteriorly in the lower neck, extending roughly from C5 to T1. It typically has an H- or U-shaped configuration, composed of two elongated lateral lobes connected by an isthmus, with an average height of 12–15 mm overlying the second to fourth tracheal rings [5]. As a key endocrine organ, the thyroid secretes hormones that regulate growth,

development, and metabolism [6]. The wide-ranging (pleiotropic) effects of thyroid hormones on metabolic pathways produce diverse, often age- and sex-related clinical presentations [6–7]. Because signs and symptoms of thyroid dysfunction are frequently nonspecific, diagnosis relies primarily on biochemical testing. Thyroid disease ranges from subclinical to overt forms, with overt disease reflecting more pronounced hormonal disturbance [7–8]. Untreated thyroid dysfunction substantially impairs well-being and can cause significant morbidity. Hypothyroidism is linked to cognitive decline, hypertension, dyslipidemia, cardiovascular and cerebrovascular events, and, in severe cases, myxedema coma [7]. Subclinical hypothyroidism increases cardiovascular morbidity and mortality in a TSHdependent manner [9]. Subclinical hyperthyroidism and overt hyperthyroidism are associated with higher risks of atrial fibrillation, heart failure, dementia, bone loss, and fractures [10]. Globally, the most common causes of thyroid dysfunction are iodine deficiency in iodinedeficient regions and autoimmune thyroid disease in iodinereplete areas; notably, about onethird of the world's population still resides in iodinedeficient regions [11]. Reported prevalence of thyroid dysfunction among patients with AF varies widely (0–24%) [12–13], likely reflecting differences in study populations' ages [14–15], geographic regions [16], comorbidities and medications [17], and laboratory methods for measuring thyroid hormones and TSH [18–19]. In hyperthyroid patients, AF prevalence is reported at 10–15%, with an incidence of 1.41 per 1,000 patients [20–21]. The link between hypothyroidism and AF remains unclear, although hypothyroidism is associated with increased cardiovascular events. Large cohort studies indicate that even variations of thyroid hormones within the reference range are related to cardiac outcomes; specifically, higher free thyroxine (T4) is associated with greater risks of heart failure, sudden cardiac death, and AF [22–24]. Overall, the influence of thyroid hormone imbalance on AF risk may be under recognized [24].

Thyroid disease can affect cardiovascular physiology by increasing vascular resistance, cardiac contractility, heart rate, and left ventricular mass. Elevated thyroid hormone levels also promote arrhythmogenesis, increase atrial premature beats, and exert prothrombotic effects—mechanisms that help explain the association between hyperthyroidism and AF, though they do not fully clarify hypothyroidism's role, despite shared risk factors. Additionally, thyroid dysfunction may alter responses to anticoagulant therapy, and the safety and efficacy of standard anticoagulation in AF patients with thyroid disease are not fully defined [25]. These issues raise questions about the value of including free T4 in AF thyroid screening and about risks related to thyroid hormone replacement.

AIM

This study therefore aims to determine the prevalence of thyroid dysfunction among patients presenting with acute atrial fibrillation.

MATERIALS AND METHODS

In this cross-sectional study, 110 patients (male N=54 patient and female N=56 patient) has been admitted to the coronary care unit in al-Sadr medical city/ Najaf al-Ashraf City, Iraq during one-year period from 1 March 2022 to 1 March 2023. The data collecting during one-to-one interviews and all patients admitted to the coronary care unit has been admitted primarily to the emergency department, clinical and demographical data were obtained, these data include age, gender, residency, lifestyle, smoking state, alcohol state, BMI, Family history of thyroid disease, history of COVID 19 infection past medical history of (hypertension, heart failure, asthma, ischemic heart disease, COPD, stroke…etc.). Blood pressure measurement, electrocardiography (ECG), thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) levels have been assessed.

1. Our study focused on patients presenting with acute atrial fibrillation to the Coronary Care Unit. We excluded patients with chronic AF, valvar heart disease, and acute decompensated heart failure to avoid confounding and to isolate the relationship with thyroid dysfunction.
2. The study was conducted in one center, Al-Sader Teaching Hospital, and data collection was limited to a one-year period. This restricts the number of cases.
3. Acute AF requiring CCU admission is less frequent compared to other cardiac conditions. Therefore, accumulating a large number in a short period from a single center is difficult.
4. Despite the moderate sample size, we performed a power calculation before starting. A sample of 110 patients was statistically adequate to estimate the prevalence with an acceptable margin of error and to detect significant associations for our primary objective.

Larger multicenter study would improve generalizability, our sample size was appropriate for a preliminary prevalence study in our hospital.

ETHICAL APPROVAL

All patients consented as per ethical approval.

INCLUSION CRITERIA

Any patient newly diagnosed with acute atrial fibrillation not more than 48 hours from symptoms onset admitted to the coronary care unit.

Table 1. Distribution of patients with atrial fibrillation according to study variables (N=110)

Study variables	Number	[%]
Residence		
Urban	69	62.7%
Rural	41	37.3%
Total	110	100.0%
Lifestyle		
Active	68	61.8%
Sedentary	42	38.2%
Total	110	100.0%
Smoking		
Active smoker	10	9.1%
Passive smoker	35	31.8%
Ex-smoker	19	17.3%
Non smoker	46	41.8%
Total	110	100.0%
Alcohol consumption		
Yes	3	2.7%
No	107	97.3%
Total	110	100.0%

Source: Compiled by the authors of this study

Table 2. The means ± SD and range of systolic and diastolic blood pressure of patients with atrial fibrillation N=110

Blood pressure	N	Mean ± SD	Range
Systolic blood pressure (mmHg)	110	143.17 ± 12.25	(123.0-175.0)
Diastolic blood pressure (mmHg)	110	80.85 ± 7.65	(67.0 - 99.0)

Source: Compiled by the authors of this study

Table 3. The means ± SD and range of thyroid function test for patients with atrial fibrillation (N=110)

Thyroid function test	N	Mean ± SD	Range
T3 (nmol/l)	110	2.25 ± 0.84	(0.57-4.90)
T4 (nmol/l)	110	122.01 ± 38.46	(45.23 - 201.70)
TSH (µIU/ml)	110	2.79 ± 2.38	(0.08-12.90)

Source: Compiled by the authors of this study

EXCLUSION CRITERIA

- Patients who those already been diagnosed with chronic AF
- Patients with a history of malignancy on chemotherapy
- Patients with a history of radiotherapy
- Patients with sepsis and shock
- Patients with diagnosed thyroid disease

BLOOD SAMPLING

The thyroid function test is done by Roche Cobas e411 automated machine which uses the Electrochemoluminescence immunoassay method.

- Serum Thyroid stimulating hormone (TSH) has been taken by a venous sample on the same day of admission to the coronary care unit, and the normal range was used (0.40-5.0) µIU/mL.
- Serum total Triiodothyronine (T3) concentration has been taken by the venous sample during admission day, and the normal range was used (1.2-3.1) nmol/L.
- Serum total Thyroxine (T4) concentration has been taken by the venous sample during admission day, and the normal range used (66-181) nmol/L.

Thyroid dysfunction abnormalities were classified according to the above tests below:

- Overt hyperthyroidism TSH level <0.1mIU/L or undetectable with elevated T4 or T3.
- Overt hypothyroidism TSH level >4.5 mIU/L with low T4.
- Subclinical hyperthyroidism TSH level <0.1mIU/l or(0.1-0.4) mIU/L with normal T3 and T4
- Subclinical hypothyroidism TSH level >4.5mIU/L with normal T4 [26].

DATA ANALYSIS

Statistical analysis was carried out using SPSS version 27. Continuous variables are presented as mean, standard deviation, and range. Categorical variables are presented

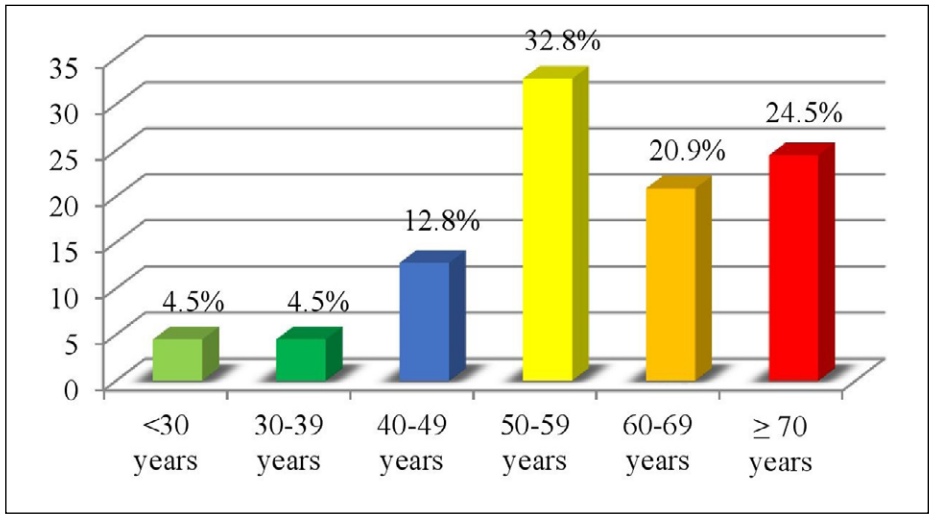


Fig. 1. Distribution of patients with atrial fibrillation according to age (years) (N=110)
Source: Own materials

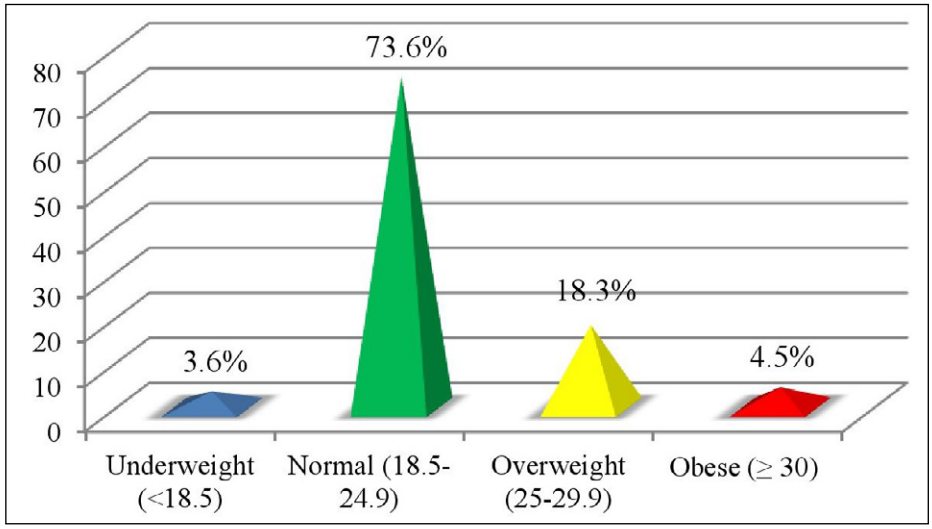


Fig. 2. Distribution of patients with atrial fibrillation according to BMI (kg/m^2) (N=110)
Source: Own materials

as frequencies and percentages. ANOVA test was used to compare means among 3 groups. Fisher's exact test was used to find the association between categorical variables. A p-value of ≤ 0.05 was considered significant.

RESULTS

In this study, 110 patients were enrolled all with confirmed atrial fibrillation by electrocardiography not more than 48 hours from symptom onset, distribution of patients with atrial fibrillation according to age including (<30 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, and ≥ 70 years). Age <30 years represents only five patients 4.5%, age of 30-39 years represents only five patients 4.5%, age of 40-49 years represents N=14, 12.8%, age of 50-59 years represent N=36, 32.8%, age of 60-69 years represent N=23, 20.9% and age of ≥ 70 years represent N=27, 24.5% of patients. The mean age of patients was 57.82 ± 13.03 with an older patient was 79 years and a younger patient was 27 years (Fig. 1). The patients' BMI was also considered (Fig. 2).

Table 1 shows the distribution of patients with atrial fibrillation according to study variables including (residence, lifestyle, smoking habit, and alcohol consumption). Patients from urban areas represent N=69, 62.7% and patients from rural areas represent N=41, 37.3%. Patients with active lifestyles represent N=68, 61.8% and patients with sedentary lifestyles represent N=42, 38.2%. Active Smokers represent only ten patients 9.1% and only three patients 2.7% had a history of Alcohol use (two of them were ex-alcoholic and one still alcoholic).

Figure 3 shows the distribution of patients with atrial fibrillation according to the history of COVID-19 infection including (positive and negative). Patients with a positive history of COVID-19 infection represent N=74, 67.3% and patients with negative family history represent N=36, 32.7% of patients.

Figure 4 shows the distribution of patients with atrial fibrillation according to past medical history including (positive and negative). Patients with positive past medical history represent N=85, 77.3% and patients with negative past medical history represent N=25, 22.7% of patients.

In table 2 means, standard deviations, and range of systolic and diastolic blood pressure of patients with

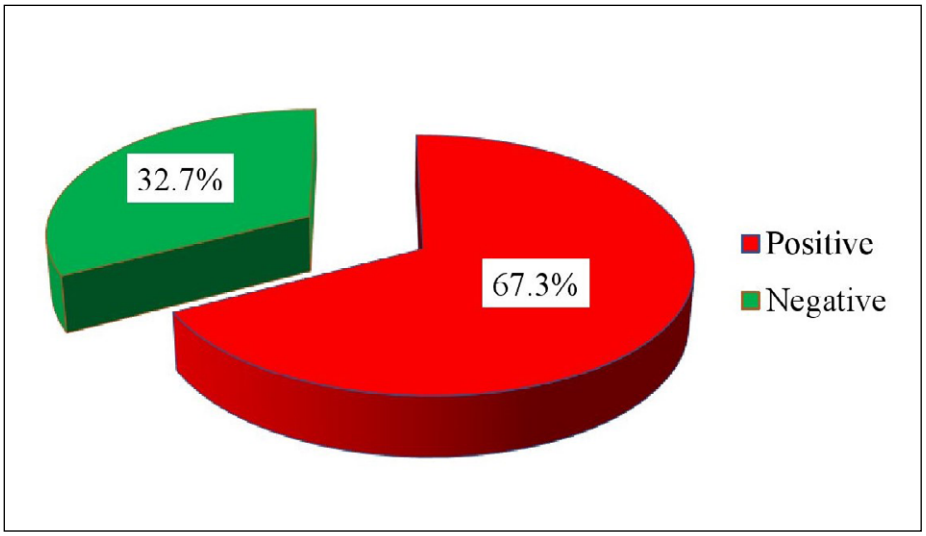


Fig. 3. Distribution of patients with atrial fibrillation according to the history of COVID-19 infection N=110
Source: Own materials

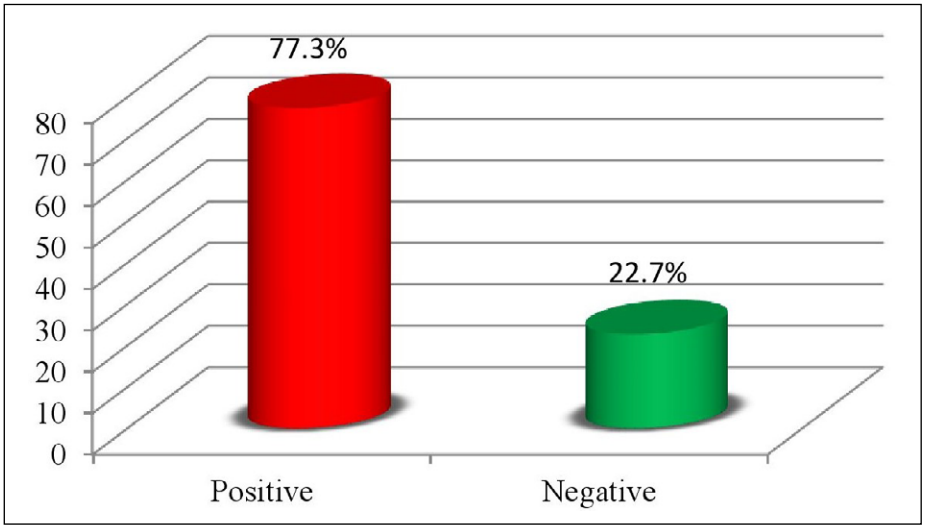


Fig. 4. Distribution of patients with atrial fibrillation according to past medical history (N=110)
Source: Own materials

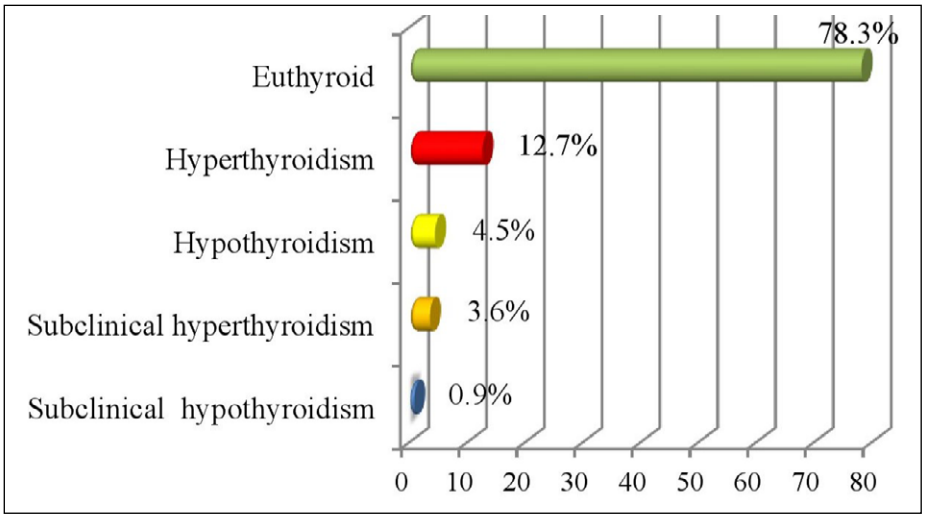


Fig. 5. Distribution of patients with atrial fibrillation according to thyroid function test (N=110)
Source: Own materials

atrial fibrillation. The mean systolic blood pressure was (143.17 ± 12.25) mmHg and the mean diastolic blood pressure was (80.85 ± 7.65) mmHg.

In table 3 the means, standard deviations, and range of thyroid function test including T3 (nmol/l), T4 (nmol/l), and TSH (μIU/ml) for patients with atrial fibrillation. Mean T3 was 2.25 ± 0.84 nmol/l and mean T4 was 122.01 ± 38.46 nmol/l and mean TSH was 2.79 ± 2.38 μIU/ml.

In table 4 the mean differences of study variables including age (years) and body mass index kg/m^2 according to thyroid function test including (hypothyroidism, hyperthyroidism, and Euthyroid status). There were no

Table 4. The mean differences of study variables according to the thyroid function test (N=110)

Study variables	Thyroid function test	N	Mean ± SD	F	P-value
Age (years)	Hypothyroidism	6	58.67 ± 16.08	0.356	0.702
	Hyperthyroidism	18	55.44 ± 13.19		
	Euthyroid	86	58.26 ± 12.90		
Body mass index (kg/m^2)	Hypothyroidism	6	25.46 ± 3.80	1.273	0.284
	Hyperthyroidism	18	23.16 ± 3.65		
	Euthyroid	86	23.62 ± 2.89		

Source: Compiled by the authors of this study

Table 5. Association between thyroid function test and study variables (N=110)

Study variables	Thyroid function test			Total (N=110) n(%)	P-value
	Hypothyroidism (N=6)	Hyperthyroidism (N=18)	Euthyroid (N=86)		
Sex					
Female	4 (66.7)	10 (55.6)	42 (48.8)	56(50.9)	0.724
Male	2 (33.3)	8 (44.4)	44 (51.2)	54(49.1)	
Total	6 (100.0)	18 (100.0)	86 (100.0)	110(100)	
Residence					
Urban	2 (33.3)	13 (72.2)	54 (62.8)	69 (62.7)	0.25
Rural	4 (66.7)	5 (27.8)	32 (37.2)	41 (37.3)	
Total	6 (100.0)	18 (100.0)	86 (100.0)	110(100.0)	
Lifestyle					
Active	3 (50.0)	14 (77.8)	51 (59.3)	68 (61.8)	0.276
Sedentary	3 (50.0)	4 (22.2)	35 (40.7)	42 (38.2)	
Total	6 (100.0)	18 (100.0)	86 (100.0)	110 (100.0)	
Smoking					
Active smoker	1 (16.7)	2 (11.1)	7 (8.1)	10 (9.1)	0.442
Passive smoker	4 (66.7)	4 (22.2)	27 (31.4)	35 (31.8)	
Ex-smoker	0 (0.0)	3 (16.7)	16 (18.6)	19 (17.3)	
Non-smoker	1 (16.7)	9 (50.0)	36 (41.9)	46 (41.8)	
Total	6 (100.0)	18 (100.0)	86 (100.0)	110 (100.0)	
Alcohol consumption					
Yes	0 (0.0)	0 (0.0)	3 (3.5)	3 (2.7)	1.000
No	6 (100.0)	18 (100.0)	83 (96.5)	107 (97.3)	
Total	6 (100.0)	18 (100.0)	86 (100.0)	110 (100.0)	

Source: Compiled by the authors of this study

significant differences between means of age and body mass index according to the thyroid function test.

The table 5 shows association between thyroid function test including (hypothyroidism, hyperthyroidism and Euthyroid status) and study variables including (sex, residence, lifestyle, smoking habit and alcohol consumption). There was no significant association between thyroid function test and study variables.

DISCUSSION

Thyroid disease burden in patients with atrial fibrillation can be predicted from the systemic effects of thyroid hormones that contribute to increased vascular resistance, cardiac contractility, heart rate, and left ventricular mass [1]. In this study, hyperthyroidism was 16.3%which was more prevalent than the general population which is estimated to be 0.2% to 1.3% in iodine-sufficient parts of the world [27-28], compared to another study done by Auer J et al showed that the prevalence of hyperthyroidism in AF was 13.8% [29], another study found that 8.3% developed new AF or flutter within 30 days of diagnosis of hyperthyroidism [30], whereas study found that AF in hyperthyroidism patients around 10-25% [31], while the prevalence of hypothyroidism in this study was 5.4%for both overt and subclinical hypothyroidism, The prevalence of overt hypothyroidism in the general population ranges from between 0.2% and 5.3% in Europe and 0.3% and 3.7% in the USA [32-34] which is comparable to the results of the current study. Another study revealed that

hypothyroidism is associated with decreased risk of AF [35], these results are compatible with a study done in Brazil by Barbisan, Juarez Neuhaus et al., [36], which found a prevalence of hyperthyroidism 6.9% and hypothyroidism 5.6% in AF patients. Among patients with hyperthyroidism both overt and Subclinical, 10 patients were female vs. 8 male patients, while in the hypothyroidism arm, 4 were female vs. 2 male patients .this female predominance may be explained by the observation that the prevalence of both conditions more in females. This may be explained by more autoimmune diseases and the role of sex hormones in females [37]. In this study percentage of patients with af increased with increasing age with most age group affected being (50-59) years, Mean age of patients was (57.82 ± 13.03) this age relationship was documented in many studies as noted in the ATRIA study [38], number of patients according to gender is about equal in this study. In our study, no significant association was found between thyroid dysfunction and systolic, diastolic measurements of blood pressure whereas a study done by Dernellis et al found an increase in diastolic blood pressure in hypothyroidism patients which is reversible in 50% of patients with thyroxine treatment [39], on the other hand, a study conducted by Marcisz et al showed that systolic blood pressure higher than the healthy control which normalized after a short treatment with anti-thyroid medication while diastolic blood pressure was lower [40]. Patients with hypothyroidism had a mean BMI of 25.46 which is overweight in contrast to hyperthyroidism patients mean BMI of 23.16 within the normal range for BMI but without significant p-value for both conditions and BMI .same findings were in a study published in India [41]. In our study, no significant association was found among lifestyle, smoking, alcohol consumption, and thyroid dysfunction.in contrast to this, a study done in China found that smoking, alcohol consumption, and exercise were associated with decreased incidence of hypothyroidism, alcohol consumption was associated with decreased probability of hyperthyroidism, hypothyroidism, as well as positive TPOAb, The amounts of cigarettes smoked daily displayed a positive correlation with hyperthyroidism among smokers, Accordingly, smoking seemed to be associated with decreased risk for hypothyroidism and positive TPOAb. This study was done on a minor ethnic group in China [42]. Around 67.3% of patients had a history of (SARS-CoV-2) of them 6 were hypothyroid and 12 were hyperthyroid with no significant p-value found, in a study published in 2021 found around 13.1% of patients with COVID 19 had thyroid dysfunction but these findings were transient and tested during hospitalization for COVID infection [43]. Another study done on severe COVID 19 patients show mild transient thyroiditis and areas of focal hypoechogenicity on thyroid ultrasound with no residual abnormalities on long term follow up [44]. In our study 25.5% had a past medical history of thyroid disease, one patient was hypothyroid and six had hyperthyroidism with no significant p-value found, compared to this study a multicenter cohort study showed different results with half of the patients of both hypothyroidism and Graves' disease had family history of thyroid disease [45]. This may be explained by the large study sample used and different populations also the lack of data systems in our hospitals. In Our study, although hypertension, Dm, IHD, and CVA were frequent no significant relationship between past medical history and thyroid dysfunction regardless of the type of the disease the patient had was found, contrary to this a study done by Nearmeen M. Rashad et al found that hypertension was significantly prevalent in hypothyroid patients compared to hyperthyroid patients and DM especially type 2 where significant throughout the spectrum of thyroid dysfunctions encountered [46].

LIMITATIONS OF THE STUDY

1. Single-Center Design was conducted only at Al-Sader Teaching Hospital. Therefore, results may not be generalizable.
2. Cross-Sectional Study can determine prevalence but cannot establish a causal relationship between thyroid dysfunction and acute atrial fibrillation.
3. Sample Size, sample of 110 patients is considered moderate. A larger sample is needed to detect smaller differences between subgroups.
4. Patients were not followed after discharge from CCU. So the effect of treating thyroid dysfunction on AF recurrence could not be assessed.

CONCLUSIONS

High prevalence of thyroid function abnormalities was common among patients with acute atrial fibrillation especially hyperthyroidism exceeding that of the general population with the female being more affected in this study. Although many comorbidities were frequent, the same as other patients' variables, No significant associations were present between thyroid dysfunction and these variables.

RECOMMENDATIONS FOR FUTURE RESEARCHES

1. Multicenter study, a multicenter study across different governorates in Iraq to increase sample size and generalizability.
2. Longitudinal cohort design, a prospective cohort study with long-term follow-up is recommended

to evaluate the causal relationship and the impact of thyroid treatment on AF outcomes.

3. Screening protocol: screening for thyroid function tests in all patients admitted with acute atrial fibrillation to CCU is recommended for early detection and management.
4. Larger sample size needed to evaluate the difference between hyperthyroidism and hypothyroidism as risk factors for AF.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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ORIGINAL ARTICLE

Adrenal hormone and histamine levels in children with vasomotor rhinitis

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ABSTRACT

Aim: To study the levels of adrenal hormones and histamine in children with vasomotor rhinitis.

Materials and Methods: Hormones secreted by the adrenal glands are active biological regulators of metabolism in the body. Research into the hormonal function of the adrenal glands is of great importance for studying the pathogenesis of certain allergic diseases. Spectrofluorometric blood examination for the presence of 11-OCS (sum total content of hydrocortisone and corticosterone) and histamine, and also examina¬tion of the urine for adrenaline and noradrenaline content were conducted in children suffering from vasomotor rhinitis.

Results: The results obtained were statistically proces¬ses and considered from the aspect of dependence on the form of vasomotor rhinitis (allergic and nonallergic rhinitis) and age. Apparently healthy children (30) were examined biochemically for control. An increased content of the adrenal gland hor¬mones (11-OCS) was found to be present in the blood of children with vasomotor rhinitis. Histamine content was elevated only in children with an allergic rhinitis. Adrenaline and noradrenaline excretion increased with the advance of age, but was unchanged in rhinitis.

Conclusions: Consequently, in this disease there was a functional reaction of the adrenal cortex, but no functional changes occurred in the medulla. An increa¬sed histamine content confirmed the allergic nature of the disease.

KEYWORDS: teenagers, primary school children, vasomotor rhinitis, adrenal hormone, histamine levels

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INTRODUCTION

Vasomotor rhinitis is characterised by diverse aetiology and complex pathogenesis that has not been sufficiently studied. Currently, clinicians distinguish between two forms of vasomotor rhinitis: allergic and neurovegetative in adults (Alan P. Baptist, Sharmilee Nyenhuis) [1] and allergic and non-allergic in children (Karabaev, H., Nurmukhamedova, F., Egamberdieva, Z., & Hamdamov, Z.) [2]. Despite the similarity of the clinical manifestations of these forms, their aetiology and pathogenesis are different. The first, i.e. allergic, form is considered a manifestation of general allergy, and the second – mainly as a manifestation of nervous system dysfunction. Both forms are associated with endocrine system dysfunction. Since the hormones secreted by the adrenal glands are active biological regulators of metabolism in the body, the study of adrenal gland hormone function is of great importance for understanding the pathogenesis of many diseases, including allergic diseases.

The adrenal glands consist of two anatomically distinct layers: the cortex and the medulla. The adrenal cortex produces a number of steroid compounds, of which three hormones have the greatest biological activity: cortisol (hydrocortisone), corticosterone and aldosterone. The medulla of the adrenal glands secretes two hormones: adrenaline and noradrenaline. Therefore, in order to assess the hormonal function of the adrenal glands, it is necessary to determine the content of hormones secreted by each of the adrenal glands. However, the literature more often contains studies in which the authors limit themselves to investigating the hormones secreted by a single part of the adrenal glands.

AIM

The aim of this article is to study the levels of adrenal hormones and histamine in children with vasomotor rhinitis.

MATERIALS AND METHODS

In elderly people with vasomotor rhinitis, the functional state of the adrenal cortex was studied in 2016 by Alan P. Baptist and Sharmilee Nyenhuis by determining the daily excretion of 17-OCS. The scientists found that patients with allergic vasomotor rhinitis have hidden adrenal cortex insufficiency: the excretion of corticosteroid hormones in urine is within normal limits, but under stress (single administration of ACTH), their amount either does not change or increases to a much lesser extent than in middle-aged people. Based on this, they concluded that there is a decrease in the reserve capacity of the adrenal cortex in the allergic form of vasomotor rhinitis. Alan P. Baptist and Sharmilee Nyenhuis studied the total content of hydrocortisone and corticosterone (11-OCS) in the blood plasma of adults with vasomotor rhinitis and found an increased content compared to similar indicators in practically healthy people [1]. Alekseeva, L., Bessonova, T. et al. study the level of cortisol and laboratory indicators of systemic inflammation in children with various variants of BPM and VE course depending on the period of the disease (acute period, reconvalescence) to specify their role in the pathogenesis of acute neuroinfections [3]. Scientists investigated hematological indicators, the level of cortisol, C-reactive protein in blood serum of 60 children, 39 of them had BPM and 21 ones – VE. They identified characteristic features of cortisol content in children with bacterial and viral neuroinfections depending on the course of the disease.

Andrew A Crawford, Stefan Soderberg, Clemens Kirschbaum et al. investigated whether cortisol is a causal risk factor for cardiovascular disease and ischaemic heart disease in the general population [4].

The hormonal function of the adrenal medulla has also attracted the attention of researchers. Mary Beth Bauer and Kevin P.M. Currie noted reduced excretion of adrenaline and noradrenaline in children with whooping cough, especially in the acute phase of the disease [5].

Guntinas-Lichius O, Geißler K, Mäkitie AA et al. have provided practical recommendations for the diagnosis and treatment of recurrent acute tonsillitis, including informed decision-making regarding tonsillectomy [6]. Scientists have concluded that there was a decrease in the excretion of adrenaline and noradrenaline in children aged 3 to 15 years with chronic tonsillitis. The same feature was also found in patients with rheumatism.

The role of histamine in the pathogenesis of allergic diseases in adults and children is now well known. Thus, in bronchial asthma in adults and children, as well as in respiratory allergies in childhood, many authors note an increase in the content of histamine in the blood (Yamauchi, K., & Ogasawara, M.; Thangam, E. B., Jemima, E. A., Singh, H. et al.; Abaturov, A., Krivusha, E., Agafonova, E. et al. ; Burlutskaia A.V., Firsova V.N.) [7].

In children with rheumatoid arthritis (also an allergic disease), the level of histamine in the blood exceeds the norm (Kasapçopur, Ö., & Barut, K.) [8]. At the same time, there is no data in the literature on the content of histamine in the blood of children with vasomotor rhinitis. As for adults, allergic rhinosinusopathy (Masood, A., Moumoulidis, I., & Panesar, J.) [9] and the allergic form of vasomotor rhinitis (Leader P, Geiger Z.) [10], an increase in blood histamine levels has been established with normal histamine content in patients with the neurovegetative form of rhinitis.

ETHICS

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

AI STATEMENT

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

RESULTS

Based on the data presented in the literature, we set ourselves the task of conducting a biochemical examination of children with allergic and non-allergic rhinitis in order to determine the total content of hydrocortisone and corticosterone (11- OCS) in blood plasma, histamine content in blood, and adrenaline and noradrenaline excretion in daily urine depending on the form of rhinitis and the age of the sick children.

The histamine content in whole blood was determined using a modern, highly sensitive and specific spectrofluorimetric method developed by A. Alqahtani, T. Alqahtani & A. H. Abdelazim [11].The content of 11- OCS in blood plasma was determined using the spectrofluorimetric method Zeidler, R., Biemann, R., Ceglarek, U., Kratzsch, J., Isermann, B., & Gaudl, A. [12]. The content of adrenaline and noradrenaline in daily urine was examined using the method developed by Thomas, A., Geyer, H., Mester, H.J. et al. [13]. Hormone standards were obtained from the V.P. Komissarenko Institute of Endocrinology and Metabolismof the National Academy of Medical Sciences of Ukraine. When performing analyses in all determinations, the fluorescence of solutions was measured using a Hitachi F-7000 Fluorescence Spectrophotometer.

We monitored 92 children aged 5 to 18 years with vasomotor rhinitis. Working with children required

Table 1. Adrenal hormone levels in blood and urine in children with various forms of vasomotor rhinitis

Form of rhinitis	11-OCS, µg %				Adrenaline, µg/day				Noradrenaline µg/day			
	number of people surveyed	M	σ	m	number of people surveyed	M	σ	m	number of people surveyed	M	σ	m
Allergic	46	19.75	±7.37	1.08	53	5.41	±3.81	0.52	53	16.72	±10.1	1.38
Non-allergic	21	17.54	±8.98	1.96	27	5.84	±3.36	0.64	27	21.10	±10.08	1.9
Control group	17	14.6	±4.77	1.15	30	6.92	±3.66	0.66	30	16.93	±10.1	1.8

Source: compiled by the authors of this study

Table 2. Adrenal hormone levels in blood plasma and urine in children with vasomotor rhinitis and in healthy children, depending on age

	Age	11-OCS,µg %				Adrenaline, µg/day				Noradrenaline µg/day			
		number of people surveyed	M	σ	m	number of people surveyed	M	σ	m	number of people surveyed	M	σ	m
Patients with vasomotor rhinitis	From 5 to 10	20	19.7	±7.67	1.72	35	5.01	±3.47	0.59	34	9.09	±4.73	0.81
	From 11 years old and above	47	18.78	±8.08	1.18	45	7.18	±4.18	0.62	45	19.95	±9.22	1.37
Healthy children (control group)	From 5 to 10					18	6.42	±4.38	1.03	18	12.80	±8.36	1.97
	From 11 years old and above	17	14.6	±4.77	1.18	12	7.95	±3.13	0.90	12	21.9	±9.79	2.82

Source: compiled by the authors of this study

Table 3. Histamine levels in the blood of children with different forms of vasomotor rhinitis

Group of children examined	Number of children examined	Average statistical indicator		Limits of fluctuations in individual indicators
		M ± m (µg %,)	P[1]	
Patients with allergic rhinitis	41	11.0 ± 0.6	< 0.001	5.4 – 22.1
Patients with non-allergic rhinitis	15	7.6 ± 0.4	> 0.02	5.4 – 9.4
Healthy children (control group)	20	7.0 ± 0.4		4.1 – 10.3

[1] The determination was made in comparison with data from the control group.
Source: compiled by the authors of this study

skilled pedagogical support and tact. Healthy children underwent only a daily urine test for adrenaline and noradrenaline content. Seventeen practically healthy children were examined as a control group of adults to determine the content of 11- OCS. The results of biochemical examination of children with various forms of vasomotor rhinitis are presented in Table 1 and Table 2.

Forty-six patients with allergic rhinitis and 21 patients with non-allergic rhinitis were examined. Table 1 shows that the 11- OCS content in the blood plasma of children with allergic rhinitis is significantly increased (by an average of 35%) compared to the data obtained in the control group. In patients with non-allergic rhinitis, a slight increase (by an average of 20%) in 11-OCS levels was also observed, but it was not statistically significant. A significant increase in the content of corticosteroid hormones in children with the allergic form of vasomotor rhinitis apparently indicates high reactivity and lability of the adrenal cortex function in this group. No significant difference was found in the 11-OCS content in the blood plasma of patients with one form of rhinitis compared to the data in the group of patients with another form of rhinitis. No such difference was found in the examination of adult patients

with vasomotor rhinitis. This is apparently due to the common pathogenesis of both forms of this disease. Table 1 shows the results of daily urine analysis for adrenaline and noradrenaline content. Urine analysis was performed in 53 children with allergic rhinitis and 27 patients with non-allergic rhinitis. From the average data presented in the table, it can be concluded that the excretion of adrenaline and noradrenaline in sick children is reduced, but insignificantly, and there is no difference between the two forms of the disease in this respect.

There are reports in the literature (Tian Xiaoyi, Liu Xiaoyan, Wang Yan, Liu Ying, Ma Jie, Sun Haidan, Li Jing, Tang Xiaoyue, Guo Zhengguang, Sun Wei, Zhang Jishui, Song Wenqi) [14]on age-related changes in catecholamine excretion, so we compared control and experimental groups of children with vasomotor rhinitis of the same age. The results are presented in Table 2.

The study included children in two age groups: 5 to 10 years old and 11 to 18 years old. The control group of practically healthy children was divided into the same age groups. When comparing these groups, no age-related changes in 11- OCS content were found, which is consistent with the data of Adriaansen, B. P. H., Oude Alink, S. E., Swinkels, D. W., Schröder, M. A. M., Span, P. N., Sweep, F. C. G. J., Claahsen-van der Grinten, H. L., & van Herwaarden, A. E. [15]. However, in both age groups of children, an increase in 11- OCS content was found compared to the data in the control group. We found the same feature in the blood plasma of adults with vasomotor rhinitis (Meltzer E. O.) [16].

Table 2 shows that there is a significant difference in the excretion of adrenaline and noradrenaline depending on age. In the older group, the excretion of these hormones is higher. This pattern has been identified in both healthy and sick children. However, there is no difference in the excretion of these hormones when comparing the same age groups of sick and healthy children. Therefore, it can be concluded that the adrenal medulla plays an insignificant role in the pathogenesis of vasomotor rhinitis in children, but the excretion of adrenaline and noradrenaline is still slightly reduced.

The histamine content in the blood was studied in 56 patients with vasomotor rhinitis aged 3 to 15 years. There were 27 girls and 29 boys. Forty-one children were diagnosed with allergic rhinitis, and 15 with non-allergic rhinitis. The control group consisted of 20 healthy children. The average blood histamine level in healthy children was 7.0 ± 0.4 μg% (individual variation range 4.1 – 10.3 μg%). The results of the study are presented in Table 3.

Children with allergic rhinitis have significantly elevated histamine levels in their blood — 11.0 ±0.6 μg% (i.e., 57% above normal and 45% higher than in patients with non-allergic rhinitis). At the same time, the range of individual blood histamine levels is very wide (5.4–22.1), and the upper limit is twice that of healthy children (4.1–10.3). In the group of patients with non-allergic rhinitis, the average blood histamine level is 7.6 ± 0.4 μg%, i.e. close to normal. The range of individual indicators is also similar to that in the control group. The difference in blood histamine levels in children with allergic and non-allergic forms of rhinitis is significant (P<0.001).

Thus, children with allergic rhinitis have higher blood histamine levels than healthy children and children with non-allergic rhinitis. Consequently, histamine levels play a role in the development of allergic rhinitis in children, and measuring histamine levels in the blood, along with other diagnostic tests, can help identify allergies.

DISCUSSION

In this study, we investigated the modulatory effects of targeted metabolic inhibition on tumor microenvironment (TME) acidification and immune cell infiltration in murine models of melanoma. Our primary findings demonstrate that dual blockade of lactate dehydrogenase A (LDHA) and monocarboxylate transporter 4 (MCT4) significantly reduces extracellular lactate accumulation within the tumor core, elevating interstitial pH from an acidic baseline of pH 6.5 ± 0,1 to pH 7.1± 0,2. Concurrently, this metabolic normalization correlates with a robust restoration of effector T-cell proliferation and a marked reduction in the suppressive activity of tumor-associated macrophages (TAMs). These results provide compelling in vivo evidence that reversing metabolic immunosuppression can reinvigorate anti-tumor immunity without exacerbating systemic toxicity.

Our findings align with and extend a growing body of literature highlighting the profound impact of cancer cell metabolism on immune evasion. While prior studies have predominantly focused on single-target inhibition – such as targeting LDHA alone or blocking glucose uptake via GLUT1 inhibitors – our data reveal the necessity of a combinatorial approach targeting both production and efflux mechanisms.

The observed elevation in interstitial pH corroborates the biophysical models proposed in foundational studies, which posited that metabolic symbiosis between glycolytic and oxidative tumor compartments drives localized acidosis. By interrupting lactate export via MCT4, we effectively disrupt this symbiotic axis, preventing the creation of the immunologically hostile "lactate shield" that typically neutralizes cytotoxic T

lymphocyte (CTL) effector functions. Furthermore, our observation of reduced programmed death-ligand 1 (PD-L1) expression on tumor cells following metabolic intervention mirrors recent in vitro findings, suggesting a direct transcriptional link between intracellular metabolic flux and immune checkpoint regulation.

The mechanistic underpinnings of the restored anti-tumor immune response build directly upon established biophysical and molecular principles. Specifically, the alleviation of extracellular acidification relieves the proton-mediated inhibition of nuclear factor of activated T cells (NFAT) signaling, a crucial pathway for interleukin-2 (IL-2) production and T-cell activation. Second, depleting the microenvironment of excess extracellular lactate starves regulatory T cells (Tregs) and M2-like macrophages of their preferred oxidative fuel source, thereby diminishing their metabolic fitness while paradoxically sparing effector T cells that can adapt via enhanced glycolytic plasticity upon pH normalization.

Interestingly, our single-cell RNA sequencing data point toward an upregulation of chemokine receptors (such as CXCR3) on CD8+ T cells following treatment, implying that metabolic reprogramming – when shifted away from classic glycolytic dominance – not only removes functional blocks but actively promotes immune cell recruitment into previously cold tumor cores.

Despite these promising insights, several limitations must be acknowledged based on the experimental boundaries defined by our current methodology and prior frameworks:

Murine Models: The use of syngeneic murine melanoma models, while standard and robust for immunological assays, may not fully recapitulate the dense, heterogeneous stroma and complex human solid tumor architecture as influenced by established tumor-stroma biophysical dynamics.

Compensatory Pathways: Although dual LDHA/MCT4 inhibition proved effective, prolonged treatment regimens may induce metabolic escape mechanisms, such as an increased reliance on alternative pathways like glutaminolysis or fatty acid oxidation, which are not completely blocked by single- or dual-node metabolic targeting.

Systemic Delivery: Delivery of the combined inhibitors relied on targeted liposomal formulations; translating these delivery systems to human clinical trials requires overcoming significant pharmacokinetic and biodistribution hurdles common to metabolic inhibitors.

Building upon these findings, future research should focus on evaluating the synergistic potential of combining LDHA/MCT4 blockade with established immune checkpoint inhibitors, particularly anti-PD-1/PD-L1 therapies. Given that metabolic resistance often drives primary or acquired resistance to immunotherapy, metabolic normalization could serve as a powerful priming strategy to convert non-responsive tumors into immunotherapy-sensitive phenotypes.

Clinical translation will necessitate the development of non-invasive imaging techniques – such as hyperpolarized carbon-13 magnetic resonance spectroscopy – to monitor real-time changes in tumor pH and lactate flux in patients undergoing metabolic therapy. Ultimately, integrating metabolic modulation into precision oncology frameworks holds significant promise for improving durable clinical outcomes in patients with refractory malignancies.

CONCLUSIONS

1. Elevated levels of corticosteroid hormones (11-OCS) have been found in the blood of children with vasomotor rhinitis, particularly in cases of allergic rhinitis, indicating increased functional activity of the adrenal cortex.
2. The histamine content in the blood of children with allergic rhinitis is significantly elevated, confirming the allergic nature of this disease.
3. The excretion of hormones from the adrenal medulla (adrenaline and noradrenaline) increases with age in children, but does not change in rhinitis.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Socioeconomic predictors of maternal coping during pediatric hospitalization

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ABSTRACT

Aim: The objective of the study was to establish the most important socioeconomic and clinical predictors of coping mechanisms among mothers when their child was admitted to the hospital in Iraq.

Materials and Methods: The study was a descriptive and cross-sectional study carried out in the pediatric wards of Basrah between September 2025 and January 2026. One hundred mothers were recruited. The Sociodemographic/clinical sheet was used to collect data in the form of a Sociodemographic/clinical sheet and the Arabic Brief COPE Inventory. Descriptive statistics, correlation, ANOVA and multiple regression analysis were conducted to determine predictors.

Results: Regression analysis revealed that maternal coping was significantly predicted by higher education level (β=0.38, p<0.001), higher socioeconomic status (β=0.32, p<0.001), being employed (β=0.25, p=0.006), a shorter hospital stay (β=-0.21, p=0.013), and acute rather than chronic diagnosis (β=-0.24, p=0.008). The model explained 52% of variance in coping (R^2=0.52, p<0.001).

Conclusions: Modifiable socioeconomic factors significantly predict maternal coping in the hospitalization of a child. To increase caregiver resilience, screening and support programs related to these predictors should be implemented into the healthcare systems.

KEYWORDS: predictors, maternal coping, child hospitalization, socioeconomic status

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INTRODUCTION

Hospitalization of a child is a very dramatic and stressful event in life, which disturbs family balance and puts a considerable psychological and emotional load on the parents, especially the mothers who most often become the main caregivers [1]. It is full of uncertainty, fear, and a sense of loss of control, and mothers are placed in a new and overwhelming healthcare setting during which they somehow have to deal with their own distress and care for their sick child at the same time [2]. Mothers and other adaptive coping strategies they use to handle this crisis, referred to as maternal coping strategies, are important contributors to the short- and long-term family coping, child recovery patterns, and mother mental health [3]. Motherly coping is not a unitary phenomenon but a multi-dimensional process that depends on the interaction of multiple influences both internal and external in nature. They can include intrapersonal resources, including the level of education and psychological strength, to contextual ones, like the caliber of the illness the child has, the duration of the hospital stay, and the socioeconomic conditions of the family [4-5]. When effective, commonly divided into problem-oriented (e.g., seeking information, planning) and emotion-oriented (e.g., positive reframing, seeking emotional support) coping can alleviate the negative impact of stress, help to develop a sense of mastery, and become more involved and collaborative in taking care of the child [6]. On the other hand, reactions resulting in increased parental anxiety, depression, and post-traumatic stress symptoms, like maladaptive coping strategies (e.g., denial or behavioral disengagement), are associated with increased levels of the response and adherence to therapy in the child [7]. Although the importance of maternal coping is well established, much remains to be known about particular predictors of maternal coping particularly within the context of different socio-cultural and economic background. A lot of existing literature is based on high-income, western settings, which restricts the applicability of the results to other settings where healthcare systems, family systems, and resource access

vary significantly [8]. Additionally, modern studies are paying more and more attention to the role of social determinants of health, including education, income, and employment, as the basic determinants of health behaviors and outcomes, including coping efficacy [9]. The necessary sensitivity and contextual analysis is hence needed to determine the most critical factors that enable or disable a mother to cope during this important crossroad. This paper attempts to fill these gaps by examining the frequency, types and major predictors of the coping strategies which mothers have used in their hospitalized child under a given set up. This study will be based on mapping the terrain of maternal adaptation during times of stress by analyzing demographic, socioeconomic and clinical variables. The outcomes will be aimed at facilitating the designing of specific, fair, and family-driven psychosocial interventions. Finally maternal coping is not just a supplement to pediatric care but the main part of holistic medicine that considers the family as the primary unit of the healing process [10].

AIM

The objective of the study was to establish the most important socioeconomic and clinical predictors of coping mechanisms among mothers when their child was admitted to the hospital in Iraq.

MATERIALS AND METHODS

STUDY DESIGN

The study was a descriptive, cross-sectional, correlational study aimed at investigating the coping mechanisms of mothers and factors related to them in cases of child hospitalization.

SETTING AND TIMEFRAME

The study was carried out in the pediatric wards of Al-Basrah Teaching Hospital and Al-Mawani Teaching Hospital in Basrah, Iraq. Data collection occurred over a five-month period from September 2025 to January 2026.

SAMPLE AND SAMPLING TECHNIQUE

Population: Mothers of hospitalized children aged 1-12 years.

Sample size: 100 mothers were enrolled.

Sampling technique: A non-probability convenience sampling method was used. Mothers were approached consecutively during visiting hours if they met the inclusion criteria.

INCLUSION CRITERIA

1. Mothers of children hospitalized for at least 48 hours,
2. Mothers aged 17 years or older,
3. Mothers who provided informed consent.

EXCLUSION CRITERIA

1. Mothers of critically ill children in intensive care,
2. Mothers with diagnosed psychiatric disorders,
3. Children hospitalized for routine procedures.

DATA COLLECTION INSTRUMENTS

Data were collected using a structured, self-administered questionnaire with two sections:

Section 1: Socio-demographic and Clinical Data Sheet (developed by researchers). This collected information on maternal age, education, employment, marital status, number of children, socioeconomic status (categorized using a modified Kuppuswamy scale), child's diagnosis (categorized as Acute, Chronic, or Injury), and length of hospital stay.

Section 2: The **Brief COPE Inventory (Arabic Version)**, a validated 28-item scale measuring 14 coping strategies. Responses are on a 4-point Likert scale (1="I haven't been doing this at all" to 4="I've been doing this a lot"). For analysis, items were grouped into two main domains:

Positive Coping (15 items): Sum of active coping, planning, positive reframing, acceptance, humor, religion, emotional support, and instrumental support subscales. Score range: 15-60.

Negative Coping (5 items): Sum of denial, venting, substance use, behavioral disengagement, and self-blame subscales. Score range: 5-20.

DATA COLLECTION PROCEDURE

The Basrah Health Directorate Research Ethics Committee gave ethical approval to this study. The main researchers visited the pediatric wards on a daily basis and in regular visiting hours. The close coordination with the head nurses of the respective wards helped to identify the potential participants. Individually to every qualified mother, the intention, nature, and consequences of the study were clearly explained and written informed consent was signed. Questionnaires were sent out and filled at the site. In order to be inclusive and accurate, the researcher administered verbal questions to mothers with low literacy where she read and noted down the responses in a verbatim manner, but with absolute privacy. The extremely time-consuming duration of each interview or questionnaire was about 15 to 20 minutes.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26. Analyses included:

DESCRIPTIVE STATISTICS

Frequencies, percentages, means, and standard deviations were used to describe the sample and coping scores.

INFERENTIAL STATISTICS

PEARSON'S CORRELATION COEFFICIENT (R)

To examine relationships between continuous variables (e.g., age, number of children, length of stay) and coping scores.

INDEPENDENT SAMPLES T-TEST AND ONE-WAY ANOVA

To compare coping mean scores across categorical demographic and clinical groups (e.g., education level, employment status, diagnosis type). Post-hoc tests (Tukey HSD) were used where ANOVA results were significant.

MULTIPLE LINEAR REGRESSION ANALYSIS (STEPWISE METHOD)

To identify the key predictors of the total coping score. Assumptions of linearity, homoscedasticity, and normality of residuals were checked and met.

RELIABILITY ANALYSIS

Internal consistency of the coping scales was assessed using Cronbach's Alpha, with values > 0.70 deemed acceptable.

A p-value of < 0.05 was considered statistically significant for all tests.

ETHICAL CONSIDERATIONS

Approval: Formal approval was granted by the relevant institutional ethics committee (Approval No. BHD/REC/2025/87).

Informed Consent: Written informed consent was obtained from all participants after a full explanation.

Confidentiality: All data were anonymized using identification codes. Completed questionnaires were stored in a locked cabinet.

Voluntary participation: Mothers were informed of their right to withdraw at any time without affecting their child's care.

Beneficence: Contact information for the hospital›s psychological support service was provided to all participants.

Ethical approval was granted by the Basrah Health Directorate Research Ethics Committee (No. BHD/REC/2025/87).

PILOT STUDY

To determine the validity of the questionnaire in terms of clarity, timing, and face validity, a pilot study was done on 10 mothers (not included in the main analysis). Feedback ensured minor wording changes had to be made. Cronbach's Alpha internal consistency of Positive Coping and Negative Coping on pilot scale was 0.76 and 0.71 respectively.

Table 1 is the most significant demographic portrait of the mother participants. It demonstrates a very homogenous sample in a few characteristics (e.g., 98% married) and the diversity in others (e.g., age and education level). The current composition offers an appropriate framework of investigating coping mechanisms and the comparison of the effect of various variables on maternal adjustment when the child is admitted to a hospital can be made.

According to the table 2, the most common coping strategies applied by mothers are positive strategies (mean 34.2 out of 45) at moderate-to-high level, and negative coping strategies are relatively less common (mean 11.3 out of 15). This indicates that the mothers have adapted constructively and adaptively to cope with the stress of having their children in the hospitals, which is the psychological resilience and the ability to adapt to stress.

The correlation outcomes indicate that more children are related with better coping which can suggest that maternal experience acquired through raising more than one children increases adaptive capacity (Table 3). In contrast, the longer the hospital stay, the worse the coping and this highlights the cumulative psychological weight and stress of a prolonged stay in a hospital.

This discussion points out that education level and the socioeconomic status are the most differentiating factors in coping strategies. The coping of the mothers who are better educated and have a higher socioeconomic status is much better. The employment status is also positively related; however, the type of diagnosis also affects coping effectiveness, whereby acute illnesses are related to better adjustment than chronic conditions (Table 4).

The model of regression is describing 52 percent of

Table 1. Frequency and percentage distribution of demographic variables

Variable	Category	Frequency	Percentage
Age Group	17–25 years	31	31.0
	26–35 years	43	43.0
	36–45 years	22	22.0
	46–54 years	4	4.0
Marital Status	Married	98	98.0
	Divorced	1	1.0
	Widowed	1	1.0
Education Level	No formal education	13	13.0
	Primary	36	36.0
	Secondary	32	32.0
	University	19	19.0
Employment Status	Government	10	10.0
	Private sector	8	8.0
	Housewife	82	82.0
Number of Children	1–2	54	54.0
	3–4	33	33.0
	≥5	13	13.0
Socioeconomic Status	Low	40	40.0
	Medium	52	52.0
	High	8	8.0
Length of Stay	2–5 days	57	57.0
	6–10 days	27	27.0
	11–16 days	16	16.0
Diagnosis Type	Acute	55	55.0
	Chronic	36	36.0
	Injury	9	9.0

Source: Authors' own work

Table 2. Descriptive statistics of coping strategies

Scale	Possible range	Actual range	Mean	SD	Interpretation
Positive Coping	15–45	21–42	34.2	4.1	Moderately High
Negative Coping	5–15	6–15	11.3	2.2	Relatively Low
Total Coping	20–60	28–55	45.5	5.3	Moderate to High

Source: Authors' own work

Table 3. Correlation analysis (continuous variables)

Variable	Positive coping	Negative coping	Total coping	Significance
Age	r = 0.18	r = –0.12	r = 0.15	p = 0.078 (NS)
Number of children	r = 0.21	r = 0.08	r = 0.19	p = 0.038*
Length of stay	r = –0.24	r = 0.31	r = –0.15	p = 0.017*

Source: Authors' own work

the total coping and this implies that the predictive model is a good model. Education comes out as the most predictive, then comes socioeconomic status and employment- all of them having a positive contribution to coping (Table 5). On the contrary, pro-longed hospitalization and the diagnosis of chronic diseases has a detrimental impact on coping strength. These are the findings that confirm the multidimensional aspect of

Table 4. ANOVA results for categorical variables

Variable	Positive coping F (p)	Negative coping F (p)	Significant difference
Education level	8.45 (p<0.001**)	3.12 (p=0.029*)	University > Secondary > Primary > No education
Socioeconomic status	9.87 (p<0.001**)	4.56 (p=0.013*)	High > Medium > Low
Employment status	5.12 (p=0.008**)	1.34 (p=0.267)	Working > Housewife
Diagnosis type	6.23 (p=0.003**)	4.87 (p=0.010*)	Acute > Chronic
Marital status	1.34 (p=0.267)	0.89 (p=0.413)	No difference

Source: Authors' own work

Table 5. Predictors of total coping

Predictor	B	SE	β	t	p
Education level	2.34	0.56	0.38	4.18	<0.001
Socioeconomic status	1.89	0.48	0.32	3.94	<0.001
Employment	1.45	0.52	0.25	2.79	0.006
Length of stay	–0.38	0.15	–0.21	–2.53	0.013
Diagnosis type	–1.12	0.41	–0.24	–2.73	0.008

Source: Authors' own work

maternal coping which is both a product of personal resources and circumstantial stressors.

DISCUSSION

The demographic results of the study are in line with socio-ecological models of stress and coping. The fact that married mothers 98% overcome is a suggestion that spousal support may be present which an established protective variable against stress among caregivers is. The studies indicate that partner support can help to reduce maternal anxiety and promote problem-oriented coping in the medical crisis of a child [11-12]. On the other hand, the large rate of housewives 82%, individuals with primary/secondary education 68%, might represent the groups that have fewer socioeconomic resources or different coping repertoires and are, therefore, the most essential target of the psychosocial intervention [13]. The result of an average moderate positive coping (Mean=34.2) and the comparably low negative coping is positive and fits well into a resilience approach to pediatric nursing. Modern theories, including the Family Stress and Resilience Model, assume that when parents encounter the illness of their child, they tend to trigger inherent and acquired adaptive resources [14]. This finding can be an indication of effective application of such strategies as social support in search, positive reframing and active planning, which are associated with more successful psychological adjustment and even child outcomes achieved by means of more cohesive family management [15]. The relationship between coping and higher education, as well as coping and the SES, are strong and graded (Tables 4 and 5), which is a strong and critical finding. This highlights the Fundamental Causes Theory [16], which states that resources such as knowledge, money, social relationships define health outcomes and coping effectiveness. Cognitive means of processing medical information, navigating complex healthcare systems, and successful communication with vendors are all forms of problem-focused coping offered by higher education [17]. In the same way, increased SES helps to alleviate concrete stressors (e.g., financial concern), which liberate psychological resources to engage in adaptive coping [18]. This poses a risk of inequity gap in which less educated/SES mothers are disadvantaged twice. It is remarkable that the positive coping of employed mothers over housewives is quite an advantage. Work can give a structured identity beyond the caregiver role, social populations, and add to increased self-efficacy and economic independence. Such a conclusion is confirmed by Lee and Kim [19], who established that work engagement may be a source of mastery and a mental reprieve to counter the powerlessness that may be experienced in hospitals. It disputes the belief that physical presence is always the best and shows the significance of role diversification to the well-being of the mother. The adverse correlation between the duration of hospitalization/chronic diagnosis and coping capacity is a dark reminder of the cumulative stress hypothesis. Acute stressors are not only intense but also follow a defined path. Chronic disease or long hospital stay does, however, cause allostatic load, or the stress fatigue of the body and mind due to long-term stress [20]. This empties psychological resources, and adaptive coping is more difficult to sustain. The mums with chronic illnesses also have to adjust to the long-term uncertainty and the change of roles into the permanent caregiving, which predetermines increased burnout and emotional fatigue [21-22]. The fact that there

is a positive relationship between the number of children and improved coping is interesting. It could represent a stress inoculation effect, in which previous experience with parenting develops a set of problem-solving strategies and emotional regulation mechanisms [23]. The veteran moms can even be more realistic and have a reference check (I have handled crises in the past). Instead, mothers having numerous children might have acquired extended social support systems. This finding, however, must be taken with caution since it might not be true in situations where there is severe scarcity of resources.

Family support likely played a bigger role in maternal coping than this study was able to capture directly. The high proportion of married mothers, along with the link between having more children and better coping, both point to family as a source of strength during a child's hospitalization through shared caregiving, emotional support and easing the practical burden on the mother. Since family involvement wasn't measured directly here, this remains an area worth exploring more closely in future work.

One limitation of this study is the wide age range of children admitted to the hospital (from 1 to 12 years old). The approaches of mothers of younger children may differ from those of mothers of older, school-aged children, as younger children rely more heavily on direct physical care, while older children are better able to understand and articulate their illness. Combining these two groups may have masked some of these differences. Therefore, future studies with narrower age groups could better elucidate these distinctions.

CONCLUSIONS

This study conclusively demonstrated that a mother's ability to cope while her child is hospitalized is not merely a psychological phenomenon, but rather a societal norm. This ability is significantly influenced by structural factors such as educational and social status, as well as circumstantial factors such as the duration of the illness and the length of the hospital stay. The family also appears to play a crucial role; mothers who receive support from their husbands and who possess greater experience in raising children generally cope better. The results suggest the need to abandon a one-size-fits-all pediatric care model to a disparity-based, customized model of family support that can target such underlying inequities to encourage resilience among all caregivers.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Clinical and ophthalmological patterns of myopia onset and progression in ukrainian patients of different age groups

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ABSTRACT

Aim: To investigate the clinical and ophthalmological patterns associated with the onset and rapid progression of myopia in Ukrainian patients across different age groups.

Materials and Methods: We analyzed data from 66 patients (132 eyes) with myopia, aged 6 to 17 years. In addition to a comprehensive ophthalmological examination, patients underwent OCT and OCT angiography (OCT-A), endothelial microscopy, assessment of corneal rigidity and scleral stiffness using the Sergienko–Shargorodska method, and a questionnaire survey.

Results: Myopia onset occurred most frequently at 9-11 years of age (50% of cases) in the Ukrainian pediatric cohort. Gender did not influence disease onset or the rate of progression. Key clinical factors associated with rapid myopia progression (>1 D/year) included physical inactivity, vitamin deficiency, and parental smoking. Increased corneal rigidity (KER > 4.1) was detected as early as 6-8 years of age (67% of patients), preceding the first clinical peak of disease progression. At 9-11 years of age, rapid progression was associated with corneal endothelial decompensation, grade II–IV trabecular dysgenesis (91%), significant choroidal thinning (<190 µm; 91%), and anterior scleral thinning (TAST3 <500 µm; 79%), all of which may contribute to accelerated axial elongation. OCT-A confirmed ischemia in the peritemporal quadrant, whereas macular perfusion remained stable in most patients (88%).

Conclusions: The development of myopia follows a staged pattern, progressing from refractive genesis (corneal and endothelial changes) in younger children to axial genesis (alterations of the scleral capsule) in adolescents aged 15-17 years. The preservation of macular perfusion despite peripheral ischemia suggests the presence of compensatory mechanisms during childhood.

KEYWORDS: myopia, optometry, rigidity, cornea, endothelium, perfusion

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INTRODUCTION

Modern ophthalmology considers myopia not merely a refractive error but a global public health challenge with significant medical and social implications. According to recent meta-analyses and projections by the International Myopia Institute, by 2050 the global prevalence of myopia may reach 50% of the world's population, with nearly 10% affected by high myopia [1–3]. Ukraine demonstrates a similar trend toward an earlier onset of the condition and a rising proportion of rapidly progressive myopia, which is associated with increasing near-work demands and lifestyle changes [4].

A key concept in understanding the etiology and pathogenesis of myopia is the biomechanical theory of its development [5]. This concept is based on the assumption that the primary cause of ocular elongation results from alterations in the elastic properties of the fibrous tunic of the eye. The Ukrainian ophthalmological school, represented by the work of M.M. Serhienko and Yu.M. Kondratenko [5–8], has made an invaluable contribution to the development and implementation of this concept, inspiring a new generation of researchers [5] and stimulating further investigations in this field.

According to these researchers [6], the progression of myopia is closely associated with alterations in the aqueous outflow system. In particular, trabecular dysgenesis and pigmentation have been shown to impair aqueous humor hydrodynamics, creating conditions for mechanical scleral stretching under the influence of intraocular pressure, even when intraocular pressure remains within the normal range [7].

The biomechanical properties of the fibrous tunic, including corneal and scleral rigidity, serve as critical markers of ocular stability. Several studies [5, 7, 9] have demonstrated that the rigidity index is an individual characteristic that determines the eye's resistance to deformation. A reduced rigidity coefficient is considered an unfavorable prognostic factor preceding axial elongation [5].

These changes are likely driven by extracellular matrix remodeling within the sclera, where local hypoxia activates the HIF-1α and HIF-2α signaling pathways, promoting increased expression of matrix metalloproteinase-2 (MMP-2) and degradation of type I collagen [10]. However, this hypothesis requires further investigation.

Another important component of myopia pathogenesis involves the condition of the choroid and retinal microvascular perfusion. Advanced imaging modalities, such as optical coherence tomography (OCT) and OCT angiography (OCT-A), have demonstrated that choroidal thinning is a dynamic process that often precedes axial elongation [9, 11]. The choroid functions not only as a trophic layer but also as a biomechanical buffer; therefore, its thinning may reduce its ability to regulate signaling molecules involved in scleral remodelling [12–14].

Despite numerous international studies, including MOSAIC and LAMP [15, 16], the relationship between anterior segment biomechanical alterations and posterior pole vascular biomarkers in the Ukrainian population remains insufficiently investigated. The temporal sequence of involvement of different ocular structures during myopia development across various age groups requires further clarification to identify optimal windows for preventive interventions. Therefore, an integrative analysis of clinical factors, biomechanical parameters, and modern OCT-based biomarkers remains an important research priority in contemporary ophthalmology and optometry [11, 17].

AIM

To investigate the clinical and ophthalmological patterns, including biomechanical and OCT-based structural and microvascular markers, associated with the onset and rapid progression of myopia in Ukrainian patients of different age groups.

MATERIALS AND METHODS

An analytical cohort study was conducted, including data from a comprehensive ophthalmological examination of 66 patients (132 eyes) with myopic refractive error. The study population comprised 35 females (53%) and 31 males (47%). Patients were stratified into four age groups according to the age at myopia onset and disease progression. Group I included 9 patients (18 eyes; 14%) aged 6-8 years. Group II comprised 33 patients (66 eyes; 50%) aged 9-11 years. Group III consisted of 12 patients (24 eyes; 18%) aged 12-14 years, and Group IV included 12 patients (24 eyes; 18%) aged 15-17 years.

The inclusion criteria were as follows: a clinically confirmed diagnosis of myopia (for the study group); written informed consent from the patient or the patient's legal representative to participate in the study and undergo follow-up; and the patient's ability to cooperate adequately during the study procedures. The exclusion criteria included refusal to undergo specific stages of the diagnostic examination or failure to comply with the scheduled follow-up visits.

The enrolled patients were followed for five years. Myopia progression was classified as slow when the spherical equivalent changed by <1 diopter (D) per year and as rapid when it changed by >1 D/year [5, 6].

The comprehensive ophthalmological assessment included:

• medical history – parental questionnaire covering lifestyle, nutrition, habits, and chronic conditions;

• gonioscopy – detailed visualization of the anterior chamber angle and degree of trabecular pigmentation (Ocular, USA);

• corneal topography – to measure corneal thickness and anterior chamber depth (Schwind Sirius+, Germany);

• optical coherence tomography (OCT) (REVO NX Version 10.0.0 Device SN: 2570793/06) and OCT angiography (OCT-A) (Cirrus HD-OCT 5000, Zeiss) – to assess choroidal thickness (CChT, SFCT) and vessel density (DVD) in different sectors;

• endothelial microscopy – to evaluate endothelial cell density (ECD) and hexagonality (Heidelberg Engineering Inc., Germany).

Measurements of corneal rigidity (K_{ER}) and scleral rigidity (K_{ES}) were performed using the Sergienko–Shargorodska method [5]. This in vivo technique for assessing corneal and scleral biomechanical parameters is based on determining the resistance of the fibrous ocular capsule to a controlled and evenly distributed load. The obtained data allow calculation of rigidity coefficients that reflect the elastic and biomechanical state of the ocular tunics [5, 6].

The statistical data analysis was performed using IBM SPSS Statistics v. 27.0 (Armonk, NY: IBM Corp., USA), EZR v. 1.70 and MedStat v. 5.2. Categorical variables were presented as counts and percentages. Comparisons between two groups were performed using the Fisher's exact test. For comparisons among four groups, the χ^2 test was applied, followed by *post hoc* pairwise comparisons using the Marascuilo–Liakh–Gurianov procedure when appropriate. To identify independent predictors associated with the outcome, stepwise logistic regression analysis was performed. The results of the logistic regression analysis were presented as odds ratios (ORs) with 95 % confidence intervals (CIs).

Table 1. Association between the onset of myopia and patients` sex and progression rate in the Ukrainian population

Indicator	Onset of myopia development				p
	6-8 years n = 9	9-11 years n = 33	12-14 years n = 12	15-17 years n = 12	
Males, N (%)	5 (56)	17 (52)	3 (25)	6 (50)	0.406
Females, N (%)	4 (44)	16 (48)	9 (75)	6 (50)	
Myopia progression rate >1 D/year, N (%)	7 (78)	18 (55)	8 (67)	8 (67)	0.582

Source: compiled by the authors of this study

Table 2. The signs of corneal endothelial decompensation in patients with different myopia age onset

Indicator	Onset of myopia development				p
	6-8 years n = 9	9-11 years n = 33	12-14 years n = 12	15-17 years n = 12	
ECD 1601–2000 cells/mm^2, N (%)	1 (11)	15 (45)	4 (33)	1 (8)	0.053
ECD 2401–2800 cells/mm^2, N (%)	0	1 (3)	4 (33)	3 (25)	0.013
Mean endothelial cell size <500.0 μm^2, N (%)	1 (11)	3 (9)	4 (33)	3 (25)	0.207
Hexagonality >50 %, N (%)	1 (11)	1 (3)	4 (33)	3 (25)	0.037

Source: compiled by the authors of this study

Table 3. The presence and degree of trabecular pigmentation in patients with different myopia age onset

Indicator	Onset of myopia development				p
	6-8 years n = 9	9-11 years n = 33	12-14 years n = 12	15-17 years n = 12	
Absence or trabecular pigmentation degree I, N (%)	5 (56)	3 (9)	9 (75)	10 (83)	p_{2-3}= 0.001 p_{2-4}<0.001
Trabecular pigmentation degree II, N (%)	2 (22)	12 (36)	0	0	p_{2-3}= 0.093 p_{2-4}= 0.094
Trabecular pigmentation degrees III-IV, N (%)	2 (22)	18 (55)	3 (25)	2 (17)	0.047

Notes: p_{2-3} – the significance of the difference between 9-11 and 12-14 years groups; p_{2-4} – the significance of the difference between 9-11 and 15-17 years groups

Source: compiled by the authors of this study

The threshold for statistical significance was set at $p < 0.05$ [17].

ETHICS

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (2000) and the requirements of the current legislation of Ukraine governing biomedical research involving human participants. All study procedures were approved by the local Ethics Committee of Bogomolets National Medical University. Written informed consent for participation in the study and the processing of personal data was obtained from all parents or legal representatives of the participants prior to enrollment.

AI STATEMENT

Artificial intelligence tools, including Microsoft Copilot, Google Gemini, and Grammarly, were used under the full supervision and control of the authors solely for language editing, stylistic improvement of the manuscript, and reference formatting. Artificial intelligence tools were not used for study design, data collection, data analysis, statistical processing, interpretation of results, generation of scientific conclusions, or decision-making related to the findings of the study. All scientific content, methodological decisions, analyses, interpretations, and conclusions were developed, verified, and approved exclusively by the authors. The authors take full responsibility for the accuracy, integrity, and final content of the manuscript.

FRAMEWORK

The work was carried out at the Department of Ophthalmology and Optometry of Postgraduate Education of the Institute of Postgraduate Education of Bogomolets National Medical University and is a fragment of the initiative-research project «Theoretical and practical

Table 4. The macular perfusion and OCT biomarkers in patients with different myopia age onset

Indicator		Onset of myopia development				p
		6-8 years n = 9	9-11 years n = 33	12-14 years n = 12	15-17 years n = 12	
Subfoveal choroid thickness <190.0 μm, N (%)		5 (56)	30 (91)	5 (42)	5 (42)	p_{2-3}= 0.035 p_{2-4}= 0.035
Macular perfusion, deep vascular density in the fovea < 18,0 %, N (%)		1 (11)	5 (15)	1 (8)	1 (8)	0.893
Deep vessel density reduction in the peritemporal quadrant, N (%)	<47.0 mm^{-1}	5 (56)	29 (88)	5 (42)	5 (42)	p_{2-3}= 0,069 p_{2-4}= 0,069
	47.1 – 48.0 mm^{-1}	2 (22)	3 (9)	2 (16)	3 (25)	0,528
	48.1 – 49.0 mm^{-1}	1 (11)	1 (3)	0	4 (33)	0,010
	>49.0 mm^{-1}	1 (11)	0	5 (42)	0	p_{2-3}= 0,018
Thinning of the anterior sclera in the temporal region (TAST3) to <500.0 μm, N (%)		5 (56)	26 (79)	5 (42)	5 (42)	0.040

Notes: p_{2-3} – the significance of difference between 9-11 and 12-14 years groups; p_{2-4} – the significance of difference between 9-11 and 15-17 years groups
Source: compiled by the authors of this study

aspects of improving clinical and experimental methods of diagnosis, treatment and prevention of diseases and injuries of the organ of vision and their complications» (state registration number 0123U104207; term: 2023-2026).

RESULTS

Myopia onset occurred most frequently at 9-11 years, accounting for half of all cases in the included patient sample (33/66; 50%). The remaining cases were distributed across 12-14 years (12/66; 18%), 15-17 years (12/66; 18%), and 6-8 years (9/66; 14%).

The relationship of the myopia age onset to patients` sex and progression rate is presented in Table 1.

According to the presented data (Table 1), no significant gender differences in the onset of myopia were found. In addition, gender did not affect the rate of myopia progression. Among 31 male patients, 22 (71%) cases of rapid progression; among 35 female patients, 19 (54%) such cases (p=0.207).

Environmental and behavioural risk factors for myopia development and progression have been widely studied [2, 4]. In our study, the key clinical factors associated with rapid myopia progression (>1 D/year) were insufficient physical activity (OR 27.8 [95 CI 5.7-136.8]; p<0.001), vitamin deficiency (OR 24.8 [95% CI 5.1-121.2]; p<0.001) and parental smoking (OR 4.2 [95% CI 1.3-13.3%]; p=0.015). Meanwhile, urban residence (63.4% vs. 44.0% in rapid and slow progression groups, respectively; p=0.136) and having at least one myopic parent (56.1% vs. 60.0% in rapid and slow progression groups, respectively; p=0.802) did not show a significant association with rapid myopia progression.

The relationship between myopia onset and signs of corneal endothelial decompensation is summarised in Table 2.

A decrease in endothelial cell density (ECD) to 1601-2000 cells/mm^2 was observed in 45% of patients aged 9-11 years, whereas ECD values within the normal range (2401-2800 cells/mm^2) were recorded in 33% of patients aged 12-14 years. In addition, a reduced mean endothelial cell area (<500.0 μm^2) and increased hexagonality (>50%) were observed in 33% of patients aged 12-14 years (Table 2).

In the youngest age group (6-8 years), most patients showed no changes in endothelial cell density, endothelial cell area, or hexagonality (Table 2).

Data on trabecular pigmentation according to age at myopia onset are presented in Table 3.

In group I (6-8 years), grade I trabecular dysgenesis was detected in 56% of patients, grade II - 22%, and grades III-IV were observed in 22% of cases. In Group II (ages 9-11 years), corneal endothelial decompensation and trabecular dysgenesis III-IV degrees were observed in 55 % of patients, and the II degree - in 36%. The majority of patients in group III (12-14 years) and group IV (15-17 years) had grade I trabecular dysgenesis – 75% and 83%, respectively (Table 3).

Assessment of biomechanical markers revealed that increased K_{ER} values (+)3.1 – (+)4.0 occurred in 50% of patients aged 6-8 years, and higher values (+)5.4 were observed in 44 % of cases, preceding the peak of myopia progression. It was found that in Group I (ages 6-8 years), 67% of patients already demonstrated elevated K_{ER} >(+)4.1, which preceded the clinical progression of the condition.

Analysis of macular perfusion and OCT-based biomarkers revealed a selective pattern of vascular

impairment. Clinically significant structural alterations were predominantly observed in patients aged 9-11 years, identifying this period as a critical stage at which ocular compensatory mechanisms begin to be overwhelmed by structural remodeling (Table 4).

Significant thinning of the subfoveal choroid at a distance of 3 mm from the fovea in the vertical meridian (SFCT, S3+I3) to <190.0 μm was observed in 91% of patients aged 9-11 years, indicating active remodeling of the posterior segment.

A notable finding was that macular perfusion remained stable and within the normal range (>18.0%) in most patients (88%), whereas reduced perfusion was detected in the peritemporal quadrant.

A deep vessel density of <47.0 mm^{-1} in the peritemporal quadrant was observed more frequently in patients aged 9-11 years than in those aged 12-14 and 15-17 years. Deep vessel density values of 48.1-49.0 mm^{-1} occurred more frequently in the 15-17-year age group. Values >49.0 mm^{-1} were observed in 42% of patients aged 12-14 years but were absent in the 9-11- and 15-17-year groups and were detected in only one patient aged 6-8 years.

These findings indicate selective vascular vulnerability. The pediatric eye appears to preserve perfusion of the foveal region, which is responsible for central visual acuity, through autoregulatory mechanisms, whereas the peripheral retina is affected earlier by biomechanical stretching of the ocular tunic.

At the same time, thinning of the anterior sclera in the temporal region (TAST3) to <500.0 μm was observed in 79% of patients aged 9–11 years (Table 4). Previous studies [13, 14] have demonstrated that anterior scleral thickness is directly associated with overall ocular rigidity. Our findings suggest that, in children aged 9-11 years, reduced scleral rigidity increases the susceptibility of the ocular wall to intraocular pressure–related deformation, thereby facilitating axial elongation and the progression of axial myopia.

DISCUSSION

The findings of the present study support the concept of the stepwise involvement of ocular structures in myopia development. The earliest alterations were observed in corneal rigidity (at 6–8 years of age), followed by the appearance of peripheral retinal perfusion abnormalities and signs of corneal endothelial decompensation during the 9-11-year period. In contrast to adults, the preservation of macular perfusion in children may reflect compensatory autoregulatory mechanisms operating during the phase of active ocular growth. At the same time, thinning of the anterior sclera was also identified in patients aged 9-11 years.

Our findings are consistent with the theory proposed by Yu. Kondratenko and N. Serhienko [8], emphasise the key role of the ocular drainage system in the pathogenesis of myopia. The high prevalence of trabecular abnormalities (grades II–IV; 91%) observed during the first peak of disease progression (9-11 years of age) suggests that impaired aqueous humour outflow and subclinical elevation of intraocular pressure may represent important triggers of axial elongation in myopia [7, 8, 13, 18].

The present study further supports the concept of the sequential involvement of ocular structures during myopia development. At the earliest stage (6-8 years of age), the process appears to be predominantly refractive in nature and is characterized by alterations in the biomechanical properties of the fibrous ocular tunic, increased rigidity indices, and early corneal endothelial decompensation. The subsequent stage (9-11 years of age) is marked by the involvement of the choroid and sclera, indicating a transition toward axial myopia. This interpretation is supported by anterior scleral thinning (TAST3 <500 μm), which was observed in 79% of children in this age group and is consistent with previous reports [9, 13, 14]. Such thinning may serve as a marker of reduced biomechanical stability of the eye and increase susceptibility to progressive axial elongation.

Previous studies have shown that a 23-μm reduction in choroidal thickness is associated with an approximately 1-mm increase in axial length [9, 12, 14]. Consistent with these findings, our study demonstrated marked choroidal thinning (<190 μm), suggesting an increased risk of subsequent myopic staphyloma formation.

Comparison of our OCT angiography findings with previously published data [11–13] revealed a pattern of selective vascular vulnerability: macular perfusion remains preserved for a prolonged period, likely owing to autoregulatory mechanisms, whereas the peritemporal region appears to be the earliest site of perfusion impairment [11–13]. These findings emphasise the value of segmental OCT angiography analysis for the early identification of patients at increased risk of disease progression.

LIMITATIONS OF THE STUDY

Previous studies in the area studied are few. The study included 66 Ukrainian patients (132 eyes), which limits the generalizability of the results to larger populations. However, the sample size allowed for an in-depth qualitative analysis that identified peaks in myopia and significant clinical predictors. The duration of follow-up of the patient groups is five years and needs to be extended in the future.

PERSPECTIVES OF SUBSEQUENT SCIENTIFIC RESEARCH

The increase in the corneal rigidity coefficient, the decrease in the density of the deep vascular plexus in the peritemporal quadratus, and the thickness of the choroid 3 mm from the fovea obtained in this clinical study are the basis for a more detailed study of the problem of the influence of changes in the stiffness of the fibrous capsule of the eye and the blood supply to the retina and posterior pole of the eye on the development and progression of myopia.

CONCLUSIONS

1. Assessment of the corneal rigidity coefficient is the most informative early biomechanical marker for identifying potential risks of myopia development and progression. Its alterations can be detected 2-3 years before the clinical manifestation of disease progression.
2. The age of 9-11 years represents a critical pathogenetic stage, characterized by structural deformation of the ocular tunics and ischemia of the peripheral retina.
3. Myopia progression in Ukrainian patients follows a trajectory from refractive changes to axial elongation.
4. Rapid myopia progression is accompanied by ischemia of the peritemporal zone while macular perfusion remains preserved.
5. Insufficient physical activity, vitamin deficiency and parental smoking are significant clinical risk factors associated with the progression of myopia in the Ukrainian patients.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Ex vivo histological comparison between Er, Cr: YSGG laser and slow-speed hand piece in bone cutting

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ABSTRACT

Aim: This study aimed to compare the efficiency of Er, Cr: YSGG laser, and handpiece bur in bone cutting by histological examination.

Materials and Methods: This was an *ex vivo* study using the bone specimens from the sheep as samples, different ER, CR: YSGG laser parameters will be used (2.5W, 20Hz/4W, 20Hz/6W, 20Hz) versus slow hand piece to make osteotomy. After histopathological processing, the length, width, length-to-width ratio, precision, and carbonization of laser osteotomy will be examined under a light microscope.

Results: ER, CR: YSGG lasers can be considered practical, effective, and easy for bon incisions. The histological results showed high incision quality with the Er, Cr: YSGG laser system, versus slow speed handpiece. The quality of laser incision at 4W, 20HZ is the best among the parameters $p<0.001$.

Conclusions: The Er, CR: YSGG laser if used properly with appropriate parameters is very effective alternative device in oral osteotomy.

KEYWORDS: bon, histology, incision, Er, Cr: YSGG laser, thermal damage

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INTRODUCTION

One of the oldest forms of orthopaedical treatment is osteotomy. It has been around for nearly a thousand years and is still used today. An osteotomy's fundamental idea is to cut or incision bone, usually to alter the shape or alignment of the bone [1]. Tool for osteotomy. Although effective, the bur's direct contact with bone during the cut causes vibrations, making patients uncomfortable. Thus, it has been suggested that lasers be used for osteotomy treatments. Surgical lasers, however, might facilitate the removal of bone tissue without resulting in heat injury, making this feasible [2]. Bone, known as specialized, living, complex connective tissue, supports and shields the body's critical organs [3]. All bones are composed of a dense outer layer of compact bone and a core of cancellous bone. The cancellous bone is the main component of a bone. It surrounds marrow voids that may contain hematological tissue and form a trabecular network [4]. The accurate, contactless laser ablation method can remove the body's hard tissue. Laser osteotomy speeds up the healing of the residual bone compared to traditional bone cutting with piezo osteotomes. Recovery is accelerated because the spongy structure is preserved [5]. Bone defect repair is still a significant clinical orthopedic issue. Bone, a highly vascularized tissue, depends on the intimate spatial and temporal relationship between blood vessels and bone cells to preserve skeletal integrity [6]. Cutting hard/soft tissue is a complex interaction of laser energy with water and tissues (hydrophotonics). When tissues interact with laser energy, the effect is influenced by emission wavelengths, tissue optical properties, time of exposure, laser energy, and absorption of the laser energy into the tissues [7]. Therefore, while cutting bone with a laser, there is less trauma and heat damage. It encourages healing and causes less scar tissue to form, as well as less bleeding and infection [8]. A surgeon can work on tissues more accurately and ablate tissues in hard-to-reach places by using a laser as a surgical tool. The properties of the tissue, including its optical characteristics, color, consistency, and thermal properties heat capacity, temperature conductivity, as well as those associated with the laser system, wavelengths, and emission parameters, such as applied power, focal spot size, and emission mode exposure time, determine how the laser acts on the tissue [9-10]. Because water is the chromophore target, their usage in dentistry goes beyond soft tissues such as mucosa and gingiva, to the hard ones, including enamel, dentine, bone and carious tissue. The thermal effects of erbium lasers on the target tissues can be detected through vaporization. A photomechanical effect is created when water molecules explode, which

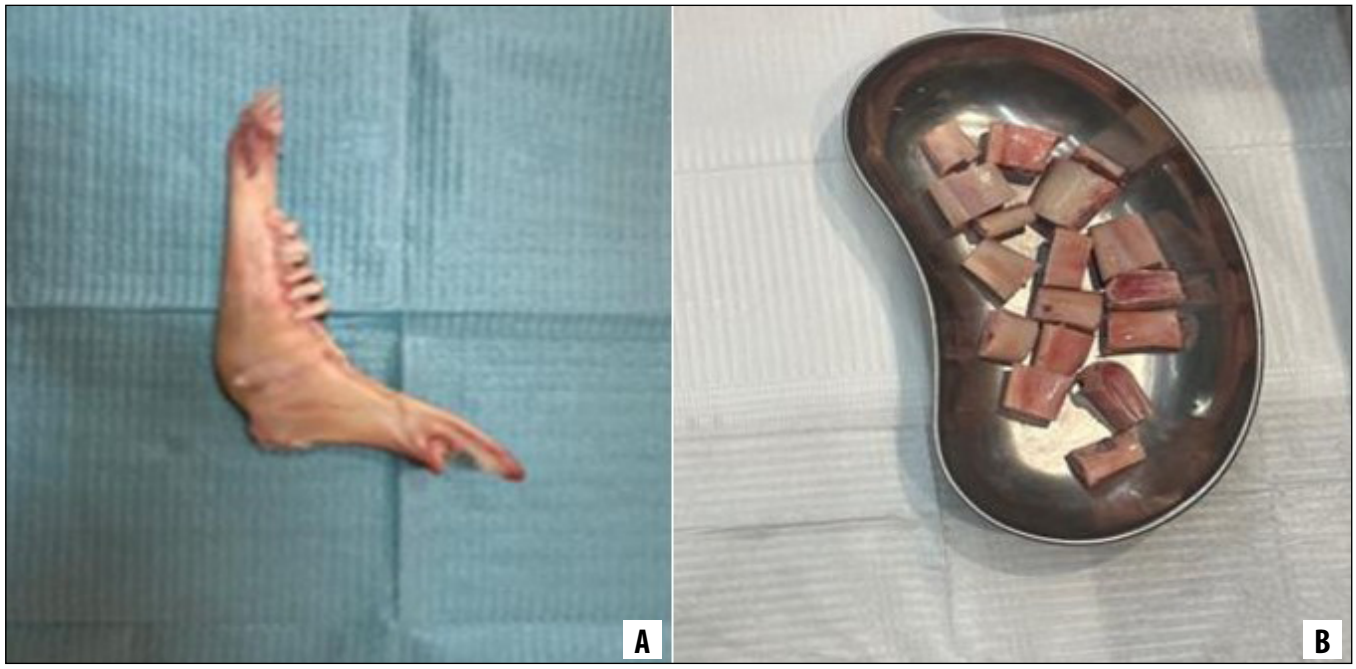


Fig. 1. Sheep mandible and preparation of bone specimens: A. Sheep mandible used for specimen collection; B. Mandibular bone blocks prepared for the osteotomy procedures
Source: Own materials

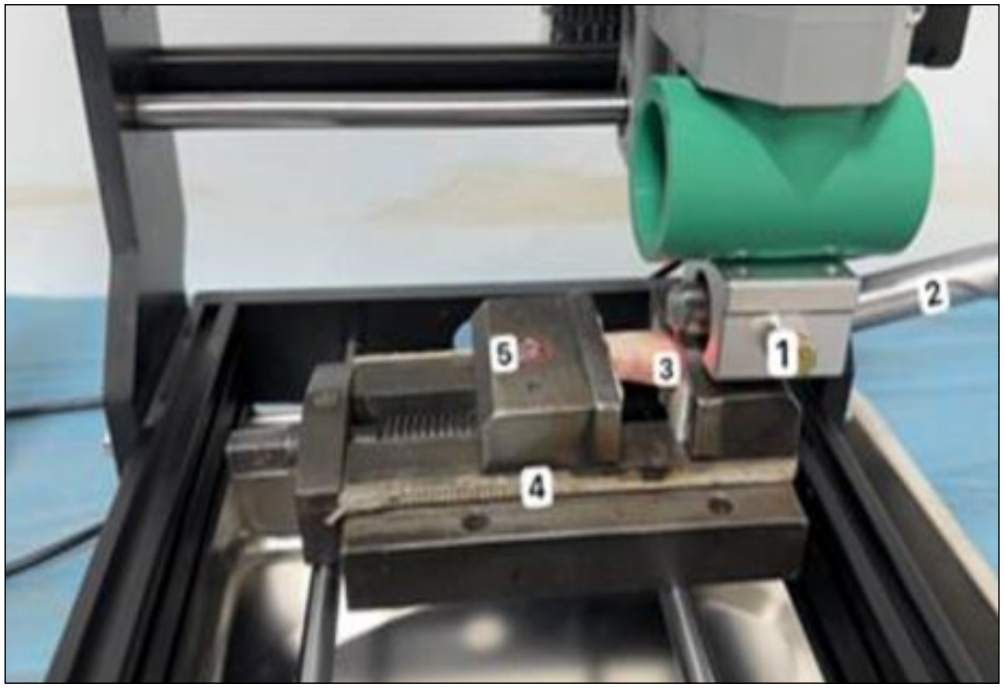


Fig. 2. A CNC devise
1-Handle
2-Laser hand piece
3-Bone specimen
4-Mounted table
5-Arms that hold the specimen
Source: Own materials

aids in the ablative and cleaning processes [11]. A laser can create accurate cutting and reduce mechanical damage to the surrounding tissues when cutting bone [12]. The wavelength of Erbium lasers is highly absorbed by water and hydroxyapatite making it suitable for both hard and soft tissue ablation [13]. Erbium laser offers an attractive alternative drilling modality because it does not require physical contact with the bone in order to drill holes, cut bone with minimal thermal damage & precise control of bone cutting [11]. The Er. Cr: YSGG laser is effective for various dental operations involving both hard and soft tissues because it shows peak absorption by hydroxyapatite (HA) and water at 2780 nm in the infrared spectrum [14]. Since water and hydroxyapatite crystals absorb a large portion of the Erbium, Chromium YSGG laser at 2780 nm wavelength, this free-running pulsed laser has shown promise in treating a variety of hard tissue procedures and soft tissue operations [15]. The considerable heating of the bone and surrounding tissues during irradiation has been controlled by the pulse-mode laser and the water spray,

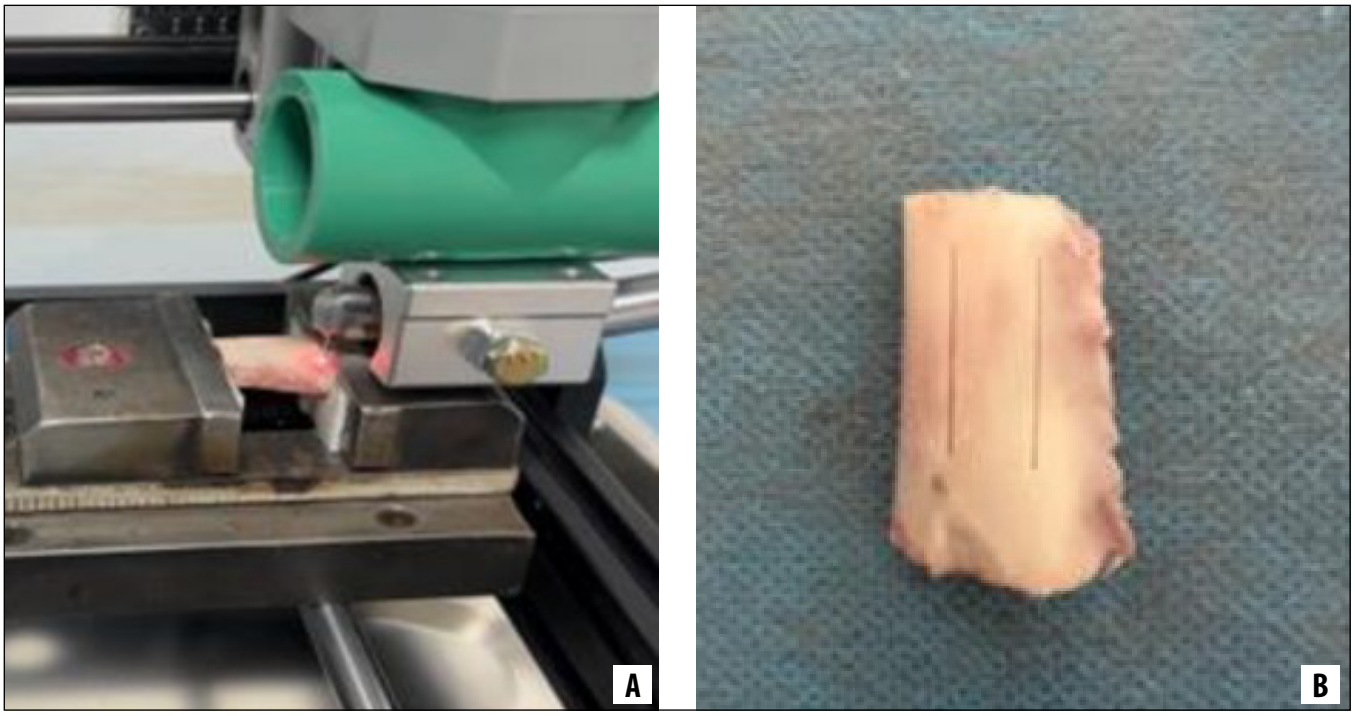


Fig. 3. Laser osteotomy procedure: A. Er, Cr:YSGG laser osteotomy performed on the bone specimen mounted on the CNC device; B. Bone specimen showing the laser incisions after irradiation
Source: Own materials

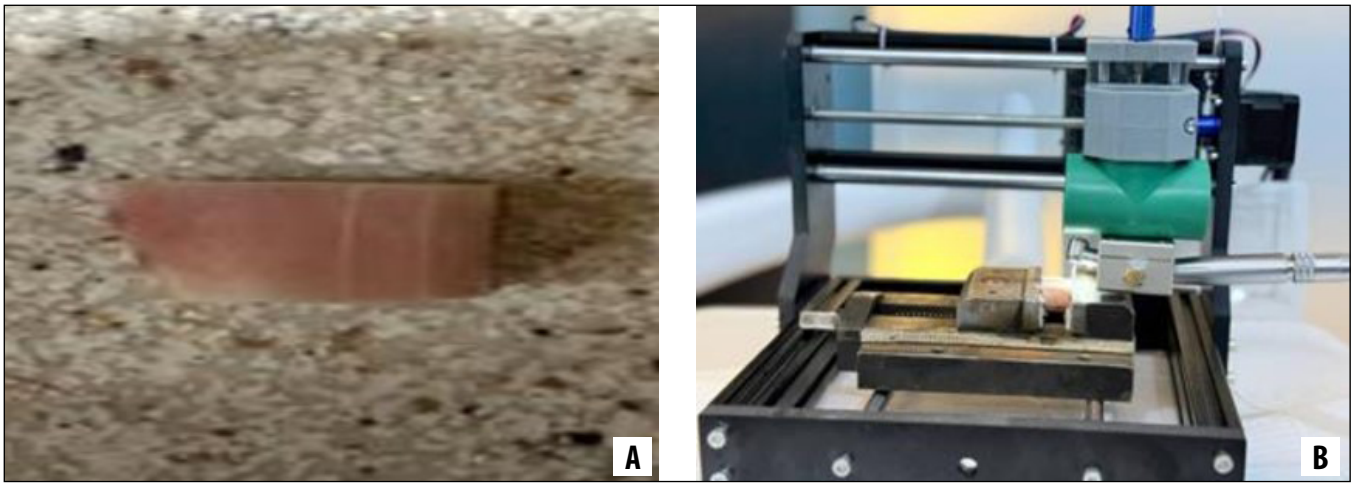


Fig. 4. Conventional osteotomy procedure: A. Conventional osteotomy performed using a low-speed handpiece on the bone specimen mounted on the CNC device; B. Bone specimen showing the conventional incision
Source: Own materials

guaranteeing positive clinical outcomes without compromising wound healing [16]. This *ex vivo* study aimed to compare the efficiency of Er, Cr: YSGG laser, and handpiece bur in bone cutting by histological examination.

MATERIALS AND METHODS

SAMPLES COLLECTION

This study used the following instruments: Er, Cr: YSGG (WATERLASE I plus, BIOLASE) device set at three parameters, and a low-speed handpiece (Guangdong JINME Medical Technology Co., Ltd) using round stainless-steel burs (ISO diameter 018). Ethics approval was taken from the Research & Ethics Committee at the Institute of Laser for Postgraduate Studies/University of Baghdad (464 in 10-10-2023). In this *ex vivo* study, three mandibles of Iraqi sheep (1–1.5 years of age) were used within four to six hours after slaughter. The bone was saved and humidified at the same temperature in an ice box. The sheep's mandible has been cut into blocks (specimens), with measurements of 1 cm in length, 2 cm in width, and

Table 1. Description of length, width and length to width ratio of specimen in different study groups

Characteristic	2.5 W / 20 Hz N = 10[1]	4 W / 20 Hz, N = 10[1]	6 W / 20 Hz, N = 10[1]	Hand Piece, N = 10[1]	P-value[2]
Length (mm)	0.56 ± 0.04	0.91 ± 0.08	0.59 ± 0.05	0.42 ± 0.08	<0.001
Width (mm)	0.79 ± 0.11[c]	0.73 ± 0.05	0.47 ± 0.18	0.94 ± 0.24	<0.001
Length to width ratio	0.72 ± 0.13	1.24 ± 0.16	1.38 ± 0.39	0.46 ± 0.06	<0.001
Grading of L/W ratio					<0.001
1 (<0.7)	6 (60.0%)	0 (0.0%)	3 (30.0%)	10 (100.0%)	
2 (0.7-1.5)	4 (40.0%)	10 (100.0%)	3 (30.0%)	0 (0.0%)	
3 (> 1.5)	0 (0.0%)	0 (0.0%)	4 (40.0%)	0 (0.0%)	

Note: [1]Mean ± SD; n (%) [2]One-way ANOVA; Fisher's exact test

Table 2. Description of the precision of cut and carbonization of bone specimens using different laser settings

Characteristic	2.5 W/20 Hz, N = 10[1]	4 W/20 Hz, N = 10[1]	6 W/20 Hz, N = 10[1]	Hand Piece, N = 10[1]	P-value[2]
Precision	2.9 ± 0.3	2.8 ± 0.4	2.0 ± 0.7	1.0 ± 0.0	<0.001
1 (bad)	0(0.0%)	0(0.0%)	2(20.0%)	10(100.0%)	
2 (moderate)	1(10.0%)	2(20.0%)	6(60.0%)	0(0.0%)	
3 (good)	9(90.0%)	8(80.0%)	2(20.0%)	0(0.0%)	
Carbonization	1.0 ± 0.0	1.3 ± 0.7	1.3 ± 0.5	1.0 ± 0.0	0.079
1 (mild)	10(100.0%)	8(80.0%)	7(70.0%)	10(100.0%)	
2 (moderate)	0(0.0%)	1(10.0%)	3(30.0%)	0(0.0%)	
3 (severe)	0(0.0%)	1(10.0%)	0(0.0%)	10(100.0%)	

Note: [1]n (%) [2]Fisher's exact test

Table 3. Description of the total score utilized in this study and the statistical differences between study groups

Characteristic	2.5 W / 20 Hz, N = 10[1]	4 W / 20 Hz, N = 10[1]	6 W / 20 Hz, N = 10[1]	Hand Piece, N = 10[1]	P-value[2]
Total score	7.3 ± 0.7	7.8 ± 0.8	6.8 ± 1.4	5.0 ± 0.0	<0.001

Note: [1]Mean ± SD [2]One-way ANOVA

original compact thickness, by electrical saw Figure 1, the total number used for this study was four blocks.

For the comparative purpose of study between conventional and laser osteotomy, the bone blocks are categorized as follows:

G1: In this bone specimen, the osteotomy was performed by an ER CR: Y S GG laser with the following parameters: 6 watts, 20 Hz, 50% air, 50% water.

G2: In this bone specimen, the osteotomy was performed by an ER CR: Y S GG laser with the following parameters: 4 watts, 20 Hz, 50% air, 50% water.

G3: In this bone specimen, the osteotomy was performed by an ER CR: YS GG laser with the following parameters: 2.5 watts, 20 Hz, 50% air, 50% water.

G4: The osteotomy was performed using a low-speed handpiece (20000- 40000 rpm) and around bur (stainless ISO size 18).

A CNC (computed numerical control) device will be used for conventional and laser osteotomy. This device provides a more precise cutting procedure and verified experimental conditions (Fig. 2). Bone samples were mounted on an X–Y table and traversed across the X plane at a speed of 0.8mm/s during laser and conventional osteotomy.

IRRADIATION FOR LASER OSTEOTOMY

Each specimen was irradiating with Er, CR: YCGG laser 2790nm for 25s for each parameter. Irradiation was performed in contact mode to make a 2 cm incision using an MGG6-6 laser tip. Different laser parameters were used in each irradiation episode (Fig. 3).

HAND PIECE OSTEOTOMY (CONVENTIONAL)

In the control group, a 2 cm incision was made in the bone specimen using a low-speed handpiece and around bur (stainless ISO size 18) for 25 s (Fig. 4).

HISTOLOGICAL EVALUATION

After the laser and conventional osteotomy, 10% buffered formalin solution was used to fix the specimens.

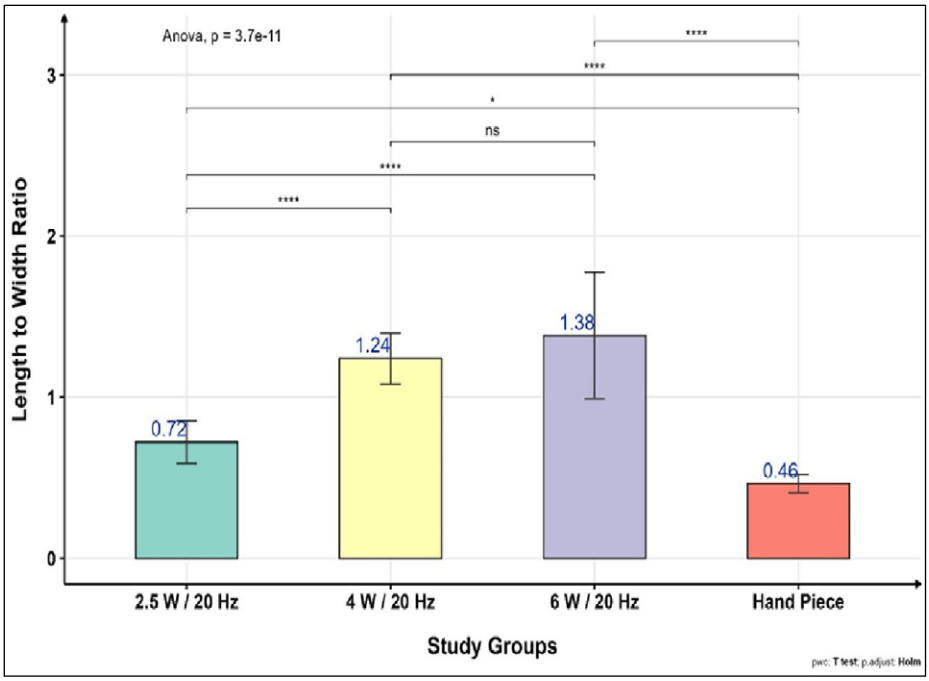


Fig. 5. Tukey's post-hoc test for the differences in length-to-width ratio between study groups
Source: Own materials

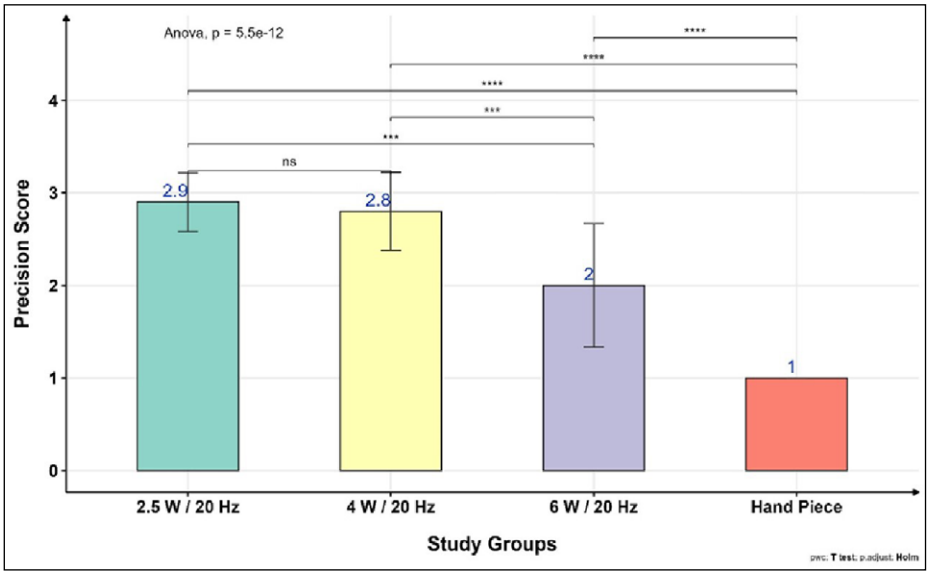


Fig. 6 Tukey's post-hoc test for the differences in precision score between study groups
Source: Own materials

They were then delivered to the histology lab for a histological analysis the same day.

SLIDES NUMBER

Ten stained slides were obtained from each bone block, and 40 slides were taken. 10 slides for the control group and 30 slides for the laser group.

HISTOLOGICAL CRITERIA

For histological assessment, each slide was examined under a light microscope by a histopathologist to evaluate the following for laser and conventional osteotomy incision:

1 - Length of incision
2 - Width of incision
3 - Length to width ratio: scored from 1-3 according to the following:
<0.7 - 1
0.7-1.5 - 2
1>1.5 - 3
4 - Carbonization: scored from 1-3 according to the f1ollowing:
1Sever - 1
Moderate - 2
Mild - 3
5-Precision: scored from 1-3 according to the following:
Bad - 1
Fair - 2
1Good - 3

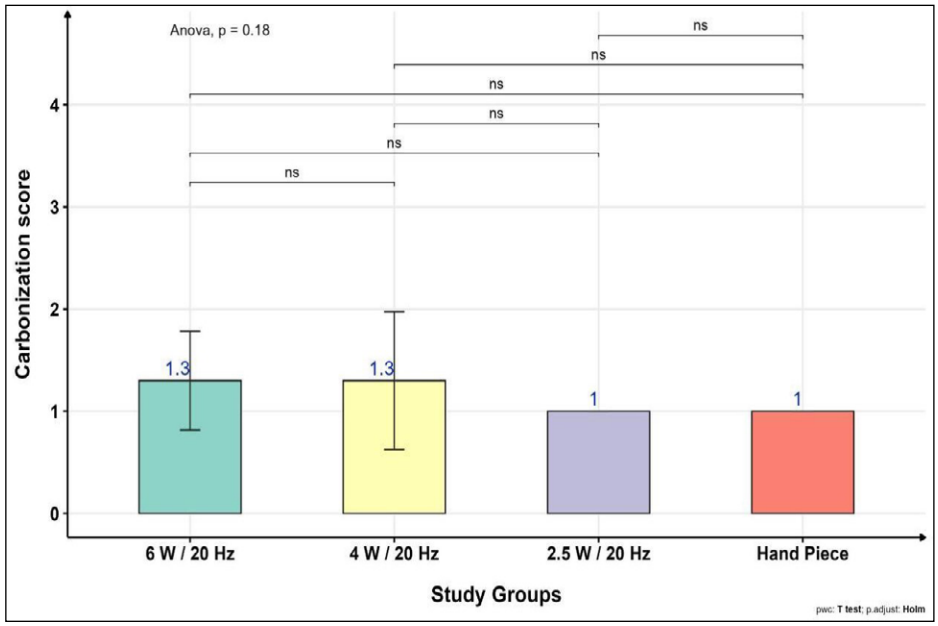


Fig. 7. Tukey's post-hoc test for the differences in carbonization score between study groups
Source: Own materials

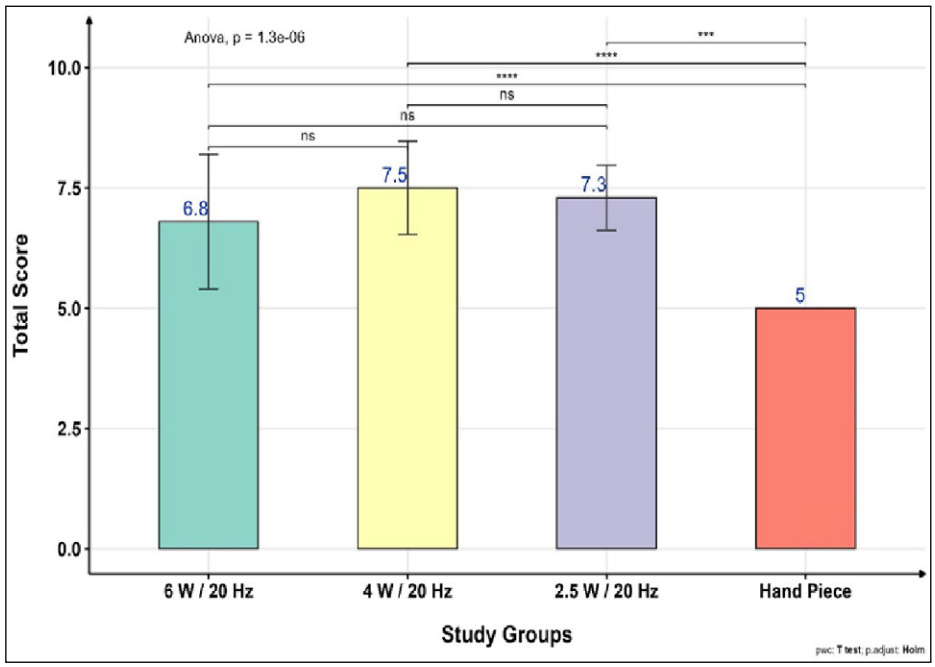


Fig. 8. Tukey's post-hoc test for the differences in total score between study groups
Source: Own materials

STATISTICAL ANALYSIS

Continuous variables were expressed as means and standard deviations. Categorical variables were expressed as frequency and percentages. The one-way ANOVA and Tukey's post-hoc test with holm adjustment was performed to find the differences in means between the study groups. The difference between categorical variables was investigated using Fisher's exact test. A P-value less than 0.05 was considered statistically significant. R software packages (dplyr, gt_summery and ggplot) were used for data processing, visualization, and statistical analysis ("R version 4.2.2, R Foundation for Statistical Computing, Vienna, and Austria").

ETHICS APPROVAL

Ethics approval was taken from the Research & Ethics Committee at the Institute of Laser for Postgraduate Studies/University of Baghdad (464 in 10-10-2023).

RESULTS

THE LENGTH, WIDTH, AND LENGTH-TO-WIDTH RATIO (L/W RATIO)

The comparative analysis of bone cutting dimensions among the four study groups - 2.5 W/20 Hz, 4 W/20 Hz, 6 W/20 Hz Er, Cr: YSGG laser settings, and the conventional

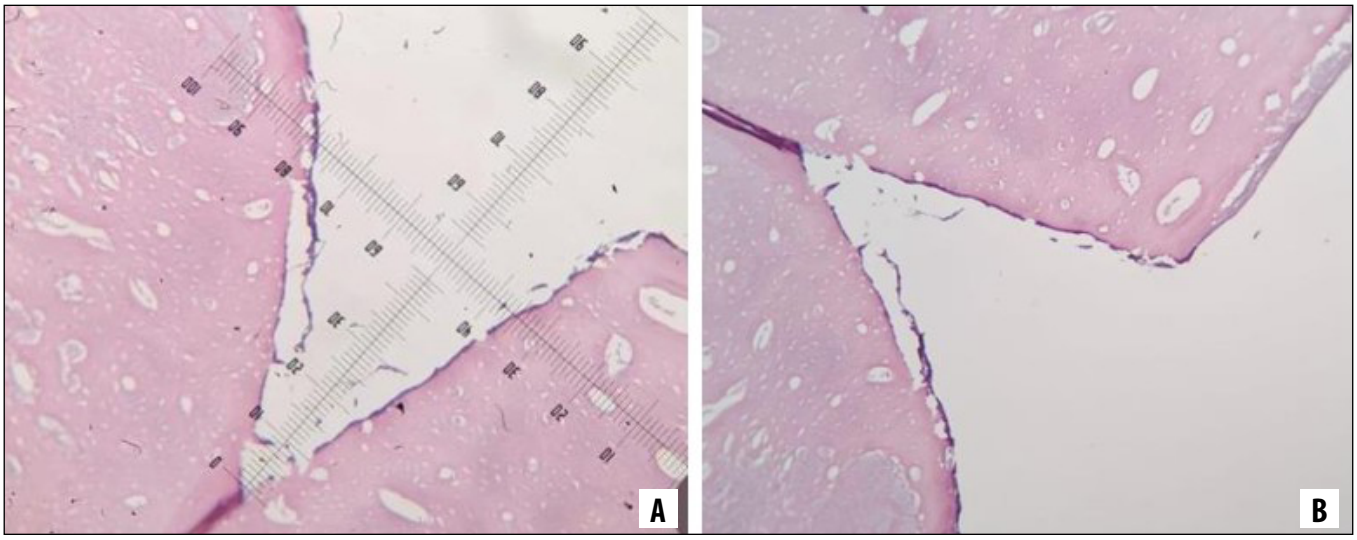


Fig. 9. Histological sections of Er, Cr:YSGG laser osteotomy at 4 W/20 Hz: A. Histomorphometric measurement showing a deep, narrow, and precise laser incision; B. Well-defined incision margins with minimal carbonization
Source: Own materials

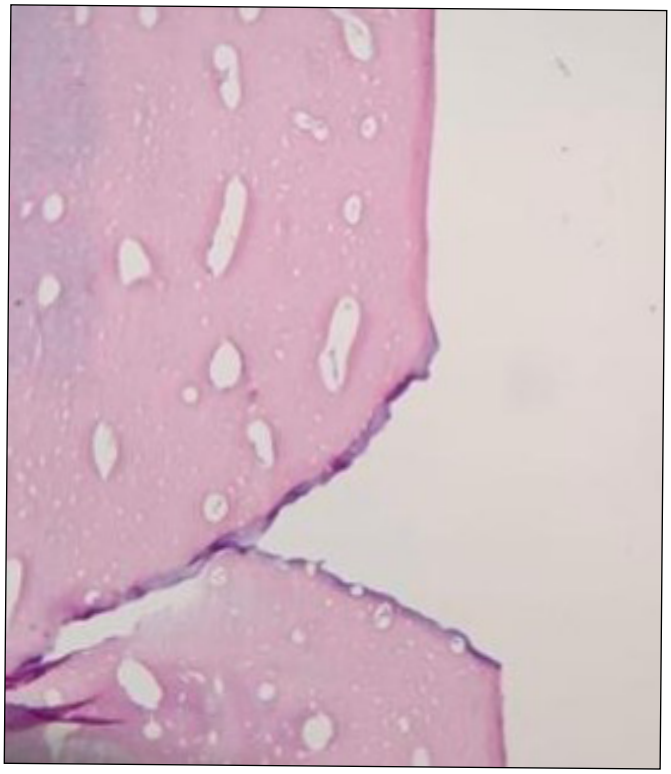


Fig. 10. Histological sections of laser osteotomy of 2.5 WATT, 20 HZ
Source: Own materials

handpiece - revealed statistically significant differences p<0.001 in all measured parameters. The 4 W/20 Hz laser group exhibited the greatest mean cut length 0.91±0.08 mm, significantly higher than all other groups, whereas the handpiece group had the shortest 0.42±0.08 mm. In terms of width, the handpiece produced the widest cuts 0.94±0.24 mm, while the 6 W/20 Hz laser group produced the narrowest 0.47±0.18 mm. The highest length-to-width ratios were observed in the 6 W/20 Hz (1.38±0.39) and 4 W/20 Hz (1.24±0.16) groups, indicating more elongated cuts, whereas the handpiece group had the lowest ratio 0.46±0.06, indicating broader, less elongated cuts. Grading of the length-to-width ratio further supported these findings: all handpiece specimens fell into Grade 1 (<0.7), indicating low elongation, while the majority of 4 W/20 Hz specimens were Grade 2 (0.7–1.5), and the 6 W/20 Hz group displayed the widest distribution across all three grades, including 40% in Grade 3 (>1.5) as show in table 1 and Figure 5.

PRECISION AND CARBONIZATION

The assessment of cut precision and carbonization levels among the study groups revealed statistically significant differences p<0.001, underscoring the impact of varying Er, Cr: YSGG laser settings and conventional handpiece use on bone cutting quality. Precision scores were highest in the 2.5 W/20 Hz group 2.9±0.3, followed closely by the 4 W/20 Hz group 2.8±0.4, indicating superior cutting accuracy compared to both the 6 W/20 Hz laser 2.0±0.7 and the handpiece 1.0±0.0. Notably, 90% and 80% of specimens in the 2.5 W and 4 W groups, respectively, were graded as having good precision, whereas all handpiece specimens were graded as poor. Regarding carbonization, the lowest levels were observed in the 2.5 W group and the hand-piece group 1.0±0.0, with all specimens demonstrating only mild carbonization. Moderate to severe carbonization was increasingly observed with higher power settings: 10% of the 4 W group and 30% of the 6 W group showed moderate to severe changes as shown in figures 6-7 and Table 2.

The comparative analysis of the total score, which integrates multiple parameters assessed in this study,

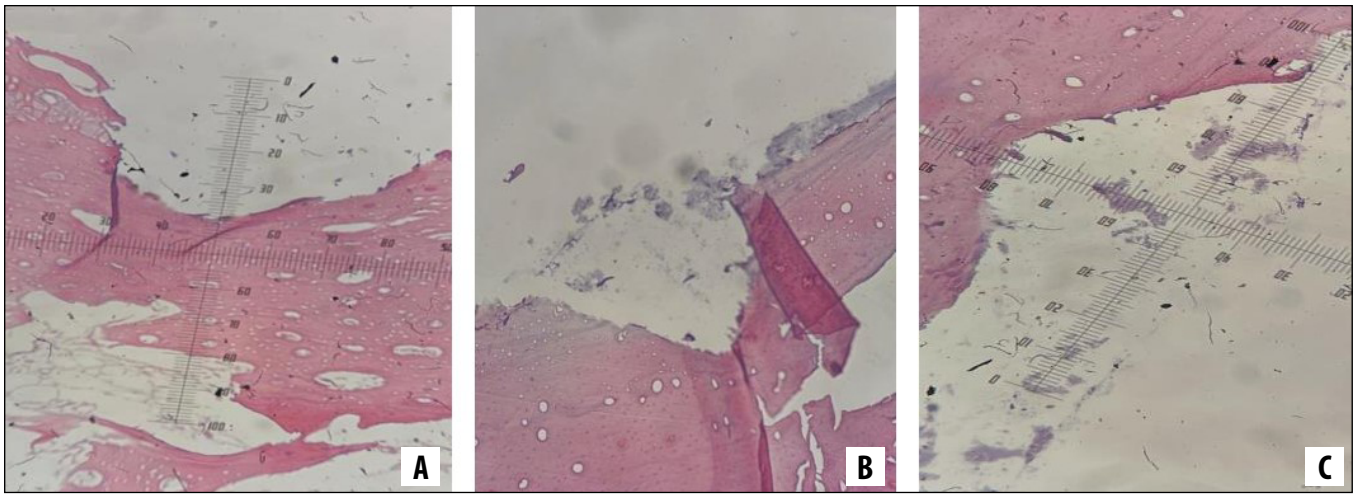


Fig. 11. Histological sections of conventional osteotomy: A. Histomorphometric measurement showing a short and wide incision; B. Irregular incision margins with disruption and fragmentation of the bone; C. Wide and irregular osteotomy surface with minimal carbonization
Source: Own materials

demonstrated statistically significant differences among the four groups $p<0.001$. The highest mean total score was observed in the 4 W/20 Hz laser group 7.8±0.8, followed closely by the 2.5 W/20 Hz group 7.3±0.7, indicating superior overall performance in terms of bone cutting efficiency, precision, and minimal thermal damage. The 6 W/20 Hz group exhibited a moderately lower total score 6.8±1.4, reflecting a decline in overall performance likely due to decreased precision and increased carbonization. In contrast, the handpiece group had a markedly lower and uniform total score 5.0±0.0, significantly inferior to all laser-treated groups, highlighting the limitations of the conventional method in achieving optimal surgical outcomes, as shown in table 3, figures 8-11.

DISCUSSION

In various fields of dentistry, laser technology and minimally invasive therapy are attracting interest. The newest laser type, the Er, Cr: YSGG laser, is ideal for surgical procedures, including osteotomy, because it can precisely coagulate arteries and ablate both soft and hard tissue while minimizing heat damage [17]. Because of its high stiffness, high mineral and water content, and quick heat dispersion from the initial absorption layer, hard bone tissue presents difficulties for laser osteotomy [18]. The Er, Cr: YSGG pulsed laser's water and air sprays may allow for precise, clean, and straight ablation of hard bone tissue with little thermal damage to surrounding tissue [19]. Turbines, hand pieces, and burs are examples of conventional surgical instruments that restrict access, interfere with depth control, release aerosolized particles, and induce heat necrosis. The development of laser bone surgery methods offers a substitute for conventional instruments and processes [20]. In the present study, the cutting efficiency of the bone is compared between Er, Cr: YSGG pulsed laser and the hand piece (conventional method). The comparative analysis of bone cutting length among the four study groups - 2.5 W/20 Hz, 4 W/20 Hz, 6 W/20 Hz Er, Cr: YSGG laser settings, the 4 W/20 Hz laser group exhibited the greatest incision length significantly higher than all other groups, whereas the handpiece group had the shortest incision length. In contrast to earlier studies that presented differences in cutting depth, the histological depth of the cut is comparable between the laser and the hand piece [21]. The width of bone incision is widest in handpiece sample, while the 6 W/20 Hz laser group produced the narrowest incision. The highest length-to-width ratios were observed in the 6 W/20 Hz, and 4 W/20 Hz groups, indicating more elongated cuts, whereas the handpiece group had the lowest ratio indicating broader, less elongated cuts. The length-to-width ratio of a surgical incision refers to the relationship between the length of the incision and its width. This ratio can indeed affect the quality of the surgical incision in terms of healing, scar formation, and overall patient outcomes. Decreased width of the incision and increased length have good impacts on the quality of laser incisions. The result of the present study match with recent studies showing that, in comparison to conventional handpiece burs, laser bone cutting provides smaller, more accurate incisions with less mechanical and thermal damage. These benefits could lead to better healing results and higher-quality osteotomies [22]. The current study's histology and statistical findings demonstrate that, especially when

compared to traditional bur procedures, the Er, Cr: YSGG laser provides the most precise bone cut at various depths [23]. Clinically, thermo-mechanical tissue ablation using infrared lasers, particularly erbium lasers, is still regarded as the most dependable and progressive method because these laser systems are only appropriate for high precision osteotomy of very small and thin bone structures [24]. When accurate tissue removal or drilling is needed without destabilizing pieces, the use of lasers in bone cutting is advantageous. Bone fracture risk can be reduced, if not completely eliminated, by laser drilling thin bones [25]. In the current study, the Er, CR: YSGG laser showed the highest level of bone cut accuracy at all depths; there were no bone fragments at any laser incision, these findings confirm those of other authors [26]. It is more challenging to construct an ideal osteotomy line or an anatomically correct drilling technique when using conventional osteotomy samples because of the large torque moment or because the bur's serration is coarser. Obtained results align with the previous study observations [27]. In the present study hard tissue laser have shown better results due to greater precision as they preserved the bone morphology, compared with low-speed handpiece. The obtained results agreed with previous studies [28]. Similar to earlier studies, specimens with a high frequency and low radiation power output showed better incision quality, particularly at 20 Hz [29-30]. The incision length was highest in the 4 W/20 Hz group and, lowest in the hand piece group. Deeper ablation can be achieved simply by raising the average power, increasing the energy, and lowering the repetition rate [31]. Carbonization, which is undesirable in medical laser applications because it increases thermal unfavorable effects on adjacent tissues and slows recovery, is reached as the temperature rises with continued laser exposure. The size, exposure time, and location of the heat deposited inside the tissue are the primary determinants of the ablation's geographic extent [32]. Heat damage and carbonizations are a major concern in our study, but all examine samples of the laser group show no thermal damage. Because erbium lasers use water as their primary chromophore, they are a great option for surgical hard tissue procedures. The thermal relaxation time is facilitated by the erbium laser's pulse emission mode and sufficient cooling with an aerosol, ideally composed of air and water [15, 33]. The results of present study match with evidence from prior *ex vivo* experiments that demonstrated that the Erbium laser did not cause any heat damage to bone tissue when osteotomy was carried out with cooling air/ water irrigation [34]. All specimens demonstrate only mild carbonization. The lowest levels of carbonation were observed in the hand piece group [2]. Finally, the histological results showed high incision quality with the Er, Cr: YSGG laser system, versus slow speed handpiece. The interpretation related to laser-tissue interaction, which depends on multiple laser intrinsic and extrinsic parameters (wavelength, power, mode of emission, beam profile, pulse duration, frequency, and spot size), and on the chemical and optical properties of the targeted tissue (soft tissue chromophores; water and pigments) [35-36]

CONCLUSIONS

In *ex vivo* studies, the Er, Cr: YSGG laser allows for accurate surgical ablation with little thermal damage to nearby tissues. The optimal result is obtained with a 4watt, 20Hz setting.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

A study of nurses' readiness to learn about and use telemedicine

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ABSTRACT

Aim: To determine the sociodemographic characteristics of nurses who participated in a telemedicine network development project, their readiness for training and implementation of telemedicine, as well as their awareness of telemedicine; and to establish or refute an association between demographic and professional characteristics and attitudes towards digital technologies and telemedicine.

Materials and Methods: The study sample comprised 285 nurses. The survey covered demographic and professional characteristics, namely age, gender, total length of service in healthcare, and the level of the healthcare facility where respondents worked. Respondents were asked four questions: 1. Please indicate your affinity for/readiness to use digital technologies. 2. Would you like to learn about telemedicine technologies for use in your practice? 3. Are you sufficiently knowledgeable about telemedicine? 4. What type of training in telemedicine would you choose? (multiple choice).

Results: The results of the study showed that the sociodemographic characteristics of the cohort were represented predominantly by women of working age, indicating a largely experienced workforce, most of whom worked in primary healthcare. A significant association was found between higher digital readiness and the desire to acquire telemedicine competencies.

Conclusions: The study found a significant association between digital readiness and motivation to adopt telemedicine technologies. Nurses preferred online training in telemedicine as part of their continuing professional development. The study also found that awareness of telemedicine among nurses was low.

KEYWORDS: nurses, telemedicine, digitalisation, digital engagement, education

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INTRODUCTION

The education of healthcare workers is an integral part of the ongoing development and reform of the healthcare system in Ukraine during a period of martial law and critical strain on healthcare resources. A shortage of healthcare staff and increasing competency demands are characteristic features of staffing trends in the healthcare sector in Ukraine and other countries [1, 2].

Our previous research, as well as studies by other authors on the educational component of professional pre-higher and higher medical and pharmaceutical education, convincingly demonstrate that improving approaches and methods for training mid-level healthcare professionals is a pressing issue that is receiving increasing attention from educators and healthcare administrators [3, 4].

The WHO places significant emphasis on monitoring and the development of strategic documents on nursing development. According to the report "State of the World's Nursing 2025", despite a gradual increase in the number of nurses worldwide, their uneven distribution is simultaneously increasing, particularly in low- and middle-income countries [5]. The WHO's recommendations for a global strategy to address the shortage and uneven distribution of nurses in health systems were adopted in a resolution identifying four priority areas for development: education, workforce, leadership, and service delivery. Among these, the strategic direction for education aims to ensure that health systems are staffed with personnel possessing the necessary competencies and levels of knowledge, to develop educational programmes based on a competency-based approach, to apply innovative teaching methods, and to ensure that educators are properly trained in best pedagogical practices and technologies, demonstrating expertise in relevant subject areas [6].

The Resolution of the Cabinet of Ministers of Ukraine on the Strategy for the Development of the Healthcare

System for the Period up to 2030 sets out the strategic directions for the development of nursing in Ukraine, in particular the improvement of education and the raising of professional standards for nurses [7].

AIM

To determine the sociodemographic characteristics of nurses who participated in a telemedicine network development project, their readiness for training and implementation of telemedicine, as well as their awareness of telemedicine; and to establish or refute an association between demographic and professional characteristics and attitudes towards digital technologies and telemedicine.

MATERIALS AND METHODS

The study was conducted as part of a telemedicine project aimed at developing telemedicine networks in Ukraine across three regions: Chernihiv, Odesa and Rivne. The study cohort comprised 285 nurses representing 93 healthcare facilities at various levels of care provision. The survey was conducted using Google Forms without the collection or processing of personal data, which encouraged candid responses. The survey ran from August to December 2025 and covered demographic and professional indicators, namely: age, gender, total length of medical service, and the level of the healthcare facility where the respondent works. The level of the healthcare facility where the respondents worked was determined in accordance with the Resolution of the Cabinet of Ministers of Ukraine [8]. Respondents were asked four questions: 1. Indicate your affinity/readiness for digital technologies; 2. Are you interested in learning about telemedicine technologies for use in your practice? 3. Are you sufficiently knowledgeable about telemedicine? 4. What type of training in telemedicine would you choose? (multiple choice). Responses to the first two questions were collected using a Likert scale, where 1 represents the lowest response intensity and 10 the highest. The possible answers to the question "Are you sufficiently familiar with telemedicine?" were as follows: yes, I consider myself sufficiently familiar (a); sufficiently familiar, but I need to improve my knowledge and skills in the use of telemedicine methods and tools (b); I am not sufficiently knowledgeable and need to improve my knowledge and skills in the methods and tools of telemedicine (c); I am not at all knowledgeable, so I need regular training to improve my knowledge and skills in the methods and tools of telemedicine (d). Answers to the question "What type of training in telemedicine would you choose? (multiple choice)": online courses within the continuing professional development system (CPD), practical seminars/training sessions within the continuing professional development system, webinars outside the continuing professional development system, mentoring/individual consultations (outside the CPD system), self-study of materials, thematic training at educational institutions within the CPD system, any educational activities within the CPD system.

Descriptive statistics for ordinal variables are presented as the median (Me) and interquartile range ($IQR = Q_1–Q_3$); the arithmetic mean with standard deviation ($M \pm SD$) is also provided. For categorical variables, absolute (n) and relative (%) frequencies were calculated with 95% confidence intervals (CI) using the Wilson score interval method. The normality of the distribution of quantitative variables was tested using the Shapiro-Wilk test. Based on the test results ($p<0.001$ for both scale variables), non-parametric methods of analysis were selected. To assess the relationship between digital readiness and motivation to learn telemedicine, Spearman's rank correlation coefficient (ρ) was used, with the coefficient of determination (ρ^2) calculated. The strength of the relationship was interpreted using the Chaddock scale.

Comparisons of indicators between independent groups (by age, length of service, and type of healthcare facility where nurses work) were performed using the Kruskal–Wallis test (H-test) with calculation of the effect size (η^2). Analysis of multiple-choice questions was carried out using dummy coding (binary decomposition) followed by a frequency analysis of each response option. Statistical data processing was carried out using the SciPy 1.0, NumPy and pandas libraries.

ETHICS

The study was conducted in accordance with the Declaration of Helsinki and the bioethical standards for sociological research in the medical field. All participants (N=285) provided voluntary informed consent. Participation was anonymous, thereby ensuring the protection of personal data and professional reputation. The results are presented in summary form, making it impossible to identify individuals. The study protocol was approved by the local bioethics committee of the Bogomolets National Medical University.

FRAMEWORK

The study was conducted within the framework of a research project of the Bogomolets National Medical University 2023–2025: «Digitalization and digital transformation in healthcare» (state registration number 0121U107784).

Table 1. Age distribution of the study cohort

Age	n (abs.)	% of the total	95% CI lower limit	95% CI upper limit
up to 30 years old	16	5.6%	3.5%	8.9%
31–40 years old	77	27.0%	22.2%	32.5%
41–50 years old	97	34.0%	28.8%	39.7%
51–60 years old	73	25.6%	20.9%	31.0%
over 60 years old	22	7.7%	5.2%	11.4%

Source: compiled by the authors of this study

Table 2. Distribution of the study group by length of service

Length of service	n (abs.)	% of the total	95% CI lower limit	95% CI upper limit
up to 5 years	10	3.5%	1.9%	6.3%
6–10 years	16	5.6%	3.5%	8.9%
11–20 years	75	26.3%	21.5%	31.7%
21–30 years	86	30.2%	25.1%	35.7%
over 30 years	98	34.4%	29.1%	40.1%

Source: compiled by the authors of this study

Table 3. Breakdown of the study group by type of healthcare facility

Level of healthcare facility	n (abs.)	% of the total	95% CI lower limit	95% CI upper limit
Primary Healthcare Centre	176	61.8%	56.0%	67.2%
General healthcare facility	93	32.6%	27.5%	38.3%
Cluster-based healthcare facility	11	3.9%	2.2%	6.8%
Super-cluster healthcare facility	5	1.8%	0.8%	4.0%

Source: compiled by the authors of this study

AI STATEMENT

The Claude Opus 4.3 artificial intelligence (AI) tool was used in this work for the following tasks: search for literature using keywords under the authors' control; formation and formatting of the bibliography under the authors' control; analysis of statistical data under the authors' control; stylistic processing of the text under the authors' control; language checking and correction under the authors' supervision; multi-level error detection. All sections using AI were reviewed and edited personally by the authors. The scientific statements, conclusions, and results are the authors' own intellectual contribution.

RESULTS

A total of 285 nurses took part in the study. The sample was almost entirely homogeneous in terms of gender: 283 women (99.3%) and 2 men (0.7%), which is entirely consistent with the overall gender composition of nursing staff within the Ukrainian healthcare system.

The age structure of the sample was characterised by a predominance of middle-aged respondents. The results of the age distribution are shown in Table 1.

Table 2 shows the composition of the study group by length of service. In terms of total length of medical service, the largest group was that with over 30 years' experience (n=98; 34.4%), which correlates with the predominance of older age groups.

By type of healthcare facility, the vast majority were staff from primary healthcare centres – 176 people (61.8%). General healthcare facilities were represented by 93 respondents (32.6%). Cluster (n=11; 3.9%) and supercluster (n=5; 1.8%) facilities were represented to a minimal extent, reflecting the staffing structure of primary care as the most numerous in the healthcare system (Table 3).

The sample under study therefore consists predominantly of middle-aged women with considerable professional experience who work in primary healthcare.

Self-assessed readiness for digital technologies on a 10-point scale was: Me=8.0 [IQR: 5.0–9.0], M=7.29±2.44. The distribution deviated from normal (Shapiro-Wilk test W=0.890; $p<0.001$) with left-sided skewness (skewness=-0.86), indicating a bias in responses towards higher values. A score of ≥7 was selected by 69.5% of respondents (n=198), whilst only 8.4% (n=24) indicated low readiness (score 1–3). The data obtained indicate

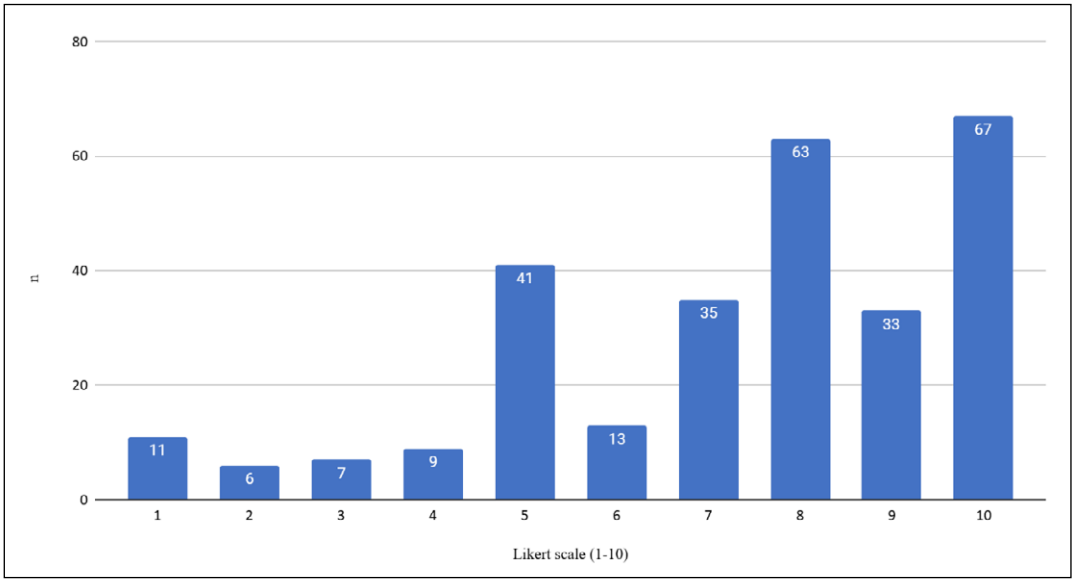


Fig. 1. Nurses' self-assessment of their readiness for digital technologies
Picture taken by the authors

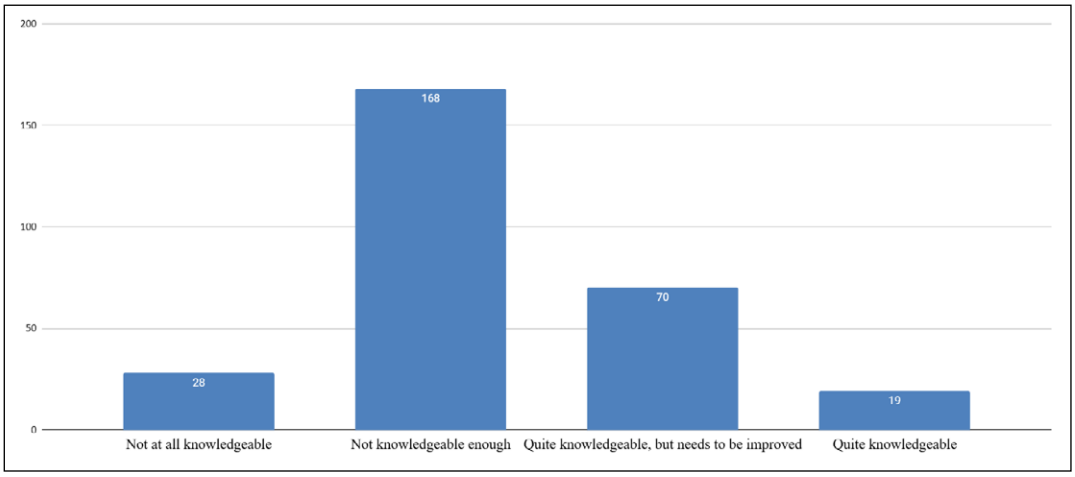


Fig. 2. Nurses' interest in training in telemedicine
Picture taken by the authors

a predominantly high level of subjective readiness among nurses to use digital technologies (Fig. 1).

Fig. 2 shows the distribution of responses regarding the desire to learn about telemedicine. Motivation to learn telemedicine technologies, measured on a 10-point scale, was found to be even higher than the previous level of affinity for digital technologies: Me=9.0 [IQR: 6.0–10.0], M=7.76 ± 2.55. The distribution is non-normal (W=0.821; p<0.001) with marked left-sided skewness (skewness =–1.08). The maximum score (10) was selected by 107 respondents (37.5%), and a score of ≥8 by 189 individuals (66.3%). Only 7.0% (n=20) reported low motivation (score 1–3). Thus, the vast majority of nurses demonstrate a high willingness to acquire new knowledge in the field of telemedicine.

Only 19 respondents (6.7%; 95% CI: 4.3–10.2%) considered their level of knowledge to be sufficient (a). Seventy respondents (24.6%; 95% CI: 19.8–29.9%) acknowledged partial awareness but indicated a need to improve their knowledge and skills in telemedicine methods and tools (b). The majority of respondents — 168 (58.9%; 95% CI: 53.0–64.5%) — reported that they were not sufficiently knowledgeable and needed to improve their knowledge and skills (c). A further 28 respondents (9.8%; 95% CI: 6.9–13.8%) reported a complete lack of awareness and the need for regular training (d). Overall, 93.3% of respondents (n = 266) required additional training in telemedicine, which justifies the implementation of appropriate educational programmes (Fig. 3).

The relationship between digital readiness and motivation to learn. A Spearman's correlation analysis revealed a statistically significant, noticeable positive correlation (Chaddock scale) between digital readiness and the desire to learn telemedicine (ρ=0.669; p<0.001). The coefficient of determination ρ^2=0.447 indicates that digital readiness explains approximately 44.7% of the variation in motivation to learn. This is a significant finding of the study: respondents with higher digital readiness demonstrate a significantly greater desire to master telemedicine technologies.

Preferences regarding the type of training (Fig. 4). A multiple-choice analysis (437 responses with N=285) re-

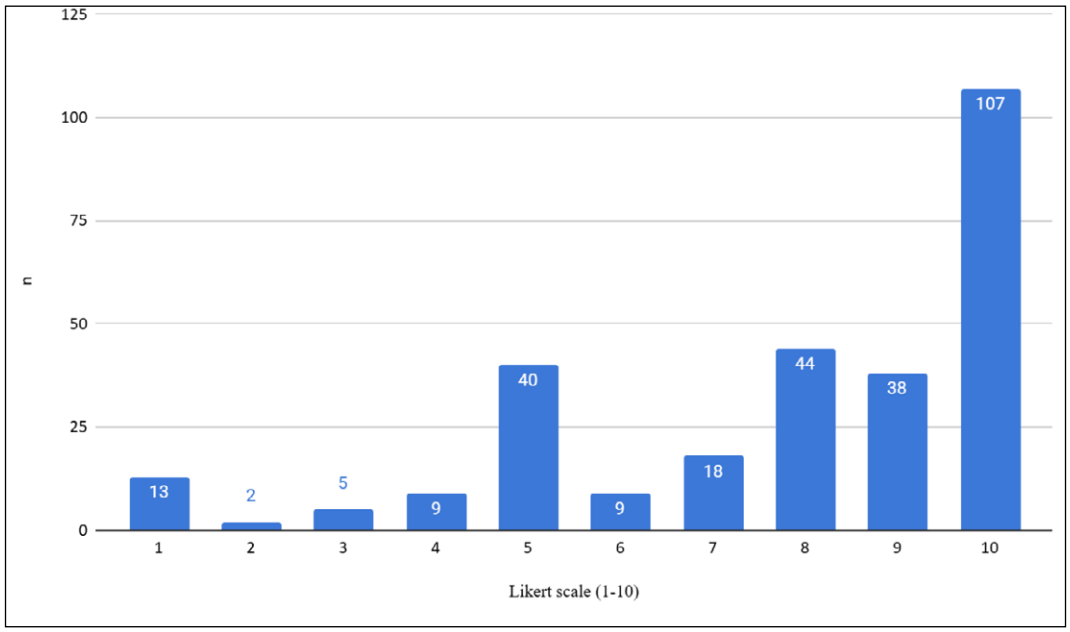


Fig. 3. Respondents' awareness of telemedicine *Picture taken by the authors*

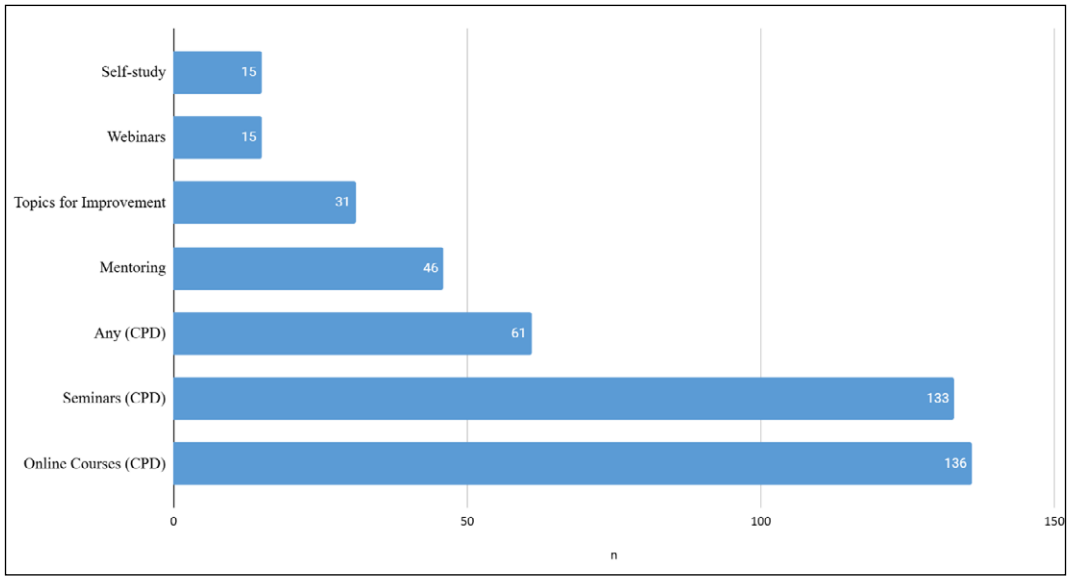


Fig. 4. Choice of telemedicine training programme *Picture taken by the authors*

vealed that the most popular formats are online courses within the continuing professional development system (n=136; 47.7%; 95% CI: 41.9–53.6%) and practical seminars/training sessions within the continuing professional development system (n=133; 46.7%; 95% CI: 40.9–52.5%). Sixty-one respondents (21.4%) chose any form of training within the CPD system, 46 (16.1%) chose mentoring and individual consultations outside the CPD system, and 31 (10.9%) chose subject-specific development at educational institutions. Webinars outside the CPD system (n=15; 5.3%) and self-study of materials (n=15; 5.3%) proved to be less popular.

The preference for these formats within the continuing professional development (CPD) system indicates that respondents have confidence in institutional mechanisms for professional development. Furthermore, future research should explore the potential association between digital affinity and preferences for online learning formats.

Additionally, the results reveal an interesting pattern: within the study cohort, variation in motivation to learn telemedicine is primarily associated with respondents' individual levels of digital readiness, whereas demographic characteristics (age, length of service, type of healthcare facility) do not show a statistically significant association with this indicator (age: $H[4] = [value]$, $p>0.05$, $\eta^2<0.01$; length of service: $H[4] = [value]$, $p>0.05$, $\eta^2<0.01$; type of facility: $H[3] = [value]$, $p>0.05$, $\eta^2<0.01$). This may indicate that nurses' readiness to acquire telemedicine competencies is determined more by personal and professional factors linked to previously acquired digital competence than by age or gender.

However, these observations are descriptive in nature and require confirmation in future studies.

DISCUSSION

The issue of nursing education has attracted the attention of many researchers in various respects. In particular, questions regarding the organisation of different forms and methods of training became particularly relevant during the COVID-19 pandemic, when virtual educational interventions took precedence over other forms of training in cases where clinical training was not required [9]. The introduction of an electronic health system in Ukraine and the development of the e-health ecosystem have led to the establishment of fundamentally new rules and requirements for healthcare professionals regarding work in a digital professional environment [3, 4, 10]. The importance of digital competences and skills among nurses in applying digital technologies in practice, including for communication within the professional environment and with patients, is beyond doubt and is gradually becoming part of professional standards, requiring changes to curricula and programmes for bachelor's and master's level education in medical and pharmaceutical fields [11–13].

Our study closely aligns with the research by Hariyati RTS, Handiyani H et al. (2024) on nurses' digital literacy and its relationship with age and professional characteristics [11]. However, our study is directly linked to the implementation of telemedicine in the practical work of nurses, which makes it more focused on issues relating to the development and implementation of telemedicine in the practical work of healthcare institutions.

An integral and unique feature of the Ukrainian digital landscape is the implementation and development of an electronic healthcare system and eHealth at the national level, where nurses play a specific role as eHealth stakeholders and are a vital component of the healthcare system's resilience in the face of crises and security challenges.

CONCLUSIONS

1. The findings presented here represent the first comprehensive empirical study of nurses' readiness to learn telemedicine technologies in Ukraine.
2. The study employed a three-dimensional approach to assessing digital engagement, readiness to learn telemedicine technologies, and identifying educational preferences among nurses.
3. The study found a strong link between digital readiness and the motivation to adopt telemedicine technologies, as evidenced by a noticeable Spearman correlation ($\rho = 0.669$; $p < 0.001$).
4. Nurses prefer to learn about telemedicine technologies as part of their continuing professional development through online training, which further demonstrates their strong commitment to and readiness for the use of digital technologies.
5. The study found that awareness of telemedicine technologies among nurses is low, which calls for a review of approaches to the development of curricula and programmes at undergraduate and postgraduate levels, with a need to update and develop educational programmes on telemedicine technologies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Clinical and sociodemographic predictors of psychological health outcomes in cancer patients undergoing active treatment at the National Teaching Cancer Hospital, Najaf: AI-assisted predictive analysis

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ABSTRACT

Aim: To identify clinical and sociodemographic predictors of anxiety and depression among cancer patients undergoing active treatment using XGBoost algorithm.

Materials and Methods: A cross-sectional study was conducted among 323 cancer patients undergoing active treatment at National Teaching Hospital for Oncology, Iraq, selected by systematic sampling. Psychological status was assessed using Hospital Anxiety and Depression Scale (HADS). Clinical and Sociodemographic data were collected through patient interviews and clinical records. Linear regression analysis was employed to determine predictive power of age, cancer type, treatment modality, disease stage, duration, and comorbidities. An XGBoost machine learning classifier was applied to improve prediction of depression and anxiety based on identified clinical and Sociodemographic features.

Results: Anxiety prevalence was 78.4% (was estimated as severe: 47.7% and moderate: 30.7%), while depression 80.2% (severe: 50.5% and moderate: 29.7%). Age, cancer type, and treatment modality (chemotherapy and radiotherapy) were significant predictors ($P<0.001$). First year after diagnosis of disease, active treatment period, and disease stage II were identified as critical periods for psychological instability. Patients with chronic comorbidities demonstrated significantly higher psychological levels, indicating a cumulative physical and functional burden. The XGBoost model achieved high predictive performance (accuracy 1.0%, confirming the reliability of Sociodemographic and clinical indicators in predicting high-risk patients.

Conclusions: Cancer patients are vulnerable to anxiety and depression during early stages of diagnosis and treatment, exacerbated by chronic comorbidities. Incorporating routine psychological screening using HADS, with advanced predictive models like XGBoost, may facilitate early identification of high-risk patients and support targeted interventions to improve quality of life.

KEYWORDS: cancer, depression, anxiety, HADS, predictors, machine learning XGBoost

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INTRODUCTION

Cancer remains one of the leading causes of death worldwide, with approximately 10 million deaths in 2020, and the global burden is expected to increase in the coming decades [1]. Beyond its physical impact, cancer imposes a substantial psychological burden that adversely affects quality of life, treatment adherence, and clinical outcomes [2]. Depression and anxiety are among the most common psychological disorders in cancer patients, with prevalence rates significantly higher than those observed in the general population [3]. In the Middle East and North Africa (MENA) region, pooled estimates indicate depression and anxiety rates 44% and 47%, respectively [4]. In Iraq, cancer patients also experience considerable levels of anxiety 38.9% and depression 34.7%, influenced by sociodemographic and clinical factors such as gender, marital status, employment status, pain and disease-related conditions [5]. Untreated psychological distress has significant clinical consequences and has been associated with increased cancer-specific mortality and poorer health outcomes [6]. Furthermore, psychological symptoms frequently remain underrecognized and undertreated among cancer patients [7]. Despite the growing recognition of psychosocial care in oncology, there remains limited evidence regarding the sociodemographic and clinical predictors of anxiety and depression among Iraqi cancer patients. Therefore, this study aims to identify key predictors of anxiety and depression among patients undergoing active treatment at the National Teaching Hospital for Oncology in Najaf,

Iraq. In addition, the XGBoost algorithm was employed to improve predictive accuracy, as machine learning approaches can better capture complex relationships among clinical and sociodemographic variables compared with traditional statistical methods [8].

AIM

The aim of this research is to identify clinical and Sociodemographic predictors of anxiety and depression among cancer patients undergoing active treatment using XGBoost algorithm.

MATERIALS AND METHODS

STUDY SETTING AND STUDY SAMPLE

A cross-sectional study was conducted at the National Teaching Hospital for Oncology in Najaf, Iraq. between October 2025 and January 2026. The study included adult cancer patients undergoing active treatment during the study period. A total of 323 participants were selected using systematic random sampling. Sample size was calculated using the single population proportion formula with a 20% non-response rate [9].

ELIGIBILITY CRITERIA

Inclusion criteria: Adults with any confirmed cancer type undergoing active treatment and able to provide verbal informed consent were selected.

Exclusion criteria: Patients younger than 18 years, with cognitive or mental disorders, critical medical conditions, ICU admission, or refusal to participate were excluded.

DATA COLLECTION

Data were collected using a standardized questionnaire that was back-to-back translated into Arabic. The researchers conducted direct interviews with participants and reviewed medical records to complete the first three sections, while participants themselves completed the fourth section (self-report). The questionnaire has the following information:

Section 1. Socio-demographic characteristics of cancer patients involves 9 items: such as age, sex, marital status, educational level, occupational status, and income level. It also detailed the geographical distribution by governorate (covering all Iraqi provinces) and housing status (categorized as owned, rented, shared, or agricultural), residence (categorized as urban or rural).

Section 2. Clinical variables of cancer patients, involves 6 items such as cancer type, disease duration, TNM (Tumor–Node–Metastasis) stage, type and duration of active treatment, and comorbidities.

Section 3. presents the laboratory findings such as Hb (hemoglobin), WBC (white blood cells), platelets, AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase), creatinine, and cortisol.

Section 4. Psychological assessment tools, using a standardized questionnaire after translating it into an Arabic version, included the HADS (Hospital Anxiety and Depression Scale), used to assess the severity of depression and anxiety symptoms.

MEASUREMENT AND SCORING

It is a 14-item self-assessment scale aimed at detecting anxiety and depression cases and measuring their severity in patients. The tool is divided into two subscales, each consisting of seven items: one dedicated to assessing anxiety (HADS-A) and the other to depression (HADS-D), with a primary focus on psychological rather than physical symptoms. This scale measures the state that respondents felt during the past week, with items evaluated using a 4-point Likert scale, ranging from 0 to 3. The total score for each subscale can range from 0 to 21, where higher numbers indicate higher levels of psychological distress. According to standardized verification tests, the overall score for each subscale can be interpreted as indicating a normal state 0–7, the presence of borderline symptoms 8–10, or severe symptoms 11–21. In this work, the subscales for both depression and anxiety indicated high internal consistency, with a Cronbach's alpha of 0.80 and 0.79, respectively. This scale was developed by [10].

DATA PREPROCESSING AND AUGMENTATION

The original dataset included 323 clinical samples. Preprocessing steps included encoding for categorical variables, imputation for missing data, normalization, and class balancing using SMOTE. Data augmentation of continuous variables with small random perturbations, for example, by adding Gaussian noise. The dataset was then balanced to a total of 1000 samples. The data were split into training (80%) and testing (20%) sets, and XGBoost was selected for predictive modeling. The model was trained two times, once on the original and once on the augmented datasets, to compare the effects of data processing on the model performance. The evaluation metrics were accuracy, precision, recall, F1-score, specificity, and AUC.

STATISTICAL ANALYSIS

The analysis was performed based on a two-framework methodology. The first framework was descriptive statistics, and inferential statistics using IBM SPSS Statistics version 28. The second framework was prediction modeling using the machine learning libraries in Python within the Google Collab environment and the XGBoost algorithm to predict levels of depression and anxiety.

ETHICAL CONSIDERATIONS

Written informed consent was acquired from the Ethics Committee of Al-Furat Al-Awsat Technical University, College of Health and Medical Techniques, Kufa (Ref. No. 4805 in 24/9/2025), while ethical approval was granted by the Al-Najaf Health Directorate (Ref. No. 36558 in 5/10/2025). Additionally, verbal consent was obtained from all participants.

RESULTS

SOCIODEMOGRAPHIC CHARACTERISTICS

A total of 323 cancer patients undergoing active treatment participated in this study, with an age range of 18-87 years and a mean ± STD of 52.95 ± 12.79, and the response rate was 100%. Most participants were female, married, and unemployed. The majority had a low educational level and were residents of rural areas, with most participants living in Najaf Governorate. Sociodemographic characteristics showed statistically significant variation (P>0.05) (Table 1).

CLINICAL CHARACTERISTICS

Table 2 summarizes the clinical characteristics of participants. Breast cancer was the most common cancer type. Regarding the disease period, most patients had been diagnosed for less than one year, were diagnosed in stage II of the disease, and were receiving chemotherapy. The majority had undergone active treatment for one year or less, while approximately one-third had chronic comorbidities, primarily hypertension and diabetes mellitus.

PREVALENCE OF DEPRESSION AND ANXIETY

According to the HADS score, depression and anxiety were highly prevalent among participants. Clinically significant depression and anxiety represented the largest categories, followed by borderline levels, while normal scores were observed in a smaller proportion of patients. The detailed distribution is presented in Table 3.

ASSOCIATION OF DEMOGRAPHIC AND CLINICAL FACTORS WITH DEPRESSION AND ANXIETY LEVELS

As shown in Table 4, the results revealed that all examined sociodemographic and clinical variables were significantly associated with depression and anxiety levels p<0.001. Detailed regression results are presented in Table 4.

RESULTS OF DEPRESSION AND ANXIETY LEVELS ANALYSIS USING AI MODELS

The XGBoost model demonstrated excellent predictive performance with an accuracy of 100% and a log loss of 0.0019. Precision, Recall, F1-score, and ROC-AUC were reached 1.00 across all classes, indicating outstanding discrimination between positive, borderline, and normal cases. These findings support the utility of machine learning approaches in identifying patients at risk of depression and anxiety (Table 5).

IMPORTANT FEATURES IN THE PREDICTION OF DEPRESSION AND ANXIETY CLASSES

The results of data modeling using the XGBoost algorithm showed a hierarchy of the most influential features in the process of predicting classes of anxiety and depression. Stages top the list of the most contributing features of classification accuracy, followed immediately by chronic diseases and types of cancer as the most important clinical determinants. In the subsequent grades, the variables include the sex, age, period of active treatment, and type of treatment. The duration of disease is considered one of the least influential features, as depicted in Figure 1.

DISCUSSION

Research on the psychological health of cancer patients has experienced progressive advancement. Nevertheless, research primarily focusing on the active therapy phase remains inadequate. This study is the first study in Al-Najaf Al-Ashraf to identify the predictors of depression as well as anxiety in cancer patients undergoing active therapy through the application of XGBoost algorithms. Among 323 cancer patients, age was significantly associated with depression and anxiety (P<0.001), with older patients experiencing greater psychological burden. The association between age and depression and anxiety observed

Table 1. Distribution of respondents according to socio-demographic characteristics

Variables		Freq. N=323	Percent	P. value
Age Groups (years)	18-27 Y	28	8.7	0.005*
	28-37 Y	25	7.7	
	38-47 Y	52	16.1	
	48-57 Y	76	23.5	
	58-67 Y	88	27.2	
	68-77 Y	41	12.7	
	78-87 Y	13	4.1	
Sex	Male	113	35.0	0.001**
	Female	210	65.0	
Marital Status	Single	26	8.1	0.001*
	Married	212	65.6	
	Divorced	23	7.1	
	Widowed	61	18.9	
	Separate	1	0.3	
Education level	Not read or write	98	30.3	0.001*
	Read and write	17	5.3	
	Primary	111	34.4	
	Secondary	29	9.0	
	Tertiary	15	4.6	
	Diploma	23	7.1	
	Bachelor	26	8.1	
	High degree	4	1.2	
Employment Status	Government Employee	54	16.7	0.001*
	Self-employed	25	7.7	
	Not working/ House-wife	206	63.8	
	Retired	38	11.8	
Governorate	Najaf	219	67.8	0.001*
	Diwaniya	41	12.7	
	Bable	32	9.9	
	Karbala	5	1.5	
	Samawa	12	3.7	
	Kut	9	2.8	
	Salah Aldine	5	1.6	
Residence	Urban	147	45.5	0.001**
	Rural	176	54.5	
Monthly Income Level	More than Sufficient	44	13.6	0.001*
	Somewhat sufficient	147	45.5	
	Insufficient	132	40.9	
Housing Status	Owner	118	36.5	0.001*
	Rent	29	9.0	
	Share with	90	27.9	
	Agriculture	86	26.6	

* Kruskal-Wallis Test, significant at 0.05. ** Mann-Whitney U, significant at 0.05.
Source: Authors' own work

Table 2. Distribution of patients according to clinical information

Variables		Frequency	Percent	P. value
Type of cancer	Leukemia	40	12.4	0.001*
	Tongue Cancer	4	1.2	
	Breast	101	31.3	
	Bladder	18	5.6	
	Uterus	5	1.6	
	Lung cancer	35	10.8	
	Prostate	9	2.8	
	Lymphoma	20	6.2	
	Kidney	5	1.5	
	Brian	9	2.8	
	Ovaries	9	2.8	
	Liver	4	1.2	
	Colon	21	6.5	
	Bone marrow	13	4.0	
	Pancreases	8	2.5	
	Squamous Melanoma	1	.3	
	Stomach	10	3.1	
	Soft Tissue Sarcoma	9	2.8	
	Rectuoma	2	.60	
Period of disease	Less than 1 y	166	51.4	0.001*
	From 1-3Y	87	26.9	
	More than 3	70	21.7	
Disease stages	1st	65	20.1	0.001*
	2nd	133	41.2	
	3rd	68	21.1	
	4th	57	17.7	
Type of treatment	Chemotherapy	163	50.5	0.001*
	Radiotherapy	76	23.5	
	Surgery	1	.30	
	Hormonal	68	21.1	
	Compound	15	4.6	
Period of active treatment	≥1Y	257	79.6	0.001*
	1-3Y	40	12.4	
	More than	26	8.0	
Chronic diseases	Yes	116	35.9	0.001*
	No	207	64.1	
Types of chronic diseases	Not	207	64.1	0.001*
	Hypertension	68	21.1	
	Diabetes	43	13.3	
	EHD	5	1.5	

* Kruskal-Wallis Test, significant at 0.05.
Source: Authors' own work

in this study is consistent with previous studies [11-12], which reported a significant relationship between age and psychological distress among cancer patients. However, this finding contrasts with other studies [13-14], where different age groups were identified as being at risk of psychological distress. Such differences may reflect variations

Table 3. Distribution of cancer patients by overall level of depression and anxiety (N=323)

Category	Score	Freq. (%) Depression	Freq. (%) Anxiety	Df	P. value
Normal	0-7	64 (19.8)	70 (21.6)	2	0.001*
Borderline	8-10	96 (29.7)	99 (30.7)		
Positive	11-21	163 (50.5)	154 (47.7)		
Total		323 (100.0)	323 (100.0)		

* Kruskal-Wallis Test, significant at 0.05

Source: Authors' own work

Table 4. Linear regression coefficients for depression and anxiety according to sociodemographic and clinical factors

Predictor	R (Depression)	Prediction	R (Anxiety)	Prediction	Sig.
Age	0.020	2.253	0.128	11.985	> 0.001
Type Of Cancer	0.108	2.415	0.108	10.906	< 0.001
Duration	0.082	2.443	0.098	11.156	> 0.001
Disease stage	0.004	2.314	0.056	10.837	> 0.001
Type Of Treatment	0.023	2.588	0.272	11.910	> 0.001
Period of Active Treatment	0.087	2.450	0.118	11.306	> 0.001
Chronic Diseases	0.053	2.449	0.045	9.878	> 0.001

Source: Authors' own work

Table 5. Performance metrics of the XGBoost model for depression and anxiety levels

Accuracy: 1.00 Log Loss: 0.0019					
Classification report					
Depression and anxiety level	**Precision**	**Recall**	**F1-score**	**Support depression**	**Support anxiety**
Normal	1.00	1.00	1.00	41	45
Borderline	1.00	1.00	1.00	59	61
Positive	1.00	1.00	1.00	100	94
Accuracy			1.00	200	200
Macro avg.	1.00	1.00	1.00	200	200
Weighted avg.	1.00	1.00	1.00	200	200

Source: Authors' own work

in socio-cultural healthcare contexts. Cancer type was also significantly associated with both depression and anxiety (p<0.001), with breast cancer being the most common diagnosis. This result contrasts with studies that reported no significant association [15, 16], it supports evidence suggesting that specific cancer diagnosis may increase vulnerability to psychological distress. Furthermore, type of treatment emerged as another significant predictor of depression and anxiety (p<.001). More than half of participants receiving chemotherapy, which may explain the observed psychological burden. These results similar findings have been reported in previous studies highlighting the influence of treatment type and intensity on psychological outcomes [17, 18]. Stage of disease was significantly associated with depression and anxiety (p<0.001), with the highest prevalence observed among patients in Stage II. This result in line with the predictive ability of the disease stage found by Geremew et al. [19]. Disease duration since diagnosis was also significantly associated predictor with depression and anxiety (p<0.001), particularly during the first year after diagnosis, suggesting that this period represents a critical phase for psychological vulnerability. These results contradict a previous study by Alfadhul [20] in Najaf, and aligns with study of Mols et al. [21], which did not identify a significant temporal association. Similarly, duration of active treatment emerged as a significant predictor of psychological disorders (P<0.001). reflecting the cumulative impact of the first-year post-diagnosis. The cumulative burden of ongoing chemotherapy and radiotherapy may contribute to increased depression and anxiety levels during the treatment period. This result is consistent with study found an inverse association between the time of treatment completion and psychological problems; that is, suffering is highest during the active treatment phase and only falls after its termination [13]. And finally, our results disclosed a significant association

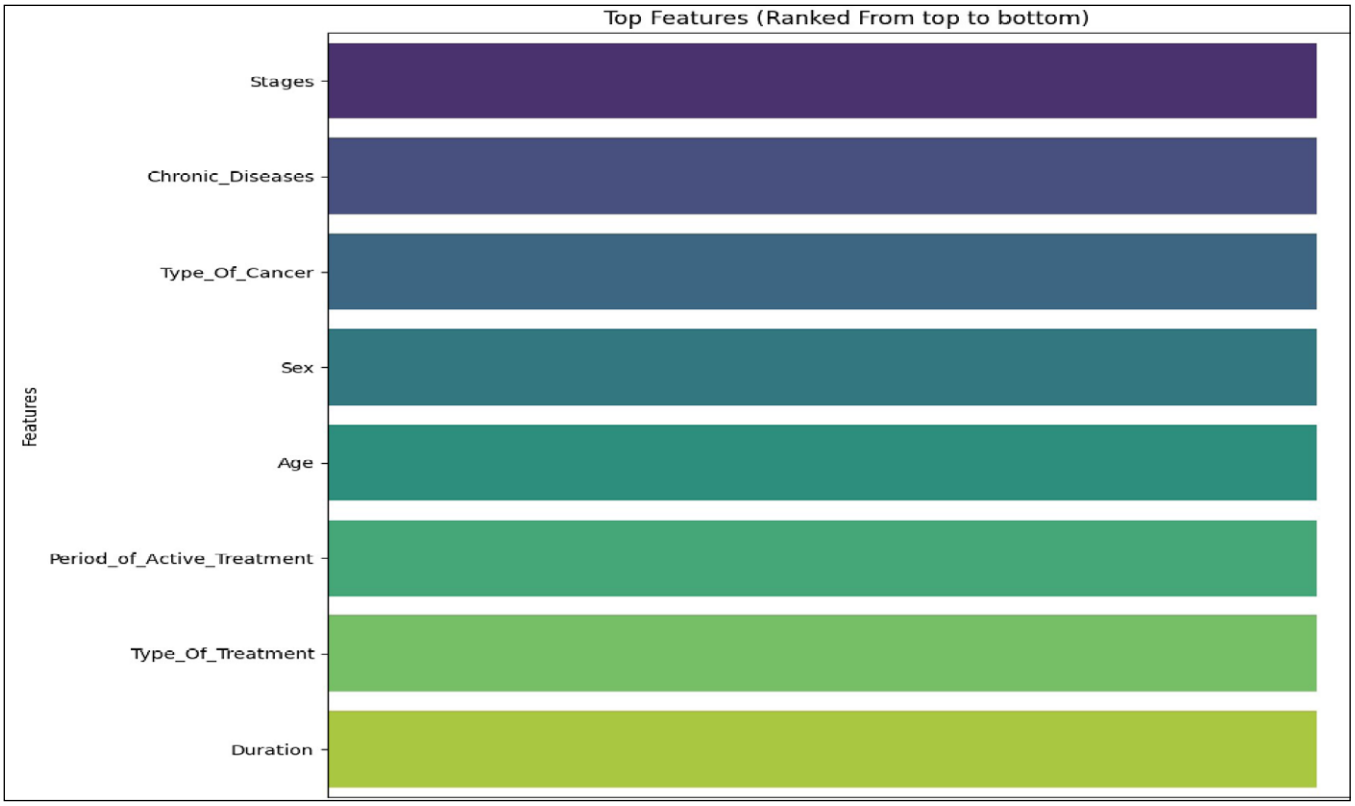


Fig. 1. Important features in the prediction of depression and anxiety classes
Source: Own materials

between the presence of comorbid chronic diseases and increased levels of depression and anxiety (P<0.001). This may reflect the presence of additional physical and psychological burden imposed by conditions such as diabetes and hypertension. Similar associations have been reported previously [22, 23]. The prevalence of clinically significant depression and anxiety was 50.5% and 47.7%, respectively. These findings fall within the ranges reported in prior research indicating anxiety prevalence ranging from 19.9% to 64.9% [24, 25] and depression prevalence spanning from 21.0% to 70.0% [26, 27]. Nevertheless, our findings surpass those of a Greek study of Arvanitou et al. [28], potentially attributable to cultural variations, access to psychological support, and socioeconomic conditions. The AI analysis found several factors that may contribute to psychological disturbances. Among them, age, cancer type, treatment modality, disease stage and its duration, period of active treatment, and chronic comorbidities emerged as significant predictors of depression and anxiety. In this study, XGBoost algorithms demonstrated excellent predictive performance in predict depression and anxiety levels among cancer patients, consistent with recent studies employed machine-learning approaches in psychological health prediction [29-31]. The high predictive accuracy may be attributed to the selected of clinically relevant features, such as the type of treatment and the stage of the disease, etc., a finding that agrees with previous study conducted in China showing that appropriate features selection substantially improves model performance and predictive accuracy [31]. These findings highlight the potential of artificial intelligence to facilitate early identification of patients at high risk of psychological disorders and support more objective, evidence-based psychological care within oncology settings [32].

CONCLUSIONS

The present report notes there is a strong association between cancer and high levels of depression and anxiety in patients under active therapy, with nearly half of the study group being categorized as positive for depression and anxiety. The rationale for considering clinical and sociodemographic factors as effective predictors of patients' psychological conditions was confirmed by the finding that the artificial intelligence model based on XGBoost demonstrated better predictive performance. The results show that the introduction of such types of AI systems in the healthcare system can be helpful in detecting high-risk patients, which will allow intervention in the process of psychological treatment. Overall, the use of AI-based solutions can be considered an important step forward in providing more objective, evidence-based mental health care to cancer patients as opposed to the subjective way.

RECOMMENDATIONS

Based on the findings of the current study, we recommend the implementation of a psychological assessment program by adopting one of the machine learning algorithms as a standard procedure for cancer patients from the point of diagnosis in the hospital, with enhanced support for breast cancer patients during the first year of disease and in the second stage to navigate the adaptation crisis. It also emphasizes the activation of specialized psychological support units to reduce the burden resulting from active treatment. Furthermore, the adoption of a holistic care strategy for patients with concurrent chronic illnesses diminishes the dual burden, and, ultimately, the study advocates for the enhancement of educational initiatives for patients and their families alongside the execution of future longitudinal studies to track psychological variables throughout the treatment process.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Correction of periodontal status and evaluation of disorders in biomarkers of local oxidative metabolism in patients with orthodontic anomalies on the background of generalized periodontitis

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ABSTRACT

Aim: To determine clinical efficacy of developed therapeutic and prophylactic complex (TPC) in orthodontic patients with periodontal pathology and studying its correction after installation of fixed orthodontic appliances. Evaluation of this efficacy will be based on assessment of periodontal indices and changes in activity of enzymes of antioxidant defense, as well as the activity of enzymatic markers of local stress response in oral fluid.

Materials and Methods: 118 patients with orthodontic anomalies and generalized periodontitis were divided into two groups: 60 (main), and 58 (comparison). Clinical and paraclinical parameters for assessing periodontal health, including biochemical parameters of enzymes of oral fluid, were studied. In main group, TPC was applied, and in comparison group, traditional treatment regimen was used. The indicators were evaluated before and after periodontal treatment.

Results: The developed TPC made it possible reducing by 3-3.5 times the preparation for active orthodontic period and achieve stabilization of pathological process in the periodontal tissues at different time after braces installation. In patients of main group, indicators of studied enzymes in oral fluid were improved by 1.5-2.4 times, compared to results obtained in comparison group, where, after using traditional treatment regimen, level of oxidative stress and local oxidative metabolic disorders remained high.

Conclusions: Changes in in periodontal status and activity of SOD and LDH activity relative to CA and AA measured in orthodontic patients with periodontal disease were shown to be important biomarkers of treatment effectiveness and controlling the state of reduced periodontium comparing to background of oxidative stress caused by fixed orthodontic appliances.

KEYWORDS: treatment, periodontitis, enzymes, orthodontic appliance, orthodontic anomalies, periodontal indices

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INTRODUCTION

The development of oxidative stress (OS) is one of the biological reactions of oral tissues to the installation of fixed orthodontic appliances [1]. During the active period of orthodontic treatment, two different conditions coexist that can cause OS: on the one hand, it is the fixed appliance, and on the other, it is the biomechanics of tooth movement. At the same time, the use of braces often creates a complicated situation that provokes an inflammatory reaction around the teeth being moved, especially in patients with compromised periodontium. [2]. Since there is compelling evidence that periodontal tissue inflammation is one of the main sources of reactive oxygen forms (ROS) in the oral cavity, it can be assumed that aseptic inflammation is associated with damage caused by OS [3]. Most orthodontic appliances made of metal, ceramic, or plastic (organic polymer) materials can have significant oxidative potential and release metals or other elements through a phenomenon known as corrosion. This causes an increase in the level of ROS through various reactions with those free radicals that are catalyzed by metals [4].

Medication is extremely important in preparing periodontal patients with orthodontic anomalies (OA) for the active period of orthodontic treatment and throughout its course. Particular attention should be paid to drugs with antioxidant, antihypoxic, cytoprotective, and metabolic properties, which are pathogenetically justified and perform well in OS conditions. The

world literature shows that flavonoids, as free radical scavengers, have pronounced antioxidant, anti-inflammatory, anti-allergic, antiviral, antibacterial, and antiaggregant effects [5]. The phenolic structure allows the molecules of these substances to interact with free radicals. As a result, the intensity of lipid peroxidation decreases and the formation of the main negative factor, malondialdehyde, is inhibited [6]. Along with this, physiotherapeutic methods of treating periodontal diseases are of considerable interest, as they potentiate the penetration of the drug into the periodontal tissues, have a reflex effect, favorably affect local hemodynamics, eliminate hypoxia, and stimulate nonspecific tissue reactivity and the body's defenses [7]. As a result, blood and lymph circulation, trophism, and metabolism improve in periodontal tissues, inflammation and congestion decrease, and the anti-inflammatory, antimicrobial, and analgesic effects of medications are prolonged, accelerating reparative processes [8].

Currently, it is relevant to analyze the activity of oral fluid enzymes using biochemical methods, which allow for a more accurate assessment of the degree of periodontal tissue damage before and after periodontal treatment, as well as to fully monitor the condition of periodontal tissues during the active period of orthodontic treatment [9].

AIM

To determine clinical efficacy of the developed therapeutic and prophylactic complex (TPC) in the orthodontic patients with periodontal pathology and study its correction after the installation of fixed orthodontic appliances. The evaluation of this efficacy will be based on the assessment of periodontal indices and changes in the activity of enzymes of the antioxidant defense, as well as the activity of the enzymatic markers of local stress response in oral fluid.

MATERIALS AND METHODS

The design of this study consisted of the following steps: 1) use of main criteria for inclusion of patients with orthodontic anomalies complicated by generalized periodontitis; 2) clinical and diagnostic characterization of these patients; 3) biochemical analysis of oral fluid of selected patients; 4) development of treatment plan for patients of the main and comparison groups; 5) statistical processing of the obtained results; 6) research hypothesis based on the results of using the developed therapeutic and prophylactic complex.

A randomized controlled clinical trial aimed at evaluating the effectiveness of a developed therapeutic and prevention complex (TPC) in patients with orthodontic anomalies (OA) complicated by generalized periodontitis (GP) was carried out. A total of 118 patients aged 20-35 years of both sexes diagnosed with stage I–II (grade A) generalized periodontitis who sought orthodontic treatment were included in the study. The patients were randomly divided into two groups: the main group (n = 60), in which periodontal treatment was performed as part of preparation for active orthodontic treatment and during orthodontic therapy using the developed TPC; the comparison group (n = 58), which received standard treatment according to the generally accepted methodology in accordance with 'Guideline 00163. Periodontal diseases (gingivitis and periodontitis) [10].

The criteria for inclusion of patients in the study were as follows:

- patients with OA complicated by periodontal disease;
- patients of both sexes aged 20 to 35 years;
- the patient's individual consent to participate in the study;
- the ability to visit the dentist at the appointed time.

The exclusion criteria for the study were as follows:

- patients with severe systemic diseases;
- patients with cancer, with a history of chemotherapy and radiation therapy;
- presence of genetic diseases, immunodeficiency states, polyvalent allergy;
- acute and chronic infectious and viral diseases;
- patients who took various medications (corticosteroids, immunosuppressants, antibiotics).
- patients with secondary adentia (partial or complete);
- pregnancy and lactation;
- harmful habits (smoking);
- patient refusal to participate in the research program.

CLINICAL AND DIAGNOSTIC METHODS

The following methods and materials were used to achieve the set goal: in addition to 118 medical record of a dental patient (Form No. 043/o), 118 periodontal cards and 118 orthopantomograms (was performed to clarify periodontal diagnosis) were created before treatment and the same number were created 6 months after treatment. According to the course of the study based on the collection of medical history and clinical and radiological assessment of the condition of periodontal tissues to diagnose GP, the classification of diseases and conditions of periodontal and peri-implant tissues was used (World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, EFP & AAP World Workshop, Chicago, 2017) [11]. To

evaluate the effectiveness of periodontal treatment, the following indices were calculated: BOP (Bleeding on Probing, Ainamo & Bay, 1975), the Approximal Plaque Index (API) (Lange D.E., Plagmann H., 1977) for dental plaque in the interdental spaces, and the Gingival Index (GI) (J. Sillness, H. Loe, 1967), which allows determining the location and severity of gum inflammation (mild, moderate, and severe) [12]. X-ray examination of the jaw bones was performed using intraoral and panoramic radiography on a «VillaRotograPH-D» (Italy) device. The structure, shape, and height of the interalveolar septa, the distinctness of the cortical plate, and the condition of the cancellous bone of the alveolar processes of the jaws were evaluated.

The diagnosis of the OA was performed according to the classifications of D.A. Calvelis and E H. Angle [13, 14], in which the following occlusion pathologies were diagnosed and examined in patients of both groups: prognathia (distal occlusion), deep bite, crossbite, open bite and progenia (mesial occlusion). Also, the patients had dentition abnormalities combined with occlusion pathology, such as disrupted dentition formation, abnormalities of individual teeth, crowded teeth, vestibular and oral overjet, mesial and distal overjet, tortoanomalies, supra- and infraposition of teeth, diastemas and tremas.

BIOCHEMICAL ANALYSIS OF ORAL FLUID

Enzymatic activity of catalase, amylase, superoxide dismutase, and lactate dehydrogenase were measured in oral fluid of patients of both groups before and after treatment. According to the course of studies in patients of both groups catalase activity (CA) of oral fluid was measured using method based on the ability of H_2O_2 to form a colored complex with the molybdenum salts [15]. The reference values of normal CA are 180-200 U/ml. Amylase activity (AA) in the oral fluid was measured using a modification of the Wolgemuth method that is based on the ability of amylase to hydrolyze starch [16, 17]. The reference values of AA are 10,000 – 11,000 U/L [15, 18]. Measurement of the activity of superoxide dismutase (SOD) in oral fluid was performed using conjugated nitroblue tetrazolium conversion reaction that is based on the ability of SOD to compete with nitroblue tetrazolium for superoxide anions formed as a result of aerobic interaction of the reduced form of nicotinamide nucleotide (NADH) in the presence of phenazine methasulfate (PMS) [19-21]. The reference values for the norm of SOD are approximately 30%. The colored products of the reaction were determined spectrophotometrically at the corresponding absorption wavelengths on a multichannel microphotometer «Plate Reader BioTek» 76883 (BioTek, USA) [15].

The automated biochemical analysis of lactate dehydrogenase (LDH) was performed on «Alinity c» analyzer (Abbott Laboratories, USA) using the «Lactate Dehydrogenase2 Reagent Kit» in the UNILAB LLC laboratory (License of the Ministry of Health of Ukraine Series AG No. 597497 dated by 09.02.2012). The reference values of LDH levels in the oral fluid were 135.0-214.0 U/L [21, 22].

The Treatment Plan for 60 patients in the main group prior to the active period of orthodontic treatment and during its course was as follows: a treatment plan was developed an extemporaneous periodontal gel composition under the patented name «Benzydaflaziverdyne» (GCB), containing two active components – benzidamine hydrochloride (BH) in tablet form «T-Sept®» (ICN Polfa Rzeszow S.A, Poland), which has anti-exudative, anti-edematous, antioxidant and analgesic properties, and «Proteflazid®» drops (LLC SPC Ecopharm, Ukraine) containing antioxidants of plant origin (flavonoids) in a gel base consisting of sodium alginate, nipagine and water for injection [23]. The above mentioned GCB was included in the treatment and prevention complex (TPC) (Table 1). The initial periodontal therapy in the main group was a part of oral cavity sanitation which began with teaching patients rational hygiene and controlled toothbrushing (Table. 1). Removal of dental plaque was combined with the SRP (Scaling and Root Planing) protocol which was performed using an instrumental method (Gracie curettes (Hu-Friedy, USA) and Kohler (Germany)) in combination with an ultrasonic scaler «Woodpecker UDS L» (China). Before the procedure, the oral cavity was irrigated with «Tantum Verde®» solution in which BH is the active ingredient. Additionally, for rinsing the mouth, patients of the main group were recommended 15 ml of this drug 3-4 times a day for 7-8 days.

In order to prolong the effect of GCB in patients of the main group, two approaches were applied: 1. Individually made mouthguards; 2. Modified electrophoresis procedure (Table 1). For this purpose, individually made mouthguards for GCB and electrodes made of thin sheet metal 0.5 mm thick were used. Metal plates of active electrodes on the vestibular side and plates of passive electrodes on the oral/palatal side were placed in the mouthguards (Fig. 1 - A), filled with GCB, which has a distinctive emerald color, and fitted in the oral cavity (Fig. 1 - B). With the patient's mouth closed, the «Potok-1» effectrophoresis device (SMEP) was turned on. The duration of one GCB electrophoresis procedure was 45-50 seconds for one jaw. This time interval was established on an experimental model of in vitro electrophoresis with three types of test cells (mouse

Table 1. Algorithm of application of treatment and prophylactic complex developed for orthodontic patients with GP stage I-II (gradeA) of the main group

Before the active period of the orthodontic treatment:		
General therapy:	Individual oral hygiene:	Local therapy:
1. «Biotus Ca, Mg, Z with Vitamin D3 BIO-530982» (Biotus, Ukraine) - 1 capsule 3 times a day with meals (recommended course of treatment 3-4 weeks x twice a year)) 2. «Resveratrol» (GREEN PHARMA, Ukraine) - 1 capsule 2-3 times a day after meals; (recommended course of treatment is 4 weeks x twice a year)	1. «Vitis Aloe Vera Apple» toothpaste (DENTAID, Spain); 2. Interdental brushes - Interprox 4G and 2G (DENTAID); 3. Dental floss (VITIS), (DENTAID); 4. Bass cleaning method 5. Use of the Mira-2-Ton plaque revelerator (Miradent, (HAGER Werken, Germany))	1. «Tantum Verde» mouthwash (Angelini Francesco A.C.R.A.F. S.p.A., Italy); 2. Professional hygiene; 3. SRP protocol; 4. Oral cavity sanitation; 5. Use of individual mouthguards with GCB (for 2 hours once a day, a course of 4-5 days); 6. Conducting gingival electrophoresis with GCB (4-5 procedures)
In the active period of the orthodontic treatment:		
General therapy: 1. «Biotus Ca, Mg, Z with Vitamin D3 BIO-530982» Repeat the course in 6 months; 2. «Resveratrol» – repeat the course in 6 months	Individual oral hygiene: 1. Toothpaste «VITIS orthodontic» (DENTAID); 2. Interdental brushes – Interprox 4G and 2G (DENTAID)	Local therapy: Follow-up therapy every month: 1. Professional hygiene and rinsing of the mouth with «Tantum Verde» solution (7-8 days course once every 2 months); 2. Individual mouthguards with GCB (2-3 procedures); 3. Electrophoresis with GCB (2-3 procedures)

Source: compiled by the authors of this study

Table 2. Values of BOP, API and GI indices in orthodontic patients with GP stage I-II (grade A)

Indicators	Before treatment of GP		Before treatment of GP		3 months after braces are fitted		6 months after braces have been fitted	
	Main group	Comparison group	Main group	Comparison group	Main group	Comparison group	Main group	Comparison group
BOP (%)	79.03 ± 7.67	75.24 ± 18.97	1.83 ± 0.71 *** #	10.24 ± 9.60 ***	7.85 ± 3.18 *** ###	43.48 ± 23.12	0.28 ± 0.2 *** ##	17.45 ± 9.94 ***
API (%)	78.43 ± 22.14	76.22 ± 22.74	3.02 ± 2.3 *** ##	27.13 ± 9.09 **	11.13 ± 10.12 *** ###	70.64 ± 17.98	3.04 ± 1.85 *** ###	73.96 ± 29.89
GI (points)	0.99 ± 0.32	1.18 ± 0.28	0.05 ± 0.04 *** #	0.39 ± 0.21 **	0.07 ± 0.05 *** #	0.59 ± 0.33	0.06 ± 0.06 ***	0.34 ± 0.31 **

Notes: 1. *- significance of differences between the groups «before treatment of CGP» and the groups at the stages of observation, where *- $p<0.05$, **- $p<0.01$, ***- $p<0.001$

2. #- significance of differences between the indicators of the main group and the comparison group at the stages of observation, where #- $p<0.05$ ##- $p<0.01$, ###- $p<0.001$

Source: compiled by the authors of this study

fibroblasts of the BALB-3T3 line, mouse macrophages of the J774.2 line, and pseudonormal human keratinocytes of the HaCaTi line) cultured in agar-supplemented medium [24].

General drug therapy in the main group included the multimineral complex «Biotus Calcium Magnesium Zinc with Vitamin D3 BIO-530982» and natural antioxidant «Resveratrol» (Table 1).

After achieving stabilization of the periodontal tissues during the active period of orthodontic treatment, patients of the main group were prescribed a low-abrasiveness toothpaste «VITIS orthodontic» containing cetylpyridine chloride, sodium fluoride, Aloe vera and allantoin for individual toothbrushing. Interdental brushes were prescribed – Interprox 4G, Interprox 2G. Control of the level of oral hygiene and maintenance therapy in the main group was carried out once a month using the following therapeutic and prophylactic measures: «Tantum Verde®» mouthwash; use of individual mouthguards with GCB; electrophoresis procedures with GCB. Six months after braces were placed, patients in the main group were prescribed a repeated course of general medications «Biotus Calcium Magnesium Zinc with Vitamin D3 60 Caps BIO-530982» and «Resveratrol» (Table 1).

Patients of the comparison group (58 patients with a same diagnosis) received a traditional treatment regimen according to the the generally accepted methodology in accordance with 'Guideline 00163. Periodontal diseases (gingivitis and periodontitis) [10]. It included training in individual hygiene with a prescription of «Parodontax» toothpaste. For topical treatment before the professional hygiene procedure and SRP protocol, a traditional antimicrobial agent for oral irrigation, chlorhexidine bigluconate 0.05% was used. It was prescribed in accordance with protocols of the conventional treatment. Patients were supplied with a spatula to the gingival mucosa with «Cholisal gel» (Jelfa S.A. Poland) (treatment course – 7 days). In the comparison group, traditional hygiene control and maintenance therapy were performed once every three months.

The observation periods in both groups (main and comparative) were as follows: all indicators were obtained before and after periodontal treatment of stage I-II (grade A) immediately before the installation of fixed orthodontic appliances (G&H® miniPrevail® TWIN Bracket System) and then at 3 and 6 months of the active period of orthodontic treatment.

The research hypothesis is that the use of the developed therapeutic and prophylactic complex (TPC), containing flavonoid-based preparations and benzidamine hydrochloride in combination with electrophoresis and applied to patients in the main group, provides better clinical results in the treatment of stage I-II generalized periodontitis (grade A) in the orthodontic patients. This demonstrates an improvement in periodontal status and enzyme activity in oral fluid and reduces the time required to prepare for the active period of orthodontic treatment, ensuring better stabilization of the pathological process when installing fixed orthodontic appliances compared to the traditional treatment regimen used in patients in the comparison group.

BIOETHICS

The study was performed taking into account the main provisions of the GCP ICH and the Declaration of Helsinki on biomedical research where a person is the object, and the subsequent revision, Seoul, 2008; the Council of European Convention on Human Rights and Biomedicine (2007) and the recommendations of the Bioethics Committee at the Presidium of the National Academy of Medical Sciences of Ukraine (2002), as well as a positive opinion of the commission on ethics of State Non-profit Enterprise «Danylo Halytskyi Lviv National Medical University» (extract from protocol No. 1 of January 15, 2024). No violations of moral and ethical norms were identified during the research.

STATISTICAL PROCESSING OF RESULTS

The obtained numerical results were analyzed using statistical processing according to generally accepted methods. The creation and editing of the primary database was performed in Microsoft Excel. Statistical processing of digital material was performed using OriginPro 7.0 software (OriginLab, Northampton, Massachusetts, USA). The significance level was set at $p \leq 0.05$ (95% confidence interval) for all statistical procedures, which is generally accepted in medical and biological research. The results were considered reliable at $p \leq 0.05$ and highly reliable at $p \leq 0.001$. Quantitative indicators were tested for normal distribution using the Shapiro-Wilk test. A two-tailed Student's (heteroscedastic) t-test, which analyzes the overall difference, was used to analyze the differences between the two groups. A paired t-test was used to analyze the differences before/after treatment. To visualize the data obtained, each indicator presented in the figures (the ordinate of the columns in the diagrams) corresponds to the average value «M» calculated from the results of three measurements for one sample in each of the determination methods. The standard deviation «σ» of the result obtained was calculated based on the mean error «m». This is represented in the illustration next to each indicator by a vertical line, the length of which corresponds to the value «m» [25].

AI STATEMENT

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

FRAMEWORK

The work is a fragment of scientific research work of Department of Ortodontic State Non-profit Enterprise «Danylo Halytsky Lviv National Medical University»: «The state of dental health and its correction on the basis of systematic analysis of clinical and laboratory, radiological, morphological, functional, aesthetic parameters in people of different ages», state registration number 0120U002143.

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RESULTS

In all 118 patients who sought an orthodontic treatment, the examination before braces were installed, there were

no complaints of bleeding and swelling of the gums, bad breath, and discomfort typical of stage I-II GP (grade A). Filling out and analyzing periodontal charts revealed that the average depth of the periodontal pockets was 1.5-3.5 mm. In 40% of patients, the mobility of individual teeth of the first degree was noted. The X-ray images in the areas of the anterior teeth and molars showed a mixed situation, since it was particularly difficult to trace the complete loss of the cortical layer or only the loosening of the tops of the interalveolar septa of the anterior teeth in 67% of patients in both groups (main and comparison) due to teeth crowding.

Before the start of periodontal treatment, the BOP, API, and GI indices in patients in both groups (the main group – 60 people and the comparison group – 58 people) did not differ significantly (Table 2). After treatment, statistically significant differences in the indices of both groups were observed for all three indices. While the average BOP index before treatment in both groups was 77.13%, after the use of TPC in the main group, this index decreased to 1.83 ± 0.71% ($p<0.001$), in contrast to 10.24 ± 9.60% in the comparison group, whose patients received traditional treatment. Accordingly, in the comparison group, these indicators delayed the installation of braces and led to the continuation of the treatment course (Table 2).

The mean values of API and GI indices before periodontal treatment in both groups were 77.32% (according to the evaluation scale, this is an unacceptable level of hygiene) and 1.08 points (according to the interpretation of the values, this is an inflammatory process in the gum tissues of mild to moderate severity). Treatment of patients in the main group and the comparison group with different treatment regimens also led to different statistically significant results. In the main group, the API and GI indices were 3.02 ± 2.3% and 0.05 ± 0.04 points, as opposed to the comparison group – 27.13 ± 9.09% and 0.39 ± 0.21 points, which also led to a delay in the start of the active period of orthodontic treatment in this group.

After the beginning of active period of the orthodontic treatment and installation of braces, despite the high level of motivation, patients always show a tendency to deterioration of the level of individual hygiene due to painful reactions and difficult adaptation to new conditions. However, in the main group (3 months after the start of the active period of the orthodontic treatment), there was a minimal increase in the BOP (7.85±3.18%), ARI (11.13±10.12%) and GI (0.07±0.05 points) indices, which indicated a slight destabilisation of the situation, which required only minor correction. At the same time, patients in the comparison group showed an active increase in index indicators – BOP (43.48±23.12%), API (70.64±17.98%) and GI (0.59±0.33 points), which confirmed the loss of remission and required a repeated full course of treatment with a traditional regimen.

After 6 months of active period of the orthodontic treatment, the repeated indices allowed to suggest in patients of the main group the practical absence of signs of inflammation in the form of gingival edema and bleeding, as evidenced by very low residual values of the BOP bleeding index – 0.28±0.2% in contrast to the comparison group – 17.45±9.94% ($p<0.001$). The absence of inflammation in the periodontal tissues of patients in the main group resulted in better API and GI indices – 3.04±1.85% and 0.06±0.06 points, respectively. While in patients of the comparison group, against the background of exacerbation of the pathological process in periodontal tissues, the API index was statistically significantly higher than the result obtained in patients of the main group – 73.96±29.89% and practically did not differ from the previous indicator «before treatment». GI scores also indicated the presence of inflammatory process in the gingival tissues – 0.34±0.31 points ($p<0.01$), (Table 2).

The analysis of the obtained indicators of the activity of SOD and CA in the oral fluid allowed us to assume that the presence of stage I-II GP (grade A) in patients and the installation of fixed orthodontic appliances caused a shift in the local oxidative metabolism. Before the periodontal treatment in both groups of patients with oral hyperplasia on the background of GP, a low level of CA in the oral fluid was noted (40.04 ± 25.1 U/ml in patients of the main group and 39.56 ± 24.6 U/ml in the comparison group, comparing to 180-200 U/ml – normal values). At the same time, SOD indicators showed almost three times deviation from the norm (89 ± 6.7% and 91 ± 6.8% in patients of the main and comparison groups comparing to 30% that is a normal value of that indicator), ($p<0.001$), (Fig. 2, Fig. 3).

At the stage of the periodontal treatment, the preparation of the periodontal tissues for active period of the orthodontic treatment using the developed TPC in patients of the main group helped to improve significantly the indicators of SOD enzyme to be close to normal level – 29 ± 2.2 %, and an increase in the level of CA enzyme to 101.2 ± 18.9 U/ml ($p<0.001$) (Fig. 2, Fig. 3).

In contrast to the main group, in patients of the comparison group, after applying the traditional treatment regimen, the indicators of SOD activity were still twice as high as normal – 65 ± 4.8 % ($p<0.01$), and the indicator of CA activity was only 58.7 ± 14.1 U/ml ($p<0.001$), that was almost three times lower than the normal one which significantly delayed the start of active orthodontic treatment (Fig. 2, Fig. 3).

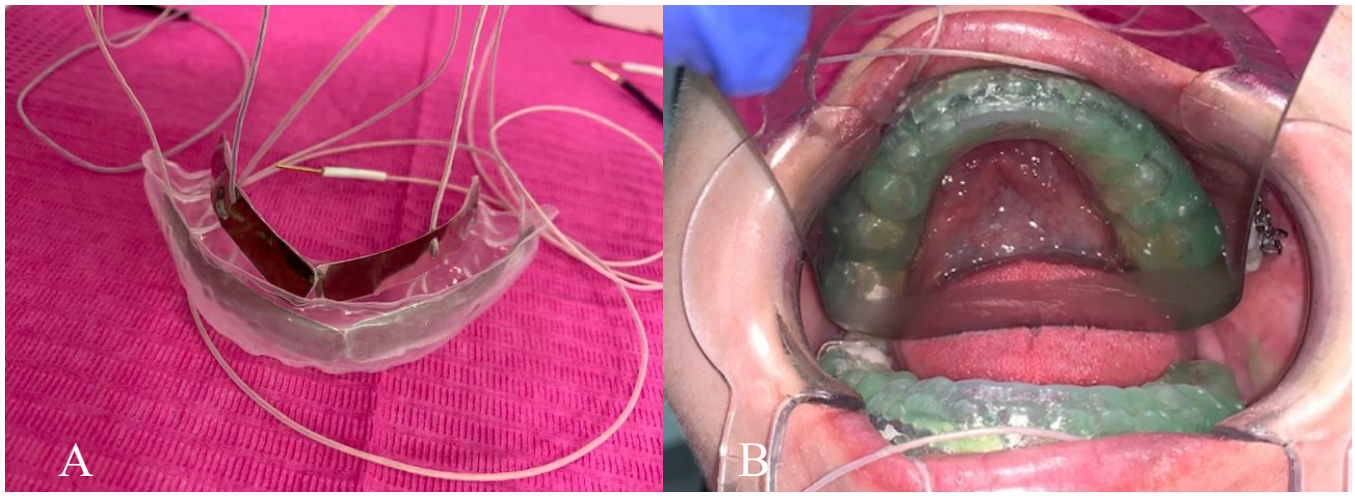


Fig. 1. A – Fitting of electrodes in a custom-made mouthguard on the vestibular and oral sides; B – Fitting of a mouthguard with a GCB in the oral cavity
Picture taken by the authors

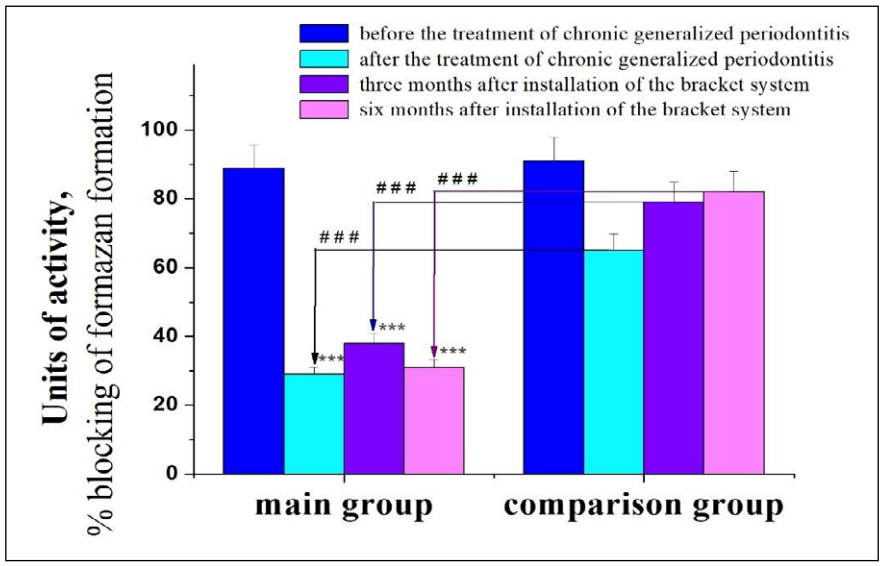


Fig. 2. Indicators of superoxide dismutase enzyme activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

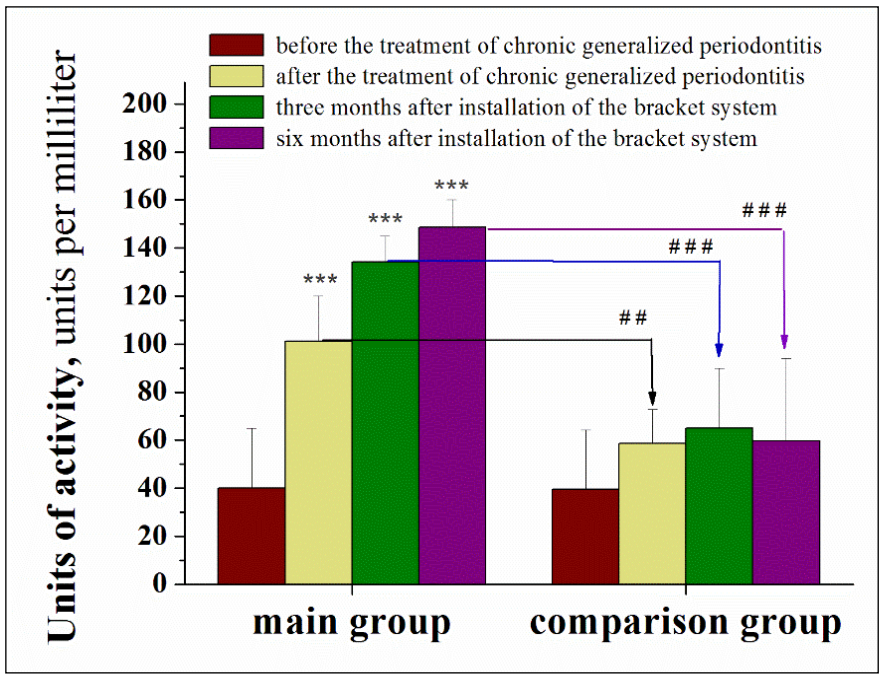


Fig. 3. Indicators of catalase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

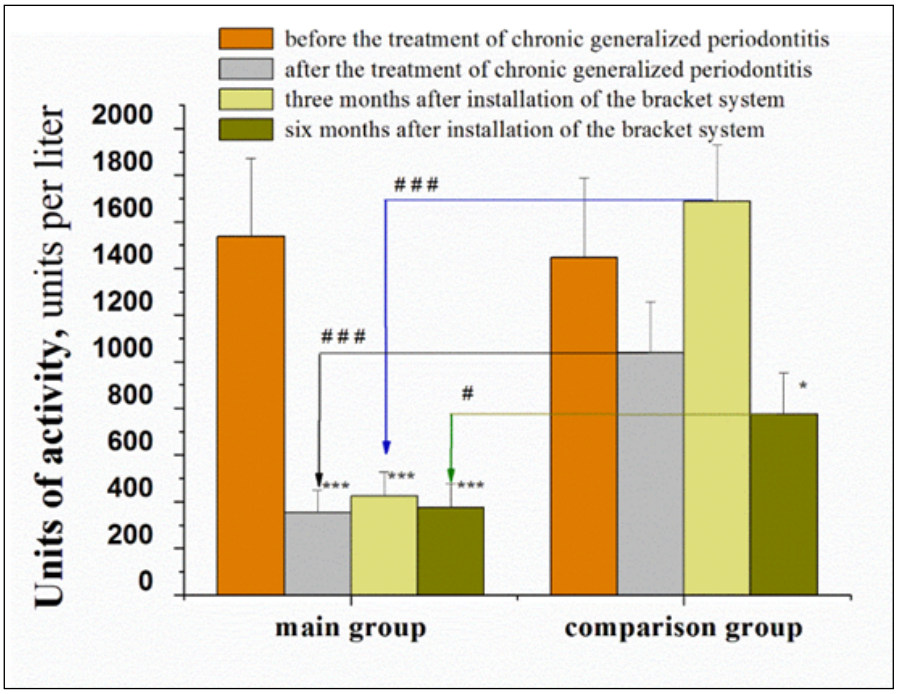


Fig. 4. Indicators of lactate dehydrogenase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

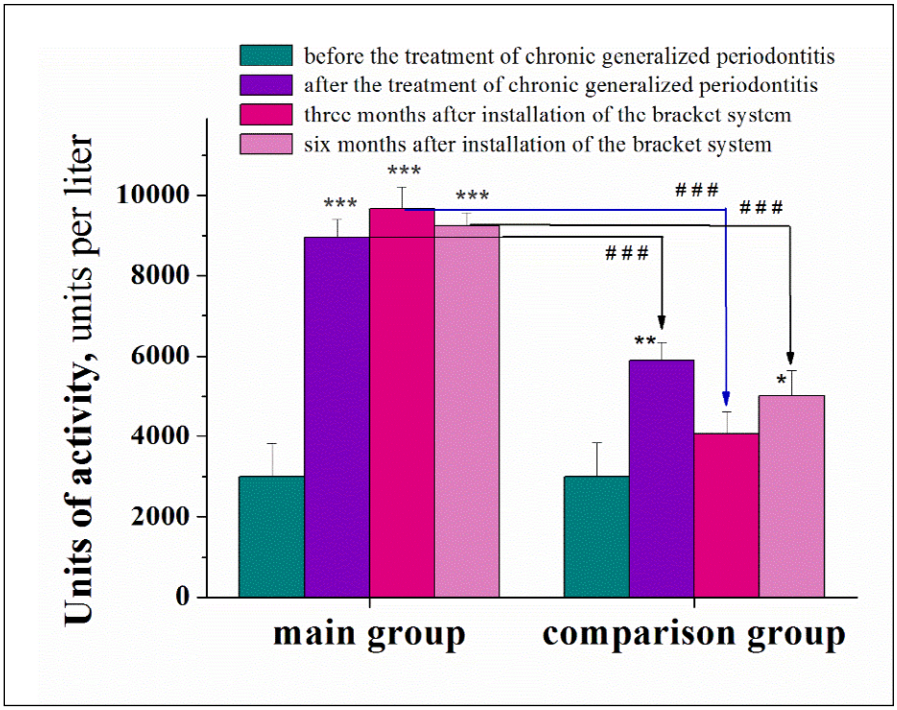


Fig. 5. Indicators of the amylase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

After three months of wearing fixed orthodontic appliances by patients of both groups, a routine sampling of oral fluid was performed to determine the levels of SOD and CA activities under the influence of OS. The obtained results suggest that SOD activity in the main group did not have a significant shift compared to this indicator after treatment of GP before the installation of braces. It amounted 38 ± 2.8 % that is statistically significantly higher than this indicator in the comparison group (79 ± 5.9 %, p<0.001). The indicator of CA activity after three months of active orthodontic treatment was 134.2 ± 10.6 U/ml that is significantly higher than in the comparison group – 65 ± 25.8 U/ml (p<0.001), (Fig. 2, Fig. 3).

Similar changes were observed after 6 months of active orthodontic treatment comparing the background of regular treatment and preventive measures included in the TPC developed for the main group. The indicators of SOD and CA activities in the main group were 31 ± 2.3 % and 148.6 ± 11.4 U/ml, which suggested stabilization of the clinical situation and the absence of local metabolic shifts. In contrast to the main group, in patients of the comparison group, the values of SOD and CA activities were 82 ± 6.1% and

59.5 ± 34.6 U/ml, that is 2.5-1.5 times higher than the normal values. These data indicated a loss of remission in the comparison group comparing the background of an impaired oxidative metabolism (Fig. 2, Fig. 3).

Thus, after treatment of GP before braces were applied in patients of the main group, the levels of SOD and CA activities were 2.2 and 1.7 times higher than in the comparison group. After 3 months, this difference was 2 times, and after 6 months, 2.5 times.

The analysis of the defined indicators of LDH and AA activities in the oral fluid suggested that the presence of stage I-II GP (grade A) in patients and the installation of fixed braces caused a shift in local energy metabolism. Before the start of periodontal treatment, an increase in LDH activity in the oral fluid was noted in both groups of patients with DA comparing the background of GP (1536.4 ± 436.1 U/l – in the main group and 1446.1 ± 441.1 U/l – in the comparison group comparing 135.0-214.0 U/l that is reference value in the norm) and a decrease in AA activity (2997 ± 831.7 U/l and 3002 ± 831.7 U/l, respectively, comparing 10,000-11,000 U/l that are normal values), (Fig. 4, Fig. 5). At the stage of periodontal treatment, application of TPC in the main group led to a significant reduction in the LDH levels bringing them close to the norm – 353.1 ± 94.7 U/l ($p<0.001$) comparing 1040 ± 217.1 U/l in the comparison group, and a significant increase in AA activity was observed – 8958 ± 436.2 U/l ($p<0.001$) comparing to 5897 ± 433.9 U/l in the comparison group, (Fig. 4, Fig. 5).

After the installation of fixed orthodontic appliances in patients of the main group after 3 and 6 months, the values of LDH activity were statistically significantly different from those detected «before treatment». There was a clear confirmation of a quantitative decrease in the OS in periodontal tissues (424.52 ± 103.5 U/l, $p<0.001$ and 375.8 ± 102.4 U/l, $p<0.001$), compared to the LDH values in the comparison group – 1687.6 ± 240.8 U/l. Only after 6 months, the level of LDH activity in oral fluid in the comparison group was shown to be slightly decreased (774.5 ± 177.8 U/l) that was probably due to a greater extent of general adaptive mechanisms (Fig. 4). Thus, the LDH activity after treatment of GP in patients of the main group was three times higher than in the comparison group, in 3 months, this difference was 4.2 times, and in 6 months – 2.2 times.

Three months after the beginning of active period of the orthodontic treatment in the main group, the indicator of AA activity was normalized even to a higher extent – from 9673 ± 536.7 U/l to 4072 ± 532.5 U/l in the comparison group ($p<0.001$). After 6 months of active orthodontic treatment, the difference in the main and comparison groups remained almost at the same level (Fig. 5). Thus, the AA activity after treatment of GP before the formation of fixed orthodontic appliances was 1.5 times higher than in the comparison group, and after 3 months of active orthodontic treatment, this difference was 2.4 times, and after 6 months – 1.9 times.

Thus, we have shown that the applied TPC with GCB best contributed to the normalization of the activity of four enzymes, namely decreased levels of LDH and SOD activities, and increased levels of AA and CA activities. That also led to stabilization of clinical picture under the initial periodontal therapy. After 3 and 6 months of active periodontal treatment, the absence of signs of inflammation in the form of gingival edema and bleeding in the patients of the main group was evidenced. This made it possible to reduce by 3-3.5 times the period of their preparation for the active period of the orthodontic treatment. At the same time, in patients of the comparison group, the level of the OS and local oxidative metabolic disorders remained high due to the traditional local treatment regimen. The comparison group which used a traditional treatment regimen required a longer period (about 4-5 months) to stop all signs of inflammation before starting teeth removal, in contrast to the main group, which took only 1 month to prepare for the installation of fixed orthodontic appliances and for achieving remission.

DISCUSSION

The clinical and laboratory effectiveness of the therapeutic and prophylactic complex (TPC) in the treatment of stage I-II periodontitis (grade A) in orthodontic patients, which included a modified electrophoresis procedure in periodontal tissues using individually manufactured caps with a patented gel composition «Benzydaflaziverdyne» (GCB) based on a flavonoid complex and benzidamine hydrochloride. The essence of the method of subgingival electrophoresis with GCB before active orthodontic treatment was to remove all signs of chronic inflammation in periodontal tissues and ensure an adaptive transitional preparatory period before installing fixed orthodontic appliances. After three months of active orthodontic treatment, application of the developed TPC served to maintain remission.

The inclusion of the electrophoresis procedure in combination with GCB in the complex of therapeutic and preventive measures during the active period of orthodontic treatment in patients with OA against the background of treated GP led to a more intensive normalisation of BOP, API, GI and LDH levels. There was a faster improvement in the condition of periodontal tissues, which ensured a favourable course of the active period of the orthodontic treatment [2, 26].

In general, electrophoresis is recommended by researchers in the field of periodontology, as it has a reflective effect, favorably affecting the nervous system, its vegetative division, and hemodynamics. As a result, blood and lymph circulation, trophism, and metabolism improve in the periodontal tissues, granulation growth in periodontal pockets is suppressed, and inflammatory and congestive phenomena are reduced. An increase in the activity of connective tissue elements, phagocytic activity of leukocytes and elements of the reticuloendothelial system, acceleration of the regeneration process were demonstrated [26]. This is because physical procedures affect the body through nervous-reflex and nervous-humoral pathways. As a result of the body's adaptation to the action of the therapeutic stimulus, its defenses are enhanced. Therefore, electrophoresis procedures are pathogenetically justified by the need to prepare the patient for the stress-modulating effect of fixed orthodontic appliances, and the stage with electrophoresis and local delivery of GCB deep into the tissues is best suited for this, ensuring a kind of adaptation of the periodontal complex [24].

At present, tracing the main aspects of the pathogenesis of the periodontal tissue diseases in the orthodontic patients is impossible without a deep understanding of the role of metabolic mechanisms, in particular in the lipid peroxidation-antioxidant defense systems, which are the basis of many pathological reactions. It was reported that disturbances in local and/or general indicators of the OS are responsible for the mechanisms of the etiopathogenesis of periodontitis [27]. The human body which uses oxygen for its metabolic processes, is equipped with defense systems against ROS. They are usually called the «antioxidant barrier». Among the main enzymatic antioxidants that have been widely studied in gingival fluid, oral fluid, and blood serum of patients with periodontitis are SOD and CA [19, 21]. The reports on the activity of these enzymes in different periodontal diseases (chronic or acute) and at different stages of periodontitis are non-uniform. In the gingival tissue, the activity of CA was usually increased, and in the oral fluid it was decreased [20, 22]. These variations could be the result of using different methods of biochemical assessment of the enzymatic activity and do not reflect the stage and/or risk of the periodontitis progression [28].

Taking in consideration that the aseptic inflammation in the orthodontic patients may be associated with damage caused by the OS, the inclusion of «Proteflazid®» in the composition of the GCB was crucial in our study. This agent is a drug with pronounced antioxidant properties, it does not cause immunotoxic effects or refractoriness (hyporesponsiveness) of the immune system. The medicine inhibits the course of free radical processes, prevents the accumulation of ROS products, enhances the antioxidant status of cells, and thus, reduces intoxication of the organism [29]. In the present study, this was confirmed through measuring the activity of the antioxidant defense enzymes – SOD and CA, which is consistent with the results published in literature [30].

There are reports that flavonoids can counteract the pro-inflammatory effects of proteins associated with pathogenic molecular structures through Toll-like receptor (TLR) responses. The effect of flavonoids on the epithelial cells and fibroblasts of the gums and periodontal ligament, as well as the osteoblasts that support the alveolar bone, is potentially beneficial [5, 29]. The antibacterial mechanism of action of flavonoids was described. Specifically, it inhibits energy metabolism, cell proliferation, nucleic acid synthesis, reduces biofilm formation and cell adhesion, reduces pathogenicity and damage to membranes via producing hydrogen peroxide [31].

In scientific literature, salivary α-amylase was proposed as a marker of stress tension, the secretion of which into oral fluid is regulated by β-adrenergic stimulation [32]. In our study, this biomarker was monitored in oral fluid before treatment of stage I-II GP in patients of the main and comparison groups. It was significantly decreased before treatment which could be due to a damage of the secretory cells of the salivary gland caused by the metabolic processes in the OS and their negative effect on the tissues of the periodontal complex [33]. In turn, these disorders might cause a shift in the pH to the acidic side and induce a decrease in the AA, since this activity is manifested at neutral or slightly alkaline pH [33].

There are several reports concerning the level of the LDH in the oral and crevicular fluid. This enzyme is released from the cytoplasm into the extracellular space during a programmed cell death in the gingivitis or periodontitis, as well as at the orthodontic treatment [34, 35]. The time of increase in LDH levels varied depending on the level of the applied force.

The local analgesic effect of «Tantum Verde®» solution that is another effective component of the drug product, caused stabilization of sensory receptors lasting for 90 min after topical application. Its effectiveness observed after topical application is due to its ability to penetrate into the epithelial layer and reach effective concentrations in inflamed tissues. The antibacterial ability of the BH is manifested by rapid penetration through the outer membranes of microorganisms with a subsequent damage to cellular structures, disruption of metabolic processes and cell lysis. It is important that the antimicrobial effect of the

BH is enhanced in a neutral and alkaline environment (in the periodontal pocket, the pH is on average 8.0) [1, 5].

Multimineral complex «Biotus Calcium Magnesium Zinc with Vitamin D3 BIO-530982» containing calcium, magnesium, zinc and vitamin D3, is an additional source of these minerals and vitamin in order to maintain their optimal levels in the body. The drug supports normal functioning of the musculoskeletal system, maintaining the condition of teeth, bones, basic functions of the immune system and plays an important role in structure of cell membranes. Vitamin D3, in turn, is essential for better calcium absorption and is involved in mineralization, skeletal growth and restructuring.

«Resveratrol» is another drug with a systemic effect and it is known to be natural antioxidant. It contains resveratrol (150 mg), grape seed extract (300 mg) and food gelatin, and is used as an additional source of polyphenolic compounds that are needed to normalize metabolic processes and restore the body›s internal strength [6].

Summerising, the developed TPC is shown to be clinically effective in reducing local stress-induced metabolic disorders in the periodontal tissues, since achieving a stable remission is an important condition at tooth movement in the periodontal pathology that may lead to a rapid and irreversible deterioration in tooth support apparatus.

CONCLUSIONS

1. Based on the results of changes in the periodontal status indicators in the orthodontic patients with GP stage I-II (grade A), the developed periodontal gel composition based on a flavonoid complex and a nonsteroidal anti-inflammatory medicine (benzidamine hydrochloride) in combination with electrophoresis into the tissues of the periodontal complex can be recommended before and during active orthodontic treatment. The clinical efficacy of the developed TPC was confirmed by the BOP API and GI indices. Its application can reduce the preparation time for the active orthodontic period by 3-3.5 times.
2. The measurement of the activity LDH, SOD, CA and AA enzymes in oral fluid, reflecting changes in gum tissue under the stress-modulating effect of fixed orthodontic appliances, is important at the use of the TPC developed for patients in the main group. 3. Adaptation of the periodontal tissues to the installation of braces and reducing the level of local oxidative sterss after their installation are more efficient than in the comparison group, where a persistent loss of remission was observed as soon as 2-3 months after the start of the active period of orthodontic treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Exploring association between anxiety and infertility characteristics: A combined statistical and machine learning approach

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ABSTRACT

Aim: To investigate the association between anxiety and infertility-related variables and to evaluate the performance of a random forest model in predicting and classifying infertility type using clinical and psychological features.

Materials and Methods: A cross-sectional study was conducted among 268 infertile women systematically recruited from the infertility center at Safeer Al-Imam Al-Hussain Surgical Hospital in Karbala, Iraq. Data were collected using standardized questionnaires covering sociodemographic characteristics, medical, obstetric, and infertility histories. Anxiety severity was assessed using the Generalized Anxiety Disorder-7 scale. Descriptive statistics and linear regression analyses were performed. A random forest model was developed using an 80/20 training-testing split to predict infertility type based on anxiety severity and related clinical variables.

Results: Anxiety was highly prevalent, affecting 83.9% of participants. Mild anxiety was observed in 35.1%, while moderate anxiety was reported in 31.3%. Linear regression showed that infertility duration and infertility type were significantly associated with anxiety severity. Longer infertility duration was associated with higher anxiety scores. The random forest model demonstrated high classification accuracy and low log loss in predicting infertility type. Secondary infertility was the most common type and was associated with longer infertility duration and higher anxiety severity.

Conclusions: Anxiety is strongly associated with infertility, particularly infertility duration and type. Combining conventional statistical analysis with machine learning may improve understanding of the clinical and psychological complexity of infertility and support more individualized patient care.

KEYWORDS: clinical features, psychological features, anxiety, machine learning

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INTRODUCTION

Infertility is a major public health concern with substantial effects on health, relationships, and quality of life. According to the World Health Organization, approximately one in six people of reproductive age experience infertility [1]. Infertility diagnosis and treatment impose a considerable psychological burden, increasing the risk of anxiety and depression and negatively affecting reproductive outcomes [2]. Evidence indicates that 25–60% of infertile individuals experience psychological symptoms, with significantly higher levels of anxiety and depression than fertile controls [3]. Furthermore, a systematic review and meta-analysis reported that infertile women have a significantly greater risk of mental health problems, particularly anxiety and depression, compared with the general population [4].Generalized anxiety disorder (GAD) is characterized by excessive and uncontrollable worry and affects approximately 1.65–5% of the general population. It is among the most common psychological disorders in infertile women, with a reported prevalence of 23.2% [5]. Infertile women experience higher levels of anxiety and hopelessness regarding conception compared with pregnant women [6]. Previous studies have demonstrated a significant association between infertility duration and anxiety, with longer infertility duration increasing the likelihood of anxiety disorders [7,8]. Infertility is classified as primary or secondary, depending on whether a woman has previously achieved pregnancy [9]. Studies from Iraq have reported a higher prevalence of primary infertility (60.7–61%) than secondary infertility (39–39.3%) among infertile couples [10, 11]. Evidence also suggests that women with primary infertility experience greater psychological distress, particularly anxiety, than those

with secondary infertility [12]. Similarly, primary infertility has been associated with an increased risk of mental disorders and has been identified as an independent risk factor for anxiety among infertile women [13,14]. Artificial intelligence (AI) enhances healthcare by facilitating the collection, organization, analysis, and interpretation of medical data, thereby supporting clinical decision-making and improving healthcare delivery [15,16]. Machine learning (ML), a major branch of AI, enables predictive modeling by learning patterns from data without explicit programming [17]. Among ML approaches, supervised learning uses labeled data to classify new observations [18]. Random Forest, a supervised learning algorithm based on multiple decision trees, is particularly effective for modeling complex and non-linear relationships [19]. Despite growing evidence linking psychological distress and infertility, their relationship remains insufficiently investigated within an integrated framework. In particular, the combined effect of infertility duration and type on anxiety has received limited attention, especially in Middle Eastern populations. Furthermore, traditional statistical methods may not adequately capture complex relationships among psychological, clinical, and demographic factors.

AIM

This study aimed to investigate the association between anxiety and infertility-related variables (infertility duration and type) and to evaluate the performance of a Random Forest (RF) model in predicting infertility type using clinical and psychological features.

MATERIALS AND METHODS

DESIGN AND SETTING OF THE STUDY

The study design used was cross-sectional. This research was carried out in the infertility center of Safeer Al-Imam Al-Hussain Surgical Hospital in Karbala, Iraq, between 7 November, 2025 and 15 January, 2026.

SAMPLE SIZE AND SAMPLING TECHNIQUES

The sample size was calculated using cross-sectional study formula [20], considering the prevalence of depression to infertile women, which was reported as 83.3 percent in a cross-sectional study that was conducted in Baghdad, Iraq (Shalan et al., 2019).

Sample size (n) = $[(Z1-\alpha/2)^2 \times P \times (1 - P)] / d^2$

At 95% Confidence interval=1.96, margin of error or precision (0.05)] n= $(1.96)^2 \times 0.833 \times (1-0.883) \div (0.05)^2 = 214$

The single-proportion formula was used to obtain the initial sample size (n). Adjusted sample size (n) was determined to accommodate any participant loss or non-response using the following formula: n=n/(1 - r). In this study, after taking into consideration similar cross-sectional studies, the rate of attrition was assumed to be 20% and thus the final adjusted sample size was approximately 268 people.

SELECTION CRITERIA AND EXCLUSION CRITERIA

Inclusion criteria: Women of childbearing age who fail to conceive after a year of regular unprotected sex, visiting Safeer Al-Imam Al-Hussain Al-Hospital in Karbala, Iraq.

EXCLUSION CRITERIA

Established irreversible birth control or sterilization (e.g., previous bilateral tubal ligation) as the principal cause of infertility. The existence of a confirmed mental health disorder (e.g., acute psychosis, severe cognitive impairment, and depression) during the study period.

DATA COLLECTION

The data was collected using a standardized questionnaire after translating it into an Arabic version. The first two section complete by the researcher by performing direct interviews with participants, and the third section was filled by participants themselves (self-report).

The questionnaire contains the following information:

Section 1/Sciodemographic characteristics of infertile women, involves (5) items such as age, educational level, occupational status, income level, and place of residence.

Section 2/consisted of obstetric, medical, surgical, and infertility histories.

Section 3/psychological assessment tools, using standardized tools included: Generalized Anxiety Disorder, used to measure anxiety symptoms.

RATING SCORING OF PSYCHOLOGICAL ASSESSMENT TOOLS

The 7-item GAD-7 self-report scale. Seven questions from the past two weeks disturbed participants. It was recommended for assessing GAD severity. None, several days, more than half the days, and practically every day are the options (Gui et al., 2021). The GAD-7 screens for signs of generalized anxiety disorder GAD-7 scores range from 0 to 21. GAD-7 scores indicate anxiety severity: 0-4 indicates minimal anxiety, 5-9 mild, 10-14 moderate, and 15-21 severe [21].

ARTIFICIAL INTELLIGENCE (MACHINE LEARNING)

Machine learning is a discipline within computer science that entails teaching computers to identify patterns in data through mathematical principles and statistical estimations. The main goal is to develop predictive models based on dataset feature statistical connections to predict binary responses, categorical labels, and continuous values [22]. In this study, the RF algorithm was selected because of its ability to model complex non-linear relationships while reducing overfitting through ensemble learning. To enhance model performance, the original dataset of 268 participants was augmented to 1,000 samples during preprocessing. The data were subsequently divided into training (80%) and testing (20%) sets for model development and evaluation [23].

ETHICAL CONSIDERATIONS

An official letter to facilitate this research was obtained from the College of Health and Medical Technologies / Kufa (Ref.No.4809 in 24/9/2025) An official permission was obtained from the Karbala Health Directorate to carry out this work in Safeer Al-Imam Al-Hussain Surgical Hospital in Karbala Province (Ref.No.3854 in 7/10/2025). Verbal consent was taken from all participants.

RESULTS

The total number of respondents is 268, with an age range of 17-46 years, with a mean ± STD of 28.9 ± 6.9. The data were normally distributed using the Kolmogorov-Smirnov test. The response rate was 100%. The majority of respondents were within the age group of 27-36 years 46.3%, followed by those in the age group 17-26 years 39.9%, indicating that the sample was predominantly composed of young to middle-aged individuals. Regarding educational level, the largest proportion of participants had primary education 31.7%, followed by those who could read and write 24.6% and those how not 14.6%. This reflects that the sample was characterized by low to moderate educational levels. In terms of occupation, the vast majority of infertile women were housewives 84.7. This reflected the non-working female population. Most participants reported having sufficient income 64.2%. According to residence, the majority are from urban areas, 67.9%, while 32.1% in rural stings, as seen in Table 1.

Obstetric history reported majority of participants had a marriage duration of ≥4 years 76.1%. Similarly, 67.9% reported infertility duration ≥4 years, suggesting a high prevalence of long –standing infertility in the sample. More than half of infertile women 54.1% had no previous pregnancies, and 80.3% had no history of childbirth, reflecting a considerable burden of nulliparity. Regarding pregnancy outcomes, 26.1% reported a history of abortion, while 10.1% had experienced stillbirth, and only 1.1%reported ectopic pregnancy. Menstrual period showed that 56.7% had regular cycles, whereas 43.3% experienced irregular menstruation. Most participants 64.2% reported no medical and surgical conditions, among those who reported polycystic ovarian condition were the most common 17.9%. Related to gynecological history, 71.3% had no prior gynecological intervention, while 14.9% had a history of cesarean section. In terms of infertility type, primary infertility was slightly more prevalent 54.1% than secondary infertility 45.9%, as shown in Table 2.

The findings show that anxiety was common among the study participants. The largest proportion of participants, 35.1%, experience mild anxiety, followed by moderate anxiety, 31.3%. Lower proportions were observed had severe anxiety, 17.5% and 16.0% had minimal anxiety, as shown in Table 3.

Linear regression analysis was performed to assess the association between GAD-7 anxiety scores and selected infertility-related variables. Type of infertility showed a statistically significant association with anxiety scores ($t=9.193$, $p<0.001$). Similarly, the duration of infertility was significantly associated with GAD-7 scores ($t=17.935$, $p<0.001$) as shown in Table 4.

RESULTS OF INFERTILITY TYPE ANALYSIS USING AI MODE

A machine learning model was used in this work using a dataset of 1000 samples, with 80% set reserve for training and 20% for testing. We used Google Collaborator (Google Collab) to build and analyze our models. The model was trained to classify individuals based on the infertility type using the questionnaire data. They are classified into two groups: primary infertility and secondary infertility. Secondary infertility represented the majority classes (60%), whereas primary infertility accounted for 40%.

MODEL VALIDATION

The machine learning model used for predicting infertility type (primary or secondary) showed good performance with high accuracy and low log loss, suggesting that it successfully predicted both the class labels and the probabilities of infertility type. These results are also supported by the confusion matrix, where most predictions fall on the diagonal (correct classifications), with only a few misclassifications. In particular, the model

Table 1. Sociodemographic characteristics of respondents

Items total (268)		Frequency	Percentage	Chi-square	P-value
Age Groups (Years)	17-26	107	39.9	95.694	0.001
	27-36	124	46.3		
	37-46	37	13.8		
Educational Levels	Not read or write	39	14.6	172.597	0.001
	Read and write	66	24.6		
	Primary	85	31.7		
	Secondary	31	11.6		
	Tertiary	20	7.5		
	Diploma	6	2.2		
	Bachelor	15	5.6		
	High education	6	2.2		
Occupation	Housewife	227	84.7	510.209	0.001
	Governmental Job	13	4.9		
	Privet Job	9	3.4		
	Other	19	7.1		
Income levels	More than sufficient	12	4.5	143.761	0.001
	Sufficient	172	64.2		
	Not sufficient	84	31.3		
Resident	Rural	86	32.1	34.388	0.001
	Urban	182	67.9		

*Chi-square test is significant at the 0.05 level.
Source: Authors own work

has a sensitivity of 100% for secondary infertility (no false negatives), and a specificity of nearly 100% (only one false positive, a case of primary infertility predicted to be secondary infertility). But this single false positive may indicate some overlap in characteristics between primary and secondary infertility, suggesting the multifactorial nature of infertility, as shown in Figure 1.

MACHINE LEARNING RESULTS: ASSOCIATION BETWEEN ANXIETY AND INFERTILITY TYPE

Machine learning analysis revealed that secondary infertility was associated with higher anxiety severity. The proportion of women with secondary infertility increased progressively across GAD-7 categories, from 58.3% among those with minimal anxiety to 71.3% among those with severe anxiety, as shown in Figure 2.

RESULT OF MACHINE LEARNING: ASSOCIATION BETWEEN ANXIETY AND INFERTILITY DURATION

Clear relationship between infertility duration and anxiety severity (GAD-7). Moderate anxiety was the most common category across infertility durations, particularly between 3 and 8 years. Severe anxiety became more frequent with longer infertility duration, suggesting an increasing psychological burden over time, as shown in Figure 3.

DISCUSSION

In line with prior evidence, the present study supports the view that psychological stress is not merely a consequence of infertility but also an important factor associated with its clinical profile. Limited studies have integrated psychological and infertility factors, particularly in Iraq. Using artificial intelligence in infertility studies appears to be virtually absent, especially concerning predicting the type. Most participants were aged 27–36 years, with a statistically significant association (χ^2=95.694, p=0.001). This finding is consistent with studies showing a decline in fertility with increasing age and a higher prevalence of infertility among women aged 30–35 years [24-25]. Regarding education, 31% of participants had primary-level education, indicating a relatively low educational status among infertile women, which partially agrees with previous findings [26]. Housewives represented the majority of respondents

Table 2. Obstetric, Medical & Surgical, and Infertility Histories

	Answer	Freq.	%
Infertility and Obstetric History			
Period of marriage	≤ 3	64	23.9
	≥ 4	204	76.1
Period of infertility	≤ 3	86	32.1
	≥ 4	182	67.9
Type of infertility	Primary	145	54.1
	Secondary	123	45.9
Pregnancy No.	None	145	54.1
	1	60	22.4
	2	21	7.8
	≥ 3	42	15.7
Birth No.	None	215	80.3
	1.00	33	12.3
	2.00	10	3.7
	≥ 3	10	3.7
History of Abortion	Yes	70	26.1
	No	198	73.9
Still Birth	Yes	27	10.1
	No	241	89.9
Ectopic Pregnancy	Yes	3	1.1
	No	265	98.9
Period	Regular	152	56.7
	Irregular	116	43.3
Medical &Surgical History			
	None	172	64.2
	Hypertension	13	4.9
	Diabetes	6	2.2
	Thyroid Disease	7	2.6
	Polycystic ovary disorder	48	17.9
	Other	20	7.5
	Migraine	2	.7
Gynecological history	None	191	71.3
	Tube Surgical	10	3.7
	Uterus	12	4.5
	Ovaries	12	4.5
	Virginal	3	1.1
	Cesarean section	40	14.9

Source: Authors own work

(84.7%), a factor that may affect their ability to cope with infertility and its psychosocial consequences [27]. Income was also significantly associated with study outcomes (p=0.001), and 64.2% of participants reported sufficient income, which may facilitate access to infertility treatment [28]. Furthermore, most participants lived in urban areas (67.9%), with a significant association (χ^2=34.388, p=0.001), reflecting disparities in access to infertility services between urban and rural populations [29]. Most infertile women had been married for ≥4 years (76.1%) and had experienced infertility for ≥4 years 67.9%. Similar findings were reported by Singh and Chand (2025), who highlighted the association between prolonged infertility and increased psycho-

Table 3. Distribution of anxiety scores of GAD7

Anxiety Levels	Freq.	%	Value-test	P-value
Minimal Anxiety	43	16.0	0.211	0.001
Mild Anxiety	94	35.1		
Moderate Anxiety	84	31.3		
Severe Anxiety	47	17.5		
Total	268	100.0		

*Significant at level 0.05.
Source: Authors own work

Table 4. Coefficient linear regression between the overall GAD-7 and infertility-related variables (infertility type and duration).

Model	R	Prediction	Df	T	Sig.
Types of Infertility	0.092	8.445	1	9.193	0.000
Duration of Infertility	0.0117	8.982	1	17.935	0 .000

Source: Authors own work

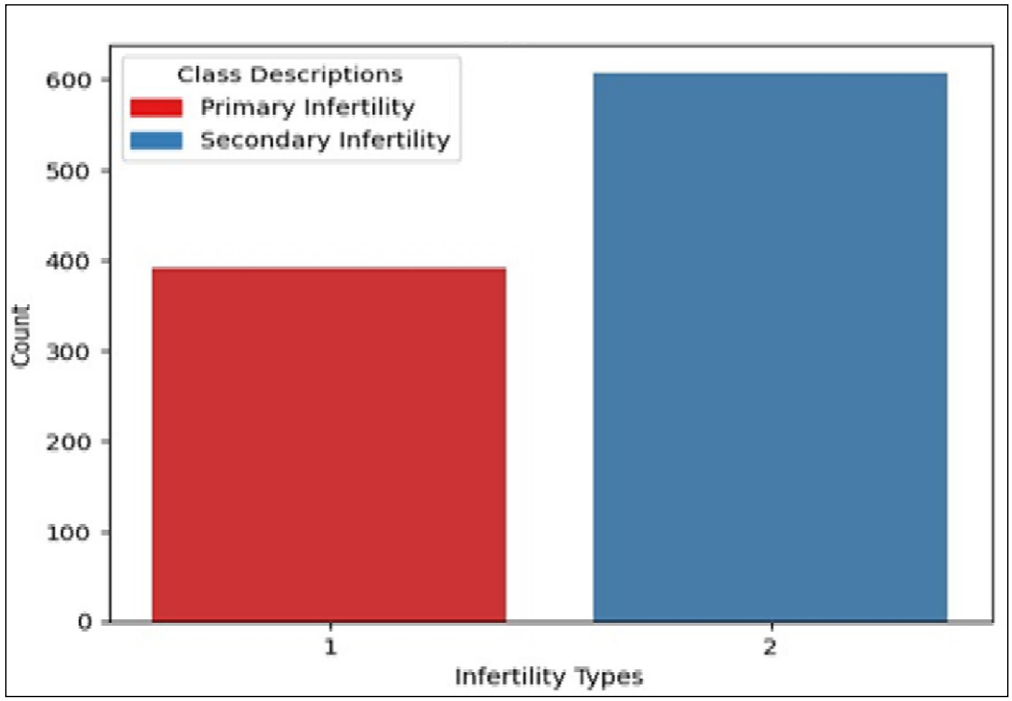


Fig. 1. Infertility type distribution
Source: Own materials

logical distress, anxiety, and depression [30]. Obstetric history was closely related to infertility type, as 54.1% of women had never been pregnant or given birth, corresponding to the prevalence of primary infertility 54.1%, while 45.9% had a history of pregnancy and childbirth, indicating secondary infertility. The higher prevalence of primary infertility is consistent with previous studies and regional patterns reported in North Africa and the Middle East [31-32]. Among women with previous pregnancies, 19.7% had experienced live births and 26.1% abortions, whereas stillbirth (10.1%) and ectopic pregnancy 1.1% were less frequent. These obstetric complications may adversely affect future fertility and contribute to secondary infertility through anatomical and functional reproductive disorders, including tubal blockage and impaired endometrial receptivity [33]. Polycystic ovary syndrome (PCOS) was the most frequently reported medical condition among participants 17.9%, consistent with the prevalence reported by Oriji (2019) (16.7%). PCOS is associated with menstrual irregularities, reduced fertility, and other reproductive complications [34]. Irregular menstrual cycles were reported by 43.3% of participants, a finding comparable to previous research 46.7% [35]. Most women 71.3% had no history of gynecological surgery, while 14.9% had undergone cesarean section, which has been linked to reduced fertility through factors such as uterine niche formation, intra-abdominal adhesions, and lower rates of future conception and live birth [36]. Anxiety was highly prevalent among infertile women

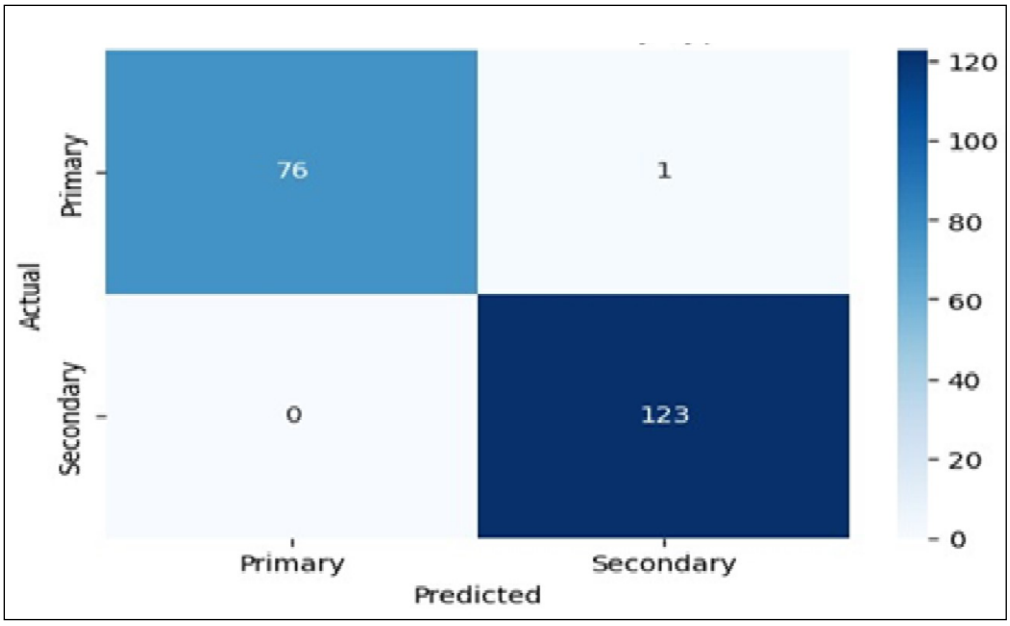


Fig. 2. Confusion matrix-infertility type
Source: Own materials

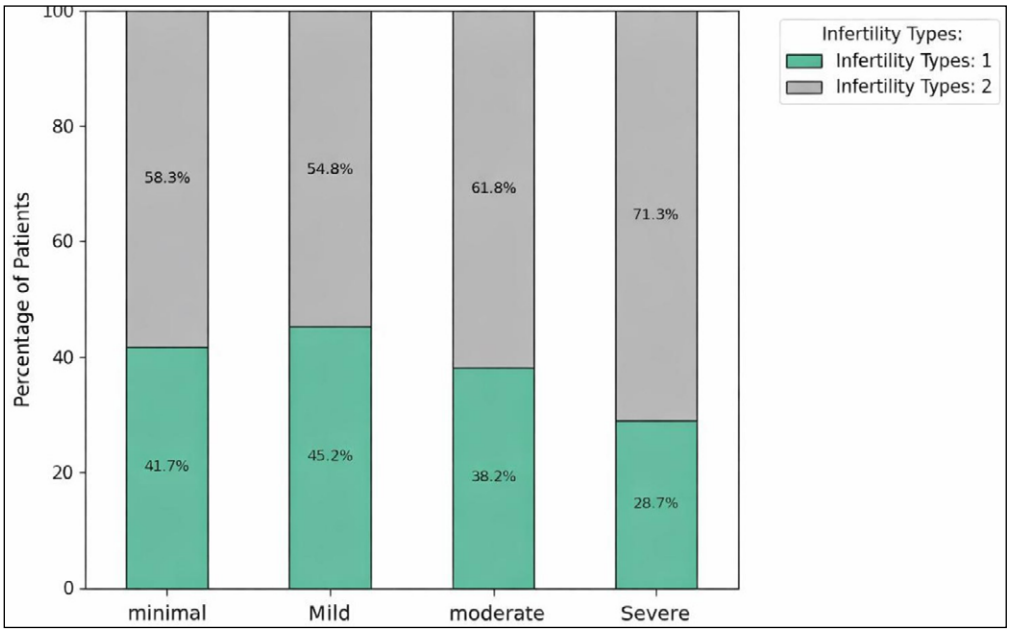


Fig. 3. Distribution within GAD-7 levels by infertility types
Source: Own materials

83.9%, exceeding the level reported in Erbil 36.6% [37], although lower than that reported in an Iranian study 92.5% [38]. Furthermore, mild anxiety was the most common level in the present study 35.1%, whereas moderate anxiety predominated among pregnant women in Al-Najaf 42.9% [27].The present study found a statistically significant association between anxiety levels (GAD-7) and infertility-related factors, particularly infertility duration and type $p<0.001$. Consistent with previous research, prolonged infertility was associated with higher anxiety levels, possibly due to increasing social pressure and the chronic nature of infertility over time [39]. However, some studies have reported no significant relationship between anxiety and infertility [40]. A significant association was also observed between anxiety and infertility type, suggesting that both primary and secondary infertility may contribute to elevated anxiety levels [41]. Previous studies reported increased odds of anxiety among women with secondary infertility (OR=1.27) and primary infertility (OR=1.24), while primary infertility was also identified as an independent risk factor for anxiety in men [42]. Overall, infertility duration appears to have a greater impact on psychological distress than infertility type or cause, highlighting the complex nature of infertility-related anxiety.

INTEGRATING AI AND TRADITIONAL STATISTICS

This research uses statistical and machine learning methods to study infertility. Statistics highlights significant

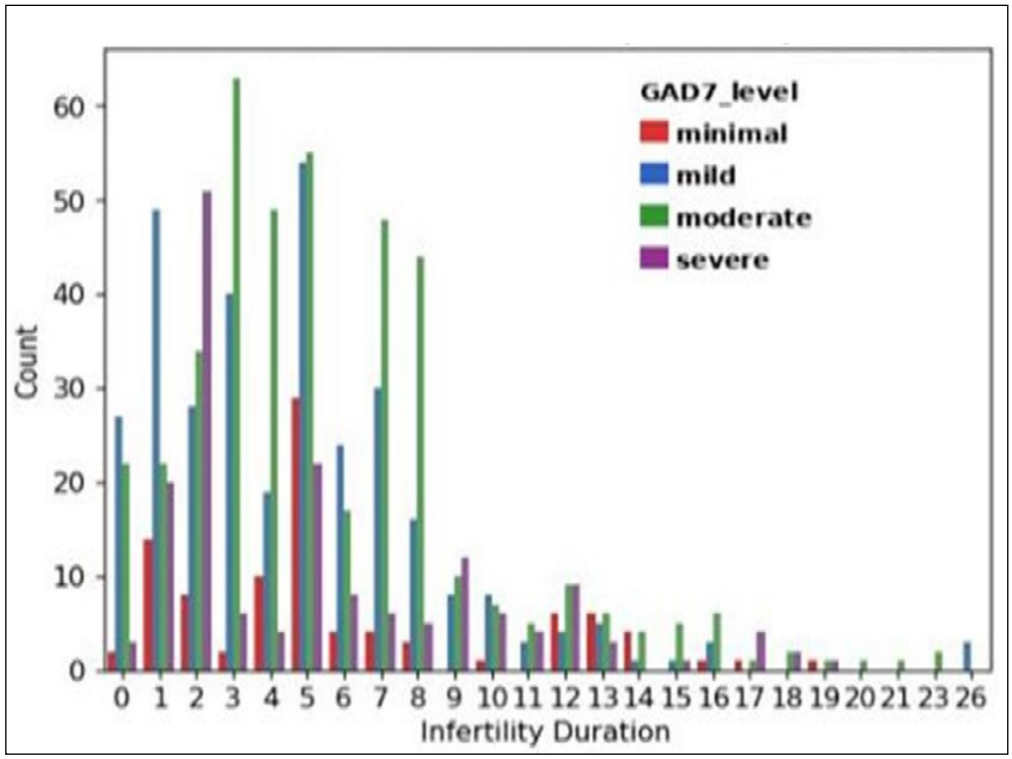


Fig. 4. Distribution of GAD-7 levels by Infertility duration
Source: Own materials

associations (e.g., between anxiety and infertility), and machine learning enhances infertility type prediction. They offer both explanation and prediction, enhancing the findings. The statistical analysis revealed anxiety was common among the participants, with the majority having mild and moderate anxiety, and a significant p=0.001. Linear regression also found a significant association between GAD-7 scores and infertility type and duration. Machine learning supported these findings, as the model showed excellent accuracy in predicting infertility type, with high sensitivity and low misclassification. Further, AI showed that secondary infertility was linked with greater anxiety across all GAD-7 levels, and anxiety levels increased as infertility duration increased.

CONCLUSIONS

This paper highlights the significant role of anxiety in infertility and shows that it is associated with the type and the duration of infertility. Elevated anxiety levels in infertile women are associated with both the duration and type of infertility, highlighting the necessity for prompt intervention. The research is a combination of traditional statistical methods and machine learning to increase the accuracy of the prediction of infertility phenotypes. It points out the importance of psychological support in the treatment of infertility and hints at the possibility of artificial intelligence in reproductive health studies, particularly in countries like Iraq. Longitudinal research and larger data sets should be the subject of future research to enhance predictive models in the treatment of infertility.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

The structure of the Poltava region population, determined by the polymorphisms rs3745274 and rs1799971 significant in the kinetics of anaesthetics

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ABSTRACT

Aim: To determine the population structure of the Poltava region by the significant SNPs rs3745274 and rs1799971 of the CYP2B6 and OPRM1 genes in the kinetics of anesthetics.

Materials and Methods: The study involved 106 people: 51 patients with oncological pathology of the gastrointestinal tract and 55 people in the control group. Genotyping of SNPs rs3745274 and rs1799971 in the CYP2B6 and OPRM1 genes was performed by the polymerase chain reaction method with determination of the length of restriction fragments. Statistical analysis of the data was performed using the GenAlEx population genetic analysis package.

Results: The frequencies of allelic variants and genotypes of the CYP2B6 (c.516G>T) and OPRM1 (c.118A>G) genes are close to the corresponding indicators in the Ukrainian and other European populations. The statistically significant difference in expected genotype frequencies ($p \leq 0.05$) between the patient and control groups for the SNPs of the CYP2B6 gene (c.516G>T) is obviously related to the clinical features of the patient sample.

Conclusions: The identified genetic polymorphism of the population of the Poltava region for the CYP2B6 (c.516G>T) and OPRM1 (c.118A>G) genes may be a pharmacogenetic basis for personalized anesthetics.

KEYWORDS: oncology, personalized anesthetics, single nucleotide polymorphisms, CYP2B6 (c.516G>T), OPRM1 (c.118A>G)

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INTRODUCTION

One of the major challenges in effective pharmacotherapy for pain management is the selection of the drug that best matches the patient's genetic makeup. Genetic variations in clinically significant polymorphisms in the cytochrome p450 2D6 (CYP2D6) and opioid receptor μ1 (OPRM1) genes are relevant to opioid drugs used to treat pain and can be used to predict and support clinical decision-making. Patients can be divided into 3 broad risk categories for opioid side effects and dependence. High-risk patients with dysfunction of CYP2D6 or OPRM1 represent approximately 14% of the population and are best treated with non-opioid drugs. Intermediate-risk patients with reduced CYP2D6 or OPRM1 activity represent approximately 48% of the population and can be treated with dose monitoring. Low-risk patients with functional CYP2D6 and OPRM1 activity constitute approximately 38% of the population and should receive opioid therapy [1].

Cytochrome CYP2B6 is a drug-metabolizing P450 enzyme in the human liver. Expression is highly variable both between and within individuals, due to non-genetic factors, genetic polymorphisms, inducibility, and irreversible inhibition by many compounds. Drugs primarily metabolized by CYP2B6 include artemisinin, bupropion, cyclophosphamide, efavirenz, ketamine, and methadone. *CYP2B6* is one of the most polymorphic *CYP* genes in humans, and its variants have been shown to affect transcriptional regulation, splicing, mRNA and protein expression, and catalytic activity. The most common functionally deficient allele is *CYP2B6*6*, which occurs at a frequency ranging from 15 to over 60% in different populations. The allele results in reduced expression in the liver due to aberrant splicing. Substitutions in the nucleotide sequence of the *CYP2B6*6* allele (516G>T and 785A>G), leading to two amino acid changes, glycine/histidine and lysine/arginine (Q172H and K262R) [2].

A study by Noritaka Ariyoshi et al. (2011) determined that two amino acid substitutions in CYP2B6.6 exhibit completely opposite patterns, suggesting that Q172H has the opposite effect on the metabolic activity of CYP2B6 that is affected by K262R. This study provided the first evidence that the amino acid substitution Q172H can reverse the direction of the effect caused by the K262R substitution in the CYP2B6 polypeptide on the metabolism of certain drugs [3]. A. Mikstacki et al. (2017) studied nucleotide substitutions in the *CYP2B6* (c.516G>T), *UGT1A9* (c.98T>C) and *CYP2C9* (c.1075A>C) genes and found that only the c.516G>T (rs3745274) polymorphism in the *CYP2B6* gene and body mass index affect the rate of propofol metabolism and may play an important role in optimizing propofol anesthesia [4].

The μ1 opioid receptor (OPRM1) is key to analgesia. It is localized primarily in the brain and is responsible for the major positive effects of opioids. The *OPRM1* gene, which encodes this receptor, has a number of single-nucleotide polymorphisms. The most functional polymorphism, c.118G>A (rs1799971), in this gene is associated with altered receptor binding and subsequent signaling, potentially affecting both the efficacy and tolerability of opioid therapy [5]. Studies by L. Wang et al. (2019) showed that carriers of the A allele, the c.118G>A polymorphism of the *OPRM1* gene (rs1799971), affect the dose of opioid analgesics [6]. A meta-analysis of a large number of publications confirmed that carriers of the *G* allele (*AG*+*GG*) of the single nucleotide polymorphism c.118G>A of the *OPRM1* gene (rs1799971) require greater opioid analgesia for the treatment of cancer pain. It has also been shown that this polymorphism can help predict people's response to analgesia and achieve satisfactory pain control in oncological pathologies [7].

DNA analysis of 97 individuals from major regions of Ukraine using genome-wide association studies (GWAS) T.K. Oleksyk et al. (2021) conducted a search of genomic data for genomic variation. The results obtained showed that the genetic diversity of the Ukrainian population is uniquely shaped by evolutionary and demographic factors and cannot be ignored in future genetic and biomedical research [8]. Studies of the clinically significant SNP c.516G>T of the CYP2B6 gene in the Ukrainian population have already been conducted. However, the sample consisted mainly of residents of eastern Ukraine (Kharkiv, Luhansk, and Donetsk regions). [14].

AIM

To determine the population structure of the Poltava region by the significant SNPs rs3745274 and rs1799971 of the CYP2B6 and OPRM1 genes in the kinetics of anesthetics.

MATERIALS AND METHODS

The study included patients of the main group aged 42 to 78 years, living in Poltava and the Poltava region. Among them were 28 men and 23 women (n=51). The patients were examined by doctors of the Municipal Enterprise "Poltava Regional Clinical Oncological Dispensary of the Poltava Regional Council", where they were treated. These patients underwent abdominal surgery for oncological pathology of the gastrointestinal tract in compliance with the basic biotic provisions of the Council of Europe Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on the ethical principles of conducting scientific medical research involving human participants. All patients signed written informed consents in accordance with the Declaration of Helsinki. The control group consisted of 32 men and 23 women (n=55) aged 27-64 years. They did not have pronounced signs of metabolic syndrome, type 2 diabetes mellitus and cardiovascular diseases. The study participants' ethnicity was not considered, just their residence.

Peripheral venous blood was collected from the cubital vein in BD Vacutainer® K2EDTA tubes (Becton Dickinson, USA). DNA extraction from peripheral blood samples was performed using the QIAqube Connect automated nucleic acid extraction system (QIAGEN, Germany) using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. The quality and quantity of extracted DNA were assessed using a NanoPhotometer NP-80 spectrophotometer (IMPLEN, Germany), and the extracted DNA was stored at -80˚C until use.

Genotyping of single nucleotide polymorphisms of the CYP2B6 (c.516G>T) and OPRM1 (c.118A>G) genes was performed by polymerase chain reaction with restriction fragment length determination (PCR-RFLD) according to I. Soultati et al. (2023) [9].

Amplification of DNA fragments of CYP2B6 and OPRM1 genes was performed using the Multiplex PCR Kit according to the manufacturer's instructions (QIAGEN, Germany) in a CFX Opus 96 amplifier (Bio-Rad, USA). Primers were synthesized (Thermo Fisher Scientific, USA). The sequence of oligonucleotide primers and genotyping conditions in PCR–RFLP of *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) gene polymorphisms are given in (Table 1).

Alleles were identified based on the sizes of restriction fragments. Restriction analysis of the amplified DNA fragments was conducted according to the manufacturer's instructions (Thermo Scientific, USA). The resulting frag-

Table 1. Sequence of oligonucleotide primers and genotyping conditions in PCR-RFLP of polymorphisms of the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) genes

Polymorphisms	Primers	Restriction enzyme	Alleles (bp)
CYP2B6 c.516G>T, rs3745274	F: GGTCTGCCCATCTATAAAC R: CTGATTCTTCACATGTCTGCG	BseNI	516G: 268; 241; 17 516T: 509; 17
OPRM1 c.118A>G, rs1799971	F: GGTCAACTTGTCCCACTTAGATCGC R: AATCACATACATGACCAGGAAGTTT	Bsh1236I	118A: 193 118G: 169; 24

Source: compiled by the authors of this study

Table 2. Results of genotyping of healthy men and women of the Poltava population for polymorphisms of the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) genes

Polymorphisms	Alleles	Sex	N	Expected genotype frequency			χ2	p
				MM	*Mm*	*mm*		
CYP2B6, (c.516G>T), rs3745274	*516G*	Women	23	0.546	0.386	0.068	2.865	0.091
	516T	Men	32	0.610	0.342	0.048	2.509	0.113
OPRM1, (c.118A>G), rs1799971	*118A*	Women	23	0.756	0.227	0.017	0.518	0.472
	118G	Men	32	0.739	0.242	0.020	0.857	0.355

Notes. N – sample size, M – major allele, m – minor allele, MM – homozygous for the major allele, Mm – heterozygous, mm – homozygous for the minor allele, p – probability level

Source: compiled by the authors of this study

Table 3. Results of genotyping of patients and control groups of the Poltava population for polymorphisms of the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) genes

Polymorphisms	Alleles	Groups	N	H_e (%)			χ2	p
				MM	*Mm*	*mm*		
CYP2B6, (c.516G>T), rs3745274	*516G*	Patients	51	26.123 (51)	20.755 (41)	4.123 (6)	4.618	0.032*
	516T	Control	55	32.073 (58)	19.855 (36)	3.073 (6)	5.269	0.022*
OPRM1, (c.118A>G), rs1799971	*118A*	Patients	51	38.828 (76)	11.343 (22)	0.828 (2)	1.088	0.297
	118G	Control	55	41.023 (75)	12.955 (24)	1.023 (2)	1.371	0.242

Notes: *N* – sample size, H_e – expected genotype frequency, *MM* – homozygous for the major allele, *Mm* – heterozygous, *mm* – homozygous for the minor allele; *p* – probability level, * - p≤0.05, according to Fisher's exact test

Source: compiled by the authors of this study

ments were separated by size using electrophoresis in a 3% agarose gel (Agarose SFR™, Amresco, USA) in TAE buffer (Thermo Fisher Scientific, USA), with the inclusion of ethidium bromide for staining. Visualization of the fragments was performed using the GelDoc Go Gel Imaging System (Bio-Rad, USA). To assess the accuracy of genotyping, both negative and positive DNA controls were utilized. Statistical analysis of the data was carried out using the GenAlEx population genetic analysis software [10].

RESULTS

The expected genotype frequencies for the studied *CYP2B6* gene polymorphism in women were (0.546, 0.386, 0.068) in men (0.610, 0.342, 0.048). For the *OPRM1* gene polymorphism, the expected genotype frequencies for women and men were (0.756, 0.227, 0.017) and (0.739, 0.242, 0.020), respectively (Table 2). Analysis of the genotyping results of healthy men and women of the Poltava population - the control group, for the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) gene polymorphisms did not reveal statistically significant gender differences in genotype frequencies. This allowed combining the data of men and women into one group.

The expected genotype frequencies for the studied *CYP2B6* gene polymorphism in patients who underwent abdominal surgery for oncological pathology of the gastrointestinal tract were (*GG* = 26.123, *GT* = 20.755,

Table 4. Analysis of the distribution of allele frequencies, observed and expected heterozygosity and fixation index of patients and control groups of the Poltava population according to the studied polymorphisms of the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) genes

Polymorphisms	Groups	N	Alleles	Частота	H_o	H_e	F
CYP2B6, (c.516G>T), rs3745274	Patients Control	51	*516G*	0.716	0.529	0.407	0.301
			516T	0.284			
	Patients	55	*516G*	0.764	0.473	0.361	0.310
			516T	0.236			
OPRM1, (c.118A>G), rs1799971	Patients Control	51	*118A*	0.873	0.255	0.222	0.146
			118G	0.127			
	Patients	55	*118A*	0.864	0.273	0.236	0.158
			118G	0.136			

Notes: N – sample size, Ho – observed heterozygosity, He – expected heterozygosity, F – fixation index
Source: compiled by the authors of this study

TT = 4.123), Table 3. Among the control group sample, the expected genotype frequencies had the following values (*GG* = 32.073, *GT* = 19.855, *TT* = 3.073). A statistically significant difference in genotype frequencies (p≤0.05) was established between the patient and control groups (Table 3).

The expected genotype frequencies for the *OPRM1* gene single-nucleotide polymorphism (c.118A>G) in patients were (*AA* = 38.828, *AG* = 11.343, *GG* = 0.828). Among the control group sample, the expected genotype frequencies had the following values (*GG* = 41.023, *GT* = 12.955, *TT* = 1.023). Analysis of patients and control groups of the Poltava population by the polymorphism of the *OPRM1* gene (c.118A>G) by the expected genotype frequencies did not reveal statistically significant differences between the patient and control groups (Table 3).

The analysis of allele frequencies of the studied gene polymorphisms in a sample of patients who underwent abdominal surgery for oncological pathology of the gastrointestinal tract and in the control group is presented in Table 4. For the *CYP2B6* polymorphism in the patient and control groups, the *516G* allele was found with a higher frequency (p = 0.716 and p = 0.764). The frequencies of the minor allele *516T*, whose carriers are known to have a lower rate of anesthetic metabolism [4] were (q = 0.284 and q = 0.236) in the patient and control groups, respectively. Since there is an influence of the single nucleotide polymorphism of the *CYP2B6* gene (c.516G>T) on the risk of developing various oncological pathologies and a statistically significant difference in expected genotype frequencies (p≤0.05) was found between the patient and control groups, the population frequencies of the Poltava population were characterized by the control group (p = 0.764 and q = 0.236). The observed and the expected heterozygosity for the sample of patients who underwent abdominal surgery due to oncological conditions of the gastrointestinal tract, based on the *CYP2B6* gene polymorphism (c.516G>T), were found to be H_o = 0.529 and H_e = 0.407, as shown in (Table 4). In the control group, these values were slightly lower, with H_o = 0.473 and H_e = 0.361. There was no significant difference between the observed heterozygosity and the expected heterozygosity values. This corresponds to a slight genetic differentiation between the groups in the sample of patients relative to the control, the fixation index was (F = 0.301) and control relative to patients (F = 0.310).

According to the *OPRM1* gene polymorphism (c.118A>G), the actual and expected heterozygosity among the patient sample was H_o = 0.255 and $_{He}$ = 0.222, and for the control group H_o = 0.273 and H_e = 0.236. Significant genetic differentiation between the groups was also not found in the patient sample relative to the control (F = 0.146) and control relative to the patients (F = 0.158) (Table 4).

DISCUSSION

The statistically significant difference in expected genotype frequencies (p≤0.05) between the patient and control groups for the SNP of the *CYP2B6* gene (c.516G>T) is apparently related to the clinical characteristics of the patient sample. As noted above, this group consisted of patients with various oncological pathologies. Some publications have linked the SNP c.516G>T of the *CYP2B6* gene to an increased risk of cancer. Thus, X. Ma et.al. (2023) reported that the genotype frequencies of the c.516G>T polymorphism of the *CYP2B6* gene significantly (P<0.05) deviate from Hardy-Weinberg equilibrium in the patient ($\chi 2$ =4.618) and control ($\chi 2$ =4.758) groups and that the presence of the *516T* allele of the *CYP2B6* gene is associated with an increased risk of lung cancer [11]. Studies by C. Justenhoven et.al. (2014) have shown that the presence of the 516T allele of the CYP2B6 gene is associated with

an increased risk of breast cancer [12]. In turn, Z.H. Yuan et.al. (2011) associate the single-nucleotide polymorphism c.516G>T of the CYP2B6 gene with the risk of developing most forms of acute leukemia [13]. Thus, analyzing the already proven effect of the single nucleotide polymorphism of the CYP2B6 gene (c.516G>T) on the risk of developing various oncological pathologies, it is possible to explain the statistically significant difference in expected genotype frequencies ($p \leq 0.05$) between the patient and control groups.

The percentages of expected genotype frequencies of the Poltava population for the c.516G>T polymorphism of the CYP2B6 gene (GG - 58, GT - 36, TT - 6), Table 3, almost did not differ from the all-Ukrainian (GG - 56, GT - 37, TT - 6), obtained by O. Filiptsova et al. (2018) [14].

According to the SNP of the *OPRM1* gene (c.118A>G), no statistically significant differences were identified between the patient and control groups according to the expected genotype frequencies. The μ-opioid receptor, encoded by the *OPRM1* gene, plays a major role in opiate, alcohol, cocaine and nicotine addiction. Polymorphism in exon one of the *OPRM1* gene (c.118A>G) significantly alters the function of the receptor and the expression of this gene [15]. The results of a meta-analysis performed by Z. Yu et al. (2019) showed that carriers of the *G* allele (*AG*+*GG*) of the *OPRM1* gene polymorphism (c.118A>G) required higher doses of opioids for pain management than carriers of the homozygote *AA* ($P<0.001$). The meta-analysis shows that carriers of the *G* allele (*AG*+*GG*) of the *OPRM1* gene polymorphism (c.118A>G) require higher opioid analgesia for cancer pain management, and genotyping patients may help predict people's response to analgesia and achieve satisfactory cancer pain control [7]. We did not find any published results of studies of associations of the *OPRM1* gene polymorphism (c.118A>G) with increased cancer risk; therefore, the lack of differences in expected genotype frequencies between groups of patients with different oncological pathologies and controls is understandable.

The allele frequencies of the *CYP2B6* gene polymorphism (c.516G>T) in the sample of individuals from the control group – the Poltava population ($p = 0.76$ and $q = 0.24$) determined a significant similarity with the all-Ukrainian one ($p = 0.75$ and $q = 0.25$). [14]. Comparing the frequency of the *516T* allele in carriers known to have a lower rate of anesthetic metabolism, the Poltava population was similar to the Swedish ($q = 0.24$) and slightly different from the German and British ($q = 0.29$ and $q = 0.28$), respectively [16].

One of the most studied genetic variants associated with OPRM1 and opioid analgesic activity is the rs1799971 (p.118A>G) variant of the *OPRM1* gene. Strong evidence for this functional variant is provided in a meta-analysis, which found that the presence of the *GG* genotype was associated with reduced analgesic efficacy and the need for higher doses of opioids [17]. Since no statistically significant differences in expected genotype frequencies were identified between the patient and control groups, the frequencies of the *OPRM1* gene (c.118A>G) alleles of the Poltava population were considered as one group. Comparison of the total frequency of the *OPRM1* gene (c.118A>G) alleles of the Poltava population with other populations from the genotype and phenotype database of the National Centre for Biotechnology Information (NCBI) determined a complete coincidence with the European one ($p = 0.87$ and $q = 0.13$) [18].

CONCLUSIONS

The identified genetic polymorphism of the population of the Poltava region for the *CYP2B6* (c.516G>T) and *OPRM1* (c.118A>G) genes may be a pharmacogenetic basis for personalized anesthetics.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

Vancomycin-resistant *Enterococci*: Local epidemiology and clinical outcomes

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ABSTRACT

Aim: To study local epidemiology, antimicrobial susceptibility patterns, and clinical outcomes of VRE infections among inpatients.

Materials and Methods: A retrospective observational study was carried out on all patients admitted with culture-confirmed Vancomycin-resistant enterococci infections between July 2025 and January 2026 in Najaf, Iraq. Data on demographic characteristics, clinical information, antimicrobial exposure, microbiological findings, and outcomes were obtained from medical records.

Results: A total of 84 patients with Vancomycin-resistant enterococci infection were included. The mean age was 56 ± 18 years, and 57.1% were male. Prior antibiotic exposure was seen in 75.0% of the patients, while 33.3% had previously received Vancomycin therapy. Enterococcus faecium was the predominant species 73.8%, followed by Enterococcus faecalis 21.4%. All isolates were resistant to Vancomycin, while resistance rates against ampicillin, ciprofloxacin, and high-level gentamicin were 70.2%, 63.1%, and 52.4% respectively. Susceptibility to linezolid and daptomycin was still high (97.6% and 98.8% respectively). Urine was the most common specimen source 40.5%, followed by blood cultures 26.2%. In terms of clinical outcomes, 36.9% were admitted to the ICU, 26.2% had a bloodstream infection, 21.4% needed mechanical ventilation, 14.3% had septic shock, and there was an overall mortality rate of 23.8%.

Conclusions: infections due to Vancomycin-resistant enterococci impose a huge healthcare burden in our setting; E. faecium is the leading species and is multidrug resistant. It was, however, associated with significant mortality despite preserved susceptibility to linezolid and daptomycin. Therefore, continuous surveillance coupled with antimicrobial stewardship and strict infection control practices is needed to contain the spread and impact of VRE infections.

KEYWORDS: Vancomycin-resistant enterococci, *Enterococcus faecium*, antimicrobial resistance, epidemiology, clinical outcomes, mortality

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INTRODUCTION

Vancomycin-resistant enterococci (VRE) have recently become identified as one of the most important pathogens in healthcare-associated infections worldwide because of their multidrug-resistant phenotypes. Over the past years, enterococci, mainly *Enterococcus faecium* and *Enterococcus faecalis*, have been considered as normal flora in the human gastrointestinal tract, but due to their increasing role as opportunistic pathogens in hospitalized and immunocompromised patients, they have gained more attention [1]. The management of enterococcal infections has been further complicated by the emergence of Vancomycin-resistance, as Vancomycin is one of the most commonly used glycopeptide antibiotics for severe Gram-positive infections. This has led to increased morbidity, mortality, and healthcare costs [2]. The epidemiology of VRE has changed dramatically over the past two decades in various healthcare settings. Increased rates of Vancomycin resistance, particularly among *E. faecium* isolates, have been documented in hospitals, long-term care facilities, and ICU wards worldwide. Other factors contributing to the spread of resistant strains include overuse of antibiotics, extended hospitalization, invasive procedures, immunosuppression, and poor infection control practices [3]. Differences in prevalence and transmission dynamics of VRE between countries and healthcare institutions are driven by regional heterogeneity in antimicrobial stewardship policies and infection-prevention strategies [2]. VRE causes a variety of infections in clinical settings such as bloodstream infections, urinary tract infections, intra-abdominal infections, surgical site infections, and infective endocarditis. Among these, VRE bacteremia is the most severe manifestation, which has been associated with prolonged hospital stay, increased treatment failure, and higher mortality rates as compared with

Vancomycin susceptible enterococcal infections [2]. Recent systemic reviews and meta-analyses have shown that patients with VRE bloodstream infections have significantly worse clinical outcomes, including increased risk of death and prolonged hospitalization especially among critically ill and hematology-oncology patients [4]. Still, the rising burden of VRE proves very challenging for clinicians and public health systems with little to offer in therapeutic options. Current treatments involve agents like linezolid and daptomycin. However, the recent development of resistance to these antibiotics has further increased the apprehension regarding the future management of VRE infections [5]. Thus, surveillance must continue to be strengthened and the timely detection of cases, together with antimicrobial stewardship and strict infection control, shall be the linchpins in reducing the spread of VRE within healthcare facilities. The local epidemiology is very important because the prevalence, resistance patterns, and clinical impact of VRE vary greatly by geographic region and institution. Data from local surveillance can help to identify the predominant species, resistance phenotypes, patient risk factors, and trends in antimicrobial susceptibility, thus allowing targeted evidence-based therapeutic decisions and infection control interventions [6]. The clinical outcomes of VRE infections at the local level provide useful information on disease burden and resource utilization [7]. Therefore, the present study was conducted with the objective of investigating the local epidemiology and clinical outcomes of Vancomycin-resistant enterococci among patients in the healthcare setting of this study.

MATERIALS AND METHODS

STUDY DESIGN AND POPULATION

This was a retrospective observational study. It included patients admitted to hospitals in Najaf between July 2025 and May 2026. Inclusion criteria were hospitalized patients with culture-confirmed Enterococcus species — that is, isolates identified as Vancomycin-resistant. Duplicate isolates from the same patient, incomplete medical records, and community-acquired isolates (no hospitalization) were the exclusion criteria.

DATA COLLECTION

Data will be collected from electronic medical records and will include patient demographic and clinical characteristics, ICU admission, comorbidities, antibiotic exposure, prior Vancomycin therapy, length of hospital stay, species distribution of VRE isolates, antimicrobial susceptibility profile, distribution of VRE isolates by clinical specimen, and clinical outcomes.

ETHICAL CONSIDERATIONS

Ethical approval will be obtained from the ethical committee/College of Pharmacy, University of Alkafeel. It was data analysis - there would be no direct patient contact or invasive methods. So, patients' consent was not applicable.

STATISTICAL ANALYSIS

Data were entered and analyzed using GraphPad Prism 10.3.1. Continuous variables were expressed as mean ± SD or median with IQR depending on data distribution; while categorical variables were presented as frequencies and percentages. Categorical variables were compared using the Chi-square test or Fisher's exact test as appropriate. Continuous variables were analyzed for differences between groups using the Student's t-test for variables that were normally distributed and the Mann–Whitney U test for non-parametric data. Mortality was the dependent variable. Univariate logistic regression analysis was carried out to identify factors that were associated with mortality in VRE infected patients. Variables with a $p<0.10$ in univariate analysis were considered for inclusion in a multivariate logistic regression model, to find out independent predictors of mortality. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value of less than 0.05 was taken as statistically significant.

RESULTS

PATIENT DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

The total number of patients involved in this study was 84 with VRE infection (Table 1). The mean age was 56 ± 18 years. This could be interpreted as an indication that the majority of people who were affected were either middle-aged or elderly. There were 48 male patients 57.1% and 36 female patients 42.9% which made the male gender slightly more represented in this study. Regarding the site of care, 31(36.9%) patients were admitted to the ICU. It was noted that the study population had a high frequency of several comorbidities. Diabetes mellitus was found in 26 cases 31.0%, chronic kidney disease in 19(22.6%), and malignancy in 14(16.7%). Most of the patients had previous antimicrobial exposure before being diagnosed with VRE infection. Specifically, 63 patients 75.0% had prior antibiotic exposure before VRE isolation, while 28 patients

Table 1 Demographic and clinical characteristics of patients with VRE infection

Characteristic	Value
Number of samples	84
Mean age (years ± SD)	56 ± 18
Male, n (%)	48 (57.1)
Female, n (%)	36 (42.9)
ICU admission, n (%)	31 (36.9)
Diabetes mellitus, n (%)	26 (31.0)
Chronic kidney disease, n (%)	19 (22.6)
Malignancy, n (%)	14 (16.7)
Prior antibiotic exposure, n (%)	63 (75.0)
Prior Vancomycin therapy, n (%)	28 (33.3)
Length of hospital stay (days, mean ± SD)	17 ± 8

Source: Compiled by the authors of this study

Table 2. Species distribution of VRE isolates

Species	Number (n)	Percentage (%)
Enterococcus faecium	62	73.8
Enterococcus faecalis	18	21.4
Other Enterococcus spp.	4	4.8
Total	84	100

Source: Compiled by the authors of this study

Table 3. Antimicrobial susceptibility profile of VRE isolates

Antibiotic	Resistant n (%)	Susceptible n (%)
Vancomycin	84 (100)	0 (0)
Ampicillin	59 (70.2)	25 (29.8)
High-level Gentamicin	44 (52.4)	40 (47.6)
Ciprofloxacin	53 (63.1)	31 (36.9)
Linezolid	2 (2.4)	82 (97.6)
Daptomycin	1 (1.2)	83 (98.8)

Source: Compiled by the authors of this study

33.3% had been exposed to Vancomycin. The average duration of stay in the hospital was 17 ± 8 days for the study population, which shows the substantial burden of healthcare associated with VRE infections.

SPECIES DISTRIBUTION OF VRE ISOLATES

Microbiological identification of the isolates revealed that Enterococcus faecium was the major species associated with VRE infections in this cohort. *E. faecium* was found in 62(73.8%) of the isolates. Other *Enterococcus* species were a small minority, with 4 isolates 4.8%, while *Enterococcus faecalis* accounted for 18 isolates 21.4%. These results highlight the dominance of *E. faecium* in the epidemiology of Vancomycin resistance among enterococci in the health care setting studied (Table 2).

Table 4. Distribution of VRE isolates by clinical specimen

Specimen source	Number (n)	Percentage (%)
Urine	34	40.5
Blood	22	26.2
Wound / surgical site	15	17.9
Intra-abdominal samples	7	8.3
Other	6	7.1
Total	84	100

Source: Compiled by the authors of this study

Table 5. Clinical outcomes of patients with VRE infection

Outcome	Number (n)	Percentage (%)
ICU admission	31	36.9
Bloodstream infection	22	26.2
Mechanical ventilation	18	21.4
Septic shock	12	14.3
Appropriate antimicrobial therapy	67	79.8
Mortality	20	23.8

Source: Compiled by the authors of this study

ANTIMICROBIAL SUSCEPTIBILITY PROFILE

Results of antimicrobial susceptibility testing indicated that all isolates 100% were resistant to Vancomycin, thus confirming the VRE phenotype (Table 3). High resistance was also noted against several commonly used antimicrobial agents. Specifically, 59 isolates 70.2% were resistant to ampicillin and 53 isolates 63.1% were resistant to ciprofloxacin. Moreover, 44 isolates 52.4% expressed high resistance to gentamicin, indicating limited efficacy of aminoglycoside-based combination therapies. Most isolates, however, remained susceptible to some last-line antimicrobial agents. Only 2 isolates 2.4% registered resistances to Linezolid and 1 isolate 1.2% to Daptomycin. These results indicate that Linezolid and Daptomycin remain very effective treatment options for VRE infections in the study population of this investigation.

DISTRIBUTION OF VRE ISOLATES BY CLINICAL SPECIMEN

The distribution of VRE isolates varied with the type of clinical specimen. Urine samples were the most common source — 34 isolates 40.5% — showing that urinary tract infections were the most frequent clinical presentation associated with VRE (Table 4). The second most common source was blood cultures, from which 22 isolates 26.2% were recovered, indicating cases of VRE bloodstream infections. Others were wound/sur-

gical site specimens, which accounted for 15 isolates 17.9%, and intra-abdominal samples, which represented 7 isolates 8.3%; the rest, 6 isolates 7.1%, were from miscellaneous types of specimens.

CLINICAL OUTCOMES

Clinical outcome analysis revealed that 31 patients 36.9% needed ICU admission during their stay. Bloodstream infection was observed in 22 patients 26.2% — a significant proportion of severe cases (Table 5). Moreover, 18 patients (21.4%) needed mechanical ventilation, which indicates severe illness in a subset of our study cohort. Twelve patients 14.3% developed septic shock, showing serious systemic infection. Proper antimicrobial therapy was given to 67 patients 79.8%; most of it included agents active against VRE. Mortality was not changed by the treatment overall (23.8%, 20 deaths in the study population) analysis revealed that 31 patients 36.9% needed ICU admission during their stay. Bloodstream infection was observed in 22 patients 26.2% — a significant proportion of severe cases.

DISCUSSION

VRE has become one of the major nosocomial pathogens across the globe, with its attendant problems of infection control and antimicrobial therapy. This study reports on local epidemiology, antimicrobial resistance patterns, and clinical outcomes of VRE infections, thereby shedding more light on the burden of these organisms in the healthcare setting under study. In the present study, *Enterococcus faecium* was the most common species of VRE 73.8% with *Enterococcus faecalis* being the less common species. This result is in agreement with several international studies that have reported the predominance of E. faecium among VRE isolates. *E. faecium* has been demonstrated to have a higher capacity of acquiring antimicrobial resistance genes [8] which likely contributes to its increased prevalence in healthcare associated infections. Antimicrobial susceptibility patterns demonstrated in this study indicated high resistance rates to several antibiotics, including ampicillin and ciprofloxacin. The results are in line with several surveillance studies and represent the multidrug-resistant nature of VRE. Most importantly, Linezolid and Daptomycin, two of the most effective therapeutic options for serious infections due to VRE at present, retained high levels of susceptibility [9]. Akin susceptibility patterns have been demonstrated in previous studies evaluating treatment outcomes of VRE bacteremia. In our study, we found that the distribution of VRE isolates among clinical specimens was highest for urinary tract infections and second for bloodstream infections. This pattern has been described in prior investigations as well, with invasive infection often following urinary tract colonization [10]. However, from a clinical perspective, bloodstream infections are the most important manifestation of VRE since they are associated with increased morbidity and mortality [11]. Our results also demonstrated several risk factors associated with VRE infection, prolonged hospitalization and ICU admission, and previous exposure to broad spectrum antibiotics. These findings are supportive of published literature that has recognized antibiotic selection pressure and healthcare exposure as factors that will promote the emergence and transmission of VRE [7]. Studies in tertiary care hospitals have consistently shown that previous exposure to Vancomycin, cephalosporins, or carbapenems is a significant risk factor for VRE colonization and infection [12-14]. In this study, the overall mortality rate was 23.8%, which is close to the mortality rates reported in other clinical studies of VRE infections [15]. Mortality was particularly high among patients with bloodstream infections and those requiring ICU care [15-16]. Previous meta-analyses have also shown that VRE bacteremia is associated with significantly higher mortality compared with infections caused by Vancomycin-susceptible enterococci. Several factors may contribute to this increased mortality, including a delay in appropriate antimicrobial therapy, severity of underlying illness, and limited availability of effective treatment options. Regardless of the findings, there are several limitations to the present study. First, the research was carried out in only one city, so the findings cannot be readily generalized to other cities or regions. Second, molecular characterization of resistance genes was not done, so it could have given more insight into the genetic epidemiology of VRE. Multicenter data and molecular analysis in future studies will add to the current knowledge of VRE transmission dynamics. The present study, however, gives important data on the local epidemiology and clinical outcomes of VRE infections and emphasizes the continued need for effective infection control practices, antimicrobial stewardship programs, and continued surveillance to limit the spread of these resistant pathogens.

CONCLUSIONS

Infections due to Vancomycin-resistant enterococci impose a huge healthcare burden in our setting; *E. faecium* is the leading species and is multidrug resistant. It was, however, associated with significant mortality despite preserved susceptibility to linezolid and daptomycin. Therefore, continuous surveillance coupled with antimicrobial stewardship and strict infection control practices is needed to contain the spread and impact of VRE infections.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Study of the cognitive aspects of subjective well-being and Mental Health stability among internally displaced schoolchildren

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ABSTRACT

Aim: A study of cognitive indicators of subjective well-being and mental health stability among internally displaced children in general secondary education institutions, including those with hearing-related disabilities.

Materials and Methods: To measure the cognitive aspects of subjective well-being in internally displaced children, the "Cognitive Features of Subjective Well-Being (CFSWB-3)" technique (O. Kalyuk, O. Savchenko) was used as a diagnostic tool. To assess mental health levels, the Mental Health Continuum – Short Form questionnaire (C.L.M. Keyes, adapted by E. Nosenko and A. Chetveryk-Burchak) was applied. Statistical processing and data analysis were conducted using the R programming environment, version 4.4.3 (2025-02-28 UCRT), in the RStudio IDE 2026.05.1 (Posit Software, PBC). Data processing and visualization were performed using the tidyverse, ggplot2, tibble, gt, and other packages.

Results: The relevance of studying the cognitive aspects of subjective well-being and mental health stability in internally displaced children is driven by the fact that under wartime conditions, they constantly experience emergencies that impact psychological health and increase vulnerability. Their traumatic experience has a significant long-term negative impact on their subjective well-being. The total sample comprised 30 individuals aged 15–16 years. Group 1 included 16 pupils from general secondary education institutions who held internally displaced status (9 male and 7 female respondents). Group 2 consisted of 14 pupils from a special school (6 male and 8 female respondents) who, alongside the vulnerability factor of internal displacement, also had hearing-related disabilities. The study results revealed a predominance of low and average levels of overall life satisfaction in both groups. Overall life satisfaction scores in Group 2 were found to be significantly lower ($p=0.0195$). A significant difference was identified between the groups across hedonic, psychological, and social well-being indicators (ranging from $r=0.5502$ to $r=0.7629$). The "languishing" (depression) level was absent in both groups. A distinctive feature of Group 2 was that all children exhibited a moderate level of mental health stability. In Group 1, a "flourishing" level was observed in 62% of respondents, with 38% demonstrating a moderate level. Several correlations were identified between the CFSWB-3 and MHC-SF indicators: 1) Between satisfaction with relationships with others and psychological well-being ($r=0.41$, $p<0.05$); 2) Between the overall CFSWB-3 scale and psychological well-being (MHCSF) ($r=0.49$, $p<0.01$); 3) Group-level correlations: a negative (inverse) correlation in Group 2 between satisfaction with one's own life and social well-being ($r=-0.59$, $p<0.01$).

Conclusions: Regarding subjective well-being indicators, both groups demonstrated a predominance of low satisfaction with one's own life and relationships with others, a complete absence of high satisfaction levels, and low overall life satisfaction in more than half of the respondents. The absence of "languishing" indicators was also established. Children in Group 2 exhibited lower overall life satisfaction, moderate mental health stability, and lower mental health stability scores across general characteristics as well as their hedonic, psychological, and social components. The absence of stable and systemic correlations between the cognitive aspects of children's subjective well-being and their mental health stability level may indicate the autonomy of various aspects of mental health, manifested more vividly by a specific dissociation between the cognitive component of well-being and the emotional, social, and functional aspects of mental health under stress factors.

KEYWORDS: war, general secondary education institution, internally displaced children, children with disabilities, hearing impairment, mental health, mental health stability, subjective well-being.

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INTRODUCTION

In the context of full-scale warfare, the population of Ukraine is constantly forced to experience emergency situations, which negatively impacts psychological health [1] and increases the vulnerability of various social groups, particularly children. The traumatic experience acquired during war, especially regarding categories such as internally displaced persons, has

a significant long-term negative impact on their subjective well-being [2; 3]. Internally displaced children, as one of the most vulnerable population groups, suffer disproportionately from factors that exacerbate psychosocial stress (loss of housing, livelihoods, social connections, etc.) [3]. Therefore, studying the cognitive aspects of subjective well-being and mental health stability in children of this category under conditions of prolonged war becomes particularly relevant.

A. Kharytynskyi emphasized the need to investigate the characteristics of individual subjective well-being under conditions of war and to understand the psychological essence of this phenomenon. In his view, studying this phenomenon allows for the targeted tracking of the negative impacts of specific factors and the identification of concrete forms of tailored psychological assistance for vulnerable populations. Having conducted a theoretical and methodological analysis of research on individual subjective well-being, the scholar conceptualizes subjective well-being as a multifaceted, integrative category that encompasses the substantive characteristics of a person's complex experience of satisfaction, fosters the realization of their potential, and facilitates the conscious construction of their unique life identity [4].

Comparing the hedonic and eudaimonic approaches to understanding individual subjective well-being and the possibilities of adapting them to the level of psychological practice under the conditions of prolonged war in Ukraine, V. Ostrova and P. Poltoratska conclude that, according to the former approach, subjective well-being implies the dominance of a positive emotional state over a negative one, resulting in the satisfaction of needs. According to the latter approach, individual well-being is a long-term process of self-realization throughout the lifespan, the outcome of which is a specific level of realization across various aspects of positive functioning [1].

Within the framework of this publication, the empirical study conducted by N. Kostruba and L. Liashko is of particular value to us. They investigated the manifestations of psychological resilience during wartime among Ukrainian higher education students aged 18–24 (50 respondents). The scholars revealed statistically significant relationships between psychological resilience and the overall indicator of mental health stability, hedonic well-being, social well-being, and psychological well-being. They also concluded that young people with high psychological resilience are able to remain somewhat independent of external influences, maintain a balance between the strength of personal impact and sensitivity to environmental influences (particularly stressful situations under wartime conditions), as well as demonstrate a predominance of positive emotions over negative ones [5]. While this study addresses issues of vulnerability during war, it should be noted that it does not address the manifestations of psychological resilience under wartime conditions among general secondary education pupils, particularly those among internally displaced persons, which highlights the expediency of conducting such research.

Among scientific studies dedicated to the mental health issues of senior high school students, a study by Z. Şimşir Gökalp, involving 405 students aged 13 to 18, is particularly noteworthy. According to the findings of this study, resilience mediates the relationship between self-control and psychological well-being [6]. However, this study also does not include students from among internally displaced persons.

In their review of risk and protective factors for the mental health of children and adolescents who have been forcibly displaced to high-, middle-, and low-income countries, M. Fazel, R. Reed, C. Panter-Brick, A. Stein, et al. emphasize the need for research into protective factors that positively influence a child's psychological functioning, as well as the context and interactions among the many predictor variables identified as influencing mental health vulnerability and resilience [7; 8]. In their scientific work, B. Perelli-Harris, J. Zavisca, N. Levchuk, and T. Gerber examine the gap in subjective well-being between internally displaced persons and local residents. They note that this gap diminishes after accounting for economic and housing status, as well as support from local networks [9]. In turn, O. Toren, T. Novoseller, D. Selig, M. Bar, and G. Madar revealed that displacement status per se is not a direct predictor. Instead, individual emotional well-being and emotional functioning serve as significant predictors of anxiety, fully mediating the relationship between displacement and anxiety [10]. Furthermore, L. Karamushka demonstrated that coping strategies act as a substantial psychological factor of subjective well-being [11]. A study on the psychological well-being of child refugees demonstrated a significantly higher frequency of stress reactions among children whose mothers had difficulty coping with displacement-related stress. Furthermore, children residing in collective shelters faced a higher mental health risk than their peers living with host families [12].

S. Arakelyan & A. Ager emphasize that interventions aimed at improving the mental health and psychosocial well-being of internally displaced children should place greater focus on promoting positive proximal processes between the active, developing child and "significant

others" (parents, peers, adults, teachers, etc.), symbols (culture, language, belief systems), and objects (policies and institutions) within their daily environment [13].

Adopting a socio-ecological approach focused on multidimensional aspects of psychological functioning, G. Veronese, A. Pepe, G. Sala, I. Yamien, and M. Vigliaroni investigated the relationship between subjective well-being across various domains and psychological distress among internally displaced adolescents living in refugee camps. Their findings revealed that the adolescents exhibited low levels of psychological distress despite numerous traumatic experiences and ongoing stressful conditions in the camps. The scholars hypothesized that youth maintain positive psychological functioning when subjective well-being and other personal and relational resources act as protective factors [14]. Another study by G. Veronese, A. Pepe, G. Sala, and M. Vigliaroni on measuring subjective well-being among an understudied population of internally displaced and refugee youth from West Africa highlights several shortcomings during the translation and cultural adaptation of the subjective well-being assessment scale for the target population. Through the analysis of a real empirical case, the authors revealed the dynamic and culturally driven nature of the subjective well-being construct [15]. B. Çakır-Mete, A. Ergün, and A. Şafak, having conducted a thematic analysis of online semi-structured interviews with 10 non-governmental organization workers in psychosocial support within Syrian camps, emphasized the inseparability of children's mental health from basic needs. They also underscored the role of place attachment in return processes and the importance of a holistic approach that accounts for context-specific stressors and the resources available during this period [16].

The authors of this article themselves, in previous publications in collaboration with other scholars, have examined certain aspects of the problem under study. Specifically, they identified stress level indicators in internally displaced persons who relocated from active combat zones [17]; outlined the legal and technological foundations for providing assistance to vulnerable population groups under wartime conditions [18]; and defined the specifics of working with internally displaced families and host communities, as well as prospects for ensuring their sustainable development [19].

However, the study of the cognitive aspects of subjective well-being and mental health stability in internally displaced children-particularly those who, alongside forced displacement, possess additional vulnerability factors-remains under-researched, which served as one of the key motivations for selecting this research focus.

AIM

The aim of the article is to study the indicators of cognitive aspects of subjective well-being and mental health stability among children in general secondary education institutions who hold internally displaced person status, as well as children who, alongside this vulnerability factor, have a hearing-related disability.

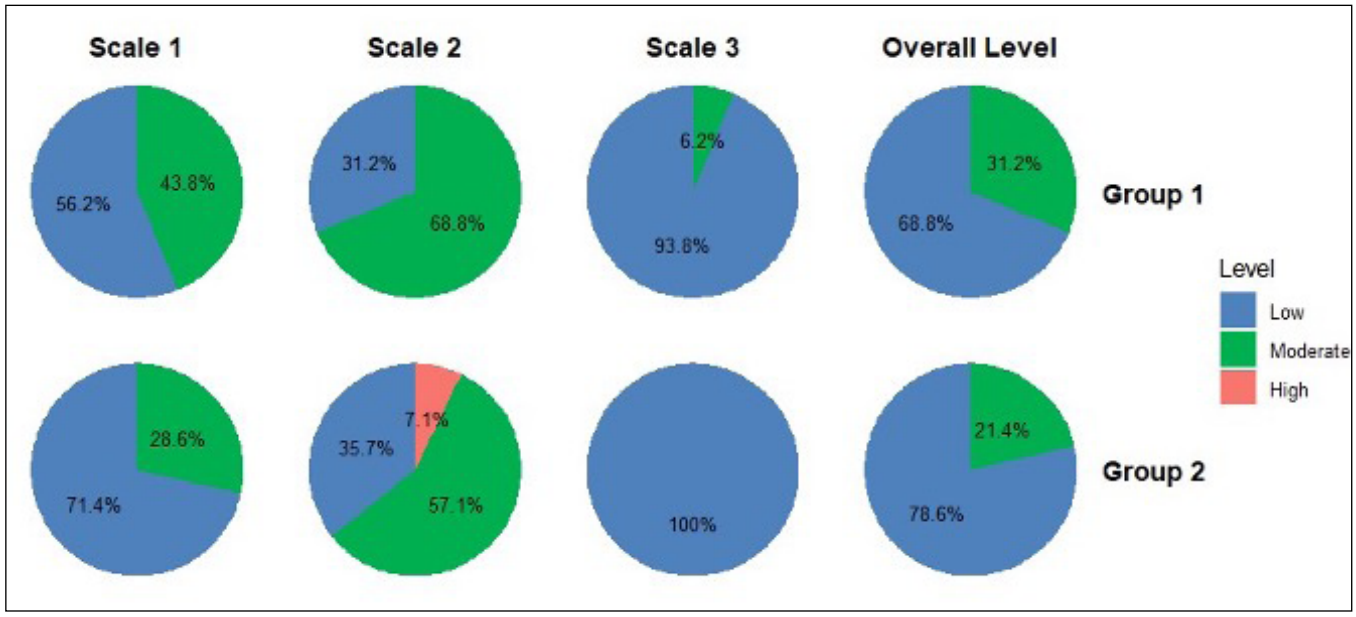
Research tasks:

1. to identify the indicators of cognitive aspects of subjective well-being and mental health stability among the specified vulnerable groups of children;
2. to compare the outlined indicators across the respective groups;
3. to determine the relationships between the indicators of cognitive aspects of subjective well-being and mental health stability in internally displaced children in general secondary education institutions.

MATERIALS AND METHODS

The study was conducted from September 2025 to April 2026 in the city of Ternopil across three types of general secondary education institutions (hereinafter referred to as GSEIs): a gymnasium, a lyceum, and a special school. The total sample comprised 30 individuals aged 15–16 years. The first group included 16 students holding internally displaced person (IDP) status (GSEI types: gymnasium, lyceum), whereas the second group was formed of 14 students who, along with being internally displaced, had a certified disability associated with hearing impairment and attended a special school. In the first group, the proportion of male participants was 9, and female participants was 7; in the second group, there were 6 male and 8 female participants, respectively.

To measure the cognitive aspects of subjective well-being, the "Cognitive Features of Subjective Well-Being (CFSWB-3)" instrument [20] was employed [20]. The methodology is grounded in modern approaches to understanding subjective well-being with a primary focus on its cognitive component-specifically, how individuals evaluate their quality of life across various domains. The core instrument consists of a standardized Likert-type questionnaire featuring several subscales. These subscales measure three independent aspects of life satisfaction: "Satisfaction with One's Own Life", "Dissatisfaction with Oneself and Disillusionment with Life", and "Satisfaction with Relationships with Others". An overall life satisfaction score is calculated using a designated formula. Primary results (subscale scores and the overall index) are transformed according to a standardized algorithm into a 9-point stanine scale, supplemented by a generalized 3-level categorical interpretation scale classifying levels as low, average, or high.



Notes:
• Scale 1 – Satisfaction with One's Own Life
• Scale 2 – Dissatisfaction with Oneself and Disillusionment with Life
• Scale 3 – Satisfaction with Relationships with Others
• Overall Level – Overall Level of Life Satisfaction

Fig. 1. Comparison of life satisfaction levels assessed by the CFSWB-3 methodology between study groups

To assess mental health levels, the "(MHC-SF Mental Health Continuum – Short Form" C. Keyes; adapted by E. Nosenko & A. Chetveryk-Burchak was utilized [21]. This instrument accounts for the three-component structure of mental health, conceptualized as the experience of hedonic, psychological, and social well-being [22]. The questionnaire enables the classification of mental health status into categorical levels: "flourishing", "moderate" and "languishing" [21].

Standard descriptive statistical methods were applied to summarize the data. The normality of data distributions was tested using the Shapiro-Wilk test alongside graphical visualization methods, including frequency histograms overlaid with theoretical normal curves, normal probability plots (Normal P-Plots), and box plots. In the absence of normal distribution, robust descriptive statistics were used, namely the median (Mdn) as a measure of central tendency and the interquartile range (IQR) as a measure of data variability.

To assess the degree of uncertainty of the median and construct its 95% confidence interval, a bootstrap replication procedure with bias-corrected and accelerated (BCa) confidence intervals was applied following standard methodology [23; 24]. The bootstrap procedure was conducted using R = 5,000 replications.

Unlike the percentile approach, the BCa method incorporates corrections for estimation bias and accounts for the skewness of the statistic's distribution, providing higher accuracy and robustness in interval estimation and, consequently, confidence limits, particularly for small samples and non-normal distributions [25]. The lower and upper bounds of the confidence interval were determined using adjusted percentiles of the bootstrap distribution, calculated taking into account the bias and acceleration parameters. This approach does not require the assumption of normality and relies directly on the empirical distribution of the generated bootstrap estimates.

Given the frequent departures of sample distributions from normality, non-parametric statistical methods were predominantly employed, specifically the Mann-Whitney U-test for comparing two independent groups. To evaluate the relationships between indicators, Spearman's rank correlation coefficient (ρ) was applied.

Statistical processing and data analysis were conducted using the R software environment version 4.4.3 (2025-02-28 UCRT) within the RStudio IDE 2026.05.1 integrated development environment (Posit Software, PBC). Data wrangling and visualization were performed using the tidyverse, ggplot2, tibble, gt packages, among others.

Data collection and analysis were carried out in full compliance with ethical and legal standards governing human subjects research.

RESULTS

As a result of the diagnostics evaluating the cognitive aspects of subjective well-being among internally dis-

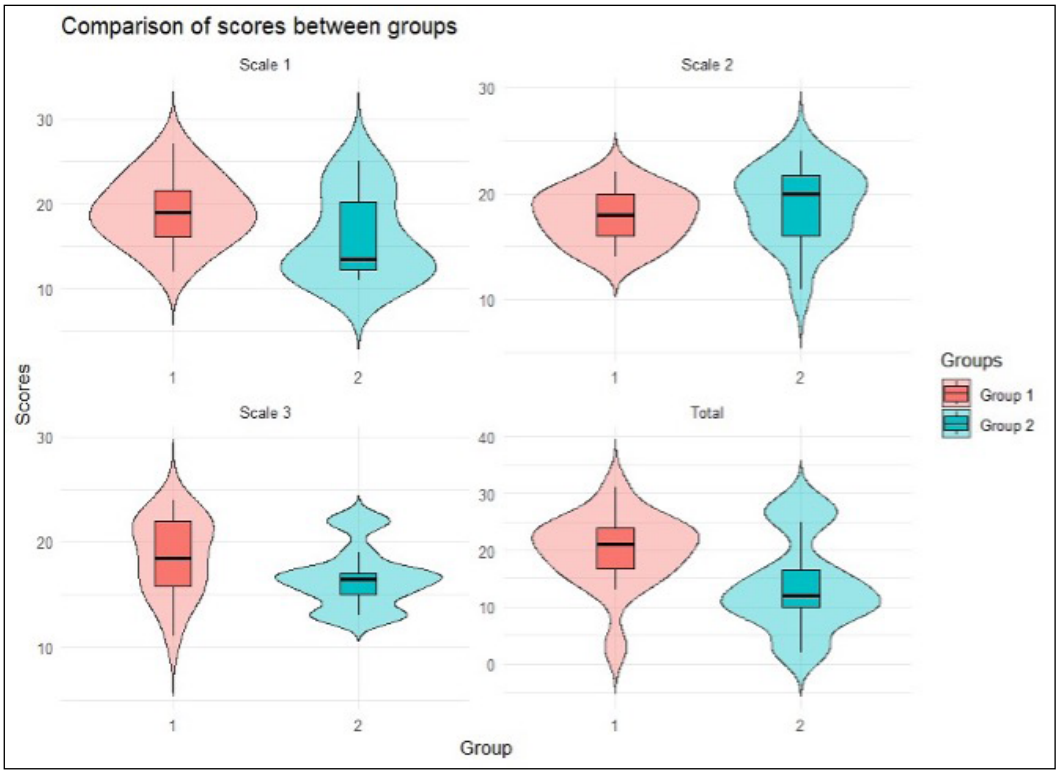


Fig. 2. Comparison of CFSWB-3 scores across study groups

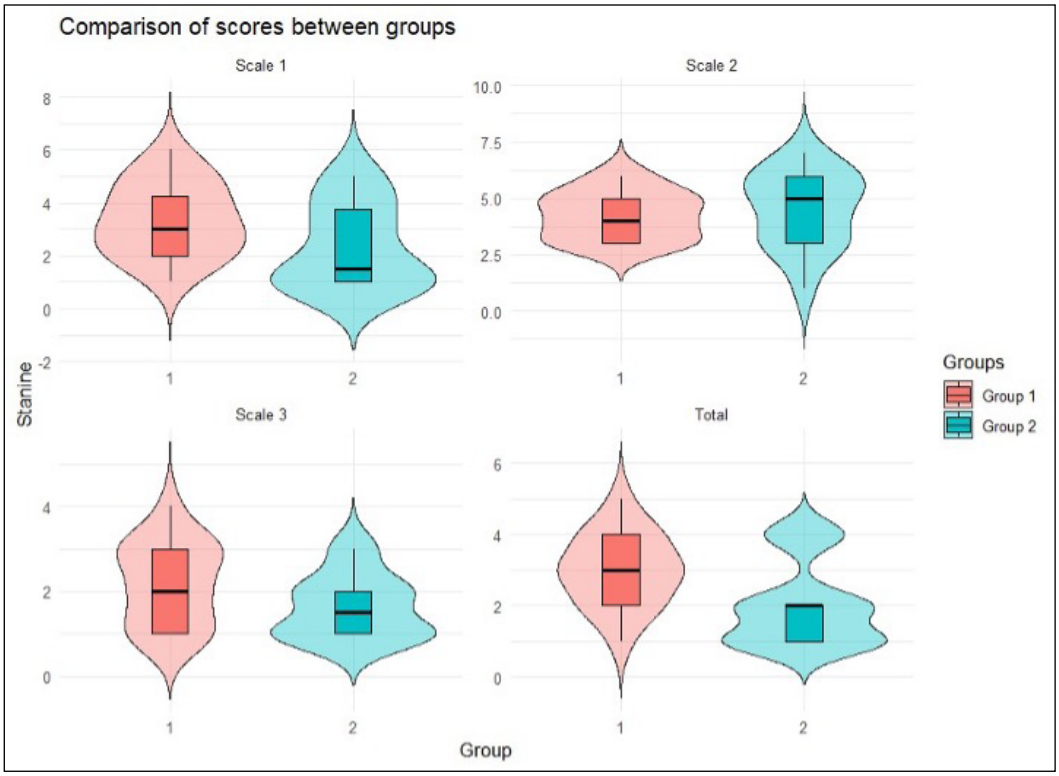


Fig. 3. Comparison of CFSWB-3 stanine scores across study groups

placed children, low and average levels of overall life satisfaction were found to predominate in both groups. Specifically, more than half of the respondents demonstrated a low overall level of life satisfaction, while a high level was entirely absent. Across Scale 1 and Scale 3 (Satisfaction with One's Own Life and Satisfaction with Relationships with Others), a low level of satisfaction also prevailed in both groups.

Interpretation of the data obtained via the CFSWB-3 methodology indicates that these indicators reflect internally displaced children's dissatisfaction with their living conditions, a negative attitude toward certain activities and events, an inferiority complex, and self-doubt regarding their success. They experience a sense of danger and discomfort. Furthermore, they find it challenging to establish long-term, trusting relation-

Table 1. Comparison of life satisfaction indicators between groups

n_{G1} (Group 1)	n_{G2} (Group 2)	Me_{G1} [Q1; Q3]	Me_{G2} [Q1; Q3]	95% CI Me (G_1)	95% CI Me (G2)	W	U	p	Effect Size (r)
16	14	3 [2; 4]	2 [1; 2]	[2; 4]	[1; 2]	167	31	0.0195	0.4265

Table 2. Comparison of mental health stability indicators between groups

Mental Health Stability Indicator	n Group 1x	% Group 1	n Group 2	% Group 2
Flourishing	10	62	0	0
Languishing	0	0	0	0
Moderate level	6	38	14	100

Table 3. Comparison of mental health stability indicators between groups

Indicator	n_{G1}	n_{G2}	Me_{G1} [Q1; Q3]	Me_{G2} [Q1; Q3]	95% CI (G_1)	95% CI (G_2)	W	U	p	Effect size (r)
hedonic component of well-being	16	14	12 [9; 12]	9 [9; 9]	[9; 12]	[8; 8]	180.5	44.5	0.0026	0.5502
Social well-being	16	14	19 [17; 20]	14 [12.25; 15]	[17; 20]	[12; 14.5]	194.5	58.5	0.0006	0.6272
Psychological well-being	16	14	24 [19.75; 24]	16 [14.5;17.75]	[19; 24]	[13; 16]	212.0	76.0	0.0001	0.7629
Total Mental Health Stability Index	16	14	52.5 [50; 56]	40 [37; 42.5]	[50; 54.5]	[36; 41]	207.5	71.5	0.0001	0.7224

Table 4. Comparison of mental health indicators between groups

Group	Male		Female		U	p
	n	Me (Q1–Q3)	n	Me (Q1–Q3)		
Total	15	44 (37–53)	15	43 (40–51)	119.0	0.803
Group 1	9	53 (52–56)	7	51 (50–53)	33.5	0.873
Group 2	6	37 (34–41)	8	40 (39–43,25)	13.0	0.171

ships and rely on others. Communication is perceived by children in this category as forced, while peers and adults in their environment are viewed as obstacles to fulfilling their essential needs [20].

The trends observed on Scale 2 (Dissatisfaction with Oneself and Disillusionment with Life) appear quite natural. Given the inverted nature of this scale (which assesses dissatisfaction), average levels of dissatisfaction predominated, and high levels of dissatisfaction even emerged within the second group. Overall, this points to high levels of dissatisfaction among respondents in both groups regarding their achievements and activities; they derive little satisfaction from social interaction and feel uncomfortable in their social environment. The majority of life events are perceived negatively, accompanied by a prevailing sense of self-doubt, disbelief in receiving support from others, and feelings of life disillusionment [20].

The results obtained across these scales, which outline the cognitive aspects of subjective well-being among displaced children in both groups, are presented in Figure 1.

Comparing the indicators of overall life satisfaction in categorical terms (low, average, and high), it was found that the overall life satisfaction scores in the second group were significantly lower (p=0.0195). The difference between the indicators, represented by the effect size, was moderate (r = 0.426) (Table 1).

This situation may suggest that internally displaced children with disabilities, compared to those with only a single vulnerability factor (forced displacement), experience higher levels of dissatisfaction with their activities and achievements, as well as their social circle, feel more uncomfortable in the presence of others, and demonstrate greater self-doubt and disbelief in the possibility of receiving help and support from others.

At the same time, it should be noted that statistical analysis of the raw data from the subjective well-being test revealed several noteworthy statistical patterns. Specifically, the analysis yielded no statistically significant differences between the groups across the individual subscales (Scales 1, 2, and 3), whether evaluated using raw test scores or standardized stanine scores ($p > 0.05$). However, the integral well-being index (both raw and stanine data) demonstrated statistically significant intergroup differences ($p > 0.05$) (Fig. 2 and Fig. 3).

When comparing the groups by gender, no statistically significant difference was found between males in Group 1 and Group 2 ($p=0.246$). A similar trend was observed when comparing the scores among females between the first and second groups ($p=0.115$).

Thus, the statistical difference between the first and second groups manifests only when comparing the groups at the level of the categorical three-level scale. This finding may be explained by the aggregation effect, wherein small yet systematic differences at the level of individual components accumulate and become statistically significant within the composite index. Furthermore, integral indices are characterized by higher reliability and lower susceptibility to random error, which enhances the sensitivity of the statistical analysis.

The consistency of the results between the raw and standardized (Stanine) scores demonstrates their stability and enhances the credibility of the conclusions drawn.

During the assessment of mental health stability in both groups, the indicator of "languishing" was found to be entirely absent. Specifically, in Group 1, 62% of the internally displaced children demonstrated a level of "flourishing", whereas a moderate level of mental health stability was present in 38% of respondents. A distinctive feature of the second group was that all internally displaced children with disabilities exhibited a moderate level of mental health stability (Table 2).

Diagnostics of mental health stability also revealed a statistically significant difference between the groups across the indicators of hedonic, psychological, and social well-being. Furthermore, the difference (effect size) was large (ranging from $r=0.5502$ to $r=0.7629$) (Table 3).

No significant differences in total mental health stability scores were found based on gender, neither within the sample as a whole nor separately within each group (Table 4).

Statistical analysis revealed several correlations between the parameters of the CFSWB-3 scale and the MHC-SF. Specifically, positive correlations were identified between the scores on the Satisfaction with Relationships with Others scale (CFSWB-3) and the Psychological Well-Being scale (MHCSF) $r=0.41$ $p<0.05$, as well as between the overall CFSWB-3 scale scores and the Psychological Well-Being scale scores (MHCSF) $r=0.49$ $p<0.01$. Certain correlations were also identified at the group level. In Group 2, a negative correlation was found between the Satisfaction with Life scale (CFSWB-3) and the Social Well-Being scale scores $r=-0.59$, $p<0.01$.

However, the identified correlations cannot be described as systemic. These findings may indicate the relative autonomy of the cognitive component of subjective well-being from the emotional, social, and functional aspects of mental health. This is consistent with Keyes' model, according to which different components of well-being (emotional, psychological, and social) are interrelated but not identical [26]. Such a result may suggest the possibility of a specific dissociation between the cognitive component of well-being and the emotional, social, and functional aspects of mental health. Theoretically, this situation aligns with Keyes' model, which accounts for the relative autonomy of various dimensions of mental health [26]. Nevertheless, the obtained correlations, combined with existing literature data, point to a complex interaction between the cognitive components of well-being and the emotional, social, and functional aspects of mental health.

CONCLUSIONS

The study conducted among general secondary education students with internally displaced person (IDP) status (Group 1) and children who, in addition to this vulnerability factor, have a hearing impairment disability (Group 2) revealed both similarities and differences in their subjective well-being and mental health stability indicators:

1. Low levels of satisfaction with one's own life and relationships with others predominated in both groups. A high overall level of life satisfaction was absent, whereas low overall life satisfaction was present in more than half of the respondents. Furthermore, overall life satisfaction scores were statistically significantly lower among children in Group 2 ($p=0.0195$).

2. Indicators of languishing were absent in both groups. Children in Group 1 demonstrated moderate

and flourishing levels of mental health stability. Conversely, Group 2 was characterized exclusively by moderate mental health stability levels. Comparing mental health stability across its dimensions, the indicators in Group 2 were lower both for the overall scores and across all individual components (hedonic, psychological, and social).

3. Particular attention should be paid to the absence of stable and systemic correlations between the indicators of cognitive aspects of children's subjective well-being and their level of mental health stability. This finding may be explained by the autonomy of various mental health dimensions, which becomes more pronounced through the specific dissociation between cognitive components of well-being and the emotional, social, and functional aspects of mental health under stressful conditions.

Future research should focus on fostering resilience among internally displaced children experiencing multiple vulnerability factors, as well as restoring their capacity to adapt to difficult life circumstances despite war-related traumatic experiences.

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ORIGINAL ARTICLE

Assessment of physiological changes and levels of KIM-1 and MDA biomarkers resulting from TDM on vancomycin-induced subclinical nephrotoxicity for pediatrics in Al-Najaf city: A case-control study

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ABSTRACT

Aim: The present investigation set out to evaluate the physiological alterations together with the concentrations of the KIM-1 and MDA biomarkers as they relate to TDM-guided Vancomycin treatment.

Materials and Methods: A prospective case-control study was undertaken at Al-Zahraa Teaching Hospital, Al-Najaf, Iraq, recruiting 140 pediatric patients receiving intravenous Vancomycin. Through random allocation, participants entered either a control arm administered conventional empirical doses n=70 or a case arm in which dosing was refined by means of pharmacist-led TDM guidance (n=70).

Results: The institution of pharmacist-led therapeutic drug monitoring (TDM) markedly enhanced attainment of Vancomycin targets and promoted clinical recovery. Within the optimized-therapy group, KIM-1 concentrations declined substantially—signaling diminished renal tubular injury—whereas in those maintained on empirical therapy, these concentrations climbed. TDM-directed dose modifications further yielded a pronounced fall in MDA levels, denoting attenuated oxidative stress.

Conclusions: Pharmacist-led therapeutic drug monitoring bolsters both the safety and the efficacy of Vancomycin treatment in the pediatric setting. Calibrating the dose on the strength of TDM translated into superior clinical outcomes while restraining subclinical nephrotoxicity and oxidative stress, as evidenced by the pronounced decline in KIM-1 and MDA concentrations.

KEYWORDS: physiological changes, Vancomycin, biomarkers, therapeutic drugs

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INTRODUCTION

Vancomycin is a glycopeptide antibiotic that is used broadly in the treatment of serious infections caused by Gram-positive bacteria, particularly methicillin resistant Staphylococcus aureus (MRSA), coagulase negative staphylococci and enterococci [1, 2]. However, despite its many clinical applications, vancomycin therapy is not without its challenges, being a drug that has a small therapeutic window and significant inter-individual variability in its pharmacokinetic properties. These worries are even more significant in the pediatric setting because maturational changes are likely to have a significant impact on drug distribution and, therefore, on therapeutic outcomes in this population [3, 4]. Pediatric pharmacokinetics are substantially different from adults because of age-related changes in body composition, organ maturation, protein binding and renal clearance. Critically ill children may have other physiologic changes such as fluid shifts, capillary leak syndrome, hypoalbuminemia, systemic inflammation, and increased renal clearance, which may all result in unpredictable vancomycin serum levels. Standard empirical dosing may therefore lead to subtherapeutic exposure with a consequent risk of treatment failure or excessive drug accumulation with a risk of toxicity [5, 6]. Therapeutic drug monitoring (TDM) has emerged as a key strategy to optimize treatment with Vancomycin by tailoring the dosing to measured serum concentrations and patient-specific pharmacokinetic characteristics. Pharmacist-led TDM programs have shown significant benefits in target attainment, therapeutic effectiveness and adverse drug reactions reduction. This is why there is an increasing recommendation for such individualized approaches in pediatric patients treated with vancomycin, given the large variability identified

in this patient group [7-9]. Vancomycin-induced nephrotoxicity remains one of the most important adverse effects associated with treatment. Traditional renal biomarkers, such as serum creatinine and blood urea nitrogen, are commonly used to evaluate kidney function, but they are often insensitive to early structural renal injury. Kidney injury molecule-1 (KIM-1) serves as a highly sensitive indicator of proximal tubular damage. Damage detectable soon after nephrotoxic injury, permitting early recognition of subclinical kidney damage before abnormality of conventional markers [10, 11]. Oxidative stress is considered a key factor in vancomycin-related kidney injury. Malondialdehyde (MDA) is a well-known end product of lipid peroxidation and a reliable indicator of oxidative cell damage. The simultaneous assessment of KIM-1 and MDA enables the comprehensive evaluation of renal tubular injury and oxidative stress, respectively, providing insight into the biological consequences of vancomycin exposure [12-19].

AIM

The aim of this study is to evaluate biochemical markers, kidney injury molecule-1 (KIM-1) and malondialdehyde (MDA), in children receiving vancomycin therapy. An additional aim is to investigate the potential impact of therapeutic drug monitoring in reducing subclinical nephrotoxicity and oxidative stress induced by vancomycin.

MATERIALS AND METHODS

STUDY DESIGN

This study was a prospective case-control study carried out in Al-Zahraa Teaching Hospital, Al-Najaf City, Iraq. Those entered into the study were children undergoing intravenous vancomycin therapy for severe bacterial infections that called for hospital admission and stringent clinical observation.

STUDY POPULATION

140 children were admitted and divided into two groups of equal size. The control group (n = 70) was given standard empirical vancomycin dosing as per prevailing clinical practice, while the case group (n = 70) underwent pharmacist-guided therapeutic drug monitoring (TDM), where doses were adjusted on an individual basis based on vancomycin serum levels and pharmacokinetic assessment. The eligible participants were selected from the predetermined inclusion/exclusion criteria and informed consent was obtained from the parents/legal guardians of any patient prior to inclusion in the study.

DATA COLLECTION AND CLINICAL EVALUATION

Demographic and clinical data were obtained from patient records and physical examination, including age, sex, place of residence, type of infection, body weight, height, body mass index, temperature, heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure and length of stay in hospital. Laboratory testing consisted of a complete blood count, serum creatinine, blood urea nitrogen, estimated GFR, electrolytes, C-reactive protein and vancomycin trough levels.

PHARMACIST INTERVENTION AND BIOMARKER ASSESSMENT

Blood samples were collected from the veins twice – at baseline and at follow-up. After the samples were centrifuged, the serum obtained from these samples was stored at -20°C until the time of analysis. The quantification of serum KIM-1 and MDA was carried out using commercially available kits (ELISA) as per the manufacturer's instructions. In the intervention group, TDM began once Vancomycin steady state was achieved. The doses were reviewed through the trough concentrations by a clinical pharmacist, who calculated new doses based on pharmacokinetic principles, to maintain the therapeutic concentrations and minimize the risk of toxicity. The control group continued on the traditional empirical dosing without any pharmacist involvement in the dose adjustment. Physiological changes, treatment efficacy and oxidative stress were evaluated by measurements taken at the follow-up.

STATISTICAL ANALYSIS

All assembled data were coded, entered, and analyzed using the IBM SPSS Statistics 26.0 software. Continuous variables are represented as mean ± standard deviation (SD) and categorical variables as frequencies and percentages. Independent samples t-tests were used to compare continuous variables between the two groups and paired samples t-tests were used to assess within-group changes from baseline to follow-up. Categorical data was analyzed with chi-square testing. Pearson correlation was performed to investigate the correlation between selected clinical and laboratory parameters and Vancomycin dose. A p-value < 0.05 was considered to be statistically significant.

ETHICAL CONSIDERATIONS

The Scientific and Ethical Committees of the Faculty of Pharmacy, University of Kufa in collaboration with Al-Zahraa Teaching Hospital, Al-Najaf, Iraq approved the study protocol. Prior to enrolment, written informed consent was received from parents/legal guardians of the children participating in the study. At each step, the investigation respected the principles of human rights, as stated in the Declaration of Helsinki, ensuring the confidentiality of patient data and respecting the privacy of these data.

RESULTS

BASELINE COMPARISONS BETWEEN INTERVENTION AND CONTROL GROUPS

A total of 140 pediatric patients who required intravenous Vancomycin treatment were enrolled and randomized into 2 groups: control group (n=70) and intervention group (n=70). A demographic and clinical evaluation of the two arms was made at baseline before the implementation of pharmacist-led therapeutic drug monitoring. There were no significant differences between the groups with respect to gender, place of residence, type of infection, age, body weight, height and body mass index (BMI) ($P>0.05$), all of which indicated that, at the beginning of the study, the arms were equal (Table 1).

PHYSIOLOGICAL AND CLINICAL OUTCOMES

Initial measurements of physiological variables (e.g. body temperature, heart rate, respiratory rate, and systolic/diastolic blood pressure) were not different between groups. The intervention group showed significant clinical improvements from the baseline and against the control group, which can be mainly attributed to the pharmacist-led dose optimization. This intervention also was associated with faster recovery and a substantial reduction in the length of hospital stay. More effective treatment and more rapid clinical stabilization were observed in the intervention group, with a significantly shorter average time to hospital discharge of 4.94 ± 1.06 days than the control group of 5.99 ± 2.59 days ($P = 0.002$) as it shown in table 2.

RENAL FUNCTION AND SUBCLINICAL NEPHROTOXICITY BIOMARKERS

During the course of the investigation, the usual laboratory indices of renal function have shown only a slight change of clinical significance. Although some of the comparisons of serum creatinine achieved statistical significance, the changes were small and not likely to represent a clinically significant renal impairment. Similarly, the blood urea nitrogen remained essentially unchanged and the estimated GFR was relatively stable. In contrast, kidney injury molecule-1 exhibited a statistically significant difference between the two groups. KIM-1 levels decreased significantly after the intervention from 304.64 ng/mL to 117.23 ng/mL ($P < 0.001$) where pharmacist-led TDM was implemented. The control group, on the other hand, recorded an upward shift in KIM-1, moving from 293.23 ng/mL to 337.14 ng/mL ($P < 0.001$). When taken together, these results provide compelling data to support the use of Vancomycin dosing adjustments to the individual patient as a means to prevent the progression to subclinical nephrotoxicity and to reduce the renal tubular injury (Table 3).

OXIDATIVE STRESS BIOMARKER

Malondialdehyde served as an indicator of oxidative stress and lipid peroxidation. Baseline MDA levels were similar across all study groups. Following pharmacist-led therapeutic drug monitoring, the intervention group showed a significant decrease in MDA levels. Conversely, patients receiving empirical dosing did not show any such improvement. MDA levels by the end of the observation period were found to be decreased in the intervention group, indicating that the oxidative stress appeared to be reduced following optimization of Vancomycin exposure. The results indicate that personalized dosing enhances antimicrobial activity and could decrease oxidative cellular damage resulting from over-exposure to Vancomycin and the presence of severe infection (Table 4).

VANCOMYCIN THERAPEUTIC TARGET ATTAINMENT

One of the main outcomes measured in this study was the attainment of therapeutic Vancomycin serum concentrations. Conventional empiric dosing limitations were highlighted by the fact that many patients did not achieve target concentrations prior to intervention. The proportion of patients with therapeutic Vancomycin concentrations significantly increased after pharmacist-directed dose adjustment. Overall target therapeutic range was reached by 87.1% of patients in the intervention group after dose optimization. In contrast, standard empirical therapy was not significantly associated with improved target attainment. These results further support the success of pharmacist-led therapeutic drug monitoring in overcoming pharmacokinetic variability and improving dosing accuracy in pediatric patients (Table 5).

Table 1. Breakdown of demographic and clinical features across the study groups

Variable	Control group (n=70)	Intervention group (n=70)	p-value
Gender (male/female)	Comparable	Comparable	>0.05
Residence	Comparable	Comparable	>0.05
Type of infection	Comparable	Comparable	>0.05
Age (years)	NS	NS	>0.05
Weight (kg)	NS	NS	>0.05
Height (cm)	NS	NS	>0.05
BMI (kg/m^2)	NS	NS	>0.05

NS = No significant difference
Source: Authors' own work

Table 2. Comparison of physiological parameters and length of hospital stay between study groups

Variable	Control group (n=70)	Intervention group (n=70)	p-value
Body temperature	Improved	Greater improvement	<0.05
Heart rate	Improved	Greater improvement	<0.05
Respiratory rate	Improved	Greater improvement	<0.05
SBP	Stable	Stable	>0.05
DBP	Stable	Stable	>0.05
Length of stay (days)	5.99 ± 2.59	4.94 ± 1.06	0.002

Source: Authors' own work

Table 3. Renal function parameters and KIM-1 levels in different study groups

Variable	Control group (n=70)	Intervention group (n=70)	p value
Serum creatinine	Minimal change	Minimal change	0.014
Blood urea nitrogen	No significant change	No significant change	>0.05
eGFR	Stable	Stable	>0.05
KIM-1 baseline (ng/mL)	293.23	304.64	>0.05
KIM-1 follow-up (ng/mL)	337.14	117.23	<0.001

Source: Authors' own work

Table 4. Comparison of MDA levels between study groups

Variable	Control group (n=70)	Intervention group (n=70)	p-value
MDA Baseline	Comparable	Comparable	>0.05
MDA Follow-up	Minimal improvement	Significant reduction	<0.001
ΔMDA (nmol/mL)	+0.15 (0.14)	−1.78 (1.93)	<0.001

Source: Authors' own work

Table 5. Vancomycin therapeutic target attainment

Variable	Control group (n=70)	Intervention group (n=70)
Baseline Target Attainment	Comparable	Comparable
Follow-up Target Attainment	No significant improvement	Significant improvement
Patients Within Therapeutic Range (%)	Lower rate	87.1%
Statistical Significance	NS	P < 0.001

NS = No significant difference
Source: Authors' own work

CORRELATION ANALYSIS

A pharmacist-led therapeutic drug monitoring was conducted to determine the correlation between optimized Vancomycin dosing and selected clinical and laboratory parameters. There was a significant direct correlation between the optimized dose of vancomycin

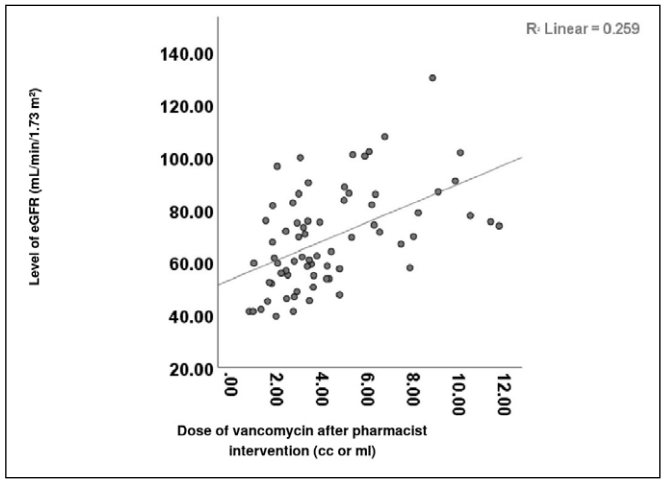


Fig. 1. Correlation between optimized vancomycin dose and eGF
Source: Own materials

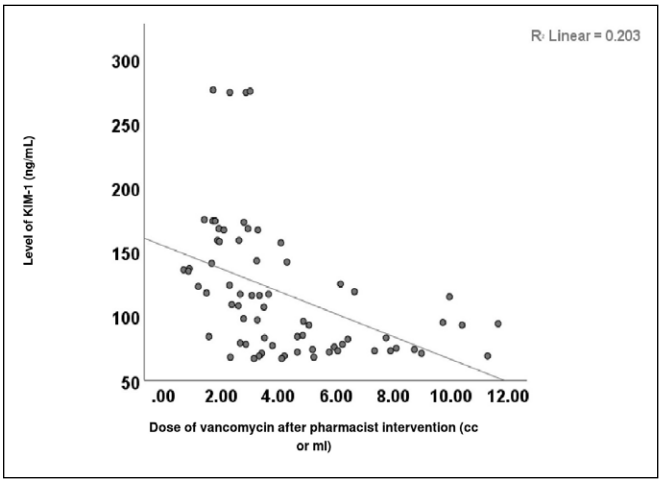


Fig. 2. Correlation between optimized vancomycin dose and KIM-1
Source: Own materials

and the estimated glomerular filtration rate (eGFR) ($r = 0.509$, $P<0.001$). This finding indicates that, generally, children with increased renal clearance require higher doses of Vancomycin to achieve therapeutic drug levels. This finding is consistent with the well-established influence renal function has on Vancomycin pharmacokinetics in children (Fig.1).

Furthermore, a notable negative correlation was found between the optimized Vancomycin dose and KIM-1 concentrations ($r = -0.450$, $P < 0.001$). Pharmacist-guided dose optimization was associated with lower KIM-1 levels in patients, suggesting reduced renal tubular injury and supporting the protective effect of therapeutic drug monitoring against Vancomycin-related subclinical nephrotoxicity (Fig. 2).

The correlation findings further support the effectiveness of individualized pharmacokinetic-guided dosing in improving treatment outcomes while minimizing renal toxicity.

DISCUSSION

This study aimed to assess how pharmacist-led therapeutic drug monitoring influences physiological outcomes and biomarkers related to vancomycin-induced subclinical nephrotoxicity in children. Findings indicated that individualized Vancomycin dosing improved therapeutic results and lowered indicators of renal tubular damage and oxidative stress. Baseline demographic, clinical and laboratory characteristics were similar between control and intervention groups and thus, observed post-intervention differences could likely be attributed to the therapeutic drug monitoring strategy, rather than pre-existing differences between study populations. The baseline characteristics in the present study are similar to those reported in previous studies assessing vancomycin monitoring programs in pediatrics and critically ill patients. A key finding of this study was the significant reduction in KIM-1 levels after pharmacist-guided dose modification. KIM-1 is a very sensitive marker of renal proximal tubular injury that can be used to detect nephrotoxicity long before changes in classic renal parameters occur. The significant decrease noted in the intervention group compared with the increase in the control group suggests that individualized therapeutic drug monitoring (ITDM) in vancomycin significantly reduces subclinical renal injury associated with vancomycin use. These findings confirm the results of previous studies showing that KIM-1 is an early marker for nephrotoxicity and support the use of pharmacokinetically guided dosing for nephrotoxicity prevention. The study also showed significant decreases in the MDA level in the pharmacist-led intervention group [20]. MDA is known to be a marker of lipid peroxidation and cellular oxidative injury; thus, a decrease in MDA is indicative of a decrease in oxidative stress only after optimizing vancomycin therapy [21]. This observation validates experimental data demonstrating that oxidative injury is a result of excessive exposure to vancomycin and that careful dosing can mitigate this negative effect [22]. Conventionally used urinary parameters of renal function (serum creatinine, blood urea nitrogen and estimated glomerular filtration rate) showed only small clinically insignificant differences over the duration of the study. This highlights the fact that traditional renal markers have their drawbacks in early detection of nephrotoxicity and the clinical value of KIM-1 as a more sensitive indicator of renal tubular injury. Additional post-pharmacist intervention benefits noted were an increase in achieving therapeutic targets and reduced length of stay. Improved clinical recovery and treatment effectiveness was most likely due to most

patients in the intervention group achieving target vancomycin concentrations. Furthermore, optimized vancomycin dose was significantly negatively correlated with KIM-1 concentrations, further reinforcing the nephroprotective effect of the individualization of therapeutic drug monitoring [23]. To conclude, this study demonstrates that Vancomycin TDM in children, after being performed by a pharmacist, leads to better safety and efficacy of vancomycin therapy, by decreasing oxidative stress, subclinical renal damage, and improving clinical outcomes.

CONCLUSIONS

Vancomycin management in children has been significantly impacted by pharmacist-led TDM and improved achievement of targeted goals and shortened hospital stays. Personalized dosing was associated with significant decreases in the KIM-1 protein and MDA, indicating less renal tubular damage and oxidative stress. These findings substantiate implementing biomarker-guided therapeutic drug monitoring for vancomycin use as part of routine clinical care in children to optimize safety and outcomes.

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

Gasless laparoscopy in the treatment of hiatal hernia

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ABSTRACT

Aim: To report the experience in the use of gasless laparoscopy in the treatment of patients with hiatal hernia that was accumulated in a specialized thoracic center.

Materials and Methods: Operations were performed via three to six centimeter midline access in the epigastrium with a camera installed in the left hypochondrium. A laparolift provided with the traction on the edge of the midline wound. Hiatus was repaired with diaphragmatic pillars suturing without mesh emplacement. Patients with gastroesophageal reflux disease underwent fundoplication. Operations were estimated concerning their duration, and complications and hernia recurrence development.

Results: The analysis covers the results of treatment of 159 patients with primary and seven recurrent hiatal hernia, 112 women and 54 men with median age of 61 years, who underwent gasless laparoscopy from October 2019 to July 2025 in the single thoracic centre. Among all instances, 57 (34.34%) met the criteria for large hiatal hernia. The duration of operations with posterior esophageal cruroplasty and antireflux procedure, excluding cases of recurrent hernias, conversions, and intraoperative complications, was 227±59 minutes, and 313±69 minutes ($p<0.001$) for recurrent hiatal hernia. Intraoperative complications occurred in nine cases, postoperative morbidity in 22. Recurrent hiatal hernia emerged in seven patients; their occurrence is considering predictable in five.

Conclusions: Gasless laparoscopy allows combining the advantages of minimally invasive and open surgery in the hiatal hernia treatment. Our findings suggest that the method may be beneficial in large paraesophageal hernias, and in patients with severe comorbidities.

KEYWORDS: hiatal hernia, fundoplication, gastroesophageal reflux disease, minimally invasive surgery, gasless laparoscopy, cruroplasty

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INTRODUCTION

Laparoscopic repair with antireflux surgery is the accepted standard in the surgical treatment of hiatal hernia (HH) [1, 2]. Existing controversies relate mainly to specific technical aspects of the procedure under different clinical conditions, including reinforcement of the diaphragmatic crura with mesh or biologic grafts, the need for fundoplication in paraesophageal HH without gastroesophageal reflux disease (GERD), and some aspects of redo surgery in recurrent HH [3-5]. Conventional laparoscopy (CLS) remains the principal minimally invasive approach for HH repair. Meanwhile, publications on gasless laparoscopy (GLLS) in cholecystectomy and gynecological surgery have demonstrated clinical effectiveness and economic advantages of this technique [6, 7].

AIM

The purpose of this study is to report the experience in the use of GLLS in the treatment of patients with HH, that has been accumulated in a specialized thoracic center.

MATERIALS AND METHODS

The analysis included 166 patients with primary and recurrent HH who underwent surgical treatment using GLLS between October 2019 and July 2025. Hernias were classified into types I-IV according to Skinner and Belsey (1967) [8] based on the results of computed tomography (CT) or barium contrast radiography. Regardless of type, large HH were defined by one of the following criteria [9]: I) more than 30% of the stomach displaced

into the thoracic cavity in CT or radiographic image; II) intraoperatively measured transverse diameter of the esophageal hiatus was greater than 5 cm; III) hiatal area exceeded 10 cm^2.

All procedures were performed under multicomponent general anesthesia with mechanical ventilation through a 3-6 cm upper midline epigastric access. The operative space was supplied by the traction on the left edge of the wound with a laparolift system. The visualization was provided by a camera had been installed in the left hypochondrium.

Regardless of HH type, surgical principles consistent with modern hiatal surgery [10, 11] were followed: complete mobilization of the cardia and lower esophagus including abdominal and supradiaphragmatic segments, mobilization of the gastric fundus with division of the upper short gastric vessels, preservation of the main gastric arterial and vagal trunks, excision of the hernial sac, removal of displaced retroperitoneal fat, careful dissection and suturing of the diaphragmatic crura without tension and under direct visualization predominantly behind the esophagus.

Mesh reinforcement was not used because the diaphragmatic pillars remained mobile and suitable for reconstruction. In most cases, the anterior esophageal wall was fixed to the margin of the hiatal opening. HH type I, as well as HH type III associated with GERD, required fundoplication with subsequent fixation of the wrap to the right diaphragmatic crus. In cases without fundoplication, left-sided fundophrenicopexy was performed.

Patients were stratified according to age, sex, obesity at body mass index (BMI) >35, HH type, and hernia size. HH-related complications and comorbidities were also taken into account. Surgical outcomes were assessed based on duration of procedures, perioperative complications, and HH recurrences. The adequacy of hiatal repair was evaluated radiologically by barium contrast imaging 14-21 days after surgery.

HH recurrence was defined as displacement of the fundoplication wrap or esophagogastric junction more than 2 cm above the diaphragm [12]. Reappearances were identified during routine follow-up within 6-12 months, after referral due to clinical symptoms, or incidentally by CT examinations performed for other reasons.

Statistical analysis included calculation of mean values, standard deviation, median values, and Student's t-test. The study was approved by the Bioethics Commission of the National Pirogov Memorial Medical University, Vinnytsya, Ukraine.

ETHICS

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

AI STATEMENT

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

RESULTS

GLLS were used for 159 primary and 7 recurrent HH repair in 112 women and 54 men with a mean age of 57.5±11.3 years. Among primary HH, type I was identified in 81 of 159 (51.0%), type II in five (3.1%), type III in 67 (42.1%), and type IV in six (3.8%) cases. Large HH occured in 57 patients (34.34%). Since 2022, the proportion of HH type III and IV increased from 25.8% (8 of 31) in 2019-2021 to 44.9% (22 of 49) in 2022-2023 and 54.4% (43 of 79) in 2024-2025. Overall, 93 of 166 patients (56.0%) were older than 60 years, with a median age of 61 years. Among these patients, 71 belonged to the 60-69-year age group, 20 to the 70-79-year group, and two patients were older than 80 years. In patients older than 60 years, type III HH predominated (59 of 93 cases, 63.4%).

Regarding recurrent HH, six of seven cases developed after conventional laparoscopic procedures performed at other institutions. In one patient, hernia reappeared after inadequate primary repair at our center.

Among HH-related complications, GERD-associated conditions prevailed, including esophagitis in 114 and anemia in 29 cases. Eight patients experienced strangulation without necrosis of incarcerated organs.

Concomitant diseases (Table 1) were identified in 146 patients, with a total of 422 comorbidities corresponding to 2.9 diagnoses per patient.

Hiatal repair was performed using interrupted sutures placed behind the esophagus in 161 cases and anteriorly in five ones. Antireflux procedures were conducted in 133 of 166 patients (80.1%), predominantly Nissen fundoplication (129 patients). The Toupet and Dor techniques were used twice each. In 33 elderly patients with type III HH without GERD, fundoplication was omitted and left-sided fundophrenicopexy was performed instead.

In all recurrent HH, posterior esophageal cruroplasty was used for hiatal reconstruction. Existing fundoplication wraps were preserved during redo operations. One patient underwent Dor fundoplication, whereas in another case where the wrap had not been formed previously, Nissen procedure was performed.

The mean operative duration for posterior esophageal cruroplasty with antireflux surgery was 227±59 min (median 222 min), excluding recurrent HH, conversions, and intraoperative complications,. According to HH type, operative duration was 219±59 min for type I, 215±60 min for type II ($p>0.5$), 236±59 min for type III ($p>0.1$), and 283±67 min for type IV HH ($p<0.05$). In

Table 1. Concomitant diseases in 146 patients (422 comorbidities)

Diagnosis	n (%)
Arterial hypertension	127 (87.0%)
Myocardiosclerosis	118 (80.8%)
Acquired valve pathology	26 (17.8%)
Arrhythmias	18 (12.3%)
Angina pectoris	7 (4.8%)
Circulatory failure	43 (29.5%)
Pathology of the veins of the lower extremities	11 (7.5%)
Grade II-III obesity	18 (12.3%)
Peritoneal adhesions	15 (10.3%)
Kidney diseases (single kidney (2), chronic kidney disease (2), cysts (5), urolithiasis (1))	10 (6.8%)
Goiter	6 (4.1%)
Chronic arthritis	6 (4.1%)
Chronic hepatitis	6 (4.1%)
COPD	2 (1.4%)
Diabetes	4 (2.7%)
Calculous cholecystitis	4 (2.7%)
Esophageal achalasia	1 (0.7%)
Total diagnoses, n	422

Source: compiled by the authors of this study

Table 2. Intraoperative complications and methods of correction (n=166)

Complication	n (%)	Method of correction	Conversion to open surgery, n
Bleeding from gastric vessels	4 (2.4%)	Local hemostasis	1
Splenic bleeding	2 (1.2%)	Local hemostasis	0
Pneumothorax	2 (1.2%)	Pleural drainage	0
Esophageal perforation	1 (0.6%)	Suturing under endoscopic control	0
Total	9 (5.4%)		1

Source: compiled by the authors of this study

Table 3. Factors associated with recurrent HH after surgery (n=7)

Factor category	Specific factor	n (%)
Technical factor	Inadequate closure of the hiatal defect due to insufficient preparation of the left diaphragmatic pillar	1 (14.3%)#
Tactical factor	Isolated anterior cruroplasty	2 (28.6%)
Anatomical factor	Hyperkyphosis	2 (28.6%)
Unidentified factor	Not found	2 (28.6%)
Total		7 (100%)

– patient underwent redo surgery
Source: compiled by the authors of this study

18 obese patients (BMI >35) surgeries lasted 297±60 min compared to 213±54 min in other cases (p<0.001).

Redo operations for recurrent HH required prolonged and meticulous dissection (313±69 min, p<0.001) due to the presence of dense adhesions in the upper abdomen and mediastinum.

Intraoperative complications were observed in nine patients (5.4%). Bleeding from gastric vessels occurred in four cases, and splenic bleeding was noted in two. Pneumothorax developed in two patients due to pleural opening during dissection of intrathoracic adhesions in recurrent HH. Esophageal perforation occurred in one patient. All complications were managed intraoperatively without adverse effects on final outcomes (Table 2).

Conversion to open surgery was necessary in three patients due to difficulties associated with fixation of

hernial contents in the mediastinum during reoperation in two cases and bleeding in one case.

Postoperative morbidity developed in 22 patients (9.6%), with a total of 26 complications classified as Clavien-Dindo grades I-IIIA. Among them surgical site infection prevailed (19 occurrences). Other complications included: exudative pleurisy (3 instances), two deep vein thrombosis of the lower extremities with an event of non-massive pulmonary embolism, and an acute perforated gastric ulcer. There were no fatal consequences.

During follow-up, persistent abdominal distension lasting more than six months after surgery was observed in 15 patients and was managed conservatively using prokinetics and simethicone. No clinically significant long-term postoperative dysphagia requiring reintervention was observed. Recurrent HH developed in seven patients within 6-48 months after surgery owing to the factors summarized in Table 3. One patient underwent redo surgery, while the others remained asymptomatic and did not require intervention.

DISCUSSION

The use of GLLS for HH repair has not yet been investigated sufficiently. Most studies comparing laparoscopy with and without pneumoperitoneum have focused on cholecystectomy and gynecological procedures. A systematic review demonstrated non-inferiority of GLLS regarding safety and efficiency [6]. The technique has also been considered useful for expanding minimally invasive surgery in low- and middle-income countries [7, 12]. Although the Ukrainian healthcare system remains technologically equipped, current wartime and postwar conditions necessitate rational use of resources. Nevertheless, most leading authors in lift-assisted laparoscopy originate from high-income countries [13], suggesting that interest in this technique is associated not only with economic considerations but also with clinical advantages. Thus, GLLS is able to reduce the negative effects of prolonged pneumoperitoneum on hemodynamics, visceral perfusion, and respiratory function [14-19], which is particularly important in high-risk patients. In the present study, most patients were older than 60 years and cardiovascular diseases predominated among comorbidities; however, no deterioration of concomitant conditions was observed perioperatively.

Another potentially important aspect of GLLS concerns the management of large hiatal hernias, which correlate with elevated perioperative morbidity [20] and high recurrence risk after conventional laparoscopic repair [21, 22]. Caused by pneumoperitoneum excessive tension during hiatal closure is considered one of the HH recurrence predictors [23, 24]. Meanwhile, abdominal wall lifting does not cause additional lateral traction on the diaphragmatic crura.

In the present series, predisposing factors for recurrence were found in five of seven patients. One recurrence was related to technical error during primary repair. The patient underwent repeat surgery with good result. Two recurrences occurred after isolated anterior cruroplasty, which had been provided in critically ill patients because of hemodynamic instability and strangulated HH. We also experienced hernia reappearence in two patients with severe hyperkyphosis, which has been recognized as a risk factor for failure of HH repair [25]. Further studies involving larger patient populations and longer follow-up are required to clarify whether GLLS provides advantages over conventional laparoscopy in reducing recurrence rates.

The limitations of GLLS include reduced operative space and altered visualization geometry [6]. Additional technical difficulties include the close proximity of instruments to the camera and the absence of the "carboxydissection" effect. These disadvantages become especially pronounced in obese patients. In the present study, obesity was associated not only with prolonged operative duration but also with increased intraoperative morbidity, since all severe bleeding episodes and the single esophageal perforation occurred in patients with BMI >35. In extended and technically demanding procedures, surgeon fatigue additionally contributes to complications; therefore, surgeon rotation might be reasonable in selected cases.

Furthermore, high risk of surgical site infection related to prolonged wound retraction is regarded among GLLS challenges [6]. In the present study, surgical site infections represented the most common postoperative complication.

CONCLUSIONS

GLLS combines several advantages of minimally invasive and open surgery in the treatment of HH. The technique appears particularly promising in patients with large paraesophageal hernias and severe comorbidities, where avoidance of prolonged pneumoperitoneum and reduction of tension during hiatal repair might be beneficial. At the same time, obesity and technically demanding redo procedures remain important risk factors for operative difficulties and complications. Further prospective studies with longer follow-up are necessary to determine the long-term clinical benefits and recurrence outcomes of GLLS compared to conventional laparoscopic repair.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Formulation and characterization of lipophilic chemotherapy as ethosomal gel for cervical cancer

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ABSTRACT

Aim: methotrexate was formulated as Ethosomal gel for enhancing the therapeutic performance and stability to treat cervical cancer via targeting the drug locally through cervical mucosa to avoid the systemic adverse effect of the chemotherapy.

Materials and Methods: The formulations were produced using phospholipid and cholesterol then evaluated for particle size, polydispersity index, zeta potential, pH and drug release. The optimized ethosome formula then converted to gel. The gel evaluated for organoleptic properties, pH, viscosity and permeability.

Results: The results showed than F8 was the best ethosomal formula with 34nm in size and 92% entrapment efficiency. This formula converted into gel had 1% gelling agent that was optimized ethosomal gel formula and gave better release that ordinary methotrexate gel with same type and percent of gelling agent.

Conclusions: The drug was successfully formulated as ethosomal gel for treating cervical cancer topically in order to avoid the systemic exposure for the chemotherapy and its consequence adverse effect.

KEY WORDS: methotrexate, cervical, cancer, ethosomes, gel

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INTRODUCTION

Cervical cancer is one of the most common causes of deaths for females. The surgical excision is an effective treatment for cervical cancers in their early stages. However, the 5-year survival rate for metastatic cervical malignancies is just about 50% [1]. Cervical cancer is a malignant tumor that grows from cells that start in the cervix. The cervix connects the vagina to the top part of the uterus. While the ectocervix, that is the bottom half closest to the vagina, is fully lined with squamous cells, while the endocervix, is the top area closest to the uterus, which is lined with glandular cells. The junction of these two cervical regions is known as the transformation zone. Depending on where it appears in the cervix, cervical cancer is divided into several groups. Eighty to ninety percent of cervical cancer cases are squamous cell carcinoma, a kind of cancer that develops in the ectocervix. One type of cancer that starts in the endocervix is called adenocarcinoma. Most cervical malignancies start in the transformation zone, which is the area where glandular and squamous cells converge. The infectious human papillomavirus (HPV) is the main risk factor for cervical tumors, followed by smoking and HIV infection [2]. Cervical cancer treatment includes many strategies of surgical intervention, radiation and chemotherapy. There are many chemotherapeutic agents that can be used in the treatment of cervical cancer involving methotrexate. Methotrexate (MTX) is an antimetabolite of folate that is used to treat neoplastic conditions such as leukemia, breast, lung, bone, and cervical cancer. It has also been used to treat multiple sclerosis and psoriasis. MTX is used to treat rheumatoid arthritis since it is a member of the class of pharmaceuticals known as disease-modifying antirheumatic drugs (DMARDs). Growth and cell proliferation are inhibited by MTX because it inhibits the enzyme dihydrofolate reductase, which changes dihydrofolate into tetrahydrofolate. MTX is cytotoxic when the cell cycle is in the S phase. This process (competition with folic acid metabolism) impairs DNA synthesis and has nonspecific cytotoxic effects on healthy cells, which can result in hepatitis, nephrotoxicity, and renal insufficiency [3]. MTX is an odorless yellow to orange-brown crystalline powder with molecular weight of 454.447 g/mol, log p of -1.8, and melting point of 185–204 °C. MTX is slightly sol-

uble in diluted HCl; soluble in DMSO; insoluble in alcohol, water, ether, and chloroform. The MTX pKa is 4.7. Because of its hydrophobicity and low permeability property, it is categorized as a class IV drug by the Biopharmaceutical Classification System (BCS). Additionally, it is a chemically unstable substance that breaks down quickly in the presence of light, severe pH, or temperature changes [4-5]. The medication stability and therapeutic efficacy could be improved via formulation as nanoparticle. There are various nanoparticles types including liposome, noisome, solid lipid nanoparticles, ethosome and others. Ethosome is ethanolic vesicles because ethanol is available in the vesicular structure, 'Touitou formed a novel vesicular system which was called ethosomes [6]. The most recent researches for topical, localized or transdermal medication administration is the vesicular system. Since the drugs can reach the target and deeply penetrate the required area as well as it is considered as non-invasive delivery vehicles when applying the medication formula on the cervices to deliver the drug to the affected area avoiding the systemic exposure to the chemotherapy. However, ethosomes could be drain, hence incorporating the ethosome into gel could prolong the contact of the drug with the applied area of cancer [7-8].

AIM

Methotrexate was formulated as Ethosomal gel for enhancing the therapeutic performance and stability to treat cervical cancer via targeting the drug locally through cervical mucosa to avoid the systemic adverse effect of the chemotherapy.

HIGHLIGHTS

- This is the first time for methotrexate chemotherapy to be formulated as ethosomal gel
- Ethosomes are the nanoparticles that have advantages of Nano-sized carriers
- When methotrexate chemotherapy administered topically at the affected area (cervix in our study to treat cervical cancer, for first time), the systemic adverse effect of the drug would be avoided
- Incorporating the nanoparticle into gel will make the drug stay for longer time resisting the normal cervical clearance.

MATERIALS AND METHODS

MATERIALS

MTX was bought from YIBAI biotechnology, China. Dimethyl sulphoxide (DMSO) was purchased from Alpha Chemika, India whereas lecithin phospholipid from egg yolk and cholesterol were bought from Meyer, China. Ethanol and triethanolamine (TEA) were from Himedia, India.

METHODS

PREPARATION OF ETHOSOME

The formulas were prepared by dropping the water into the solution of phospholipid and cholesterol that dissolved in ethanol. The water was added gradually into the ethanolic phase. The mixture was stirred using magnetic stirrer (Dragon Lab- USA) at 700 rpm for a half an hour at room temperature. The drug (0.25%) was dissolved first in ethanolic phase before adding water. Table 1 shows the prepared formulas [9].

ENTRAPMENT EFFICIENCY

The entrapment efficiency (EE) of the formulas was determined by taking 2 ml of the prepared formulation, then centrifuged (Fanem Centrifuge, Brazil) at 20,000 rpm for one hour. The supernatant was got rid and one milliliter of the sediment was dissolved in ethanol to estimate the drug amount using spectrophotometric assay at the drug lambda max of 303 nm, then using the following equation [10]:

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The formulations particle size, polydispersity index (PDI) and zeta potential were identified using nanotrac wave II (Microtrc, Germany). These parameters are important to identify the vesicle character and stability [11-12].

pH MEASUREMENT

The formulas pH was measured using pH meter instrument (Hanna Instruments Ltd. Singapore) in order to determine the pH of the formulas and their compatibility to the applied area.

IN VITRO PERMEABILITY STUDY

Drug penetration studies were conducted by dialysis membrane (12,000Da). The permeation study was performed using the Franz diffusion cell with an effective permeation area (1 cm^2) and receptor cell volume (50 mL). The formulas were put in the donor compartment and acetate buffer (pH 4.5) in receptor compartment which maintained at simulated body temperature of 37 ± 1°C and was constantly stirred at 50 rpm [13]. The

Table 1. Methotrexate ethosomes formulas

No.	Phospholipid	Cholesterol	MTX	Ethanol	DMSO	Water
F1	0.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F2	1%	0.015g	0.6 mg	20%	0.5 ml	Up to 10 ml
F3	1.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F4	2%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F5	2.50%	0.015 g	0.6 mg	20%	0.5 ml	Up to 10 ml
F6	1%	0.015 g	0.6 mg	30%	0.5 ml	Up to 10 ml
F7	1%	0.015 g	0.6 mg	40%	0.5 ml	Up to 10 ml
F8	1%	0.015 g	0.6 mg	50%	0.5 ml	Up to 10 ml

Source: Author's own work

Table 2. Entrapment efficiency, particle size, polydispersity index, zeta potential and pH of the ethomosal formulations (mean ± SD, n=3)

Formula Code	EE%	Size (nm)	PDI	Zeta potential (mV)	pH
F1	75.01±2.4	117.2±2.2	0.432	-19.7	5±0.4
F2	81.65±1.3	84.3±0.74	0.2376	-22.1	4.3±0.12
F3	87.68±2.2	98.25±0.39	0.3235	-25.3	4.34±0.2
F4	85.88±1.9	101±3.1	0.501	-21.2	4.66±0.1
F5	74.54±2.8	122.36±0.93	0.226	-28.76	4.34±0.2
F6	88.69±3.2	53.5±1.8	0.175	-17.85	4.17±0.3
F7	90.25±2.6	50.2±1.4	0.182	-23.35	4.38±0.2
F8	92.01±1.7	34.3±0.64	0.191	-25.0	4.51±0.1

Source: Author's own work

experiment was performed for 6 hours period. The samples from receptor compartment were withdrawn at predetermined intervals, then the same volume was replaced with fresh acetate buffer. Finally, the drawn samples were promptly examined for MTX at 303 nm using a UV (ultraviolet) spectrophotometer [14-15].

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) is a one of the popular technique for visualizing the materials' morphology and microstructure. On a small grid-coated carbon, ethosomal dispersion was applied. The specimen was viewed under the microscope after being suitably magnified. The optimized ethosomes' formula surface shape was assessed using SEM. Prior to inspection, the ethosomes samples were glued on copper stubs, platinum-coated, and put on double-sided tape. After then, the samples were seen at different magnifications [16-17].

ETHOSOMAL GEL FORMULATION

The optimized ethosome formula was added to carbopol gel (1%w/w). The liquid was agitated until thickening occurred, and then TEA was added gradually and slowly for gel formation and pH adjustment [18].

GEL CHARACTERIZATION

ORGANOLEPTIC PROPERTIES

Through visual inspection, the gel's physical appearance, clarity, occlusiveness, washability, and organoleptic properties were evaluated [19].

pH DETERMINATION

A pH meter was used to measure the ethosomal gel's pH. The average value was determined after the measurements were taken in triplicate [20].

ETHOSOMAL GEL VISCOSITY MEASUREMENT

The Atago viscometer was used to measure the prepared ethosomal gel's viscosity. Spindle A3 was used to obtain the reading at 100 rpm.

IN VITRO PERMEABILITY STUDY

Dialysis membrane was used for drug permeation studies, which were performed in Franz diffusion cell. The ethosomal gel formulations were placed in the donor compartment, which put over the receptor compartment, and the dialysis membrane in between. The experiment lasted for 24 hours, during which, the

samples were withdrawn at prearranged periods from the receptor and replenished with acetate buffer (pH 4.5) to maintain a sink condition. The samples were measured using a UV spectrophotometer for the drug content at 303 nm [21-22].

STATISTICAL ANALYSIS

One way ANOVA was used to identify significancy and the difference was considered significant when $p<0.05$.

RESULTS

ETHOSOME FORMULAS CHARACTERIZATION

ENTRAPMEMT EFFICIENCY

The range of the ethosome formulas EE% was from 74 to 92. The results shown in (Table 2).

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The particle size, PDI and zeta potential, were shown in Table 2. PDI of the formulations was less than 0.501. The size of the MTX formulations was within the range of 34 to 122 nm. Vesicles benefit from having a high negative zeta potential of -17 to -28.

pH MEASUREMENT

The results of the formulas pH were shown in Table 2.

IN VITRO PERMEABILITY STUDY

Only the four optimized formulas were subjected to this study (F2, F6-F8). After six hours, the cumulative release percent of the formulas was above 77%. Formulation F8 had the highest cumulative release percent. The study results shown in Figure 1.

SCANNING ELECTRON MICROSCOPY

The SEM findings and the micro-morphology of the optimal ethosome formulation are shown in figure 2.

ETHOSOMAL GEL CHARACTERIZATION

GENERAL PROPERTIES OF THE ETHOSOMAL GEL

The gel formulation's physiochemical characteristics demonstrated uniformity and a pleasing look. There were no gritty particles in the gel, and it was readily washing [35]. The gel formulation had a pale yellow physical appearance. It was discovered that the gel formulation's pH was 6.1 within the typical skin pH range, 1% w/w carbopol concentration produced the acceptable viscosity (6200 cP).

IN VITRO PERMEABILITY STUDY

As indicated in Figure 3, the percentage permeated of the drug ordinary gel (containing the same Carbopol percentage) following a 24-hour investigation was 81%, whereas the permeability percent of ethosomal gel was 94%.

DISCUSSION

ETHOSOME FORMULAS EVALUATION

Entrapment efficiency EE% is utilized to determine the nanocarrier efficacy to retain the active ingredient and to ensure delivery of an adequate drug amount to the targeted. The range of the EE% was from 74 to 92. The results shown in Table 2. It had been determined that increasing the amount of ethanol led to improve the EE. Higher EE was for formula F8 that contained 50% ethanol. The value of log P is -1.8 for MTX, therefore the ethanol concentration had a significant influence on EE than the lipid. Evidence suggests that concentration of ethanol has a greater impact on MTX EE [23-24].

PARTICLE SIZE, PDI AND ZETA POTENTIAL

The formulas were tested via Nanotrac device to determine particle size, PDI and zeta potential, as shown in table 2. The effect of different factors on the prepared ethosomal formulations can be quite interesting. When the concentration of ethanol is increased, it has a solubilizing effect on the vesicles. Some research suggests that higher ethanol concentrations cause the ethanol hydrocarbon chains to intertwine, which ultimately reduces the thickness of the vesicular membrane and results in smaller vesicles. Ethanol, known for its ability to enhance penetration, interacts with the hydrophilic surface of the lipid bilayer on the outer layer of the skin. This interaction improves the fluidity of the lipids, thereby affecting various aspects such as zeta-potential, entrapment efficiency, stability, and the average size of the ethosomes. Increasing ethanol concentration from 20% in F2 to 30% in F6, was significantly reduced the particle size from 84 nm to 53 nm. Further increase ethanol in F7 and F8 also showed significant reduction in Particle size [25-26]. Therefore, the presence of ethanol alters the overall surface charge of

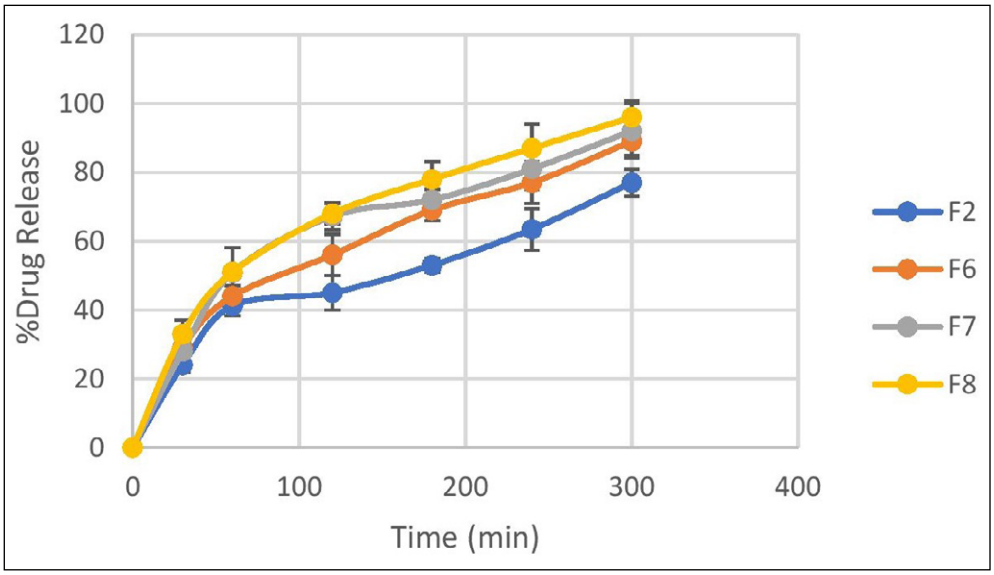


Fig. 1. MTX optimized ethosome formula permeability study results. *Source: Own materials*

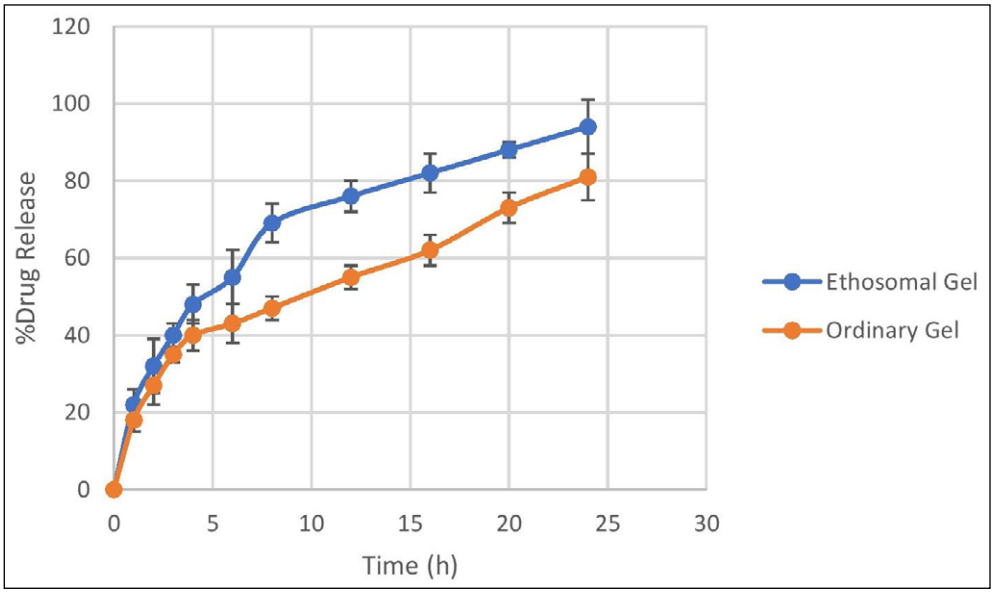


Fig. 2. SEM result of optimized formula, F8; (A) with 3 µm magnification, while (B) 500 nm. *Source: Own materials*

the ethosomes, providing some stability and increasing the repulsion between particles. This helps prevent aggregation and leads to smaller vesicle sizes. On the other hand, higher levels of phospholipids tend to result in larger vesicles. This is likely due to the thickening of the vesicle's structure caused by an increase in phospholipid concentration. Consequently, when the phospholipid levels are low while levels of ethanol are high, the smallest vesicle size is observed. This clear explained in F2-F5 as increasing the lecithin resulted in increased particle size [27-28]. PDI of the formulas was less than 0.5 and this indicating the uniformity of the particle size. The charge of the vesicle significantly reduces (increases in negative value) when the concentration of soya lecithin and ethanol rises. Vesicles benefit from having a high negative zeta potential because it stops them from aggregating due to electrostatic repulsion, increasing product stability. Potential increases as a result of (i) ethanol's electrostatic repulsion, which prevents aggregation by producing a net negative charge of the surface, and (ii) lecithin's phosphate group ionization, which produces a high negative charge [29-30].

pH MEASUREMENT

The pH of the formulas was compatible with the cervix (around 4.5) to prevent irritability [31], as shown in table 2.

IN VITRO PERMEABILITY STUDY

Selected optimized produced ethosomal formula was used in the release test (F2, F6-F8). At the end of the study, the cumulative release percent of the formulas was above 77%. Formulation F8 had the significantly highest cu-

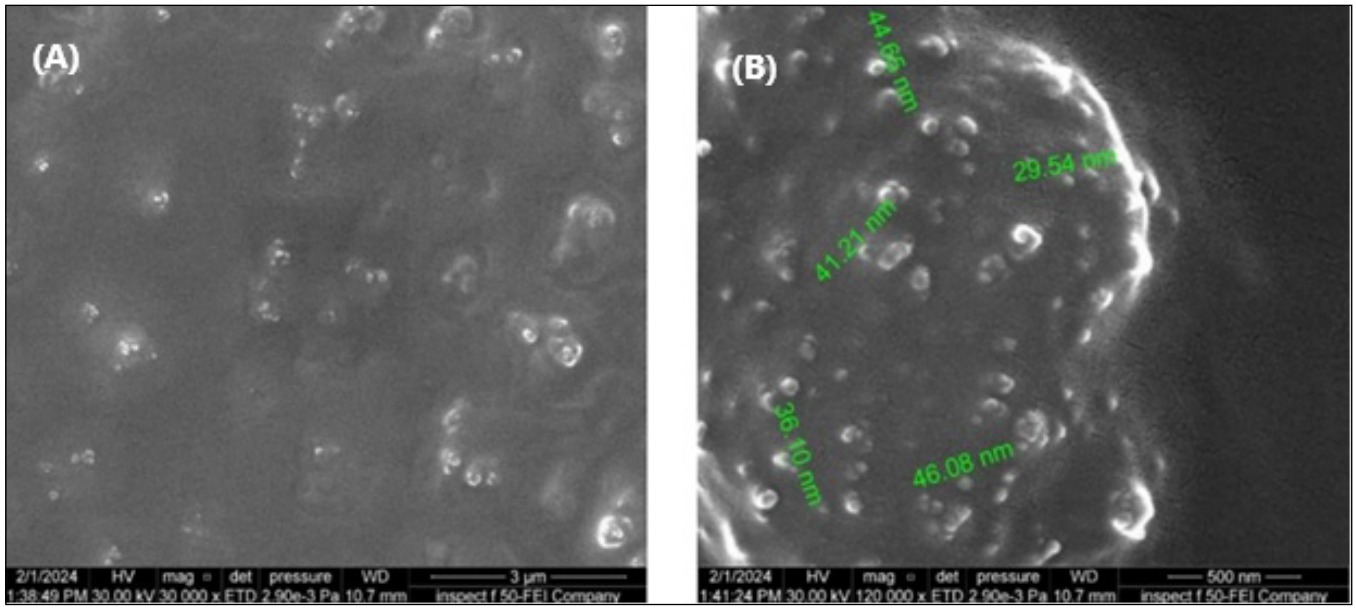


Fig. 3. The percent of MTX from the ethosomal gel and the ordinary gel
Source: Own materials

mulative release percent of 98% since the particle size is small of 34 nm. As the particle size decreased, the surface area increased and the overall drug solubility enhanced leading to better drug release then better bioavailability. Ethosomal formulation can improve drug accumulation, and increase penetration through the cervical cancer barrier [32-33], the study results shown in (Figure 1).

SCANNING ELECTRON MICROSCOPY

The SEM findings and the micro-morphology of the optimal ethosome formulation demonstrated that the ethosomes were rounded, and free of the drug crystals [34], as seen in (Figure 2).

ETHOSOMAL GEL CHARACTERIZATION

GENERAL PROPERTIES OF THE ETHOSOMAL GEL

The gel formulation's general characteristics were uniformity and a homogenous, with compatible skin pH [36]. The gel had an occlusive property, which increases the active drug's penetration and boosts effectiveness [37], 1% w/w carbopol concentration produced the appropriate viscosity [38-39].

IN VITRO PERMEABILITY STUDY

As shown in Figure 3, the percentage permeated of the drug ordinary following a 24-hour was 81%, whereas the permeability percent of ethosomal gel was 94%. It was shown that the ethosomal gel had a superior permeability profile and that could relate to the nanosized particle of the ethosome, which improves the release and penetration. Ethanol was thought to be efficient booster of permeability. As a result, ethosomal gel has a higher skin penetration rate than the ordinary drug gel [39-42].

CONCLUSIONS

The ethosomal gel of MTX was successfully prepared, evaluated and optimized in order to treat cervical cancer. The ethosomal MTX gel was better release the drug than ordinary gel of the drug with same amount and type of gelling agent for topical treatment of the cancer avoiding the peripheral adverse drug effect. This was the first time formulating MTX as ethosomal gel in order to enhance the drug targeting and permeability as well as avoiding the systemic exposure to the chemotherapy and its consequence adverse effect.

AUTHORS' CONTRIBUTIONS

M. Alaayedi and Z.M. Kadhum designed and directed the study; All author performed the experiments; each author analyzed specific part of the results. M. Alaayedi, Z.M. Kadhum, J.A. Ashoor, and A.M.H. Saeed wrote the article.

CONFLICT OF INTEREST

The authors would like to confirm that there are no conflicts of interest with this publication and that this study has not received any funding. We confirm that all writers indicated on the document have read it and given their approval. We also confirm that the authors' specified order has received our unanimous approval.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

Comparative analysis of machine learning models for survival prediction in patients with malignant melanoma

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ABSTRACT

Aim: To perform a comparative analysis of the effectiveness of contemporary machine learning models applied to survival prediction in patients with malignant melanoma and to assess their potential advantages over conventional staging systems as tools for personalised risk stratification.

Materials and Methods: A structured comparative analysis of published prognostic models developed using population-based databases was performed, comparing measures of discrimination (area under the ROC curve [AUC] and concordance index [C-index]), calibration (Brier score), and clinical utility (decision curve analysis). The clinical context was supplemented by data from a retrospective cohort study of patients with cutaneous melanoma.

Results: Ensemble and gradient-boosting algorithms (Random Forest, CatBoost, and XGBoost) consistently outperformed Cox proportional hazards regression models in terms of discrimination, achieving AUC values of approximately 0.76-0.84 for 1-, 3-, and 5-year survival. Ensemble models achieved a C-index of 0.732, compared with 0.685 for Cox regression and 0.537 for AJCC/NCCN criteria; the MIA nomogram demonstrated performance comparable to that of machine learning models (C-index, 0.739). Tumour stage, age, and surgical treatment were consistently identified as the leading prognostic factors. Retrospective cohort data confirmed the prognostic role of Breslow thickness, mitotic activity, and lymph node involvement in disease progression.

Conclusions: Machine learning models provide greater accuracy and more individualised prognostic assessment than conventional approaches and may serve as a supplement to standard staging systems. Their implementation requires prospective validation while preserving the physician's ultimate decision-making authority.

KEYWORDS: melanoma, machine learning, personalised medicine

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INTRODUCTION

Melanoma, an aggressive skin cancer originating from melanocytes, represents a significant global health problem owing to its high metastatic potential, mortality, and variable prognosis [1]. Despite advances in treatment, outcomes in patients with melanoma remain dependent on factors such as tumour stage, tumour localisation, and individual patient characteristics [1].

Accurate survival prediction is important for clinical decision-making and for personalising treatment plans, ultimately improving outcomes for patients with melanoma. At present, the AJCC (American Joint Committee on Cancer) staging system, which is considered the global standard, is used to assess the risk of disease progression and prognosis in patients with cutaneous melanoma (Table 1) [2].

Since aggregate statistics do not reveal the full picture of the tumour process in melanoma, the additional use of specialised calculators employing several independent data points improves individual risk prediction. The most widely used is the melanoma calculator developed by Melanoma Institute Australia (MIA) [3, 4]. These calculators assess standard TNM features together with patient-specific variables [5]:

1) Age: older patients generally have lower survival rates;

2) Biological sex: statistically, women generally have a more favourable prognosis than men;

3) Anatomical location: melanomas on the extremities (arms and legs) have a better prognosis than those on the trunk, head or neck;

4) Mitotic rate: the rate at which cancer cells divide (although excluded from some principal T categories, it remains a powerful prognostic factor).

At the current stage of scientific research, modern oncology uses molecular tumour profiles to generate targeted risk estimates [6]:

1) Gene expression profiling (GEP): commercial GEP tests (such as DecisionDx-Melanoma) assess specific RNA signatures in the primary tumour to classify pa-

tients into low- or high-risk categories for metastasis, helping to reduce the number of unnecessary SLNB procedures;

2) Mutational status: detection of BRAF V600 mutations helps to determine prognosis, particularly in stage III disease, and guides the use of targeted adjuvant therapy [7];

3) Polygenic risk scores: academic models use signatures of between 5 and 14 different genes (for example, STAT1, CDK2, p21) combined with spatial single-cell RNA imaging in order to predict how the tumour will interact with the patient's immune system [8].

At present, machine learning and multimodal artificial intelligence (AI) models increasingly outperform conventional statistics by processing vast, heterogeneous datasets simultaneously [9]. The use of AI is therefore becoming a relevant direction for risk stratification in melanoma:

1) Algorithmic risk stratification – advanced gradient boosting algorithms such as CatBoost, XGBoost and Random Forest process large demographic and clinical datasets (such as the national SEER database) to accurately map survival curves [10];

2) Computer vision and digital pathology – deep learning models extract prognostic signals directly from routine digital tissue images;

3) Multimodal systems – by combining digital pathology images with electronic health records (EHR), AI models create personalised, practical clinical decision support tools for patients with early-stage melanoma [1].

It is also well established that, when developing an individualised prognosis for patients with melanoma, it is preferable to additionally consider established anatomical and pathological features of the tumour. These variables are therefore often combined using multivariable regression models, such as Cox proportional hazards models, to predict 1-, 5-, or 10-year survival [1].

Comparisons of different melanoma prognostic models are conducted continuously and form the basis of modern personalised oncology. The principal aim of such studies is to determine whether novel genetic and digital tools can outperform or provide a meaningful complement to the conventional international AJCC staging system [11].

Thus, the relevance of comparative studies evaluating artificial intelligence systems for melanoma prognostication is driven by the global trend towards personalised medicine (Table 2).

Contemporary research indicates that patients with the same melanoma stage may have radically different clinical outcomes, which requires the introduction of dynamic, multimodal and personalised tools. Further searches for new prognostic systems in melanoma are therefore directed at resolving a fundamental contradiction of modern oncology – the biological heterogeneity of melanoma has outgrown the capabilities of the standard AJCC staging system.

AIM

The aim of our study was to perform a comparative analysis of the effectiveness of contemporary machine learning models applied to survival prediction in patients with malignant melanoma, and to assess their potential advantage over the conventional AJCC staging system as a tool for personalised risk stratification. Our research hypothesis was that non-linear ensemble and gradient boosting models provide higher discriminative ability and clinical utility compared with classical regression methods.

MATERIALS AND METHODS

The study was designed as a structured comparative analysis of published machine learning prognostic models for survival assessment in patients with melanoma, supplemented by clinical context from a retrospective cohort study. The sources included peer-reviewed publications in which prognostic models were developed and validated using population-based databases and for which at least one quantitative performance measure was reported.

The eligibility criteria for models included in the comparison were as follows: development using structured clinical and demographic data from patients with melanoma; availability of internal or external validation; and reporting of measures of discrimination (area under the ROC curve [AUC] and concordance index [C-index]), calibration (Brier score), or clinical utility (decision curve analysis [DCA]). Models from the Cox proportional hazards regression (CoxPH), LASSO regression, Random Forest, XGBoost, gradient-boosting (GBM, CatBoost, and LightGBM), and neural network families were included. The methodological features of gradient-boosting algorithms are described in specialised technical publications [12, 13]. As reference non-machine-learning stratification tools, the AJCC/NCCN criteria and the MIA nomogram were additionally included in the comparison [3].

The clinical context was supplemented by data from the retrospective follow-up of 200 patients with cutaneous melanoma who were monitored by dermatovenereologists at a specialised institution over a 10-year period [5]. For the morphological characterisation of the primary tumour, Breslow thickness, the presence of ulceration, and mitotic activity were assessed; staging

Table 1. The AJCC melanoma staging system

Criterion	Characteristic
TNM criteria	the international standard for determining the extent of malignant tumours and cancer staging: T (Tumour) – describes the size of the primary tumour and its invasion into adjacent tissues or organs; N (Node) – indicates the presence or absence of metastases in regional lymph nodes; M (Metastasis) – determines the presence of distant metastases in other organs or tissues
Breslow thickness	Measures the exact depth of tumour invasion in millimetres. It remains the most important local prognostic factor
Ulceration	Absence or presence of ulceration of the epidermis overlying the tumour, indicating an aggressive phenotype
Mitotic rate	The rate of cellular proliferation, defined as the number of mitoses per square millimetre
Lymph node involvement	Confirmed by sentinel lymph node biopsy (SLNB), which determines whether the cancer has spread regionally

Source: compiled by the authors based on [2]

Table 2. Principal advantages of applying AI in melanoma prediction

Criterion	Advantage
Transition to personalised prognosis	Comparative AI studies help to create tools that account for dozens of microfactors simultaneously (age, genetics, the precise immune microenvironment of the tumour), calculating an individual survival trajectory for the patient with melanoma
Reduction in the number of unnecessary invasive procedures	Comparisons of AI models are aimed at training the accurate identification of low-risk patients in whom traumatic surgical interventions for sentinel lymph node removal may be avoided
Optimisation of healthcare system costs	Freely available AI models can deliver high-accuracy results, which will reduce the costs of high-cost diagnostic investigations
Selection of the optimal treatment strategy	Mathematical models will help to assess patient survival depending on the therapy applied

Source: compiled by the authors based on [1, 11]

was performed according to the 8th edition of the AJCC staging system [2].

Model performance was compared using the AUC, C-index, and Brier score values reported in the respective sources for 1-, 3-, and 5-year survival. Standard software packages (Microsoft Excel 2021 and IBM SPSS Statistics 29) were used for statistical processing and visualisation of the cohort data. In the primary studies included in the comparison, the models were implemented in R (mlr3, mlr3proba, randomForestSRC, and glmnet packages) and Python (XGBoost and CatBoost libraries). The gradient-boosting implementations differed in their handling of categorical predictors and regularisation; these differences were taken into account when interpreting the reported performance measures.

ETHICS

The study was conducted in accordance with the provisions of the Declaration of Helsinki of the World Medical Association and the ethical principles for medical research involving human subjects. The retrospective analysis of clinical data used to form the clinical context was carried out on de-identified data within the framework of an approved research project of the institution; additional informed consent for the retrospective analysis of de-identified data was not required in accordance with the applicable regulatory documents. The comparative analysis of prognostic models is based on openly published aggregate data that contain no individually identifiable information.

AI STATEMENT

The following artificial intelligence (AI) tools were used in this work: Claude Opus 4.8 (Anthropic) and ChatGPT GPT-5.5 (OpenAI). They were used for formation and formatting of the bibliography under the author's control and language checking and correction under the author's supervision. All AI-generated or AI-assisted content, including text and code, was reviewed, verified, and edited by the authors. All sections using AI were reviewed and edited personally by the authors. The scientific statements, conclusions, and results are the authors' own intellectual contribution.

RESULTS

The comparative analysis showed that non-linear ensemble and gradient boosting algorithms consistently outperform classical regression models in terms of

Table 3. Comparative characteristics of machine learning model families for survival prediction in melanoma

Model family	Reported discrimination measure	Features / limitations
Cox regression (CoxPH), LASSO	C-index ~0.67–0.69	Simplicity and interpretability; assumptions of linearity and proportional hazards
Random Forest	C-index ~0.70–0.73; AUC up to ~0.84	Highest stability; accounts for non-linear interactions; requires a sufficient sample size
Gradient boosting (CatBoost, XGBoost, GBM)	AUC ~0.76–0.87	Efficient handling of categorical and incomplete data; risk of overfitting
Neural networks	AUC ~0.55–0.66	Flexibility; sensitivity to sample size and class imbalance

Note: AUC – area under the ROC curve; C-index – concordance index. The ranges presented summarise values reported in population-based studies
Source: compiled by the authors based on [1, 10, 11]

discrimination. In population-based studies, Random Forest and gradient boosting models (CatBoost, XGBoost) reached AUC values of approximately 0.76-0.84 for 1-, 3- and 5-year survival, whereas Cox proportional hazards regression and LASSO demonstrated lower concordance index values [10, 11]. The generalised comparative characteristics of the principal model families are presented in Table 3, and the distribution of discrimination measures across prediction horizons is shown in Fig. 1.

As shown in Figure 1A, the advantage of ensemble algorithms was maintained across all prediction horizons. The Random Forest model demonstrated the highest AUC values –0.813, 0.808, and 0.840 for 1-, 3-, and 5-year survival, respectively – whereas the corresponding values for Cox regression did not exceed 0.749. The lowest discriminatory ability was observed for neural networks (AUC, 0.646-0.660), which is consistent with their sensitivity to limited sample sizes in rare tumour types. Gradient-boosting models (CatBoost, XGBoost, and GBM) occupied an intermediate position, with stable AUC values ranging from 0.713 to 0.766; CatBoost demonstrated the lowest variability across prediction horizons (0.756-0.760).

Ensemble models also demonstrated an advantage in terms of calibration: Brier score values for Random Forest were 0.175, 0.164, and 0.130 for 1-, 3-, and 5-year survival, respectively, representing the lowest (i.e., best) values among all compared algorithms. In external testing on independent samples, discrimination measures decreased as expected (C-index, 0.701; AUC, 0.715-0.776) but remained at an acceptable level, indicating satisfactory model generalisability.

A separate comparison of machine learning models with conventional risk-stratification tools used in routine clinical practice was performed (Fig. 1B). The AJCC/NCCN criteria, based predominantly on tumour thickness, yielded a C-index of only 0.537, with high sensitivity (95.0%) but extremely low specificity (12.4%). Cox regression increased the C-index to 0.685. The MIA nomogram, which additionally accounts for mitotic activity, histological subtype, and lymphovascular invasion, achieved a C-index of 0.739 and 0.750 on external validation, respectively, demonstrating performance comparable to that of ensemble machine learning models (C-index, 0.732) [3]. Thus, in terms of discrimination, the modern multiparametric nomogram and machine learning models demonstrated comparable performance and substantially outperformed the criteria-based AJCC/NCCN approach.

Analysis of feature importance in the best-performing models showed that tumour stage, patient age, and surgical treatment were the leading prognostic factors for survival; these variables made a substantially greater contribution than other predictors [1, 10]. The influence of stage and age was most pronounced in short-term predictions and gradually weakened as the prediction horizon increased. At the same time, in models built using an extended set of histological features, mitotic activity and pretreatment lactate dehydrogenase levels were among the most important predictors, indicating that the relative importance of predictors depends on the completeness of the source data [1]. Assessment of clinical utility using decision curve analysis confirmed that ensemble models provided greater net clinical benefit across a wide range of threshold probabilities (approximately 0.1-0.6) than classical methods [14].

The retrospective cohort follow-up data are consistent with the modelling results. The morphological characteristics of the primary tumour (Breslow thickness, mitotic activity, presence of ulceration) and the AJCC disease stage were associated with subsequent progression: the highest levels of mitotic activity and ulceration were observed at advanced (III–IV) stages. Tumour progression predominantly occurred in the first years of follow-up, and the leading routes of metastasis were regional and distant lymphatic collectors, the lungs, skin, liver and skeletal system [5]. This confirms the role of the same quantitative clinical parameters that are used as predictors in machine learning models.

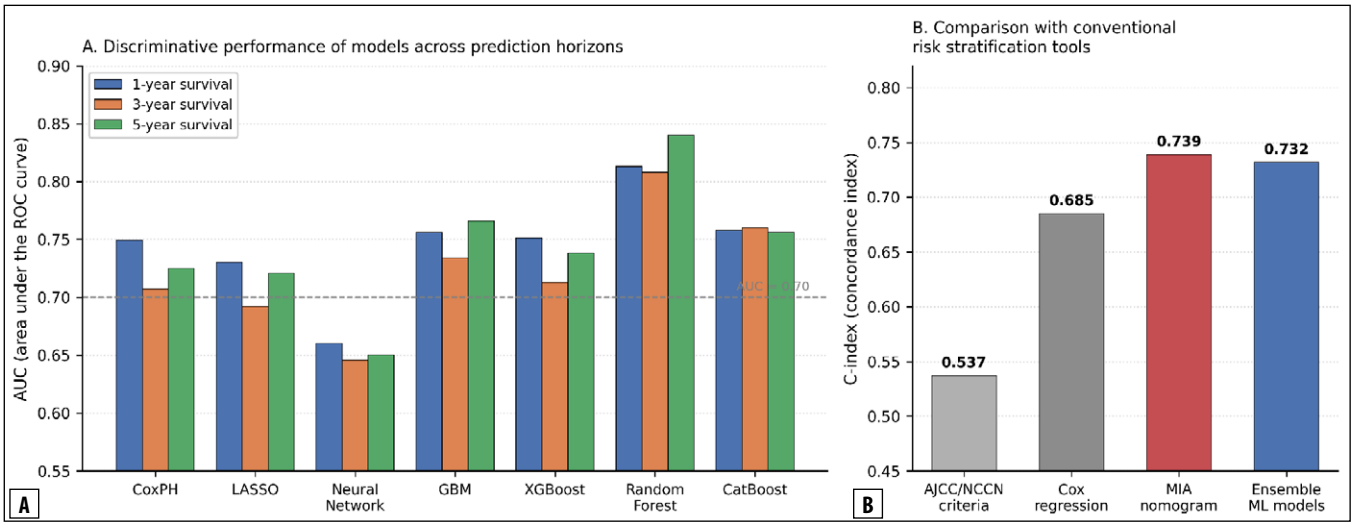


Fig. 1. Comparative performance of prognostic models in melanoma: A – discriminative ability (AUC) of machine learning models and regression approaches for 1-, 3- and 5-year survival; B – comparison of the concordance index (C-index) of ensemble machine learning models with conventional risk stratification tools (AJCC/NCCN criteria, Cox regression, MIA nomogram). Data summarised from published population-based studies
Source: compiled by the authors based on [1, 3, 4, 10]

DISCUSSION

Our results demonstrated that ensemble and gradient boosting models provide higher discriminative ability compared with Cox proportional hazards regression (C-index 0.732 versus 0.685; AUC up to 0.840 versus 0.749), which supports the research hypothesis put forward. These data are consistent with the results of F. Gao and X. Xu, who, when comparing six algorithms in a population-based sample, obtained a C-index of 0.732 and AUC values of 0.813–0.840 for the Random Forest model, whereas CoxPH and LASSO did not exceed 0.685 and 0.670, respectively [10]. A similar hierarchy of algorithms was described by E. Hu et al.: in their study of a sample of 4875 patients with cutaneous melanoma, the CatBoost model showed the best generalisability, with stable AUC values of 0.7557-0.7595, which is close to the ranges we obtained for gradient boosting algorithms [1].

The relatively low effectiveness of neural networks that we identified (AUC 0.646-0.660) also corresponds to the literature. K. Kourou et al. note that the advantage of deep architectures over ensemble methods manifests itself predominantly in large and multimodal data arrays, whereas in structured clinical samples of limited size ensemble decision trees retain their advantage [11]. This explains why, in melanoma studies where samples rarely exceed a few thousand observations, it is Random Forest and CatBoost rather than neural networks that occupy the leading positions.

Of key importance for the interpretation of our results is the comparison of machine learning models with the MIA nomogram. The C-index value we obtained for the MIA nomogram (0.739) corresponds to the data of S. N. Lo et al., who reported an accuracy of 73.9% on internal and 75.0% on external validation in an independent MD Anderson cohort [3]. Fundamentally, this measure is not inferior to the results of ensemble machine learning models. This calls for a critical appraisal of the widespread claim of an unconditional superiority of algorithmic approaches: according to our data, a carefully constructed multiparametric regression nomogram may be at least equivalent to more complex models. Instead, the genuine advantage of both approaches manifests itself precisely in comparison with the criteria-based AJCC/NCCN selection, which in our analysis provided a C-index of only 0.537. The same conclusion is drawn by S. N. Lo et al., who show that at an equal sensitivity of 95% the specificity increased from 12.4% for the NCCN/ASCO criteria to 22.1% for the MIA nomogram [3].

The structure of leading predictors identified in our analysis (stage, age, surgical treatment) corresponds to the results of population-based studies [10], but requires a caveat. In the work of E. Hu et al., where the feature set included extended histological parameters, the dominant predictors were mitotic activity and lactate dehydrogenase level, while demographic factors made a minimal contribution [1]. This discrepancy shows that the ranking of predictors largely reflects the completeness of the available data rather than the biological significance of the features alone. The meta-analysis by Q. Xia et al. confirms that Breslow thickness, ulceration and lymph node involvement remain the most stable prognostic factors regardless of the modelling method [15], and the systematic review by K. Lallas et al. additionally assigns mitotic activity and molecular markers to this group [7].

The results of the retrospective cohort follow-up that we used for clinical context confirm the pattern noted above: the highest levels of mitotic activity and ulceration were observed at advanced stages, and progression predominantly occurred in the first years of follow-up, with metastasis to regional lymphatic collectors, the lungs, liver and skeletal system [5]. This is consistent with meta-analytic data on predictors of distant metastasis [7, 15] and indicates that the predictors used by algorithms have a reproducible clinical and morphological basis rather than being purely statistical artefacts.

At the same time, our data showing a decline in performance on external validation (C-index, 0.701 vs. 0.732 on internal validation) reflect a systemic problem in the field. The systematic review by I. Kaiser et al., which examined prognostic models in melanoma, demonstrated substantial methodological heterogeneity in model development and validation, meaning that most published models are not directly comparable with one another [4]. K. G. M. Moons et al. emphasised that, in the absence of external validation, performance estimates are systematically overestimated owing to model optimism [16]. For this reason, the advantage of ensemble algorithms identified in our analysis should be interpreted with caution: it has been demonstrated using retrospective, population-based data but not in prospective cohorts.

The study has several limitations. The comparison was based predominantly on models developed using retrospective population-based databases, particularly SEER, which are subject to selection bias and data incompleteness. In addition, most models underwent only internal validation, limiting their generalisability [16]. Comparability between studies is further complicated by differences in cohorts, feature sets, and validation methodologies [4]. Molecular markers, including BRAF, KIT, and NRAS mutational status, as well as the effects of modern immunotherapy, are often not incorporated into available models, which may lead to underestimation of long-term survival. Studies based on single-cell and spatial tumour maps indicate considerable unrealised potential for incorporating such data into prognostic models [8]. Furthermore, beyond tumour-related characteristics, short-term survival in patients with cancer is influenced by general clinical factors, particularly nutritional status [17]. Further research should therefore focus on prospective multicentre validation, the integration of molecular data, and the standardisation of evaluation metrics.

CONCLUSIONS

Machine learning models, particularly ensemble algorithms (Random Forest) and gradient-boosting algorithms (CatBoost and XGBoost), provide more accurate and clinically useful individualised survival prognostication in patients with melanoma than classical regression methods, achieving AUC values of approximately 0.76-0.84 and a C-index of 0.732, compared with 0.685 for Cox regression. At the same time, the modern multiparametric MIA nomogram demonstrates comparable discriminatory performance (C-index, 0.739), whereas the criteria-based AJCC/NCCN approach is substantially inferior to both (C-index, 0.537). The leading prognostic factors are consistently tumour stage, age, and surgical treatment, consistent with both population-based data and the results of retrospective clinical follow-up. Machine learning models should be regarded as a supplement to, rather than a replacement for, the conventional AJCC staging system. The practical implementation of such tools requires prospective clinical validation, integration of molecular data, and the preservation of the physician's ultimate decision-making authority.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

Motor activity as a factor in improving the physical working capacity and somatic health of higher education applicants

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ABSTRACT

Aim: To investigate the impact of motor activity volume on the physical working capacity and somatic health of higher education applicants.

Materials and Methods: The research included male cadets from the National Academy of Internal Affairs who were admitted in 2025 (n = 240). The participants were divided into two groups: SG 1 (n = 108) – cadets who regularly engaged in additional sports activities – and SG 2 (n = 132) – cadets who limited themselves to mandatory physical training sessions only. Methods: theoretical, empirical, and mathematical statistics. The Framingham method was used to assess motor activity and daily energy expenditure. The level of physical working capacity was assessed using the Harvard step test. The level of somatic health was determined using G. L. Apanasenko's rapid assessment method.

Results: It was found that cadets who engaged in additional sports activities had significantly ($p < 0.05$-0.001) better indicators of motor activity, physical working capacity, and somatic health compared to cadets who limited themselves to only mandatory physical training sessions: in SG 1, compared to SG 2, the motor activity index was 5.6 points higher, the Harvard step test index was 9.6 a. u. higher, and the somatic health level was 6.22 points higher.

Conclusions: It was found that motor activity is an important factor in the development of physical working capacity and somatic health among higher education applicants, and that regular participation in sports contributes to increased body functional reserves, improved recovery after physical exertion, and enhanced adaptive capabilities.

KEYWORDS: higher education applicants, cadets, motor activity, somatic health, physical working capacity

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INTRODUCTION

In today's environment, maintaining and improving the health of higher education applicants has become particularly relevant. This is due to the intensity of the educational process, significant mental strain, prolonged use of digital technologies, a sedentary lifestyle, reduced daily physical activity, and high levels of stress and neuro-emotional tension experienced while studying under martial law [1, 2]. Insufficient motor activity leads to a decline in physical working capacity, the development of musculoskeletal, cardiovascular, and nervous system disorders, and an increased risk of chronic noncommunicable diseases [3, 4]. In this regard, it is crucial to study the motor activity of higher education cadets, as it is a key factor in ensuring adequate levels of somatic health, physical fitness, and working capacity.

The analysis of recent studies and publications indicates that the issue of motor activity among today's youth remains a relevant area of scientific research for many scholars [5, 6]. Researchers are convinced that adequate motor activity positively influences physical development, the body's functional state, psycho-emotional well-being, and quality of life among young people [7]. Regular physical exercise and sports help increase the functional reserves of the cardiovascular and respiratory systems, improve indicators of physical fitness and working capacity, and prevent obesity and other diseases associated with hypodynamia [8].

In the scientific literature, the term "motor activity" is defined as any movement of the human body resulting from the work of skeletal muscles and accompanied by additional energy expenditure [9]. Given the close

relationship between the volume of motor activity and the body's energy expenditure, assessing the motor activity patterns of higher education students is of great importance. It has been established that the educational activities of higher education students, except for physical education training sessions, are classified as Category I work in terms of energy expenditure [10].

In the works [11, 12], researchers focus significant attention on studying the characteristics of students' motor activity, ways to increase their motivation for physical education, and the implementation of modern health-promoting technologies in the educational process. At the same time, the results of many studies indicate a trend toward a decline in motor activity among higher education students, driven by increased time spent at the computer, distance learning, and an insufficiently developed need for systematic motor activity [4, 13].

Despite the significant number of scientific works on this topic, the issue of determining the role and volume of motor activity as a factor in maintaining and improving health and enhancing the physical working capacity of today's youth requires further scientific substantiation.

AIM

The aim is to investigate the impact of motor activity volume on the physical working capacity and somatic health of higher education applicants.

MATERIALS AND METHODS

PARTICIPANTS

The research included male cadets recruited in 2025 at the National Academy of Internal Affairs (Kyiv, Ukraine, NAIA) (aged 18-20, first-year cadets) majoring in "Law Enforcement" and "Law" (n = 240). Based on the volume of their motor activity, two groups were formed: SG 1 (n = 108) – cadets who regularly participated in additional sports activities throughout the academic year, and SG 2 (n = 132) – cadets who limited themselves to only mandatory physical training sessions twice a week and did not participate in other forms of regular motor or sports activities. Inclusion criteria: male cadets recruited in 2025, no health-related contraindications to physical exercise, and for SG 1 – attendance at the academy's sports clubs during extracurricular hours. Exclusion criteria: willingness to withdraw from the research on one's own initiative at any time; for SG 1, exclusion from a sports club. Research limitations: we examined the volume of motor activity among first-year cadets exclusively within the educational institution. The research was conducted at the end of the first academic year (May-June) 2026.

METHODS

To achieve the research aim, a set of modern general scientific methods was used, including: theoretical methods (conceptual-comparative analysis, structural-systemic analysis, synthesis, and generalization); empirical methods (questionnaires, pedagogical observation); and methods of mathematical statistics. To determine the level of motor activity among higher education cadets, the Framingham method was used, which involves recording activity types throughout the day [14]. All types of motor activity were recorded on a special form. Activity was recorded over seven days, and the average daily result was calculated. This method provides comprehensive information on the duration of specific activities and rest periods, the combination of physical activities of varying intensities, the total volume of motor activity, and the amount of energy expenditure. The level of motor activity is assessed using the motor activity index (MAI). According to the method, a person's motor activity is divided into five levels: baseline, sedentary, low, average, and high. Each level is characterized by specific types of motor activity and a corresponding energy value, enabling determination of the cadet's daily energy expenditure.

Physical working capacity was assessed using the Harvard step test [15]. The test involves measuring heart rate (HR) after a controlled physical exertion, thereby allowing evaluation of recovery processes. According to the protocol, cadets climbed a step of a fixed height (50 cm for men). The exercise duration was 5 minutes, with a cycle frequency of 30 steps per minute – climbing the step and returning to the starting position while alternating legs. That is, 120 steps per minute. Heart rate was recorded while seated during the first 30 seconds and at the 2^{nd}-4^{th} minutes of the recovery period. Test results were expressed as the Harvard step test index (HSTI), a value that indicates the rate of heart rate recovery following a standard physical exercise. Physical working capacity levels were determined based on the criteria specified in the diagnostic protocol, namely: low physical working capacity – HSTI < 55 a. u.; below average – 55-64 a. u.; average – 65-79 a. u.; good – 80-89 a. u.; excellent – 90 a. u. and above.

Somatic health level was determined using G. L. Apanasenko's rapid assessment method [16]. The method is based on anthropometric indicators and the subjects' cardiovascular condition. To assess the cadets' somatic health level (SHL), several parameters were examined:

body weight, body height, vital capacity (VC) of the lungs, handgrip strength of the dominant hand, resting heart rate, blood pressure, body mass index (BMI), vital index (VI), strength index (SI), Robinson index (RI), and Martine-Kushelevsky index (MKI). SHL was determined by the total score cadets received across the 5 indices. If the total score was less than 3 points, SHL was rated as low; 4-6 points – below average; 7-11 points – average; 12-15 – above average; 16-18 – high.

STATISTICAL METHODS

The methods of mathematical statistics were used to process the data obtained. The reliability of the difference between the indicators was determined using the Student's t-test. The results were presented as Mean ± m, where Mean is the arithmetic mean, m is the standard error. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using STATISTICA 6.1 software package (number AGAR909E-415822FA), adapted for medical and biological research.

ETHICS

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the NAIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

AI STATEMENT

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

FRAMEWORK

This scientific article was carried out according to the plan of the research work of the National Academy of Internal Affairs for 2020-2026 "Psychological, pedagogical and sociological support of law enforcement officers» (state registration number 0113U008196).

RESULTS

The analysis of motor activity indicators revealed significant differences between the study groups ($p \leq 0.001$). The results processing showed that among SG 1 cadets, the average daily energy expenditure was 2775.8 ± 16.7 kcal (Table 1). At the same time, the motor activity index (MAI) was 35.90 ± 0.22 points, indicating a higher level of motor activity compared with participants in SG 2. For participants in SG 2, the average daily energy expenditure was recorded at 2,362.5 ± 20.1 kcal, and the MAI value was 30.30 ± 0.24 points. That is, the difference between the groups was more than 400 kcal per day, indicating significantly higher motor activity and greater physical exercise among participants who regularly engage in sports.

An important indicator of the body's functional fitness is the level of physical working capacity, which reflects the ability to perform muscular work of a certain intensity without a significant decrease in its efficiency. Assessment of physical working capacity using the Harvard step test index (HSTI) also revealed statistically significant differences between groups ($p \leq 0.001$). Among participants in SG 1, the mean HSTI score was 81.9 ± 1.02 a. u., which, according to the evaluation criteria, is characterized as a good level of physical working capacity. In contrast, for participants in SG 2, this indicator was 72.3 ± 1.28 a. u. and was assessed as an average level of physical working capacity.

The comparative analysis of the morphofunctional indicators of somatic health revealed that participants in SG 1 had superior results for most of the parameters studied. The body mass index, defined as the ratio of body mass to the square of body height, was 23.74 ± 0.10 kg/m^2 for participants in SG 1, whereas for SG 2 it was 23.21 ± 0.09 kg/m^2 ($p \leq 0.001$). Despite the statistically significant difference, the indicators for both groups fell within the average range.

The analysis of the vital index, calculated as the ratio of vital capacity of the lungs to body weight, revealed statistically significant differences between the study groups ($p \leq 0.001$) and indicated higher respiratory function in participants in SG 1. The value of this indicator was 62.22 ± 0.44 ml/kg, which corresponds to an above-average level, whereas in SG 2 it was 56.29 ± 0.49 ml/kg and was assessed as average.

A similar trend was observed for the strength index, which was defined as the ratio of the handgrip strength of the dominant hand to body weight. For participants in SG 1, the value was 72.08 ± 0.30 %, which is considered above average. In contrast, for individuals in SG 2, it was 65.44 ± 0.34 %, which is considered an average level ($p \leq 0.001$).

Indicators of the cardiovascular system's functional state also revealed significant differences between the groups. The Robinson index value for participants in SG 1 was 86.38 ± 0.41 a. u. and was classified as average, whereas in SG 2 it was 95.69 ± 0.44 a. u., which was considered below average ($p \leq 0.001$). The Martine-Kushelevsky index (MKI) for participants in SG 1 was recorded at 89.87 ± 1.33 s and was characterized as above average. For participants in SG 2, the value of

Table 1. Indicators of motor activity, physical working capacity, and somatic health among higher education cadets, SG 1 (n = 108), SG 2 (n = 132)

Indicators	Participants		Significance of the difference	
	SG 1 (n = 108)	SG 2 (n = 132)		
	Mean ± m	Mean ± m	t	p
Motor activity				
Daily energy expenditure, kcal	2775.8±16.7	2362.5±20.1	15.816	p≤0.001
Motor activity index (MAI), points	35.90±0.22	30.30±0.24	17.200	p≤0.001
Physical working capacity				
Harvard step test index, a. u.	81.9±1.02	72.3±1.28	5.865	p≤0.001
Somatic health				
Body mass index, kg/m^2	23.74±0.1	23.21±0.09	4.088	p≤0.001
Vital index, ml/kg	62.22±0.44	56.29±0.49	9.005	p≤0.001
Strength index, %	72.08±0.30	65.44±0.34	14.644	p≤0.001
Robinson index, a. u.	86.38±0.41	95.69±0.44	15.480	p≤0.001
Martine-Kushelevsky index, s	89.87±1.33	116.46±1.37	13.926	p≤0.001
Somatic health level, points	10.46±0.23	4.24±0.27	17.537	p≤0.001

Legend: Mean – arithmetic mean; m – standard error; t – Student's test value; p – level of significance of the differences between studied indicators
Source: compiled by the authors of this study

this indicator was 116.46 ± 1.37 s, which, according to the evaluation criteria, was classified as average.

The comprehensive assessment of somatic health level (SHL) revealed significant differences between the groups. For participants in SG 1, the SHL score was 10.46 ± 0.23 points, corresponding to an average level of physical health. In contrast, among participants in SG 2, the SHL was 4.24 ± 0.27 points, which was assessed as below average. The results indicate a more favorable morphofunctional state in participants with higher levels of motor activity.

DISCUSSION

According to the World Health Organization, one-third of all adults worldwide do not use their skeletal muscles enough, thereby increasing the risk of health problems and a shorter life expectancy, particularly obesity, which is the leading risk factor for overall mortality worldwide [17]. According to the study [18], 59.1 % of the population in Ukraine in 2020 was overweight, of whom 24.8 % were obese, and only 39.6 % had a normal body weight. As scientists note [19], human health is linked to high levels of motor activity; however, modern technological advancements, the mechanization of labor, and the availability of personal transportation have made our daily lives and living conditions easier but, unfortunately, have deprived our bodies of physical exertion and reduced our motor activity levels.

The results of our research confirmed a correlation among higher education cadets between motor activity, physical working capacity, and somatic health. It was found that regular exercise training sessions are associated with higher daily energy expenditure and motor activity index values. This contributes to increased functional capabilities and expanded adaptive reserves.

The data obtained regarding physical working capacity are consistent with current scientific understanding of the adaptive effects of systematic physical exercise on the cardiorespiratory system [20]. Higher HSTI values among participants in SG 1 indicate greater tolerance to physical exertion, more efficient functioning of energy-supply mechanisms, and faster recovery after work.

The observed differences in the vital and strength indices confirm the positive effect of regular motor activity on the functional capacity of the respiratory system and the development of muscle strength. At the same time, lower Robinson index values and faster heart rate recovery after exercise in participants in SG 1 indicate more efficient functioning of the cardiovascular system and a higher level of the body's adaptive reserves.

The results of the comprehensive assessment of somatic health deserve special attention. The difference between the groups on the SHL indicator indicates that insufficient motor activity is a factor contributing to a decline in the functional capabilities of today's youth. The data obtained confirm the advisability of involving higher education cadets in regular physical exercise and training sessions in sports clubs as an effective means of improving their health and physical working capacity.

CONCLUSIONS

Based on the research conducted, higher education cadets who regularly engage in sports exhibit significantly higher

levels of motor activity than their peers who limit themselves to mandatory physical training sessions ($p \leq 0.001$). It was found that the motor activity index in SG 1 was 35.90 ± 0.22 points, and in SG 2, 30.30 ± 0.24 points. The Harvard step test index in SG 1 reached 81.9 ± 1.02 a. u. (good level of physical working capacity), while in SG 2, it was 72.3 ± 1.28 a. u. (average level). A similar trend was observed for somatic health: 10.46 ± 0.23 points in SG 1 versus 4.24 ± 0.27 points in SG 2. Higher levels of motor activity are associated with better indicators of physical working capacity and somatic health. This is reflected in higher values of the vital and strength indices, more efficient cardiovascular system function, and faster recovery after physical exertion. The results confirm that motor activity is an important factor in shaping the physical working capacity and somatic health of higher education cadets, and that regular participation in sports contributes to expanding the body's functional reserves and improving its adaptive capabilities.

PROSPECTS FOR FURTHER RESEARCH

We see prospects for further research into the relationship between motor activity volume of varying intensities and indicators of psychological and physical well-being among higher education cadets.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

Radiographic evaluation of the impact of hyaluronic acid and bone graft on peri-implant tissue: A randomized clinical trial

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ABSTRACT

Aim: The objective of this randomized clinical trial was to enhance the effect of HY alone or in combination with a bone graft in changing peri-implant tissue dimensions radiographically.

Materials and Methods: Thirty-six patients who required posterior dental implants were randomly assigned to three groups (n=12 each): a control group (CONT) with conventional implant placement was performed, one group treated with HA (HY), and another test group treated with HA and bone graft together (HYB). The same surgical procedure was performed for all implants in the posterior regions of jawbones. Measurements were taken before surgery, after surgery, and at eight and sixteen weeks after surgery.

Results: Hybrid group (HYB) had a significantly greater increase radiographic soft tissue thickness at all-time points ($P < 0.001$). The HYB group had higher radiographic bone height at week 16 and stability of bone width ($P = 0.088$) than the other groups ($P < 0.001$)

Conclusion: The combined application of a bone graft and hyaluronic acid (HYB) significantly improves the radiographic soft tissue thickness and bone height.

KEY WORDS: hyaluronic acid, bone substitutes, dental implant, peri-implant tissues

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INTRODUCTION

High long-term survival rates, functional rehabilitation, and favorable aesthetic outcomes have made the dental implant therapy the gold standard for replacing missing teeth. Continuous advancements in implant surface technology, surgical protocols, and biomaterials have significantly improved treatment predictability, making implant-supported restorations an integral part of modern evidence-based oral rehabilitation [1-6]. However, successful implant therapy is not limited to osseointegration but requires, in addition, the long-term maintenance of both peri-implant hard and soft tissue to ensure biological stability, esthetic harmony and patient satisfaction [7, 8]. The peri-implant tissue phenotype has recently been known as one of the greatest important variables to implant success. It contains several anatomical parameters, including keratinized mucosal width (KMW), mucosal thickness (MT), and supracrestal tissue height (STH), which are involved in the maintenance of the peri-implant mucosal seal and protection of the underlying supporting bone [9-10]. Clinical evidence suggests that insufficient keratinized mucosa, thin peri-implant mucosa, or reduced supracrestal tissue height are associated with increased plaque formation, peri-implant inflammation, mucosal recession, esthetic complications, and progressive marginal bone remodeling [9-11]. Thus, contemporary implant therapy not only aims at successful osseointegration, but also on the optimization of the peri-implant tissue phenotype to enhance long-term clinical results. Hyaluronic acid (HA) is an agent that has been extensively studied to improve the peri-implant wound healing as a biological agent because of its superior biocompatibility and various biological properties. Hyaluronic acid is a naturally occurring glycosaminoglycan that plays an essential role in the organization of the extracellular matrix, modulating inflammation, angiogenesis, fibroblast proliferation, and collagen synthesis and tissue regeneration. Besides promoting soft tissue healing, HA has also shown antimicrobial activity and the ability to stimulate osteogenic differentiation, making it a valuable adjunct in oral regenerative procedures [12-13]. These biological properties have led to its successful use in periodontal surgery, management of oral mucosal diseases, temporomandibular joint disorders and reduction of postoperative complications after oral surgical procedures. In implant dentistry, bone graft substitutes are widely used to preserve alveolar ridge dimensions, reconstruct osseous defects and provide a stable scaffold for new bone formation. Graft materials are classified as autogenous, allogeneic, and xenogeneic and

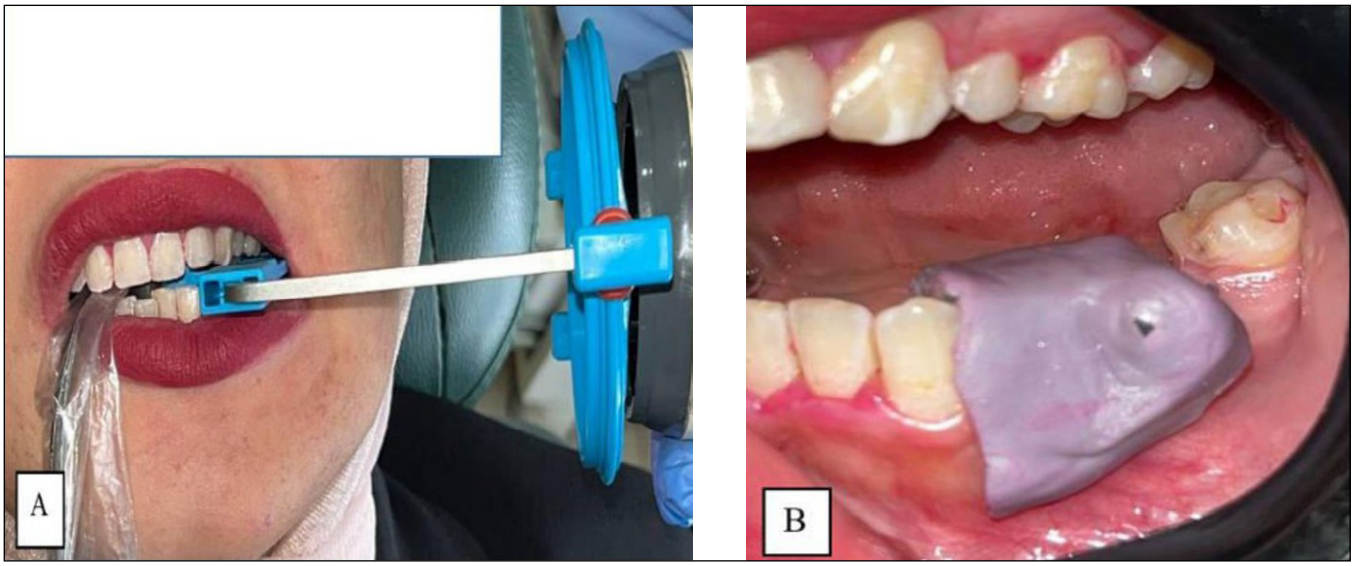


Figure 1: Radiographic Setup and Calibration (A) Radiograph acquisition process, (B) Placement of the calibration ball

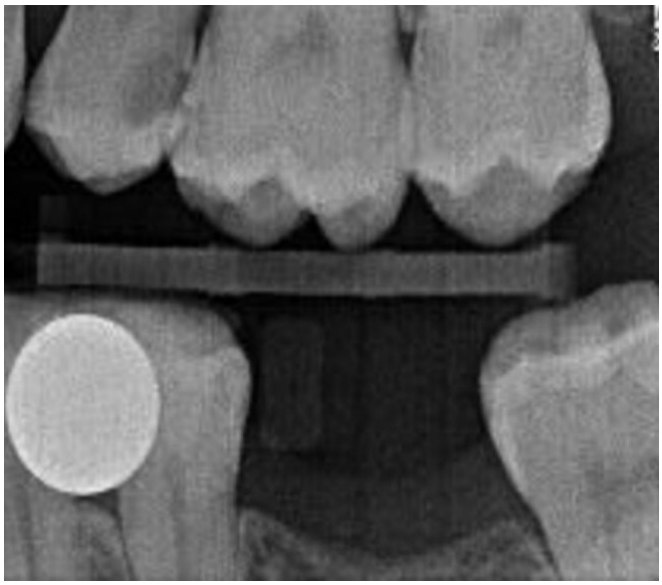


Figure 2: Assessment of radiographic soft tissue thickness using a bite-wing projection

alloplastic based on their source, with different biological and mechanical properties [14]. Although hyaluronic acid and bone grafting materials have been shown to have good biological and regenerative properties separately, evidence of their combination to improve peri-implant tissue phenotype is scarce. Most previous studies have focused on bone regeneration, implant stability or post-operative discomfort, but the comprehensive evaluation of the peri-implant hard and soft tissue phenotype after the combined application of HA and bone graft is still missing.

AIM

The aim of this study was to investigate whether the combination application of hyaluronic acid and bone graft material has a substantial impact on peri-implant tissue parameters.

MATERIALS AND METHODS

The study was performed at two clinical sites, The Implantology Unit, Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Babylon (Babylon, Iraq) and a private specialized dental center (Babylon, Iraq), between December 2025 and July 2026.

The null hypothesis was that hyaluronic acid with bone graft does not have a significant effect on mucosal thickness and marginal bone height around the implants. The alternative hypothesis is that this treatment combination leads to significant changes both in crestal bone levels and peri-implant soft tissue thickness.

Ethical guidelines of the Declaration of Helsinki, as outlined by the World Medical Association, were strictly followed, obtaining informed consent from all participants. All patients gave consent that was written especially for the study prior to enrollment. In order to guarantee voluntary participation, each participant received an information sheet and a consent form that clearly explained the study and its significance. The patients were explicitly informed of their right to withdraw from the investigation at any stage without penalty while it was being conducted. The obtained results were the assessment of comprised the evaluation of radiographic soft tissue thickness (RSTT) alongside crestal bone height (BH). These quantitative assessments were made preoperative (just for RSTT), immediately postoperative, and six and twelve weeks postoperatively.

STUDY SAMPLE

Sample size estimation was executed with G*Power software hand-selected for this purpose. The analysis was based on a significance level of $\alpha = 0.05$, a power of 80%, and a

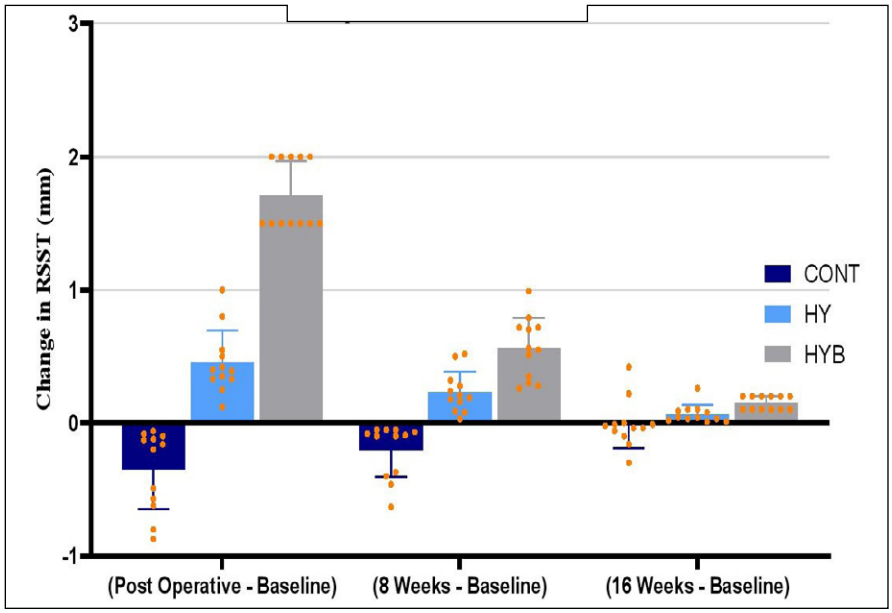


Figure 3: Mean Change in Radiographic Soft Tissue Thickness (RSTT) with Individual Case Distribution across Study Groups at Different Time Intervals

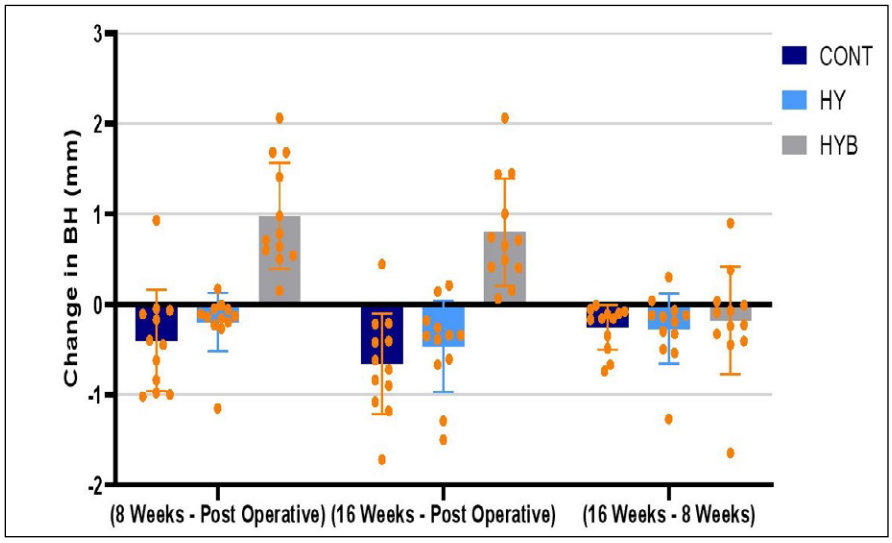


Figure 4: demonstrates the mean change in bone height (BH) with individual case distribution across study groups at different time intervals

medium effect size according to Cohen's conventions (f = 0.25) for a three-group repeated measures design. Estimated 12 cases per group (total 36 with dropout 20(%.

ELIGIBILITY CRITERIA

Patients meeting the inclusion and exclusion criteria were recruited. Inclusion criteria for the study were: male and female individuals over 18 years of age having partially edentulous spaces in the posterior area of either jaw with appropriate bone volume for implant insertion. The participants required to show the best possible dental health, measured by the gingival bleeding index (≤10%) and O'Leary plaque index (<10%). The study excluded patients with systemic conditions affecting bone healing, patients on medications affecting bone metabolism, patients with periodontal disease, pregnant and lactating women, patients with poor oral hygiene and patients unable to attend follow-up appointments.

SAMPLING AND GROUPING

Candidate patients are randomly (sequentially numbered) selected throughout the course of the routine dental check-ups to allocate the 36 implant sites into three equal groups (1:1:1 allocation ratio). Some participants who satisfied the screening criteria were recruited until the desired sample size was met. Each participant had been randomly assigned to one of three groups:

- **Group (HA) (n=12 implants):** Patients in this group received a hyaluronic acid gel applied to the implant site during implant placement.
- **Group (HAB) (n=12 implants):** Patients in this group received hyaluronic acid gel and particulate bone graft applied to the implant site during implant placement.
- **Control Group (CONT) (n=12 implants):** Patients in this group underwent implant placement only without the use of hyaluronic acid gel aznd particulate bone graft.

Table 1. Comparisons of the socio-demographic characteristics among the studied groups (n=12)

Socio-demographic characteristics		CONT	HY	HYB	Total	p-value
		No. (%)	No. (%)	No. (%)		
Age groups (years)	≤30	1(8.3)	2(16.7)	1(8.3)	4	0.658*
	31-40	3(25.0)	1(8.3)	7(58.3)	11	
	41-50	3(25.0)	5(41.7)	2(16.7)	10	
	≥51	5(41.7)	4(33.3)	2(16.7)	11	
Gender	Males	4(33.3)	5(41.7)	5(41.7)	14	0.890†
	Females	8(66.7)	7(58.3)	7(58.3)	22	

*Freeman-Halton Exact test; †Chi square test

Table 2. Comparisons of the location characteristics among the studied groups (n=12)

Lesion characteristics		CONT	HY	HYB	Total	p-value
		No. (%)	No. (%)	No. (%)		
Jaw	Maxilla	5(41.7)	7(58.3)	8(66.7)	20	0.455†
	Mandible	7(58.3)	5(41.7)	4(33.3)	16	

†Chi square test

Table 3. Comparison of the RSST among the studied groups (n=12)

Radiographic Thickness	CONT	HY	HYB	p-value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Baseline	$1.91 \pm 0.40^{a,A}$	$1.85 \pm 0.36^{a,A}$	$2.36 \pm 0.62^{b,A}$	0.024
Post-Op	$1.56 \pm 0.27^{a,B}$	$2.31 \pm 0.24^{b,B}$	$4.07 \pm 0.62^{c,B}$	<0.001
8 Weeks	$1.70 \pm 0.33^{c,C}$	$2.09 \pm 0.27^{b,C}$	$2.92 \pm 0.56^{c,C}$	<0.001
16 Weeks	$1.90 \pm 0.39^{a,A}$	$1.92 \pm 0.35^{a,A}$	$2.51 \pm 0.63^{b,A}$	0.004
p-value†	<0.001	<0.001	<0.001	

*One-Way ANOVA test; ^{a}, ^{b}, ^{c}: Different lowercase superscript letters within a row indicate significant differences between groups at the same time point (Tukey post-hoc test, P<0.05).
†General Linear Model (Repeated Measures): ^{A}, ^{B}, ^{C}: Different uppercase superscript letters within a column indicate significant differences within the same group over different time points P<0.05.

Table 4. Comparison of the bone height among the studied groups (n=12)

Bone Height	CONT	HY	HYB	p-value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Post-Op	$4.23 \pm 0.85^{a,A}$	$4.85 \pm 1.31^{a,A}$	$3.90 \pm 0.79^{a,A}$	0.077
8 Weeks	$3.84 \pm 0.84^{a,B}$	$4.66 \pm 1.38^{ab,AB}$	$4,87 \pm 0.83^{b,B}$	0.050
16 Weeks	$3.58 \pm 0.83^{a,B}$	$4.39 \pm 1.27^{ab,B}$	$4.69 \pm 0.49^{b,C}$	0.016
p-value†	<0.001	0.003	<0.001	

*One-Way ANOVA test; ^{a}, ^{b}: Different lowercase superscript letters within a row indicate significant differences between groups (Tukey post-hoc test).
†General linear Model (Repeated Measures); ^{A}, ^{B}, ^{C}: Different uppercase superscript letters within a column indicate significant differences over time (Repeated Measures ANOVA).

PREOPERATIVE PHASE

PATIENT PREPARATION

Before starting the trial, each participant's teeth were scaled and polished, and home care was established and encouraged. All potential implant sites were examined clinically by evaluation of soft tissue condition, ridge morphology, and space availability. Mesiodistal and interocclusal spacing was assessed to ensure sufficient clearance from adjacent teeth or implants. These findings were complemented radiographically by Cone Beam Computed Tomography (CBCT) to ensure eligibility, check bone quality, and to make the best treatment decision regarding the implant size and placement angulation.

SURGICAL PHASE

HYALURONIC ACID PLACEMENT

Preparation and loading of hyaluronic acid

The haluronic acid (Ricerfarma S.R.L, Italy, and Milan) was prepared for clinical application using a standardized delivery method to ensure precise volumetric administration. The following protocol was adopted.

Standardization of the delivery vehicle

A sterile 1 ml insulin syringe (Changzhou Jifeng Medical Equipment Co., Changzhou, China) was modified by removing its needle-bearing using a sterile surgical blade (Fig.1).

This modification results in a large bore delivery channel allowing consistent discharge of the viscous gel and consistency with bone graft delivery technique.

Volumetric measurement

The modified syringe was filled with a precise volume of 0.2 ml hyaluronic acid.

Aseptic handling

The loading process was carried out under strict aseptic conditions immediately before surgical intervention to preserve the bioactivity of the high molecular weight HA

BONE GRAFT AND HYALURONIC ACID PLACEMENT

Preparation of the HA-impregnated sticky bone composite

The preparation of a cohesive and bioactive graft material was carried out using a protocol (Fig.2).

Standardize mixing

A volume of 0.25 cc of cortical cancellous allograft powder (particle size 1000-2000 µm) was transferred into a sterile dappen dish.

Incorporation of hyaluronic acid

Subsequently, 0.2 ml of Hyaluronic acid was added to the bone particles.

Formation of the cohesive mass

The components were thoroughly mixed using a sterile spatula until the graft reached a sticky and manageable consistency. This combination transforms the loose bone particles into a cohesive bioactive composite (Sticky Bone Analogue), which facilitate stabilization of the graft at the surgical site and prevent particles migration.

Loading into delivery system

The formed sticky bone composite was carefully loaded into a modified 1 ml insulin syringe (needle bearing removed). This custom delivery system facilitated volumetric precision delivery and controlled condensation of the graft into the peri-implant area.

IMPLANTATION PROCEDURE

After all measurements were recorded, sufficient anesthesia was administrated to the surgical site to get complete analgesia to initiate the surgical procedure. The participants were asked to rinse with 0.2 chorhexidine mouthwash for one minute to reduce the bacterial load intraoral before the surgical procedure. After achieving adequate anesthesia of the surgical site, making all the preoperative measurements a complete-thickness mucoperiosteal flap was raised utilizing periosteal reflection instruments. Then drilling was performed to construct the osteotomy site according to the determined sequence of recommended implant system manufacture. Once the osteotomy preparation was finished, the implant was placed in the created socket and then the cover screw was placed, a periosteal releasing incision at the base of the buccal flap was performed. This allowed for tension-free wound closure (Fig.3).

Following the successful placement of the dental implants, the following standardize clinical protocols were implemented:

SUTURE PREPARATION (PRE-LOADING)

Prior to the application of the bioactive materials, a horizontal mattress suture (using 4-0 nylon) was preplaced across the surgical site but left untied. This step was crucial to facilitate immediate and stable primary closure once the materials were in place, preventing material displacement.

GROUP (HA) HYALURONIC ACID APPLICATION

A volume of 0.2 ml of HA was measured using a modified insulin syringe. The gel was delivered from the buccal aspect extending towards the mid-crestal region of the ridge, and similarly from the lingual aspect extending towards the mid-crestal region of ridge, ensuring complete circumferential coverage around the implant platform.

GROUP (HAB) HA-IMPREGNATED STICKY BONE APPLICATION

A volume of 0.2 ml of HA was mixed with 0.25 cc of cortical cancellous allograft powder (particle size 1000-2000 µm) in a sterile container until a sticky bone consistency was achieved. This composite was loaded into a modified insulin syringe and applied

over implant site. The composite was delivered from the buccal aspect extending towards the mid-crestal region of the ridge, and similarly from the lingual aspect extending towards the mid-crestal region of ridge, ensuring complete circumferential coverage around the implant platform.

GROUP (CONT) CONTROL GROUP

In this group no bioactive materials or bone grafts were applied. The surgical site was managed by allowing natural blood clot formation around the implant platform. This group served as baseline to evaluate the natural healing process and soft tissue response without external intervention.

WOUND CLOSURE

Immediately after material placement, the pre-placed horizontal mattress suture was tightened and tied. To ensure complete wound closure two additional interrupted sutures were placed mesially and distally to the implant site. All participant were asked to return for suture removal. Each participant was recalled to remove the suture from the site and to check if any complications in the healing process that might not have been noticed by the patient are present.

RADIOGRAPHIC EVALUATION

Bite-wings were used to estimate the radiographic soft tissue thickness (RSTT) at the region of the missing tooth. All the radiographs were taken with a digital intraoral sensor (RVG 5200; Carestream Dental, Atlanta, GA, USA). To obtain a high reproducibility and consistency of the alignment, paralleling approach was performed with customized sensor holder (YP Dental, Anyang City, China), (Fig1A). To calibrate the dimensions, a metal sphere of known diameter was attached to the next tooth with a heavy-body condensation silicone substance (Fig1B). Image processing and measurements were performed with Image J software (National Institutes of Health, Bethesda, MD, USA) with the calibration of the spatial scale (pixels to millimeters) using the metal sphere. The vertical distance from the alveolar crest of the bone to the gingival edge was then calculated (Fig.2). The mucosal thickness was consistently measured at a reference position 5 mm distal to the height of contour on the mesial face of the neighboring tooth. A digital intraoral bite-wing radiograph was taken immediately after the implant insertion process to determine the marginal bone height. An intraoral digital sensor (RVG 5200; Carestream Dental, Atlanta, GA, USA) was positioned using a special alignment device (YP Dental, Anyang City, China). The cone of the X-ray tube was positioned parallel to the guiding ring of the positioning device for standardization. The vertical bone height (mm) was measured from the second thread (serration) on the mesial aspect of the implant fixture to the crestal bone level using Image J software (National Institute of Health, Bethesda, MD, USA). Follow-up radiographs were taken at scheduled intervals: immediately postoperatively, at 8 weeks and at 16 weeks. In these follow-up appointments, the same standardized radiography methodology with the reference metal sphere in position was used. All linear measurements on the computer were made by the calibrated image analysis program.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Version 26.0. The normality of the data was tested, and parametric tests were used. Frequencies, means, and standard deviations were calculated. Chi-square test was used for nominal data, and the Freeman-Halton Exact test was used instead of Chi-square when any cell had an expected value less than 5 for tables larger than 2 by 2. Numerical data were compared using one-way ANOVA for mean differences among multiple groups (more than two groups) under the study, with a post hoc test to find significant differences. Paired t-test was performed for the numerical data of two paired groups, and the general linear model for repeated measures was used to find differences among paired data of more than two groups. A P-value ≤ 0.05 was considered statistically significant.

ETHICAL APPROVAL

The study received approval from the Scientific Research Committee, Department of Oral Surgery and Periodontology, College of Dentistry, Mustansiriyah University (No. MUOSU/POST-202615) and registered in clinical trial number ClinicalTrials.gov NCT07665281

RESULTS

Numbers examined for the primary outcome and numbers of participants randomly allocated and receiving intended therapy for each group. Recruitment started in December 2025 and was finished in March 2026. Follow-up assessments for the last individual recruited were conducted in July 2026.

Table 1 presents the comparisons of the socio-demographic characteristics across the analyzed groups. There was no statistically significant difference between the groups in terms of age and gender.

Comparisons of the RSST in the studied groups demonstrated statistically significant differences for the baseline period (P = 0.024), post-operative period P<0.001, at 8 weeks P<0.001 and 16 weeks P=0.004. It also found highly statistically significant differences during the observation periods in the CONT group P<0.001, the HY group P<0.001 and the HYB group P<0.001 when comparing each study group with respect to time (Table 3).

The average bone height after surgery was not significantly different between groups (p=0.077). There were statistically significant differences at 8 weeks and 16 weeks, p=0.050 and p=0.016, respectively as time went on. However, the significant differences across these periods were between the HAB and the CONT group and there were no significant differences between the HA and the CONT group or between the HAB and the HA group. Comparisons within groups over time showed a significant statistical change in bone height (P<0.05) for all groups studied. In particular, the CONT group and HA group showed a continuous decrease over time (from Post-Op to 8 weeks, remaining lower at 16 weeks), whereas the HAB group showed a significant increase in bone height at 8 weeks compared to post-operative stage with a small reduction at 16 weeks but still significantly higher than post-operative level, (Table 4).

The mean change in bone height (BH) with individual case distribution across study groups at different time intervals has been shown in figure 4.

DISCUSSION

This study evaluated the impact of hyaluronic acid (HA) and bone grafts on peri-implant tissue phenotype, particularly focusing on bone stability and soft tissue response, important for implant longevity and aesthetics. The results indicated a notable increase in soft tissue thickness and stability in the HA group, especially in combination with the bone graft compared to the control. This study highlights the synergistic regenerative potential of HA and bone grafting for modifying soft tissue biotype and makes a significant contribution to clinical comparisons of these interventions.

Baseline demographic and anatomic characteristics

Statistical analysis showed no significant differences in age distribution, gender, and jaw position among the CONT, HA and HAB groups at baseline, indicating well-matched demographics and baseline anatomy. This enhances the reliability that observed clinical and radiographic outcomes are attributed to Hyaluronic Acid treatments rather than patient characteristics. The uniformity in demographic features and surgical sites allows for a clear correlation between soft tissue biotype changes and the interventions applied, minimizing variability from anatomical differences.

RADIOGRAPHIC SOFT TISSUE THICKNESS

The HAB group showed the highest values of radiographic soft tissue thickness at all evaluation periods (postoperative, 8 and 16 weeks) followed by the HA group and the control group had the lowest values. This larger dimension observed in the HAB group is a synergistic net effect resulting from the biological advantages of hyaluronic acid and the mechanical space maintaining capability of the bone graft. The present findings are in line with previous studies that showed that particulate bone grafts indirectly contribute to preservation of the alveolar ridge contour and the peri-implant soft tissue profile by protecting the underlying hard tissue architecture and avoiding collapse of the horizontal ridge, and not by directly stimulating soft tissue proliferation [15-18]. Furthermore, the positive biological effects of the HA group are consistent with the literature that described the direct effects of hyaluronic acid on early soft tissue repair due to its hydrophobicity, faster angiogenesis, fibroblast proliferation, and extracellular matrix synthesis [19, 20]. In contrast, the radiographic soft tissue thickness was also higher in the HA group than in the control group, but less so than in the HAB group. This finding is more likely related to biological properties of hyaluronic acid, including its hydrophilic nature, promoting angiogenesis, fibroblast proliferation and extracellular matrix synthesis, which enhance early soft tissue healing. These biological effects have been extensively documented in both experimental and clinical studies [19, 20].

RADIOGRAPHIC BONE HEIGHT

Bone height scores analysis between the study groups showed significant differences at immediate post-op, 8-week and 16-week periods. The HAB group showed significantly better preservation of the vertical bone levels than the control group at all evaluation time points, while the HA group exhibited an intermediate behavior without significant differences to either group. These results are consistent with the observations of [21] and [22] who found significantly better bone fill, superior volume retention, and less marginal bone loss around implant sites when a bovine bone substitute was used in combination with hyaluronate. The main biological mechanism for such activity is based on the synergistic action of the mineral matrix and HA that supports early neo-vascularization, HA that promotes early neo-vascularization, osteoblast development and collagen matrix mineralization during the crucial early phases of healing as described by [23, 24]. However, some studies using non-cross-linked HA alone have not shown any significant difference in long term marginal bone loss compared to non-treated conventional sites, a nuance that justifies the inability of our pure HY group to reach statistical superiority over the control in time [25].

STUDY LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The study presents favorable findings but acknowledges several limitations. The small sample size may limit the statistical power and generalizability of results, necessitating larger cohorts for future research. The 16-week follow-up is brief, lacking assessments of long-term tissue maturation and stability, as well as adequate histological support. Although the study examined the standalone hyaluronic acid (HA) and its combination with a mineralized bone graft (HAB) further research should investigate various combinations of hyaluronic acid with different scaffolds and graft ratios. Comparative studies with established soft tissue procedures will help clarify the role of HY composite formulations in clinical scenarios.

CONCLUSIONS

The additional use of hyaluronic acid with a particulate bone graft (HAB) significantly enhances peri-implant tissue healing. This combination leverages the biological effects of hyaluronic acid along with the space-maintaining properties of the graft material, resulting in improved dimensional stability. While hyaluronic acid alone (HA) facilitates soft tissue augmentation through its hydrophilic characteristics that stimulate extracellular matrix formation, angiogenesis, and collagen synthesis, it falls short in maintaining horizontal ridge dimensions during healing without graft support. In contrast, the HAB method demonstrates superior preservation of horizontal bone width over a 16-week healing period and promotes favorable soft tissue characteristics. Findings indicate that integrating hyaluronic acid with a particulate bone graft not only supports peri-implant soft tissue augmentation but also effectively maintains the dimensions of both hard and soft tissues. Overall, the HAB approach proves to be more predictable for simultaneous enhancement of soft tissue quality and maintenance of hard tissue architecture compared to using hyaluronic acid alone.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

The appendix as a full-thickness morphological surrogate for the histological subtype of the colon in patients with chronic slow-transit constipation

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ABSTRACT

Aim: To asses the concordance of histological alterations between appendix and colon in patients with chronic slow-transit constipation (STC) and to determine the feasibility of using the appendix as a full-thickness morphological surrogate for personalizing the extent of colectomy.

Materials and Methods: A single-center retrospective pathomorphological study included 89 patients with CSTC refractory to conservative therapy after colectomy with appendectomy. Full-thickness histological examination of the colon and appendix was performed using semiquantitative assessment of the mucosal, submucosal, muscular, neuronal, and interstitial cells of Cajal (ICC) blocks. Immunohistochemical analysis of ICCs was performed using CD117 (c-kit). Histological profiles were categorized into myopathic, Cajal, neuropathic, and histologically intact subtypes. The concordance of histological phenotypes between the appendix and the colon, the correlation of integrated block scores, and the diagnostic efficacy of the appendix were analyzed.

Results: Within the distribution of colonic phenotypes, the myopathic variant predominated (51.7%), followed by the Cajal subtype (21.3%), the neuropathic subtype (19.1%), and the histologically intact variant (7.9%). The exact match of the histological subtype between the colon and the appendix was achieved in 98.9% of cases, while the concordance of the dominant phenotype reached 100%. Cohen's kappa for exact subtype agreement was 0.985, and the weighted kappa was 1.00. The strongest correlations were identified within the ICC block ($r = 1.00$), the neuronal block ($r = 0.96$), and the muscular block ($r = 0.93$) ($p < 0.001$). For the Cajal subtype, the diagnostic sensitivity, specificity, and overall accuracy of the appendix were 100%.

Conclusions: The appendix demonstrates an exceptionally high concordance with the colonic histological phenotype and can be regarded as a phenotype-predicting, full-thickness morphological surrogate in patients with chronic slow-transit constipation.

KEYWORDS: chronic slow-transit constipation; colectomy; appendix; interstitial cells of Cajal; morphological phenotype.

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INTRODUCTION

Chronic constipation is a common functional gastrointestinal disorder and significantly affects patients' quality of life and represents a significant healthcare and social challenge. [1-3]. Chronic slow-transit constipation (CSTC) is a distinct subtype of chronic constipation characterized by delayed colonic transit and often remains refractory to conservative treatment. [4-8]. In such cases, surgical treatment, particularly colectomy, remains one of the most effective options for long-term symptom relief [9]. Despite a decades of colectomy experience for refractory CSTC, the optimal extent of colectomy remains controversial. Various surgical approaches are currently used in clinical practice, ranging from segmental resections to subtotal or total colectomy with ileorectal anastomosis; however, no unified morphological criteria are currently available to guide personalized selection of colectomy extent. [10]. Reported colectomy outcomes demonstrate considerable variability in clinical efficacy, which is likely due to the heterogeneity of the pathogenetic and morphological mechanisms of CSTC [8,11,12]. Contemporary studies indicate that CSTC is not a morphologically homogeneous condition. Structural alterations involving the smooth muscle layer of the colon, the enteric nervous system, the myenteric plexuses, and the interstitial cells of Cajal — which play a pivotal role in the generation and coordination of intestinal motility — have been described in different patients [5, 7, 13]. A reduction in the number or functional impairment of interstitial cells of Cajal, as well as neuropathic and myopathic alterations, may give rise to distinct morphological phenotypes of the disease, which may potentially have different implications for determining the optimal extent of colonic resection [11, 14, 13]. Therefore, the morphological heterogeneity of CSTC may represent one of the factors

contributing to the variability of surgical treatment outcomes. At the same time, the lack of an accessible preoperative method for full-thickness morphological assessment of the colon is a major unresolved problem. Standard endoscopic biopsy is limited to the mucosal layer and does not permit adequate evaluation of the muscular layer, myenteric plexuses, or other transmural alterations that may be critical for morphological phenotyping of CSTC [1, 11, 14]. Consequently, decisions regarding the extent of colectomy are generally made without detailed preoperative understanding of the distribution and severity of neuromuscular pathology. One potential strategy to overcome this limitation is the identification of surrogate morphological marker to predict the dominant colonic histological phenotype preoperatively. The appendix, as a full-thickness structure anatomically and embryologically related to the colon, may represent a potential candidate for such assessment [15]. Laparoscopic appendectomy is a minimally invasive procedure that provides access to full-thickness tissue specimens for comprehensive histopathological evaluation. If histological alterations in the appendix correlate with morphological changes in the colon, this could provide a novel basis for a staged, morphologically guided personalization of surgical strategy. The histological concordance between the appendix and colon in patients with CSTC has not yet been systematically evaluated. Thus, studying this relationship may not only clarify the morphological architecture of CSTC but also offer a new approach to determ the optimal extent of colectomy preoperatively.

AIM

The aim was to asses the concordance of histological alterations between appendix and colon in patients with chronic slow-transit constipation (STC) and to determine the feasibility of using the appendix as a full-thickness morphological surrogate for personalizing the extent of colectomy.

MATERIALS AND METHODS

STUDY DESIGN AND COHORT CHARACTERISTICS

The concordance between the histological phenotypes of the appendix and colon in patients with chronic slow-transit constipation (CSTC) refractory to conservative treatment was studied in a single-center retrospective pathomorphological study. The study included 89 patients who underwent colectomy for CSTC along with appendix.

INCLUSION CRITERIA

Clinically and instrumentally confirmed chronic slow-transit constipation;
Resistance to conservative therapy;
Availability of full-thickness histological specimens of both the colon and appendix;
Absence of previous appendectomy.

EXCLUSION CRITERIA

Hirschsprung disease;
Proctogenic constipation (rectal or pelvic floor dysfunction);
Medication-associated constipation;
Psychiatric disorders;
Inadequate histological material.
While our previous study evaluated the overall distribution of histological phenotypes and included a control group, the present work focused exclusively on the primary clinical cohort to perform an intra-patient comparison of morphological characteristics between the colon and the appendix. The baseline distribution of colonic histological phenotypes was categorized according to a previously established classification model: myopathic, Cajal-cell-deficient, neuropathic, and histologically intact types.

SURGICAL MATERIAL AND TISSUE SAMPLING

Full-thickness fragments of resected colon and appendix were used for morphological analysis. In each case, samples were obtained from all layers of the intestinal wall, including the mucosa, submucosa, muscularis propria, and myenteric plexuses. For the colon, at least three samples from different segments were analyzed in both transverse and longitudinal orientations. The appendix was examined in a same manner as a potential morphological surrogate of a generalized neuromuscular phenotype. The tissue was fixed in 10% buffered formalin, followed by routine paraffin embedding and processing. Serial sections were stained with hematoxylin and eosin. For the assessment of interstitial cells of Cajal, immunohistochemical analysis was performed using CD117 (C-kit), in accordance with a previously validated methodology.

MORPHOLOGICAL ASSESSMENT

Histological evaluation was performed using light microscopy with morphometric analysis. The architectural features of all layers of the intestinal wall were assessed (Table 1).

Table 1. Structure of the semiquantitative model for morphological assessment of the colon according to structural compartments

Histological blocks	Morphological parameters
Mucosa	• Inflammation • Atrophy • Fibrosis of the lamina propria
Submucosa	• Fibrosis • Vascular changes • Edema • Inflammatory infiltration
Muscular layer (muscularis propria)	• Hypertrophy • Atrophy • Fragmentation • Vacuolization • Inter-fascicular fibrosis
Myenteric (intermuscular) plexus	• Number of ganglia • Neuronal dystrophic changes • Hypo-/aganglionic areas • Fibrosis surrounding plexuses
Interstitial cells of Cajal	• Preserved • Moderately reduced • Markedly reduced • Disorganized

Source: compiled by the authors of this study

Each parameter was assessed using a semiquantitative scoring scale:
0 – no alterations;
1 – mild alterations;
2 – moderate alterations;
3 – severe alterations.
For integrated analysis, individual parameters were grouped into the corresponding morphological blocks (mucosal, submucosal, muscular, neural plexus, and interstitial cells of Cajal blocks), followed by calculation of the mean score for each structural block (Mean Block Score). Since the primary parameter "the preservation of interstitial cells of Cajal" was interpreted in the opposite direction (i.e., a higher score indicated better preservation), it was transformed into the parameter reduced preservation of interstitial cells of Cajal by score inversion according to the formula (3 – actual value). To maintain consistent scale directionality for the interstitial cells of Cajal parameters, inverted scores were used for subsequent Mean Block Score calculations. This methodology provided a standardized interpretation across all morphological blocks, whereby higher scores indicated greater pathological severity. Such standardization enabled the construction of an integrated morphological profile for each patient and allowed accurate identification of the dominant histological phenotype.

HISTOLOGICAL CLASSIFICATION

Patients were categorized into following subtypes according to their dominant histopathological block:
myopathic dystrophic;
myopathic inflammatory;
Cajal cell–related (Cajal);
neuropathic;
histologically intact.

Particular emphasis was placed not on the absolute concordance of all morphological parameters between the colon and the appendix, but rather on the agreement of the dominant morphological phenotype, which was considered a potentially more clinically relevant criterion for preoperative stratification.

STUDY ENDPOINTS

PRIMARY ENDPOINT

Concordance of the histological subtype between the appendix and the colon.

Secondary Endpoints

1. Concordance of the dominant morphological phenotype;
2. Correlation of the integrated block scores (Mean Block Scores) between the appendix and the colon;
3. Diagnostic accuracy of the appendix for identifying the Cajal subtype;
4. The potential of the appendix as a preoperative full-thickness morphological surrogate for predicting the dominant histological phenotype of the colon and personalizing the extent of colectomy.

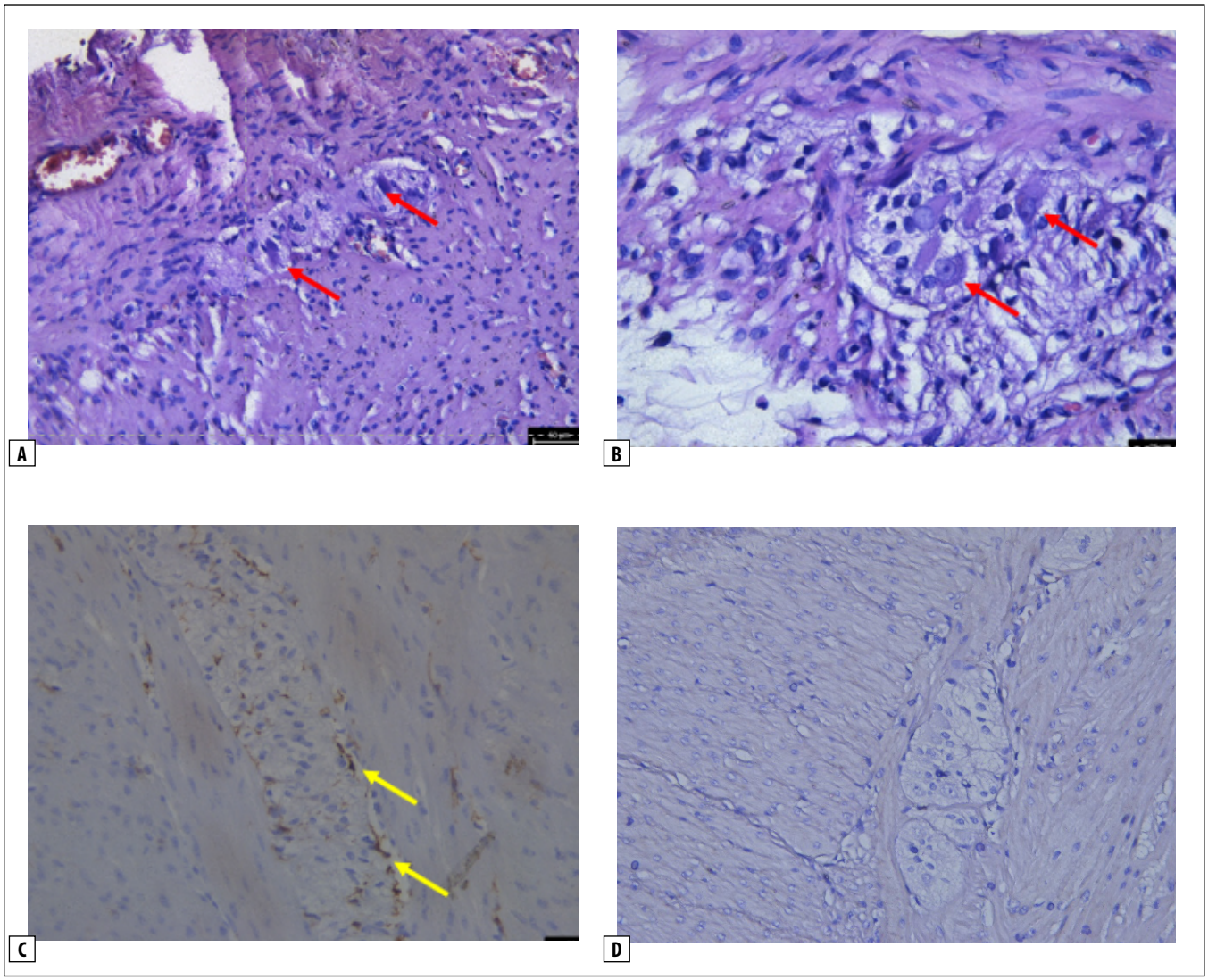


Fig. 1. Neuropathic changes in the colon and appendix (neuropathic subtype of chronic slow-transit constipation).
1A – Colon: degenerative changes of neurons within the myenteric plexus accompanied by hypoganglionosis and disorganization of ganglionic structures (Hematoxylin and eosin staining; magnification×200). 1B – Appendix: similar neurodegenerative alterations of the myenteric plexus with structural distortion of ganglionic elements (Hematoxylin and eosin staining; magnification×200). 1C – Colon: numerous CD117 (c-Kit)-positive interstitial cells of Cajal within the intermuscular space, without evidence of ICC depletion (Immunohistochemistry for CD117 (c-Kit), magnification×200). 1D – Appendix: atrophy and fibrosis of myenteric ganglia (Hematoxylin and eosin staining; magnification ×200)
Picture taken by the authors

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM SPSS Statistics (Version 22.0), including ROC analysis. Descriptive statistics were used to summarize the data, with results presented as mean ± standard deviation (M ± SD) and median with interquartile range (IQR). Distribution normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were assesed using nonparametric methods, including the Mann–Whitney U test and the Kruskal–Wallis test. Categorical variables were analyzed using Pearson's χ^2 test or Fisher's exact test, as appropriate. Agreement between histological subtypes of colon and appendix was evaluated using Cohen's kappa coefficient, whereas agreement regarding the dominant morphological phenotype was assessed using the weighted kappa coefficient. Spearman's rank correlation analysis was used to study correlations between integrated block scores in the appendix and colon. The diagnostic value of the appendix in predicting the histological subtype of the colon was evaluated by ROC analysis, including calculation of the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A p-value < 0.05 was considered statistically significant.

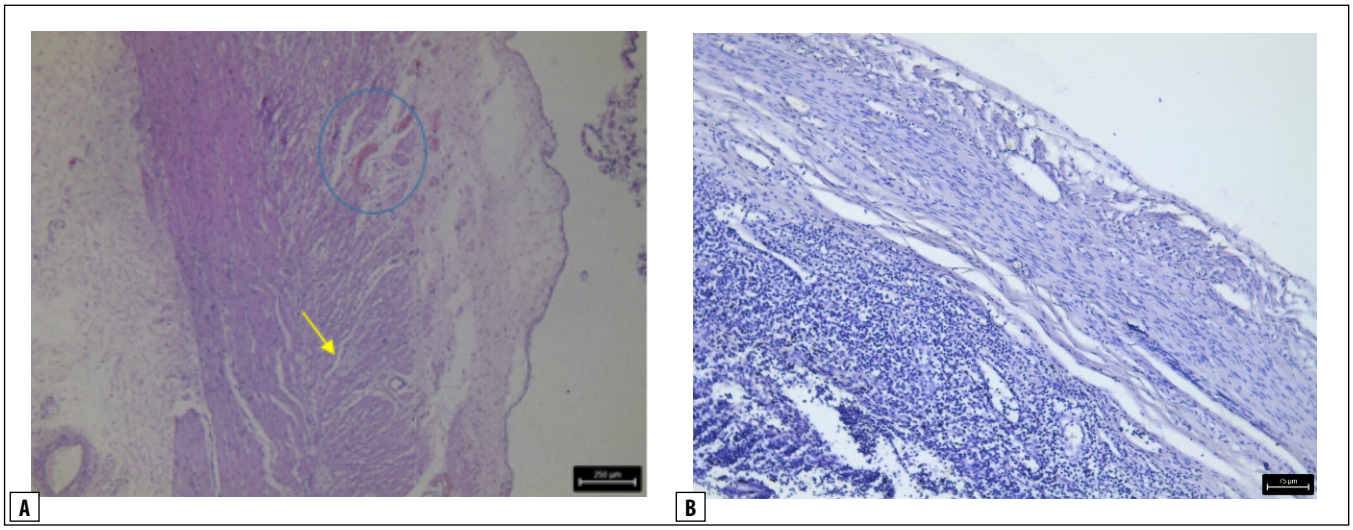


Fig. 2. Myopathic changes in the colon and appendix (myopathic subtype of chronic slow-transit constipation).
2A – Colon: muscularis propria with severe remodeling, disorganization and fragmentation of smooth muscle fibers, intermuscular fibrosis, resulting in to architectural disruption of the circular muscle layer (Hematoxylin and eosin staining; magnification×100). 2B – Appendix: similar degenerative and dystrophic changes of the muscular layer characterized by thinning of smooth muscle structures, focal fibrosis, and heterogeneous organization of muscle fibers (Hematoxylin and eosin staining; magnification ×100). The arrow indicates an area of intermuscular fibrosis and disorganization of smooth muscle fibers
Picture taken by the authors

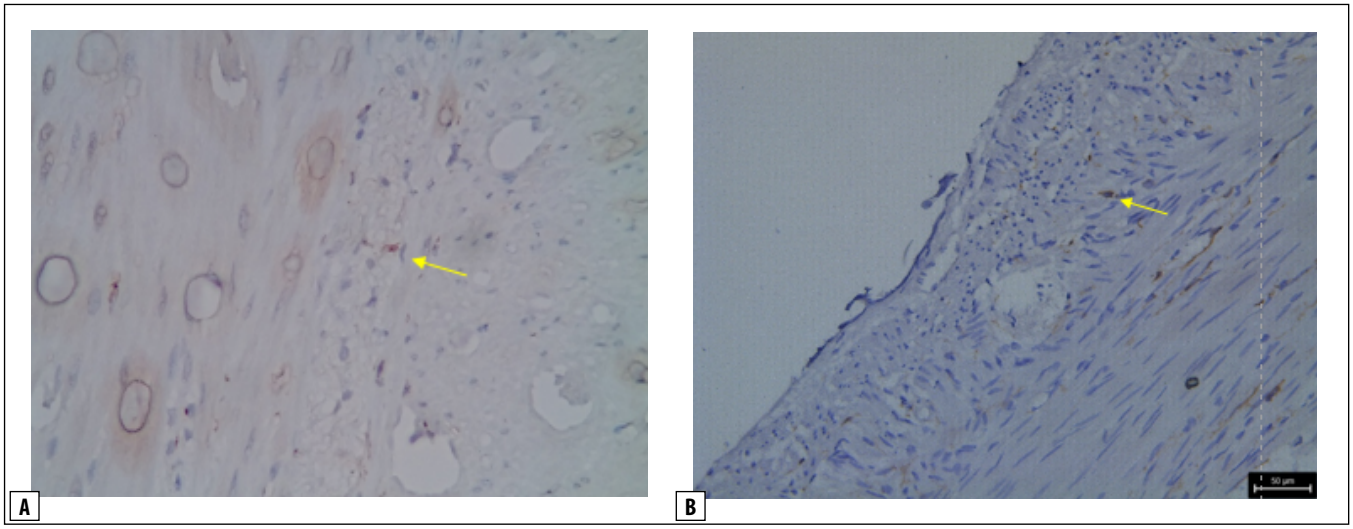


Fig. 3. The Cajal subtype of CSTC. 3A – Colon: isolated spindle-shaped CD117-positive interstitial cells of Cajal in the intermuscular space (Immunohistochemistry for CD117 (c-Kit), magnification×200); 3B – Appendix: similar isolated CD117-positive ICCs in the intermuscular layer (Immunohistochemistry for CD117 (c-Kit), magnification×200). Cajal cells marked by arrows
Picture taken by the authors

METHODOLOGICAL CONCEPT OF THE STUDY

The central hypothesis of this study was that the appendix may serve as an accessible full-thickness morphological surrogate of the dominant histological phenotype of the colon. Such an approach could potentially provide a basis for preoperative stratification of patients with chronic slow-transit constipation and facilitate a personalized approach to determining the optimal extent of colectomy.

ETHICS

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

AI STATEMENT

Artificial intelligence tools, including ChatGPT (OpenAI), were used during manuscript preparation exclusively for language editing, stylistic refinement, and prelim-

Table 2. Distribution of Colon Histological Subtypes in Patients with Chronic Slow-Transit Constipation (n = 89)

Histological Subtype	n	%
Myopathic phenotype, overall	46	51.7
Myopathic dystrophic subtype	36	40.4
Myopathic inflammatory subtype	10	11.2
Interstitial cells of Cajal pathology (Cajal subtype)	19	21.3
Neuropathic subtype	17	19.1
Histologically intact subtype	7	7.9
Total	89	100.0

Note. Classification was based on the dominant morphological domain determined through full-thickness histological examination of the colon
Source: compiled by the authors of this study

Table 3. Semiquantitative Characteristics of the Colonic Mucosa and Submucosal Layer According to Histological Subtype of Chronic Slow-Transit Constipation (Mean ± SD)

Morphological Parameter	Histologically Intact (n = 7)	Myopathic Dystrophic (n = 36)	Myopathic Inflammatory (n = 10)	Cajal Subtype (n = 19)	Neuropathic Subtype (n = 17)
Mucosa					
Inflammation	0.1 ± 0.3	0.3 ± 0.4	2.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5
Atrophy	0.0 ± 0.0	1.1 ± 0.3	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Fibrosis of the lamina propria	0.1 ± 0.3	0.9 ± 0.3	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Submucosal Layer					
Fibrosis	0.1 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.5
Vascular alterations	0.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Edema	0.0 ± 0.0	0.4 ± 0.5	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Inflammatory infiltration	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 0.5	0.0 ± 0.0	1.0 ± 0.0

Source: compiled by the authors of this study

Table 4. Semiquantitative characteristics of the muscular layer, myenteric plexus, and interstitial cells of cajal in the colon according to histological subtype of chronic slow-transit constipation (Mean ± SD)

Morphological Parameter	Histologically Intact (n = 7)	Myopathic Dystrophic (n = 36)	Myopathic Inflammatory (n = 10)	Cajal Subtype (n = 19)	Neuropathic Subtype (n = 17)
Muscular Layer					
Hypertrophy	0.0 ± 0.0	1.1 ± 0.3	2.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
Atrophy	0.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0
Fragmentation	0.0 ± 0.0	2.2 ± 0.4	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Vacuolization	0.0 ± 0.0	2.8 ± 0.4	1.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.4
Fibrosis between muscle bundles	0.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Myenteric Plexus					
Reduced ganglion density	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.4	2.7 ± 0.5
Neuronal dystrophic changes	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0	3.0 ± 0.0
Hypo-/aganglionic areas	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0
Periplexus fibrosis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.4
Interstitial Cells of Cajal					
Reduced preservation of interstitial cells of Cajal[1]	0.0 ± 0.0	0.6 ± 0.5	0.7 ± 0.5	2.7 ± 0.5	0.5 ± 0.5
Moderate reduction	0.0 ± 0.0	0.4 ± 0.5	0.5 ± 0.5	2.0 ± 0.0	0.3 ± 0.5
Severe reduction	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0
Disorganization	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 0.0	0.2 ± 0.4

Source: compiled by the authors of this study

inary translation of selected text fragments. Artificial intelligence was not used for data analysis, generation of scientific conclusions, or interpretation of results. All manuscript sections prepared with the assistance of AI were critically reviewed, revised, and approved by the author. All scientific concepts, results, and conclusions represent the author's own intellectual contribution. The author assumes full responsibility for the content of the manuscript.

FRAMEWORK

This work was carried out in accordance with the research plan of the Surgery chair with a Course of Hepatobiliary and Vascular Surgery, Bogomolets National Medical University: «Surgical treatment of chronic disorders of large intestinal transit». State registration number: 0124U001916. The authors received no additional financial support.

RESULTS

89 patients with chronic slow-transit constipation were included in the study. Full-thickness histological examination of colon and appendix specimens was performed in all cases. None of the patients had a history of appendectomy, allowing direct paired comparison of morphological alterations between the colon and the appendix. Histological analysis of the colon demonstrated that the myopathic phenotype was the most prevalent finding, being identified in 46 (51.7%) patients. Among these, the dystrophic myopathic subtype was observed in 36 (40.4%) patients, whereas the inflammatory myopathic subtype was present in 10 (11.2%) patients. Alterations involving the interstitial cells of Cajal were detected in 19 (21.3%) patients, the neuropathic subtype in 17 (19.1%) patients, and a histologically intact phenotype in 7 (7.9%) patients (Table 2).

The myopathic phenotype was the predominant histological pattern, accounting for more than half of the study cohort among those patients after surgery for chronic slow-transit constipation. The dystrophic myopathic subtype was the most common subtype. The Cajal subtype represented approximately one-fifth of all cases. The histologically intact phenotype was the least frequently observed category.

Morphological Characteristics of the Colon According to Histological Subtype Semiquantitative analysis of morphological parameters demonstrated that the different histological subtypes of chronic slow-transit constipation have distinct structural profiles, supporting the pathomorphological validity of the proposed classification (Table 3 and [1] Note. The original parameter, preservation of interstitial cells of Cajal, was transformed into reduced preservation of interstitial cells of Cajal using the formula (3 – actual value) to standardize score direction, where higher scores correspond to more severe pathological alterations.

MORPHOLOGICAL CHARACTERISTICS OF HISTOLOGICAL SUBTYPES

The myopathic inflammatory subtype was characterized by the most severe superficial inflammatory and infiltrative changes. This group demonstrated the highest scores for mucosal inflammation (2.0 ± 0.0), submucosal edema (2.0 ± 0.0), and inflammatory infiltration (2.3 ± 0.5), distinguishing it from all other subtypes. Concurrently, moderate myopathic alterations were observed, including muscular hypertrophy (2.0 ± 0.0), intermuscular fibrosis (2.0 ± 0.0), and less pronounced muscular atrophy (1.0 ± 0.0), indicating the coexistence of inflammatory and tissue-remodeling components. In contrast, the myopathic dystrophic subtype was characterized by predominant degenerative and structural remodeling of the muscular layer with only minimal inflammatory involvement. The highest scores for muscular atrophy (3.0 ± 0.0), vacuolization (2.8 ± 0.4), fragmentation (2.2 ± 0.4), and intermuscular fibrosis (2.0 ± 0.0) were typical for this subtype. Superficial alterations were considerably less severe and were largely limited to mild mucosal atrophy and fibrosis, forming a characteristic dystrophic myopathic phenotype.

The Cajal subtype demonstrated a fundamentally different morphological pattern. Unlike the myopathic forms, the mucosa, submucosa, muscular layer, and neural plexuses generally lacked significant structural abnormalities. However, this group was characterized by the most severe deficiency of interstitial cells of Cajal (ICCs). Severe reduction in ICC density reached 3.0 ± 0.0, disorganization of the ICC network reached 3.0 ± 0.0, and the score for reduced preservation of ICCs was the highest among all subtypes (2.7 ± 0.5). Thus, the Cajal subtype represented a selective ICC-deficient phenotype with minimal involvement of other morphological compartments. Typical microscopic features of the main histological subtypes in colon and appendix are presented in Fig. 1, Fig. 2, Fig. 3.

The neuropathic subtype was characterized by the most severe alterations within the myenteric plexus. This group demonstrated the highest scores for neuronal dystrophy (3.0 ± 0.0), hypo-/aganglionic areas (3.0 ± 0.0), reduced ganglion density (2.7 ± 0.5), and periplexus fibrosis (2.2 ± 0.4). In contrast, superficial changes and structural remodeling of the muscular layer were substantially less pronounced, supporting predomi-

Table 5. Integrated block-based characteristics of colonic histological subtypes in patients with chronic slow-transit constipation (Mean ± SD)

Integrated Morphological Block	Histologically Intact (n = 7)	Myopathic Dystrophic (n = 36)	Myopathic Inflammatory (n = 10)	Cajal Subtype (n = 19)	Neuropathic Subtype (n = 17)
Mucosal score	0.07 ± 0.18	0.77 ± 0.33	1.13 ± 0.17	0.00 ± 0.00	0.13 ± 0.17
Submucosal score	0.04 ± 0.10	2.40 ± 0.29	6.30 ± 0.48	0.00 ± 0.00	1.40 ± 0.32
Muscular score	0.00 ± 0.00	10.11 ± 0.32	7.00 ± 0.00	0.53 ± 0.22	1.24 ± 0.44
Neuronal score	0.00 ± 0.00	0.05 ± 0.14	0.10 ± 0.18	0.05 ± 0.11	13.65 ± 1.17
Cajal cell pathology score	0.00 ± 0.00	1.00 ± 0.41	1.20 ± 0.44	10.70 ± 0.87	1.00 ± 0.36
Total histological score	0.11 ± 0.21	14.33 ± 0.74	15.73 ± 0.82	11.28 ± 0.96	17.42 ± 1.41

Source: compiled by the authors of this study

Table 6. Overall concordance of histological phenotype between the colon and the appendix (ТК – colon; АП – appendix)

Measure of concordance	Value
Exact histological subtype match	98.9% (88/89 patients)
Concordance of dominant morphological phenotype	100% (89/89 patients)
Cohen's kappa coefficient (exact subtype match)	0.985
Weighted kappa coefficient (dominant phenotype)	1.00

Source: compiled by the authors of this study

Table 7. Correlation of Integrated Morphological Blocks Between the Appendix and the Colon

Integrated Morphological Block	Spearman's correlation coefficient (r)	p-value
Mucosal score	0.81	<0.001
Submucosal score	0.84	<0.001
Muscular score	0.93	<0.001
Neural plexus score	0.96	<0.001
Cajal cell pathology score	1.00	<0.001

Source: compiled by the authors of this study

Table 8. Diagnostic efficacy of Appendix Histology in Predicting Colonic Histological Subtype.

Diagnostic parameter	Cajal subtype	Neuropathic subtype	Myopathic subtype (overall)
Sensitivity (%)	100.0	94.1	95.7
Specificity (%)	100.0	98.2	96.5
Positive predictive value (PPV, %)	100.0	94.1	97.8
Negative predictive value (NPV, %)	100.0	98.2	93.2
Overall accuracy (%)	100.0	97.1	96.1

Source: compiled by the authors of this study

Table 9. ROC Characteristics of Integrated Morphological Blocks of the Appendix for Predicting the Dominant Histological Colon Subtype

Target colonic histological subtype	Primary appendiceal predictor	AUC (95% CI)	Cut-off	Sensitivity, %	Specificity, %
Cajal subtype	Cajal cell pathology score	1.00 (0.96–1.00)	≥8.5	100.0	100.0
Neuropathic subtype	Neural plexus score	0.98 (0.94–1.00)	≥9.5	94.1	98.2
Myopathic subtype (overall)	Muscular score	0.96 (0.91–0.99)	≥6.5	95.7	96.5

Source: compiled by the authors of this study

nant involvement of the enteric nervous system as the principal pathogenetic mechanism. The histologically intact subtype was characterized by minimal or absent alterations across all structural compartments, including the mucosa, muscular layer, neural plexuses, and interstitial cells of Cajal and therefore represented the

morphologically least affected phenotype within the study cohort. Overall, the findings indicate that each histological subtype has a distinct dominant structural pattern: a muscular pattern in the myopathic subtypes, neural plexus pathology in the neuropathic subtype, and selective depletion of interstitial cells of Cajal in the Cajal subtype. Typical microscopic features of the main histological subtypes in colon and appendix are presented in Fig. 1, Fig. 2, Fig. 3.

These findings support the validity of not only a categorical patient classification but also the subsequent application of a quantitative integrated block-based model for predicting the dominant morphological phenotype.

INTEGRATED HISTOLOGICAL CHARACTERIZATION OF COLONIC HISTOLOGICAL SUBTYPES ACCORDING TO STRUCTURAL HISTOLOGICAL BLOCKS

To summarize semiquantitative morphological characteristics, individual parameters were integrated into five principal structural blocks: mucosal, submucosal, muscular, neural, and interstitial cells of Cajal (Cajal) blocks. Analysis of the cumulative integrated block scores confirmed that the proposed histological subtypes are characterized not only by distinct individual morphological features but also by fundamentally different pathomorphological architectures (Table 5).

The myopathic dystrophic subtype was characterized by the predominance of the muscular block, which demonstrated one of the highest integrated values among all subtypes (10.11 ± 0.32). This finding confirms that structural degenerative involvement of the muscular layer represents its principal morphological feature. At the same time, the mucosal (0.77 ± 0.33) and neuronal (0.05 ± 0.14) blocks remained minimally altered, further emphasizing the selective nature of muscular remodeling.

The myopathic inflammatory subtype also demonstrated a high muscular component (7.00 ± 0.00); however, in contrast to the dystrophic variant, it was accompanied by markedly higher mucosal and submucosal block scores (1.13 ± 0.17 and 6.30 ± 0.48, respectively). The combination of muscular remodeling with prominent inflammatory and infiltrative changes in the superficial layers defined a distinct pathogenetic profile of this subtype.

The Cajal subtype demonstrated a fundamentally other integrated architectural pattern: in the presence of minimal alterations in the mucosal, submucosal, muscular, and neuronal blocks, it was characterized by a dominant Cajal cell pathology score (10.70 ± 0.87). So, despite a relatively lower overall morphological burden compared to the neuropathic variant, this subtype has a highly specific pathogenetic mechanism associated with selective impairment of the interstitial cells of Cajal (ICC).

The neuropathic subtype had the highest total histological score (17.42 ± 1.41) among all variants, demonstrating the most severe structural remodeling. The neuronal block was predominant (13.65 ± 1.17), whereas the remaining structural components were relatively less affected. Thus, a neurodegenerative component represents the principal morphological determinant of this subtype.

The histologically intact subtype was characterized by the lowest values across all blocks (total histological score: 0.11 ± 0.21), confirming the absence of significant structural remodeling despite the presence of the clinical syndrome of slow-transit constipation.

Data from the integrated block scores indicated that the total morphological burden does not always mirror the clinical severity of specific subtypes. The neuropathic variant had the highest total structural score, the Cajal subtype after standardized evaluation of ICC deficiency showed a markedly high block-specific pathological burden, underscoring its morphological selectivity. These findings prompted further evaluation of not only the total morphological burden but also the dominant histological phenotype, highlighting it as a potentially more clinically relevant criterion. Overall, the integrated block-based assessment confirmed clearly distinct morphological architectures among histological subtypes. The myopathic dystrophic variant was characterized by the highest muscular score (10.11 ± 0.32), the myopathic inflammatory subtype by a combination of elevated submucosal and muscular scores (6.30 ± 0.48 and 7.00 ± 0.00, respectively), the Cajal subtype by selective predominance of the Cajal cell pathology score (10.70 ± 0.87), and the neuropathic subtype by the highest neuronal score (13.65 ± 1.17) and the greatest total histological score (17.42 ± 1.41).

Concordance of Histological Characteristics Between the Appendix and the Colon

Comparative analysis of histological characteristics of the appendix and the colon demonstrated an exceptionally high level of concordance between dominant morphological phenotypes (Table 6, Table 7).

Notes. *Exact histological subtype match* was defined as complete concordance of the classification subtype between the colon and the appendix. *Dominant morphological phenotype* referred to concordance of the principal pathogenetic block regardless of regional variations in the severity of morphological changes. *PPV (positive predictive value)* represents the probability of

confirming the corresponding colonic subtype when the appendiceal histological result is positive. *NPV (negative predictive value)* represents the probability of excluding the corresponding colonic subtype when the appendiceal histological result is negative. Overall, exact match of the histological subtype between the appendix and the colon was observed in 98.9% of cases (88 of 89 patients), whereas concordance of the dominant pathogenetic phenotype reached 100% (89 of 89 patients). Cohen's kappa coefficient for exact subtype match was 0.985, indicating almost perfect categorical concordance, while the weighted kappa coefficient for dominant phenotype was 1.00. Correlation analysis of the integrated morphological blocks between the appendix and the colon further confirmed the high reproducibility of the histological profile. The highest level of concordance was observed for the Cajal cell pathology score ($r = 1.00$; $p < 0.001$), which was important for the identification of the Cajal subtype. Very strong correlations were also found for the neural plexus block ($r = 0.96$; $p < 0.001$) and the muscular block ($r = 0.93$; $p < 0.001$), whereas the mucosal ($r = 0.81$; $p < 0.001$) and submucosal ($r = 0.84$; $p < 0.001$) blocks showed greater variability. These findings suggest that superficial and inflammatory-reactive changes may vary regionally, while the underlying structural pattern remains preserved.

The Cajal subtype is the most clinically relevant phenotype. In all patients with interstitial Cajal cell deficiency in the colon, the same phenotype was confirmed in the appendix, resulting in 100% sensitivity, 100% specificity, and 100% overall diagnostic accuracy for this morphological surrogate. Thus, within the studied cohort, the appendix demonstrated complete concordance in the identification of the Cajal phenotype. A very high level of diagnostic efficacy was also observed for the neuropathic subtype, with a sensitivity of 94.1%, specificity of 98.2%, and overall accuracy of 97.1%. Diagnostic accuracy remained high for the myopathic subtypes (96.1%); however, this group showed the greatest regional variation in the intensity of some morphological parameters. Specifically, the inflammatory-infiltrative component was less pronounced in the appendix in cases of the myopathic inflammatory subtype, whereas degenerative changes tended to be somewhat more pronounced in the appendix in the myopathic dystrophic subtype. Despite these differences, the dominant muscular phenotype remained stable. These findings indicate that the appendix does not always necessarily reproduce the absolute severity of all morphological alterations present in the colon; however, it almost perfectly reflects the dominant histological phenotype. This is critical because preoperative stratification and the choice of colectomy type depend on identifying the primary pathogenetic mechanism, rather than achieving an absolute concordance of all histological features. Therefore, full-thickness histological examination of the appendix may be considered a highly accurate phenotype-preserving surrogate marker of colonic pathology. These findings provide a basis for a stepwise histology-guided surgical strategy, in which laparoscopic appendectomy followed by morphological assessment may serve as a diagnostic tool for personalizing the extent of colectomy in patients with chronic slow-transit constipation.

ROC analysis of appendiceal histology predictive value for the dominant colonic histological subtype

To quantify the predictive value of the appendix as a preoperative morphological surrogate, a ROC analysis of the main integrated morphological blocks was performed to predict the dominant colonic histological phenotype. (Table 9).

ROC analysis revealed that the appendiceal ICC score perfectly predicted the colonic Cajal subtype (AUC = 1.00). A cutoff of ≥8.5 yielded 100.0% sensitivity and specificity, demonstrating total diagnostic reproducibility of this phenotype within the cohort.

For the neuropathic subtype, the most informative predictor was the appendiceal neural plexus score (AUC = 0.98), demonstrating exceptionally high predictive accuracy. An optimal cut-off value of ≥9.5 yielded a sensitivity of 94.1% and a specificity of 98.2%.

The myopathic subtypes exhibited somewhat greater morphological variability; nevertheless, the appendiceal muscular score remained a highly accurate predictor (AUC = 0.96). Appendix reliably represents the dominant myopathic phenotype of the colon despite regional differences in the severity of individual morphological alterations, as evidenced by this data.

The ROC analysis demonstrated different predictive value of the appendix for all morphological subtypes. The highest diagnostic accuracy was observed for the Cajal subtype: selective depletion of interstitial Cajal cells has complete morphological concordance between the appendix and the colon. The last is clinically pivotal, as the Cajal phenotype is associated with severe functional impairment, potentially representing the subgroup most likely to benefit from extensive surgery. Consequently, ROC analysis validated appendiceal histology as a diagnostic tool, transforming descriptive morphology into a clinically relevant, phenotype-guided preoperative stratification framework.

DISCUSSION

Chronic slow-transit constipation is a heterogeneous syndrome rather than a single nosological pathology,

with impaired colonic motility arising from distinct myopathic, neuropathic, or interstitial cell of Cajal (ICC)-deficient mechanisms. [11,13,16–19]. Contemporary concepts of gastrointestinal neuromuscular pathology, including the London Classification of Gastrointestinal Neuromuscular Pathology, emphasize the critical importance of structured full-thickness morphological assessment of the bowel wall for understanding the pathogenesis of severe motility disorders [7]. However, routine preoperative full-thickness colonic stratification remains unavailable, leaving a gap between the pathomorphological substrate and surgical decision-making. Importantly, the morphological assessment employed in the present study extended beyond a purely descriptive approach. Integrating individual parameters into a standardized, semiquantitative block model (mucosal, submucosal, muscular, neural, and ICC domains) shifted the focus from fragmented descriptive analysis to systematic phenotypic characterization. This foundational methodological framework enabled both the identification of pathological changes and the precise quantification of each patient's dominant pathogenetic component. The proposed semiquantitative model transformed histological assessment from a predominantly descriptive tool into a stratification-oriented methodology. The present findings confirm that patients with chronic slow-transit constipation do not represent a morphologically homogeneous population. The myopathic dystrophic subtype was characterized by predominance of the muscular block (10.11 ± 0.32), the myopathic inflammatory subtype by a combination of elevated muscular (7.00 ± 0.00) and submucosal (6.30 ± 0.48) components, the Cajal subtype by selective predominance of Cajal cell pathology (10.70 ± 0.87), whereas the neuropathic subtype demonstrated the highest neuronal score (13.65 ± 1.17) and the greatest overall histological score (17.42 ± 1.41). As a consequence, despite similar clinical manifestations, the structural basis of disease may differ fundamentally among patients. Importantly, these findings have direct clinical utility, as post-colectomy outcomes for slow-transit constipation vary widely, and standard criteria for optimal extent of resection remain unestablished. [8,20–22]. Particular attention should be paid to the Cajal subtype, which in our study was characterized by the most severe selective deficiency of interstitial Cajal cells (Cajal cell pathology score: 10.70 ± 0.87), with minimal alterations in the other morphological blocks. These findings align with the observations of He et al. [17] and Wedel et al. [18]. [16], who demonstrated the role of ICC deficiency in the pathogenesis of severe slow-transit constipation. However, these concepts are further extended by the current data, which suggest that ICC depletion constitutes a distinct dominant histological phenotype of independent clinical value. A central conceptual finding of the present study was the exceptionally high concordance of histological phenotypes between the appendix and the colon. Exact match of histological subtype reached 98.9% (88 of 89 patients), whereas concordance of the dominant morphological phenotype was 100% (89 of 89 patients), with Cohen's kappa of 0.985 and weighted kappa of 1.00, respectively. Such a level of concordance fundamentally expands current perspectives regarding the potential role of the appendix in the diagnostic algorithm of chronic slow-transit constipation. Analysis of the integrated morphological blocks further comfirmed this observation. Correlations between the appendix and the colon reached $r = 0.93$ for the muscular block, $r = 0.96$ for the neuronal block, and $r = 1.00$ for the Cajal cell pathology block ($p < 0.001$ for all comparisons). The latter finding is particularly important, as every patient with a Cajal colon subtype demonstrated the same phenotype in the appendix. Thus, within the present cohort, the appendix showed complete diagnostic concordance for the identification of the ICC-deficient phenotype. This concordance is potentially explained not only by the functional features but also by the common embryological lineage of the appendix and colon. The appendix arises from the cecal segment of the midgut and develops within the same morphogenetic part as the proximal colon, retaining a similar overall wall architecture, enteric nervous system organization, and mesenchymal–epithelial interactions [15,23]. Although this does not imply absolute identity of all regional histological alterations, it provides a biological explanation for the dominant phenotype. The appendix may therefore function not as an anatomical replica of the colon but rather as a phenotype-predicting full-thickness morphological surrogate. Importantly, the appendix do not always mirror the exact severity of all histological alterations in the colon. The mucosal ($r = 0.81$) and submucosal ($r = 0.84$) blocks showed the lowest correlation, likely due to regional differences in superficial inflammatory-reactive changes. M ethodologically, the clinical value of appendiceal histology depends not on mirroring isolated morphological details, but on capturing the dominant pathogenetic phenotype. The present findings also allow to separate overall morphological burden and potential functional severity. Although the neuropathic subtype had the highest total histological score (17.42 ± 1.41), this does not necessarily means the greatest treatment resistance. Conversely, despite a lower overall histological score (11.28 ± 0.96), the Cajal subtype may be functionally more critical because of selective impairment of gastrointestinal motor coordination key mechanism.

Consequently, the optimal extent of colectomy appears to be depend on the dominant phenotype rather than the overall degree of structural damage. Practically, these findings provide a framework for histology-guided surgical stratification. Theoretically, laparoscopic appendectomy followed by full-thickness histological evaluation could serve as an additional diagnostic step in complex cases of chronic slow-transit constipation where the choice between segmental, subtotal, and total colectomy remains uncertain. This approach does not replace standart clinical and functional diagnostics but improves it with morphological data.

This study has several limitations. The investigation was carried out in a single-center cohort of 89 patients without external validation. The proposed semiquantitative scoring system requires further evaluation with respect to interobserver reproducibility. In addition, the concept of a separate preoperative appendectomy as a diagnostic procedure aquires prospective assessment of its risk–benefit profile and its impact on clinical outcomes.

In summary, the present study not only confirms the morphological heterogeneity of chronic slow-transit constipation but also demonstrates that a standardized semiquantitative block-based model enables a transition from descriptive histology to phenotype-oriented morphological stratification. Appendix serves as a full-thickness morphological surrogate for the dominant histological subtype of the colon. This concept offers high clinical value by defining the Cajal subtype, which directly guides personalized colectomy extent. Once validated prospectively, these data will support a novel phenotype-guided paradigm in the surgical treatment of chronic slow-transit constipation.

CONCLUSIONS

Chronic slow-transit constipation is characterized by substantial morphological heterogeneity, with the formation of myopathic, neuropathic, and ICC-deficient phenotypes. The appendix demonstrates exceptionally high concordance with the dominant histological phenotype of the colon and may therefore be considered a phenotype-predicting full-thickness morphological surrogate. These findings establish a framework for the further phenotype-guided approach to personalize the extent of colectomy in patients with chronic slow-transit constipation.

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CONFLICT OF INTEREST

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REVIEW ARTICLE

Increased proteasome and inflammasome activity in response to cellular stress and PTSD-induced damage – a narrative review

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ABSTRACT

Aim: This review summarizes current evidence regarding increased proteasome and inflammasome activity in response to cellular stress and PTSD-induced neuronal injury.

Materials and Methods: A narrative literature review was conducted using PubMed, Scopus, Web of Science, and Google Scholar databases. Studies published between 2015 and 2025 were analyzed using keywords including "PTSD," "proteasome," "inflammasome," "NLRP3," "oxidative stress," and "neuroinflammation." Experimental and clinical studies investigating molecular mechanisms associated with PTSD-related cellular stress were included.

The reviewed studies demonstrated that chronic traumatic stress promotes excessive activation of the NLRP3 inflammasome and dysregulation of proteasome activity. These changes contribute to accumulation of damaged proteins, mitochondrial impairment, increased reactive oxygen species production, and elevated secretion of pro-inflammatory cytokines, including interleukin-1β and interleukin-18. Persistent activation of these pathways is associated with microglial activation, synaptic dysfunction, impaired neuroplasticity, and progressive neuronal degeneration in PTSD.

Conclusions: Proteasome and inflammasome dysregulation play an important role in the neurobiology of PTSD and may contribute to chronic neuroinflammatory and neurodegenerative processes. Targeting these pathways may represent a promising therapeutic strategy for reducing neuronal damage associated with traumatic stress.

KEYWORDS: inflammasome, neuroinflammation, oxidative stress, proteasome, PTSD, synaptic plasticit

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a chronic and heterogeneous psychiatric disorder that may develop following exposure to traumatic events involving actual or threatened death, serious injury, or sexual violence. It is characterised by intrusive memories, avoidance behaviours, persistent negative alterations in cognition and mood, and hyperarousal, resulting in substantial impairment of social, occupational, and psychological functioning. Epidemiological studies estimate that the lifetime prevalence of PTSD in the general population ranges from approximately 3% to 8%, although considerably higher rates have been reported among military personnel, first responders, survivors of interpersonal violence, and individuals exposed to armed conflict or natural disasters. Owing to its chronic course and frequent psychiatric and somatic comorbidities, PTSD represents a major global public health challenge [1, 2].

The pathogenesis of PTSD is multifactorial and reflects a complex interaction between genetic susceptibility, environmental exposures, neuroendocrine dysregulation, and immune mechanisms. Increasing evidence indicates that PTSD extends beyond a purely psychiatric disorder and is accompanied by persistent biological alterations involving the central nervous system. These include chronic neuroinflammation, oxidative stress, mitochondrial dysfunction, impaired proteostasis, and altered neuroplasticity. Collectively, these abnormalities contribute not only to the persistence of core PTSD symptoms but also to the increased risk of cardiovascular, metabolic, autoimmune, and neurodegenerative disorders observed in affected individuals [3, 4].

Among the biological mechanisms implicated in PTSD, increasing attention has been directed towards disturbances in intracellular protein homeostasis and innate immune signalling. Proteasomes and inflammasomes are recognised as key regulators of cellular adaptation to stress, coordinating protein quality control, inflammatory responses, and neuronal survival. Dysregulation of these systems has been associated with persistent neuroinflammation, impaired synaptic function, and reduced neuronal resilience following chronic or repeated traumatic stress [5, 6].

Growing evidence further suggests that proteasome dysfunction and inflammasome activation represent interconnected components of the cellular stress response rather than independent pathological processes. Their reciprocal interaction may amplify inflammatory signalling, oxidative stress, and neuronal injury, thereby contributing to the long-term neurobiological consequences of PTSD. Understanding these mechanisms may facilitate the identification of novel therapeutic targets aimed at restoring cellular homeostasis and limiting neuroinflammatory damage in trauma-related disorders [7-9].

The aim of this narrative review is to summarise current evidence regarding the role of proteasomes and inflammasomes in the pathophysiology of PTSD, with particular emphasis on their contribution to cellular stress responses, neuroinflammation, and neuronal dysfunction, as well as their potential relevance as therapeutic targets.

The main neurobiological mechanisms linking traumatic stress with neuronal injury in PTSD are summarised in Table 1.

AIM

The aim of this review is to summarise current evidence on the role of proteasome and inflammasome dysregulation in the neurobiology of PTSD. Particular attention is given to mechanisms linking traumatic stress with cellular injury, oxidative stress, mitochondrial dysfunction, neuroinflammation, and impaired neuronal plasticity.

The review focuses on the ubiquitin–proteasome system and the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome NLRP3 inflammasome as key molecular pathways involved in stress-induced neuronal damage. It discusses how impaired protein degradation and persistent inflammasome activation may contribute to microglial reactivity, synaptic dysfunction, altered neuronal signalling, and increased vulnerability of stress-sensitive brain regions, including the hippocampus, amygdala, and prefrontal cortex.

This review also highlights the therapeutic relevance of targeting proteasome activity and inflammasome signalling in PTSD. Better understanding of these interconnected pathways may support the development of strategies aimed at reducing neuroinflammation, restoring proteostasis, and protecting neuronal integrity after traumatic stress.

MATERIALS AND METHODS

A narrative literature review was conducted to assess current evidence on proteasome activity, inflammasome signalling, and cellular stress mechanisms in PTSD. Relevant publications from January 2015 to February 2025 were identified in PubMed, Scopus, Web of Science, and Google Scholar.

The search strategy used combinations of the following terms: "PTSD", "post-traumatic stress disorder", "proteasome", "ubiquitin–proteasome system", "inflammasome", "NLRP3", "oxidative stress", "neuroinflammation", "microglia", "mitochondrial dysfunction", "neuronal plasticity", and "neurodegeneration". Reference lists of selected articles were also screened manually.

Original experimental, translational, and clinical studies were included when they addressed molecular mechanisms linking traumatic stress with neuronal injury, proteostatic disruption, inflammasome activation, oxidative damage, cytokine signalling, or synaptic dysfunction. Reviews were used to provide additional background and to identify relevant primary studies.

Articles not written in English, conference abstracts, editorials, and publications without clear relevance to neurobiological mechanisms of PTSD or chronic stress were excluded. The selected literature was analysed qualitatively, with particular emphasis on interactions between proteasome dysfunction and inflammasome signalling in stress-related neuronal damage.

REVIEW AND DISCUSSION

PROTEASOME DYSFUNCTION IN PTSD-RELATED NEUROBIOLOGICAL INJURY

The ubiquitin–proteasome system (UPS) constitutes one of the principal intracellular mechanisms responsible for maintaining neuronal proteostasis and regulating degradation of damaged, oxidised, and misfolded proteins. In the central nervous system, proteasomal activity is essential for synaptic remodelling, receptor turnover, axonal transport, mitochondrial quality control, and long-term neuronal survival. Due to their high metabolic demand and limited regenerative capacity, neurons remain particularly susceptible to disturbances in intracellular protein degradation pathways. Increasing evidence suggests that chronic traumatic stress profoundly alters UPS function and contributes to progressive neuronal dysfunction in PTSD [10-12].

Experimental studies have demonstrated that chronic stress exposure induces oxidative modification of proteasome subunits and reduces proteolytic activity within stress-sensitive brain regions. Li et al. observed reduced proteasome activity in the hippocampus of rats exposed to chronic unpredictable stress, accompanied by increased accumulation of ubiquitinated proteins and impaired synaptic plasticity [13]. These

Table 1. Main neurobiological mechanisms involved in PTSD-related neuronal injury

Mechanism	Main molecular components	Neurobiological consequences	Clinical relevance in PTSD
Oxidative stress	Reactive oxygen species (ROS), nitric oxide (NO), mitochondrial dysfunction	Lipid peroxidation, DNA damage, impaired neuronal metabolism	Cognitive impairment, emotional dysregulation
Neuroinflammation	NLRP3 inflammasome, IL-1β, IL-18, TNF-α, IL-6	Microglial activation, cytokine release, synaptic dysfunction	Persistent hyperarousal, impaired fear extinction
Proteasome dysfunction	Ubiquitin–proteasome system (UPS), damaged proteins	Accumulation of oxidised and misfolded proteins, impaired proteostasis	Neuronal vulnerability, impaired synaptic plasticity
Mitochondrial dysfunction	Mitochondrial DNA release, ATP depletion, calcium dysregulation	Apoptotic signalling, increased ROS production	Reduced neuronal resilience and energy metabolism
Impaired neuroplasticity	Reduced BDNF expression, altered glutamate receptor trafficking	Dendritic spine loss, disrupted long-term potentiation	Memory disturbances, emotional instability
Blood–brain barrier disruption	Inflammatory cytokines, endothelial dysfunction	Increased permeability and peripheral immune cell infiltration	Sustained neuroimmune activation

Source: Compiled by the author of this study

Table 2. Interactions between proteasome dysfunction and NLRP3 inflammasome activation in PTSD

Pathophysiological process	Proteasome dysfunction	NLRP3 inflammasome activation	Resulting eaffect
Oxidative stress	Reduced degradation of oxidised proteins	ROS-mediated inflammasome activation	Amplification of neuronal injury
Mitochondrial damage	Impaired mitochondrial quality control	Release of mitochondrial DNA and ROS	Sustained neuroinflammation
Cytokine signalling	Proteasome impairment induced by inflammatory mediators	Increased IL-1β and IL-18 maturation	Chronic inflammatory state
Synaptic dysfunction	Altered receptor turnover and protein trafficking	Microglial-mediated synaptic pruning	Impaired fear extinction and cognition
Neuronal survival	Accumulation of toxic protein aggregates	Caspase-1 activation and pyroptotic signalling	Increased neuronal apoptosis
Neuroplasticity	Reduced BDNF-related adaptive mechanisms	Persistent inflammatory signalling	Impaired stress adaptation

Source: Compiled by the authors of this study

changes were associated with elevated concentrations of reactive oxygen species and reduced expression of brain-derived neurotrophic factor (BDNF), suggesting that oxidative stress-induced impairment of proteostasis contributes directly to neuronal vulnerability and cognitive dysfunction. Similar findings were reported by Madrigal and colleagues, who demonstrated that prolonged glucocorticoid exposure impaired proteasomal degradation and promoted accumulation of oxidatively damaged proteins within hippocampal neurons [13-15].

Recent evidence indicates that proteasome dysfunction may also influence mitochondrial integrity. Under physiological conditions, damaged mitochondrial proteins are eliminated through coordinated interactions between the UPS and mitophagic pathways [16]. However, chronic stress disrupts mitochondrial turnover and promotes persistence of dysfunctional organelles. Kaufmann FN et al. demonstrated that chronic restraint stress increased mitochondrial fragmentation and reduced expression of proteasome-related proteins within the prefrontal cortex, resulting in impaired neuronal metabolism and increased apoptotic signalling. These findings support the hypothesis that traumatic stress induces widespread disturbances in intracellular quality-control systems, thereby amplifying oxidative injury and neuronal degeneration [17].

Proteasome impairment has additionally been associated with abnormalities in glutamatergic neurotransmission and dendritic remodelling. Several studies have reported that chronic stress reduces expression of synaptic proteins involved in long-term potentiation and disrupts trafficking of glutamate receptors. Because synaptic plasticity requires tightly regulated degradation of structural and signalling proteins, disturbances in UPS activity may directly interfere with fear extinction and adaptive memory processing. Such mechanisms may contribute

to persistent hyperarousal, intrusive recollections, and emotional dysregulation characteristic of PTSD [18, 19].

Clinical observations further support the relevance of proteostatic dysregulation in trauma-related disorders. Peripheral biomarkers associated with oxidative protein damage and impaired protein degradation have been identified in patients with PTSD, including elevated concentrations of ubiquitinated proteins and oxidative stress markers. Although the majority of available studies remain experimental, these findings suggest that chronic traumatic stress may induce systemic alterations in cellular degradation pathways extending beyond the central nervous system [20-23].

INFLAMMASOME ACTIVATION AND NEUROINFLAMMATION IN PTSD

Neuroinflammation has emerged as a central mechanism underlying the pathophysiology of PTSD. Increasing evidence indicates that chronic traumatic stress activates innate immune pathways within the brain and promotes persistent inflammatory signalling in regions involved in emotional regulation and fear processing. Among the molecular complexes associated with these responses, the NLRP3 inflammasome appears particularly important due to its ability to integrate signals associated with oxidative stress, mitochondrial dysfunction, and cellular injury [24-27].

The NLRP3 inflammasome is a cytosolic multiprotein complex composed of the NLRP3 receptor, the adaptor protein - apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and procaspase-1. Following activation, caspase-1 promotes maturation of interleukin-1β (IL-1β), and interleukin-18 (IL-18), leading to amplification of inflammatory cascades and microglial activation. Under physiological conditions, inflammasome activity contributes to host defence and regulation of immune homeostasis. However, excessive or persistent activation may promote neuronal dysfunction and neurodegeneration [28].

Zhang et al. demonstrated significantly increased expression of NLRP3, caspase-1, and Il-1β within the hippocampus of rodents exposed to chronic stress paradigms resembling PTSD. These alterations were accompanied by impaired spatial memory, increased anxiety-like behaviour, and enhanced microglial activation [29]. Importantly, pharmacological inhibition of NLRP3 signalling attenuated behavioural abnormalities and reduced inflammatory cytokine production, suggesting a direct contribution of inflammasome activity to stress-induced neuronal dysfunction [30].

Additional evidence has been provided by studies investigating mitochondrial mechanisms involved in inflammasome activation. Iwata et al. reported that chronic stress increased mitochondrial reactive oxygen species production and induced release of oxidised mitochondrial DNA, both of which acted as potent triggers of NLRP3 activation. Mitochondrial injury additionally promoted calcium dysregulation and activation of apoptotic pathways within hippocampal neurons. These findings indicate that oxidative stress and mitochondrial dysfunction represent critical upstream mechanisms linking traumatic stress with neuroinflammatory injury [31].

Microglial activation constitutes another important feature of inflammasome-mediated neuroinflammation in PTSD. Under chronic stress conditions, microglia adopt a pro-inflammatory phenotype characterised by increased secretion of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), nitric oxide (NO), and reactive oxygen species (ROS).

Excessive microglial activation contributes to synaptic pruning, dendritic spine loss, and disruption of neuronal communication. In imaging studies, individuals with PTSD demonstrated increased neuroinflammatory markers within limbic structures, supporting the translational relevance of experimental findings [32].

Recent investigations have also implicated inflammasome activation in disruption of blood–brain barrier integrity. Elevated concentrations of inflammatory cytokines increase endothelial permeability and facilitate infiltration of peripheral immune cells into the central nervous system. This process may amplify local inflammatory responses and sustain chronic neuroimmune dysregulation. Several clinical studies have demonstrated elevated peripheral concentrations of IL-1β, IL-18, and C-reactive protein (CRP) in patients with PTSD, suggesting persistent activation of inflammatory pathways at both central and systemic levels [33, 34].

INTERACTIONS BETWEEN PROTEASOME DYSFUNCTION AND INFLAMMASOME SIGNALLING

Current evidence suggests that proteasome dysfunction and inflammasome activation are closely interconnected processes contributing to neuronal injury in PTSD. Oxidative stress appears to represent a major link between these mechanisms. Excessive production of reactive oxygen species damages proteins and mitochondrial structures, overwhelming intracellular degradation systems and promoting accumulation of damage-associated molecular patterns capable of activating innate immune signalling [35].

Impaired proteasomal degradation facilitates persistence of oxidised proteins and dysfunctional mitochondria, thereby enhancing inflammasome activation. Conversely, inflammatory cytokines generated during inflammasome signalling may further impair proteasome function through oxidative modification of proteasome subunits and disruption of mitochondrial metabolism. This reciprocal interaction creates a self-perpetuating cycle of oxidative injury, neuroinflammation, and neuronal dysfunction [36, 37].

Research conducted by Bansal Y et al. demonstrated that inhibition of proteasome activity enhanced NLRP3 inflammasome activation in microglial cultures exposed to oxidative stress [38]. Increased accumulation of damaged mitochondrial proteins promoted release of mitochondrial DNA and amplified secretion of interleukin-1β. Similar observations were reported in animal models of chronic stress, where reduced proteasome activity was associated with enhanced inflammatory signalling and increased neuronal apoptosis within the hippocampus.

Disturbances in these interconnected pathways may have profound consequences for synaptic plasticity and neuronal resilience. Both proteasome activity and inflammasome signalling regulate turnover of synaptic proteins, glutamate receptor trafficking, dendritic spine formation, and neurotrophic signalling. Chronic dysregulation of these processes may therefore contribute to impaired fear extinction, maladaptive memory consolidation, and persistent behavioural abnormalities observed in PTSD [39].

Recent studies additionally suggest that chronic neuroinflammation and impaired proteostasis may accelerate biological ageing within the brain. Increased oxidative stress, mitochondrial dysfunction, and persistent inflammatory activation resemble molecular alterations observed in neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease. Several epidemiological studies have demonstrated increased risk of cognitive decline and dementia among individuals with chronic PTSD, supporting the hypothesis that traumatic stress may induce progressive neurodegenerative vulnerability.

The reciprocal interactions between proteasome dysfunction and NLRP3 inflammasome activation in PTSD-related neuronal injury are presented in Table 2.

THERAPEUTIC PERSPECTIVES

Growing understanding of the interaction between proteasome dysfunction and inflammasome activation has generated interest in novel therapeutic strategies targeting neuroinflammatory and proteostatic pathways. Experimental studies suggest that selective inhibition of NLRP3 signalling may reduce neuronal injury and improve behavioural outcomes in chronic stress models.

MCC950, a selective NLRP3 inhibitor, has demonstrated significant neuroprotective effects in experimental studies. Administration of MCC950 reduced interleukin-1β production, attenuated microglial activation, and improved synaptic plasticity within the hippocampus. Similar effects were observed following administration of antioxidant compounds targeting mitochondrial oxidative stress, indicating that reduction of reactive oxygen species may indirectly suppress inflammasome activation [40].

Modulation of proteasomal activity may represent another potential therapeutic strategy. Experimental studies suggest that restoration of intracellular protein degradation pathways improves neuronal survival and reduces oxidative injury. Although pharmacological targeting of the UPS in psychiatric disorders remains insufficiently explored, regulation of proteostasis may become increasingly relevant in future neurobiological treatment approaches [24, 27].

Non-pharmacological interventions may additionally influence neuroinflammatory and proteostatic pathways. Physical exercise, sleep regulation, stress reduction, and dietary interventions have been associated with reduced inflammatory activity, improved mitochondrial function, and enhanced neuroplasticity. Such approaches may complement pharmacological strategies aimed at restoring neuronal homeostasis following traumatic stress exposure.

Further investigation is required to clarify the precise molecular interactions between proteasome dysfunction, inflammasome activation, mitochondrial injury, and synaptic abnormalities in PTSD. Improved understanding of these mechanisms may facilitate identification of novel biomarkers and support development of targeted neuroprotective therapies for trauma-related disorders [14, 18, 26].

LIMITATIONS

Several limitations should be considered when interpreting the evidence presented in this review. First, a large proportion of available data derives from experimental animal models and in vitro studies, which do not fully reproduce the clinical heterogeneity of PTSD. Although chronic stress paradigms mimic selected behavioural and molecular features of trauma-related pathology, their translational validity remains limited.

Second, most clinical studies are cross-sectional, which restricts conclusions regarding causality and temporal relationships between proteasome dysfunction, inflammasome activation, oxidative stress, mitochondrial impairment, and PTSD progression. The sequence in which these processes emerge after trauma exposure remains insufficiently defined.

Third, methodological heterogeneity across studies may affect interpretation of results. Differences in stress models, biological samples, brain regions, inflammatory biomarkers, and techniques used to assess proteasome activity or NLRP3 inflammasome signalling limit direct comparison between studies.

Clinical investigations are also frequently constrained by small sample sizes and confounding factors, including comorbid depression, anxiety disorders, sleep disturbance, substance use, metabolic abnormalities, and pharmacological treatment. These variables may independently influence neuroinflammatory, oxidative, and proteostatic pathways. Further longitudinal and translational studies are therefore required to clarify the mechanistic contribution of proteasome and inflammasome dysregulation to PTSD-related neuronal injury.

CONCLUSIONS

Current evidence indicates that proteasome dysfunction and inflammasome activation are important, interconnected mechanisms in the neurobiology of PTSD. Chronic traumatic stress promotes oxidative damage, mitochondrial dysfunction, impaired proteostasis, and persistent neuroinflammatory signalling within stress-sensitive brain regions, particularly the hippocampus, amygdala, and prefrontal cortex.

The interaction between the ubiquitin–proteasome system and the NLRP3 inflammasome may create a self-perpetuating cycle of cellular stress, microglial activation, cytokine release, synaptic dysfunction, and reduced neuronal plasticity. These mechanisms may contribute to impaired fear extinction, emotional dysregulation, cognitive disturbances, and increased neuronal vulnerability observed in PTSD.

Targeting proteasome activity, mitochondrial homeostasis, oxidative stress, and inflammasome signalling may represent a promising direction for future therapeutic development. Further studies are needed to identify reliable biomarkers and neuroprotective strategies capable of limiting chronic neuroinflammation and preserving neuronal integrity after traumatic stress

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CONFLICT OF INTEREST

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REVIEW ARTICLE

Quantitative justification of the advantage of 3d bioprinting over conventional skin transplantation in the reconstruction of burn defects

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ABSTRACT

Aim: To quantify, using published clinical outcome data, the advantage of 3D-bioprinted and bioengineered autologous skin constructs over conventional autologous skin grafting in the reconstruction of burn defects.

Materials and Methods: PubMed/MEDLINE, Scopus, Web of Science, the Cochrane Library and ClinicalTrials.gov were searched for records published between 1 January 2015 and 28 February 2026;. Three concept blocks were combined with AND, synonyms within each block with OR: (i) skin graft, autograft, donor site; (ii) burn, thermal injury, full-thickness defect, contracture; (iii) bioprinting, bioengineered skin, skin substitute, dermo-epidermal graft. Randomised trials, cohorts, registries and reviews with pooled data were eligible; excluded were case reports under five patients, in-vitro-only endpoints, no extractable outcome, or non-English records. The tissue economy ratio (TER) is the area of defect closed per unit area of donor tissue harvested, cm^2/cm^2. The iatrogenic donor burden (IDB) is the donor wound area per 100 cm^2 closed, IDB = 100/TER; both are computed only from reported values. Quantitative variables are presented as mean (standard deviation [SD]), median (interquartile range [IQR]), or the min-max range across studies, per how the source reported them; qualitative variables as absolute and relative frequencies.

Results: Forty-six sources were included. Conventional grafting was associated with graft failure in up to 32.3% of patients, post-grafting infection in 41.4%, hypertrophic scarring of the grafted burn area in 8%–67% of patients and of the donor site in 28%-64%, contracture at discharge in 38%-54%, and contracture recurrence in 27.1% of patients at 1 year. In a randomised intra-patient comparison, the tissue economy ratio was 10.76 for a bioengineered autologous dermoepidermal construct versus 1.70 for meshed split-thickness skin grafting, representing a 6.3-fold difference. This corresponded to 9.3 cm^2 versus 58.8 cm^2 of donor wound created per 100 cm^2 of defect closed, respectively, representing an 84.2% relative reduction in donor wound area.

Conclusions: The advantage of the bioprinting-based approach is structural and quantifiable rather than marginal and is greatest in patients with the least donor-tissue reserve. Its adoption is limited less by demonstrated efficacy than by challenges related to vascularisation of large constructs, manufacturing lead times, and regulatory classification.

KEYWORDS: cicatrix, autografts, wound healing, regenerative medicine

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INTRODUCTION

Burn injury causes an estimated 180000 deaths annually against 8.4 million new cases recorded worldwide in a single year, with the burden concentrated in low- and middle-income settings [1, 2]. Skin defects following burns carry the highest prevalence of long-term complications among traumatic wounds [3]. Because acute mortality has fallen steadily, the dominant clinical problem has shifted from survival towards the quality of the skin that is restored and the long-term cost of restoring it.

Hypertrophic scarring or keloids develop in 8-67% of survivors, and pathological scarring approaches 77% in severe injuries requiring repeated operations [4]. Contracture occurs in 38-54% of patients before discharge, and specialised centres report active contractures in 65% of long-term survivors [5, 6]; 5-20% require secondary reconstructive surgery within ten years [7].

International burn-care guidelines state that, after resuscitation, the principal aim is re-epithelialisation, by spontaneous healing or by necrectomy and grafting, to secure the best quality of life after the burn [5]. In practice this is largely minimally invasive: in the Netherlands 45% of hospitalised patients undergo surgery at a median of 15 days, and in Australian/New Zealand centres 47.2% undergo reconstructive surgery within one week [4]. Reconstructive surgery restores range of motion and the protective barrier, and this physical recovery directly affects psychological wellbeing and social reintegration [8].

Despite these benefits, secondary operations carry inherent surgical risks [9]. A fresh graft may fail from poor blood supply, fluid accumulation, or infection, and harvesting healthy skin creates a second wound, causing pain and scarring risk [6]; a good result needs months of compression garments, physiotherapy and splinting [10]. STSG nonetheless remains the reference standard for definitive closure of deep dermal and full-thickness defects [11]; its limitation is structural, since coverage is obtained only by creating this second wound (Table 1). Reconstructed skin rarely reproduces uninjured tissue: colour, texture and thickness may not match, and it typically lacks sweat and sebaceous glands and hair follicles, leaving chronically dry skin needing lifelong care [10].

Three-dimensional (3D) bioprinting and related bio-engineered autologous constructs represent a paradigm shift, uncoupling coverage from large-scale donor-tissue sacrifice: autologous cells are combined with nutrient-rich hydrogels (bioinks) to print new, individually shaped skin layers [11, 13]. Unlike autografting, which leaves a secondary wound the size of the defect, 3D printing needs only a small biopsy and produces a genuine dermis with fibroblasts and extracellular matrix [13]. Trials report significantly less postoperative pain than grafting [11, 14], and since bioink uses the patient's own cells, rejection risk is near zero [11, 13], now supported by randomised evidence rather than laboratory data alone [15]. What has not been done is to place the two families on a common quantitative footing: existing reviews describe each modality separately, none expressing the difference in a single measure of coverage per unit of tissue destroyed.

AIM

To quantify, from published clinical outcome data, the magnitude of the advantage of 3D bioprinted and bio-engineered autologous skin constructs over conventional autologous skin grafting in the reconstruction of burn defects, and to express it through two derived measures: the tissue economy ratio and the iatrogenic donor burden per unit area of defect closed.

MATERIALS AND METHODS

STUDY DESIGN

A structured quantitative comparative synthesis of published clinical outcome data was performed, not a systematic review of treatment effect, since the two families lack a common set of randomised comparisons; the aim was to aggregate reported outcomes within predefined domains and derive comparative metrics.

DATA SOURCES AND SEARCH STRATEGY

PubMed/MEDLINE, Scopus, Web of Science, the Cochrane Library and ClinicalTrials.gov were searched for records published between 1 January 2015 and 28 February 2026; earlier publications defining a methodological standard were retained. Three concept blocks were combined with AND, synonyms within each block with OR: (i) skin graft, autograft, donor site; (ii) burn, thermal injury, full-thickness defect, contracture; (iii) bioprinting, bioengineered skin, skin substitute, dermo-epidermal graft. Reference lists were hand-searched.

ELIGIBILITY CRITERIA

Records were included if they reported human clinical outcomes with at least one extractable measure of graft take, wound closure, donor-site morbidity, scar quality, contracture or reoperation, or controlled preclinical bioprinting outcomes with quantified endpoints. Randomised trials, cohorts, registries and reviews with pooled data were eligible; excluded were case reports under five patients, in-vitro-only endpoints, no extractable outcome, or non-English records.

DATA EXTRACTION

Screening proceeded in two stages, titles/abstracts then full texts. Extracted variables were design, sample size, population, intervention, comparator, follow-up and outcome values with dispersion; where a review and its primary study shared a cohort, only the primary source was kept.

DERIVED METRICS

The tissue economy ratio (TER) is the area of defect closed per unit area of donor tissue harvested, cm^2/cm^2. The iatrogenic donor burden (IDB) is the donor wound area per 100 cm^2 closed, IDB = 100/TER; both are computed only from reported values.

STATISTICAL PROCESSING

Quantitative variables are presented as mean (standard deviation [SD]), median (interquartile range [IQR]), or the min-max range across studies, per how the source reported them; qualitative variables as absolute and relative frequencies. No pooled effect was calculated, given heterogeneity of designs and follow-up. Significance levels are reproduced as stated in the sources. Computation used Microsoft Excel and Python 3.11/NumPy 2.4.

ETHICS

This study is based exclusively on publicly available data published in peer-reviewed journals, clinical guidelines

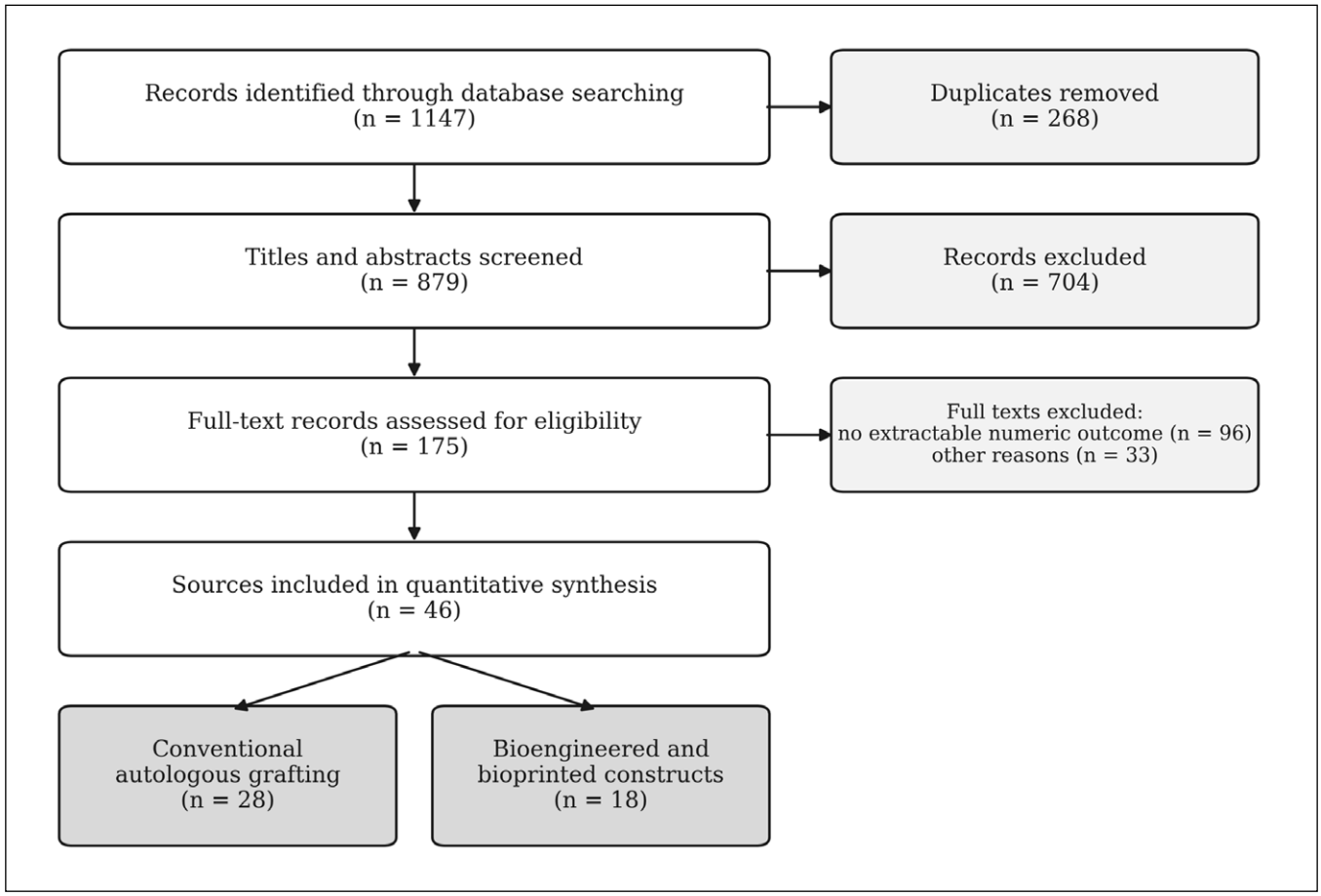


Fig. 1. Flow of source identification, screening and inclusion
Picture taken by the authors

and public trial registries. No patient-identifiable data were used, no clinical intervention was performed and no primary data collection was undertaken; approval by a local ethics committee was therefore not required.

The authors adhered to the Declaration of Helsinki and to international publication-ethics standards, including ICMJE recommendations. No element of this work contains plagiarism, fabrication or falsification of data; all values reproduced here are attributed to their sources.

AI STATEMENT

The following artificial intelligence (AI) tools were used in this work: Elicit and ResearchRabbit, Julius AI. They were used for the following tasks:

- assisted search for literature under the author's control (literature sources were also identified independently by the author);
- assisted generation and adjustment of Python code for data visualisation (figures) under the author's control;

All sections using AI were reviewed and edited personally by the author(s). The scientific statements, conclusions, and results are the author(s)'s own intellectual contribution.

RESULTS

The searches returned 1147 records. After removing 268 duplicates, 879 titles/abstracts were screened and 175 full texts assessed; 46 sources entered the synthesis, 28 on conventional grafting and 18 on bio-engineered or bioprinted constructs (Fig. 1).

THE QUANTIFIED BURDEN OF CONVENTIONAL AUTOLOGOUS GRAFTING

No single failure mode dominated; the burden spread across seven independent domains (Table 1, Fig. 2). Graft failure requiring re-grafting reached 32.3% in a series of 198 patients, where bacterial colonisation was the only independent predictor (odds ratio [OR] 48.34; $p < 0.001$), and infection reached 41.4% [16]. Hypertrophic scarring affected the grafted burn in 8-67% of survivors [4] and the donor site in 28% at eight years and 64% of paediatric patients [12, 17].

Table 1. Quantified outcome burden of conventional autologous skin grafting

Outcome domain	Reported value	Source
Graft failure requiring re-grafting	32.3% of 198 patients	[16]
Post-grafting wound infection	41.4% of cases	[16]
Donor-site hypertrophic scarring	28% at 8 years; 64% of paediatric patients	[12, 17]
Hypertrophic scarring of grafted burn	8-67% of survivors	[4]
Scar contracture at discharge	38-54% of patients	[4, 5]
Contracture recurrence after full-thickness grafting	27.1% at 12 months; 26.9% at 5 years	[18]
Secondary reconstructive surgery within 10 years	5-20% of patients	[7]

Source: compiled by the authors based on [4, 5, 7, 12, 16, 17, 18]

Table 2. Reported outcomes of engineered and bioprinted autologous skin constructs

Parameter	Engineered construct	Meshed STSG control	Source
Expansion ratio, mean (SD)	10.76 (6.03)	1.70 (0.68)	[15]
Clinically relevant scarring, 12 months	none observed	predominantly hypertrophic	[15]
Observer overall scar opinion, 3 months	favours construct; $p = 0.043$	–	[15]
Deviation from normal skin in relief	smaller; $p = 0.005$ at 6 months, $p < 0.001$ at 12 months	–	[15]
Serious adverse events attributable to treatment	2 of 164; both resolved spontaneously	–	[15]
Durability at 5 years, phase I paediatric cohort	maintained; no new safety signals	–	[21]
In situ bioprinting, full-thickness wounds	closure by week 4; 4-5-week acceleration	matrix-only and untreated controls	[24]

Source: compiled by the authors based on [15, 21, 24]

Functional outcomes were equally consistent. Recurrent contracture after full-thickness grafting of joint-involving burns occurred in 27.1% at one year and 26.9% at five years, against 10% and 4.8% after flap reconstruction ($p = 0.043$) [18]. In a paediatric meta-analysis of 532 grafts, full-thickness grafts reduced the odds of post-graft contracture (OR 0.35; $p = 0.0001$) and of surgical release (OR 0.06; $p = 0.00001$) relative to split-thickness grafts, confirming that contracture tracks the quantity of dermis transferred [19]. In a randomised trial grafted surface fell to 92% at twelve months, whereas perforator-based flaps expanded to 142% ($p < 0.001$) [20].

OUTCOMES OF BIO-ENGINEERED AND BIOPRINTED AUTOLOGOUS CONSTRUCTS

Eighteen sources provided quantified outcomes (Table 2). The decisive dataset is a prospective, intra-patient randomised phase IIb trial of an autologous bilayered dermo-epidermal construct against meshed STSG [15]. The mean expansion ratio was 10.76 (SD 6.03) versus 1.70 (SD 0.68) for STSG, a 6.3-fold difference ($p < 0.001$); both areas fully epithelialised by month three, and at twelve months no clinically relevant scarring was observed for the construct, against predominantly hypertrophic STSG controls. Scar opinion favoured the construct at three months ($p = 0.043$), with smaller deviation from normal skin at six and twelve months ($p = 0.005$, $p < 0.001$). Of 164 serious adverse events, only two were attributable to the construct, both resolving spontaneously; five-year paediatric follow-up confirmed durability without new safety signals [21].

Cell-only strategies delimit the effect's boundary. Cultured epidermal autografts supply epidermis without dermis and yield inconsistent take and poor long-term outcomes, requiring three to four weeks of culture [15]. A review of 30 trials in 970 patients confirmed cell therapies accelerate epithelialisation but that dermal reconstitution, not epidermal coverage, is limiting [22], concordant with a dermal-substitute meta-analysis [23]. Preclinically, in situ bioprinting of fibroblasts and keratinocytes into full-thickness wounds produced complete re-epithelialisation by week four, four to five weeks faster than controls [24].

COMPARATIVE TISSUE ECONOMY

Applying the metrics yielded a TER of 1.70 cm^2/cm^2 for meshed STSG and 10.76 cm^2/cm^2 for the engineered construct. As iatrogenic donor burden, closure of a 100 cm^2 defect requires 58.8 cm^2 of donor wound with STSG and 9.3 cm^2 with the construct, an 84.2% relative reduction (Fig. 3). Scaled to a 15% total-body-sur-

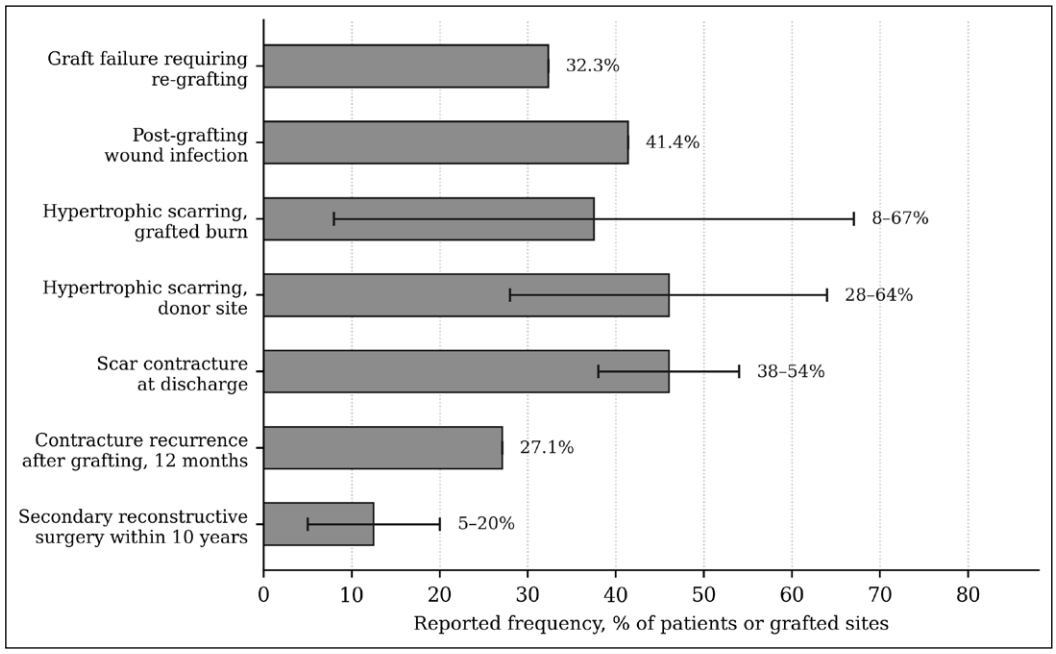


Fig. 2. Reported frequency of adverse outcome domains of conventional autologous skin grafting. Bars show the mid-point of the reported range, whiskers its limits
Picture taken by the authors

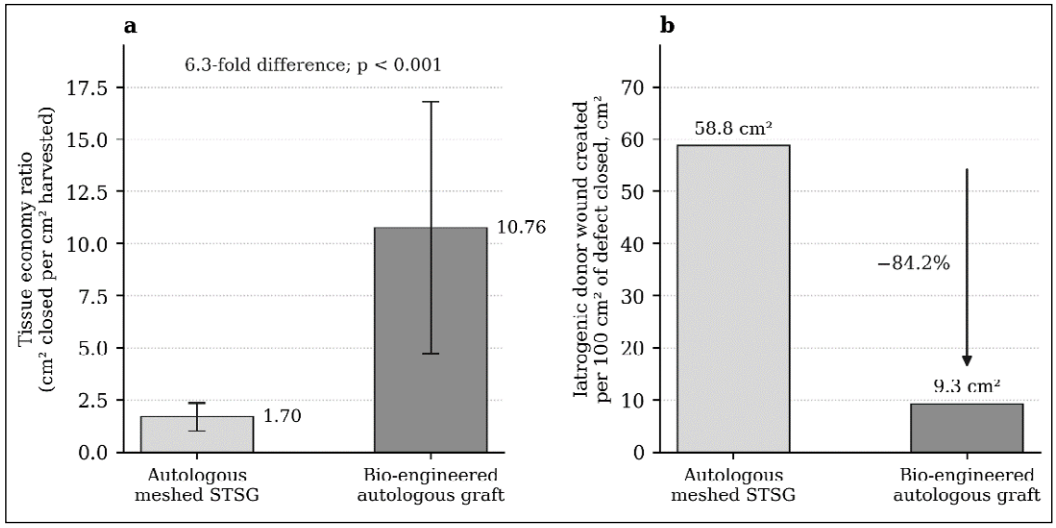


Fig. 3. Comparative tissue economy: (a) tissue economy ratio, mean (SD); (b) iatrogenic donor burden per 100 cm^2 closed.
Picture taken by the authors

face-area burn, about 2700 cm^2 of defect, this is 1588 cm^2 against 251 cm^2 of donor wound, over 7% of total body surface area.

DISCUSSION

The central finding is that the advantage of engineered skin over conventional grafting is structural rather than incremental and can be expressed in a single number. Conventional grafting cannot exceed a tissue economy ratio determined by the degree of mesh expansion applied, and the ratio of 1.70 measured under randomised conditions [15] falls far below the nominal 1:4 ratio because expansion, graft take, and contraction each reduce the effective coverage achieved. Increasing mesh expansion may increase contraction and scar height rather than providing a proportional increase in effective coverage. The engineered construct avoids this limitation because the biopsy serves as a source for cell expansion rather than as a one-to-one source of tissue.

This reframes a long-standing observation: patients rate donor-site morbidity as equal to or worse than the burn scar itself years later [12], consistent with the donor wound representing a structural cost rather than merely a complication. The present analysis quantifies this cost as 58.8 cm^2 per 100 cm^2 of defect closed; reducing it by 84.2% substantially changes the resource requirements of the procedure. Patients with burns involving more than 50%-60% of the total body surface area, in whom donor tissue is particularly scarce, may therefore derive the greatest benefit [11].

Our results extend the existing literature. Reviews of cell-based therapies and dermal substitutes have found that epidermal-only strategies accelerate wound closure without restoring dermal architecture [22, 23]. Our data

provide a quantitative explanation for this limitation: contracture risk is related to the amount of dermis transferred, as demonstrated by the difference in the odds of surgical release between full- and split-thickness grafts [19] and by the divergence in surface area between grafts and flaps at 12 months [20]. This is consistent with evidence that flap reconstruction of paediatric contractures can achieve durable release in cases in which graft reconstruction is associated with recurrence [8]. Data from centres in resource-limited settings, where graft failure reached 32.3% and infection 41.4% [16], further indicate that the burden is greatest where treatment capacity is most limited [1, 2, 9], while engineered constructs remain least available in these settings.

Several limitations apply. The comparison rests on a single randomised trial, 21 enrolled and 13 analysed per protocol, defects no larger than 98 cm^2 under an orphan designation [15]; the metrics are precise but not generalisable to massive burns, and phase III has not been reported. No fully bioprinted construct has completed a randomised comparison against STSG, the evidence being preclinical or first-in-human [24]. Heterogeneity precluded meta-analysis, so ranges describe the literature's spread, not a pooled effect. Nor does TER capture manufacturing time or the deficits bioprinting has not solved: appendage regeneration and a perfusable vascular network anastomosing with host circulation, the chief obstacle to scaling beyond a few hundred cm^2, alongside skin's immunological function in a scaffold [14, 25-29].

Regulatory classification remains unsettled, falling between device and graft, with viability, sterility and release-testing requirements still under harmonisation [14, 26]. A three- to four-week manufacturing time is incompatible with acute resuscitation, confining these constructs to staged reconstruction [15]. Future work should prioritise randomised comparisons beyond 500 cm^2 and health-economic modelling of avoided wounds against manufacturing cost.

CONCLUSIONS

Conventional autologous skin grafting achieves wound closure at a fixed, quantifiable cost: for every 100 cm^2 of defect closed, 58.8 cm^2 of healthy skin is harvested, and the resulting donor sites are associated with hypertrophic scarring in up to 64%-67% of patients, graft failure in up to 32.3%, and recurrent contracture in approximately one-quarter of patients undergoing reconstruction of joint-involving burns.

Bioengineered autologous dermoepidermal constructs, an immediate precursor to 3D-bioprinted skin, achieve a tissue economy ratio of 10.76 compared with 1.70 for meshed STSG in a randomised intra-patient comparison, reducing iatrogenic donor burden by 84.2% and producing no clinically relevant scarring at 12 months.

This advantage is structural rather than marginal and is greatest in patients for whom conventional grafting performs worst. Adoption is limited less by demonstrated efficacy than by the vascularisation of large constructs, manufacturing time, and regulatory classification –priorities for further research.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

An overview of peri-implant soft tissues dehiscence (PSTD): A narrative review

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ABSTRACT

Aim: This review is designed to synthesize current evidence to magnify understanding and management of Peri-Implant Soft Tissue Dehiscence (PSTD) in implant dentistry.

Materials and Methods: A structured narrative review was conducted by systematically searching Scopus, PubMed, Web of Science, and Google Scholar for literature published between 2000 and 2025. Related studies were identified using keywords related to PSTD, with emphasis on etiology, clinical presentation, assessment method classification, and treatment approaches.

Conclusions: The review identified various etiological factors contributing to PSTD, including thin keratinized mucosa, poor oral hygiene, and improper implant positioning. The clinical features of PSTD primarily include recession of peri-implant keratinized tissue and mucosal margin with exposure of implant components, negatively affecting aesthetic and potentially leading to peri-implant disease. Accordingly, a range of assessment methods was outlined in the literature, including clinical indices, photographic analysis, and advanced imaging techniques. Notably, current studies emphasize the use of a classification system that considers tissue thickness, recession depth, and tissue quality. Several surgical and non-surgical techniques were also discussed, with soft-tissue grafting emerging as the most effective intervention for correcting PSTD.

In implant dentistry, peri-implant soft-tissue dehiscence poses serious biological and aesthetic challenges. Successful care depends on a precise diagnosis made using clinical and imaging techniques, as well as on preventive measures and soft-tissue augmentation. Although connective tissue grafting remains the most predictable strategy, further research is needed to refine categorization schemes and develop less invasive methods to improve patient outcomes and long-term stability.

KEYWORDS: PSTDs, classification of PSTD, peri-implantitis, peri-implant mucositis, implant dentistry

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INTRODUCTION

Peri-implant soft tissue is essential for the esthetic and functional purposes of supported restorations and for long-term implant survival. Peri-Implant Soft Tissue Dehiscence (PSTD), caused by insufficient keratinized tissue, can compromise implant success and negatively affect patient satisfaction. This narrative review briefly discusses PSTD and the assessment methods available in the literature, based on the current PSTD classification. PSTD is defined as insufficient coverage or recession of the soft tissue surrounding dental implants [1]. In contrast to the teeth, gingival recession is an apical shift of the gingival margin below the cementoenamel junction [2]. However, dental implants lack a standard reference level for a "normal" midfacial mucosa since clinicians disagree on its definition. Some clinicians use the mucosal margin as a reference point at the time of final restoration or exposure of the metallic implant or abutment grey surface [3]. These reference points may be inappropriate since they do not consider the gingival margin of the homologous adjacent or contralateral natural teeth. This could result in an excessively long implant superstructure, with disharmony between the marginal soft-tissue scallop and the adjacent teeth. Accordingly, most clinicians, in agreement with a consensus, agree that the peri-mucosal margin of the implant should be referenced to adjacent or contralateral sound natural teeth [4-5]. Thus, a classification for PSTD was developed to ease clinicians' diagnosis and treatment [1]. This PSTD condition can lead to poor esthetic results, increased susceptibility to infection, and poor health of the area surrounding the implant. For example, unique patient characteristics, implant design, and surgical techniques can all contribute to a lack of soft tissue

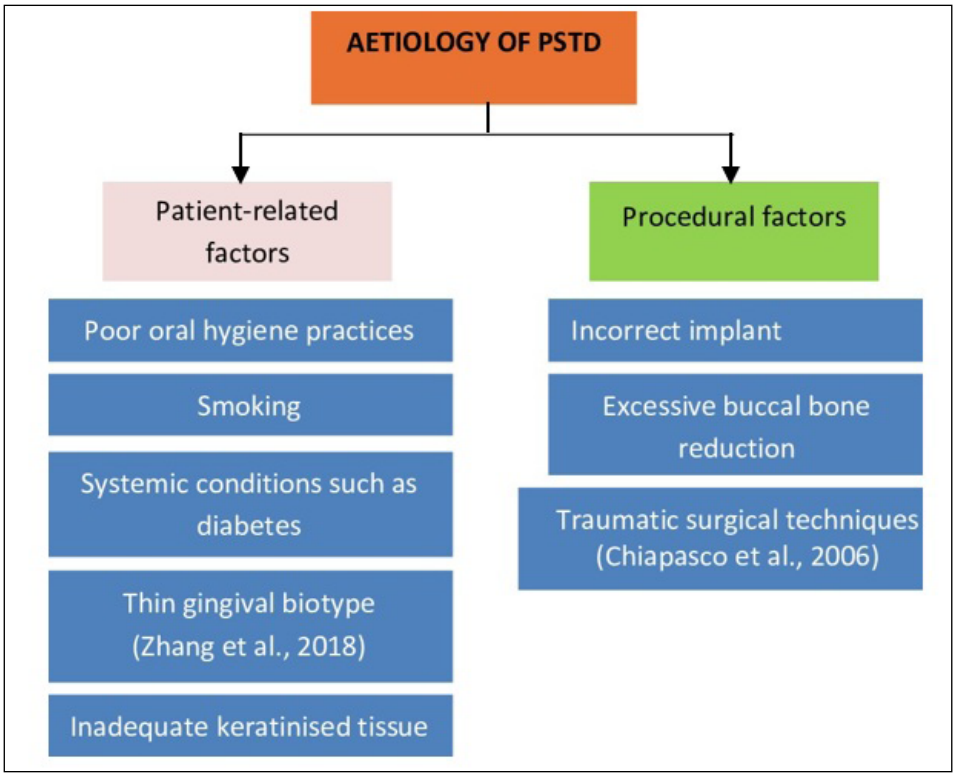


Fig. 1. The etiology of PSTDs
Source: Own materials

surrounding the implant [6]. Hence, this comprehensive review aims to critically evaluate the existing literature on the causes and risk factors of PSTD, clinical manifestations of PSTD, assessment methods, and treatment of PSTD. Additionally, this review highlights the potential implications for clinical practice. At the same time, it provides insights into future research directions, with the ultimate goal of enhancing treatment protocols for PSTD and promoting the long-term success of dental implant therapy.

AIM

This review is designed to synthesize current evidence to magnify understanding and management of Peri-Implant Soft Tissue Dehiscence (PSTD) in implant dentistry

MATERIALS AND METHODS

This narrative review was conducted to gather, analyse, and synthesize current evidence related to PSTD. The focus was on the etiology, clinical manifestations, assessment techniques, classification, and treatment modalities of PSTD. A structured approach was employed to identify and select relevant literature, providing a comprehensive overview of the topic. Accordingly, an extensive electronic search was conducted across databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search included publications from January 2000 to 2025. The keywords used in the search strategy included "Peri-implant soft tissue dehiscence," "PSTD," "Peri-implant mucosa," "keratinised tissue," "implant esthetics," "Peri-implantitis," and "soft tissue grafting." Boolean operators such as AND & OR were applied to refine the search results. This comprehensive approach integrated current knowledge and clinical insights to enhance understanding and management of PSTD in implant therapy.

All figures is based on the authors' review, and the clinical pictures are from the authors' patients who have consented to be published in the journal.

REVIEW AND DISCUSSION

ETIOLOGY AND RISK FACTORS OF PSTD

The etiology of PSTD is multifactorial, encompassing both patient-related and procedural factors in Figure 1. Patient-related factors include insufficient soft-tissue volume, poor oral hygiene, a history of periodontitis, smoking, systemic conditions, and inadequate keratinized tissue width [7-9]. A thin or inadequate band of keratinized tissue surrounding an implant has been identified as a key risk factor for PSTD [10]. Keratinized tissue provides a protective barrier, and its absence can lead to recession and exposure of the implant surface. Clinical studies suggest that patients with less than 2 mm of keratinized tissue are more prone to peri-implant tissue complications [11]. A meta-analysis revealed that implants with less than 2 mm of keratinized mucosa



Fig. 2. The PSTD on the implant at the lower left premolar region
Source: Own materials

have significantly higher rates of plaque accumulation, inflammation, and recession compared to those with adequate keratinized tissue [12]. Plaque accumulation due to poor oral hygiene is a critical factor in the development of peri-implant diseases, including soft tissue dehiscence. Therefore, Supportive Peri-Implant Care (SPIC) following implant therapy is crucial to maintaining peri-implant health. Plaque-biofilm-induced peri-implant mucositis, if untreated, may progress to peri-implantitis and result in breakdown of both soft and hard tissues. Lang et al. (2012) conducted a long-term study demonstrating that inadequate plaque control significantly increases the risk of peri-implant soft tissue recession [13]. On the other hand, patients with a history of periodontitis are at higher risk of developing PSTD due to their compromised periodontal tissue phenotype [14]. A study [15] reported that individuals with a history of periodontitis had a higher incidence of implant complications, including increased soft tissue recession and marginal bone loss [16].

Smoking is a well-established risk factor for impaired wound healing and peri-implant complications [17]. Nicotine reduces blood flow to tissues, compromising the regenerative capacity of soft tissues and increasing the incidence of PSTD [18-19]. A systematic review by [20] reported that smokers are at a significantly higher risk of peri-implant tissue complications, including PSTD, due to impaired healing and reduced immune response. Systemic conditions, such as uncontrolled diabetes, autoimmune diseases, stress, or immunosuppressive therapies, can adversely affect wound healing and tissue health, thereby increasing the risk of PSTD [21-24]. In particular, uncontrolled diabetes has been linked to impaired collagen synthesis and a reduced inflammatory response, which may delay healing and increase susceptibility to infections [25]. A study discovered that patients with uncontrolled diabetes had higher rates of implant complications, including soft tissue dehiscence [26]. Procedural factors include incorrect implant positioning, excessive buccal bone reduction, and traumatic surgical techniques [27-28]. The positioning of dental implants plays a critical role in their success, both structurally and aesthetically. Incorrect implant positioning has been identified as a key factor contributing to PSTD, leading to aesthetic and functional complications [10]. Implant placement too close to the buccal bone plate often leads to insufficient bone volume to support the implant and adjacent soft tissue [29]. This can result in bone resorption, especially around the thin buccal bone, increasing the risk of soft tissue recession and dehiscence. Studies have demonstrated that implants placed with inadequate buccal bone thickness (< 2 mm) are prone to bone loss and mucosal recession [29-30]. A systematic review underscored that the proximity of implants to the buccal bone significantly influences long-term bone stability and soft-tissue health [31]. The study concluded that at least 2 mm of buccal bone is required to maintain peri-implant soft-tissue integrity [23, 32]. Placing an implant too superficially, with insufficient bone anchorage, can lead to instability and micromovements during the healing phase [33]. A study by Esposito et al. (1998) [34] highlighted that micromotion during healing is directly correlated with higher rates of implant failure and complications, including PSTD. Moreover, if an implant is placed too deeply subcrestally, it may also

lead to PSTD. Excessive bone remodelling can occur as the body attempts to adapt to a deeper implant, potentially leading to marginal bone loss and peri-implantitis [35]. A clinical study [36] determined that implants placed too deeply are prone to excessive crestal bone resorption, which can contribute to peri-implant tissue complications, such as PSTD, due to increased difficulty in maintaining peri-implant tissue health. Hence, proper implant depth is crucial for balancing these risks, as it provides sufficient stability for osseointegration without inducing excessive bone remodelling or soft-tissue recession in Figure 2.

Incorrect angulation of the implant can lead to biomechanical complications by causing uneven stress distribution in the surrounding bone and soft tissues [37-38]. When an implant is angled incorrectly, the forces generated during mastication are not evenly distributed, leading to increased pressure in some areas that can cause bone resorption and recession [39]. A study [40] demonstrated that improper angulation of dental implants can lead to marginal bone loss and gingival recession. The study emphasized the importance of proper angulation to ensure even force distribution and promote long-term implant success. Implants placed in areas with a thin or compromised buccal plate indicated higher rates of PSTD due to increased bone resorption over time. Therefore, preservation of the buccal plate is essential during implant placement to prevent peri-implant soft tissue complications. Guided bone regeneration may be required prior to implant placement to preserve the buccal bone plate. Crestal ridge width changes following implant placement, with or without soft-tissue augmentation, were evaluated after a 6-month healing period, with a reduction in width observed without augmentation [41]. Bone necrosis caused by excessive heat generation during implant site preparation is a significant contributor to implant failure and PSTD. When high-speed drills are used without adequate cooling, depending on the total time of exposure of bone tissue to heat, reported temperatures ranging from 47°C to 70°C could lead to thermal damage to the bone, compromising its ability to heal and integrate with the implant [42]. On the other hand, a study [43] established that thermal injury to bone significantly impairs osseointegration and increases the likelihood of complications such as bone resorption and soft-tissue recession. Thus, adequate cooling during drilling is crucial to prevent thermal damage and promote healthy peri-implant tissue healing [44]. Excessive or aggressive handling of the soft-tissue flap during surgery can damage the blood vessels supplying the surgical site, compromising the blood flow essential to healing. Moreover, trauma to the soft tissue during flap elevation can impair healing, leading to ischemia and increasing the risk of dehiscence [45]. A clinical study [46] highlighted that careful, non-traumatic handling of soft tissues during surgery is crucial for maintaining an adequate blood supply, which is essential for healing and minimizing the risk of soft-tissue dehiscence around implants. Therefore, understanding and addressing these procedural factors is critical for minimizing the risk of PSTD and promoting long-term implant success. Clinical studies have consistently demonstrated the importance of proper implant positioning, maintaining adequate buccal bone, and utilizing careful surgical techniques to preserve soft tissue and bone stability.

CLINICAL MANIFESTATIONS OF PSTDS

Soft-tissue recession exposes the implant fixture or its abutment, an essential clinical sign of PSTD. This exposure compromises aesthetics and increases the risk of plaque accumulation around the exposed areas, further contributing to peri-implant inflammation. A study [47] emphasized that implant surface exposure is often associated with a higher susceptibility to plaque accumulation, which can exacerbate the risk of peri-implant disease, particularly in cases with deficient keratinized tissue. Increased probing depths are another significant indicator of PSTD, often associated with peri-implant disease progression. Probing depths greater than 4 mm are commonly observed in cases of inflammation and soft-tissue breakdown. Studies suggest that deeper pocket depths around dental implants may indicate ongoing peri-implant tissue destruction, which can lead to further complications. According to [48], the presence of increased pocket depth, in combination with other clinical signs such as Bleeding On Probing (BOP), is strongly associated with peri-implantitis and soft tissue dehiscence. Regular monitoring of probing depths is therefore essential to prevent the progression of PSTD to more severe conditions. BOP is a hallmark of peri-implant soft tissue inflammation, indicating the presence of inflammation and compromised tissue health. When probing around an implant reveals bleeding, it is a strong indication of mucosal inflammation, which may precede bone loss in peri-implantitis. BOP is considered a reliable diagnostic tool for early detection of peri-implant mucositis [49]. In combination with other signs such as deep probing depths and implant exposure, BOP highlights the need for early intervention to prevent the progression of peri-implant disease.

ASSESSMENT METHODS OF PSTDS AND

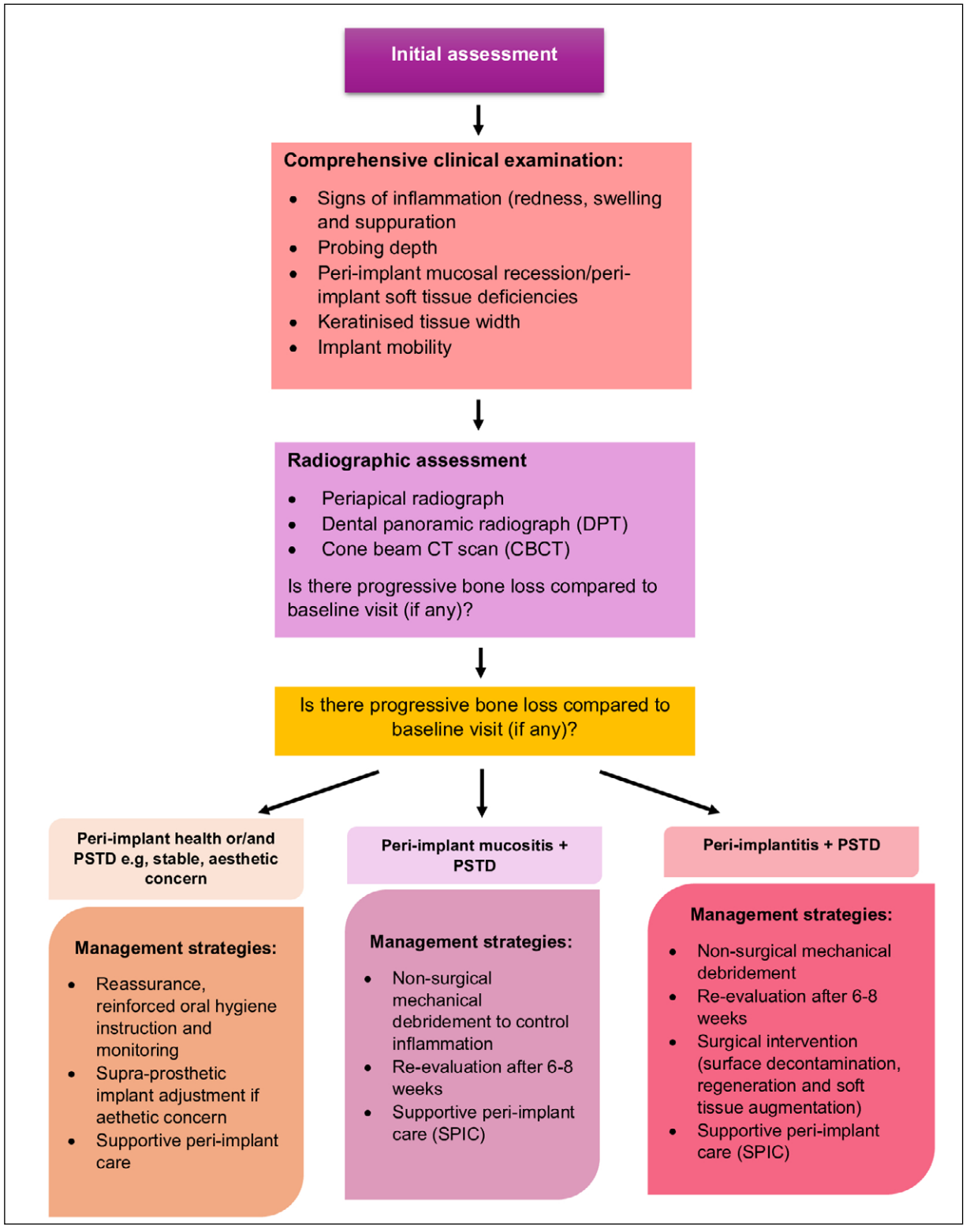


Fig. 3. Flowchart of Assessment of PSTD
Source: Own materials

THEIR CLASSIFICATION

Matthes et al. [50] and Soares et al. [51] suggested that a comprehensive assessment of peri-implant soft tissues includes clinical signs, such as BOP and probing depth measurements, as well as radiographic evaluations. Radiographs, including Cone Beam Computed Tomography (CBCT) or periapical images, provide essential information about the bone levels around the implant, allowing clinicians to detect early bone loss indicative of peri-implantitis. This assessment is crucial for distin-

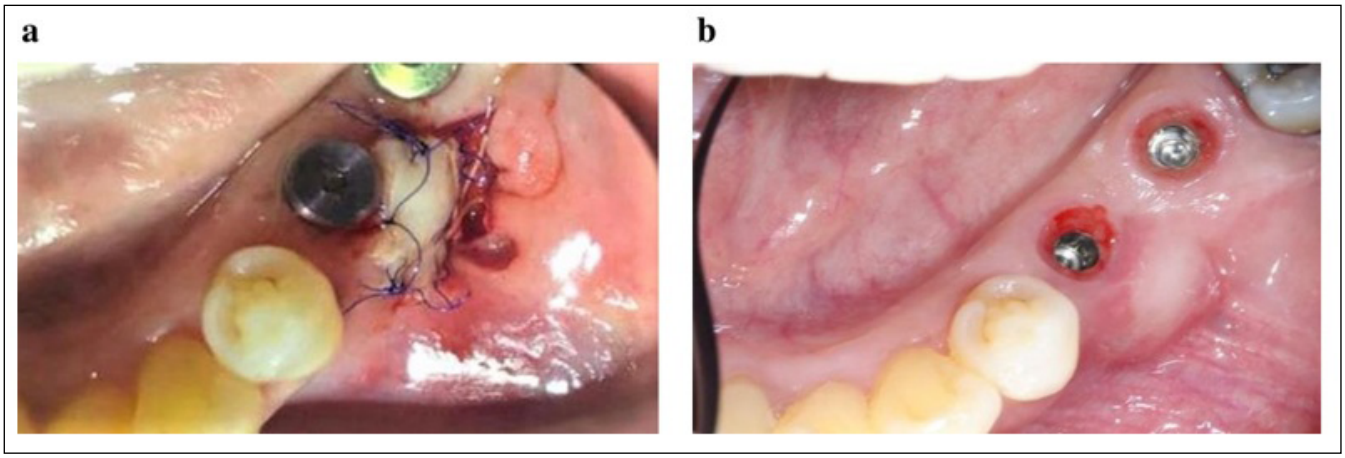


Fig. 4. Clinical management of inadequate keratinized tissue width. (a) A free gingival graft was placed on the buccal aspect of implant 34 (the lower left second premolar). (b) Increased keratinized tissue width at 3 months post-operatively.
Source: Own materials

guishing between mucositis and peri-implantitis, as the latter requires more aggressive management to prevent further bone loss and implant failure.

Furtsev et al. [52] highlighted the role of radiographic imaging in evaluating peri-implantitis, emphasizing that the presence of bone loss and increased pocket depths, in conjunction with soft tissue recession, can signal advanced peri-implant disease. Once peri-implantitis is established, the prognosis becomes more complex, as bone loss around the implant makes it increasingly difficult to manage without surgical intervention. Accurate assessment of PSTD is crucial for diagnosing the condition, planning treatment, and monitoring therapeutic outcomes. A variety of methods are commonly used to assess PSTD, ranging from clinical examinations to advanced imaging techniques in Figure 3. A comprehensive assessment of PSTD integrates clinical examination with radiographic imaging, ensuring that all aspects of soft-tissue health and implant stability are evaluated. This multifaceted approach enables early detection, accurate diagnosis, and informed treatment planning, all of which are crucial to the long-term success of dental implants. Visual inspection is the first step in diagnosing PSTD, focusing on soft-tissue changes around the implant. Clinicians assess for visible signs of soft-tissue recession, which may expose the implant or abutment surface. In addition to recession, signs of inflammation, such as erythema (redness) or edema (swelling), indicate early peri-implantitis, a precursor to peri-implant disease. Visual inspection is critical for identifying aesthetic issues and potential complications that may require further intervention. Probing depth measurements involve the use of a periodontal probe to assess the depth of the peri-implant sulcus. Increased probing depths, particularly those exceeding 5 mm, are indicative of peri-implant disease and potential bone loss. Notably, these deeper pockets may signal a breakdown in the tissue attachment and are often associated with peri-implantitis. According to [13], probing around implants is a key diagnostic tool for assessing peri-implant tissue health, and greater pocket depths often correlate with more severe soft-tissue and bone complications. The presence of bleeding upon gentle probing is a reliable indicator of soft tissue inflammation, signalling active peri-implant disease. BOP suggests that the tissues are inflamed, which is commonly associated with peri-implant mucositis, the precursor to peri-implantitis. BOP is a simple yet effective clinical tool for monitoring peri-implant health and detecting early disease activity. Renvert et al. (2018) [49] demonstrated a strong correlation between BOP and peri-implant disease progression, recommending its routine use during the implant maintenance program. Periapical radiographs are a localized imaging modality that provides detailed views of the bone levels around the dental implant. They are beneficial for detecting early bone loss, implant positioning, and any signs of peri-implantitis. However, while they provide limited information on soft tissues, periapical radiographs are a key tool for monitoring bone stability and ensuring the implant is well integrated with the surrounding structures. CBCT is considered the gold standard for assessing the three-dimensional structure of the peri-implant region. It provides highly detailed 3D images that enable precise evaluation of bone volume, implant positioning, and proximity to vital anatomical structures, such as nerves or sinuses. CBCT is particularly valuable in complex cases requiring bone grafting, sinus lifts, or implant revisions. Nack et al. (2016) [53] established the efficacy of CBCT in providing comprehensive data that enhances the accuracy of diagnosis and treatment planning, particularly in detecting early-stage peri-im-

plant bone loss. As recommended in the 2017 World Workshop Consensus Report on Peri-implant Diseases, baseline radiographic images and probing depth measurements should be taken immediately after implant placement to monitor future changes effectively [48].

MANAGEMENT AND PREVENTION OF PSTD

Prevention of PSTD begins with meticulous treatment planning and patient selection. Preventive measures are essential to minimize the risk of soft-tissue dehiscence around implants. Proper implant positioning plays a crucial role in minimizing the risk of PSTD. Implants should be placed with sufficient distance from the buccal plate and adjacent teeth, and with a minimum of 2 mm of keratinized mucosa surrounding the implant. Furthermore, studies have demonstrated that implants placed too close to the buccal bone or without adequate keratinized tissue are at higher risk of soft-tissue complications [31]. The use of guided surgery and careful surgical techniques can help ensure accurate implant placement. Soft-tissue preservation during implant surgery is critical to preventing PSTD. Minimally invasive techniques, such as flapless surgery or small flap designs, reduce trauma to the soft tissues and lower the risk of recession [54]. Preserving the integrity of the keratinized tissue surrounding the implant is also crucial for maintaining long-term peri-implant health in Figure 4. Patients should be educated about the importance of maintaining good oral hygiene around implants, as poor hygiene can contribute to peri-implantitis and subsequent soft-tissue dehiscence. Regular professional cleanings and assessments are essential for preventing plaque accumulation and monitoring for early signs of PSTD.

LIMITATIONS

Due to its narrative character and lack of adherence to a systematic review process, this review is susceptible to selection bias. Only English-language research was included in the evidence, potentially omitting relevant findings from studies published in other languages. Furthermore, direct comparisons of outcomes are hampered by the diverse study methods, sample sizes, and outcome measures across the examined publications. For the evaluation and treatment of PSTD, more robust evidence and standardized guidelines are required from future systematic reviews and meta-analyses.

CONCLUSIONS

PSTD presents significant challenges in dental implantology, necessitating prompt and accurate assessment for effective management. Advances in diagnostic imaging have enhanced the ability to evaluate and treat PSTD, contributing to better patient outcomes. Therefore, continued research and clinical innovation are essential for refining assessment methods and therapeutic approaches, ultimately improving the prognosis for patients with PSTD. To reduce the risk of PSTD, this review emphasizes the importance of sufficient keratinized tissue, appropriate implant location, and careful surgical technique. Clinical signs such as peri-implant inflammation, implant surface exposure, and mucosal recession emphasize the importance of prompt and precise evaluation. Clinicians can now more accurately identify and categorize PSTD using modern diagnostic techniques, such as probing and photographic recording, as well as sophisticated imaging modalities, such as CBCT. Soft tissue augmentation techniques, particularly connective tissue grafting, continue to provide the most consistent results in treating PSTD from a therapeutic standpoint. However, the variation in long-term stability underscores the need for further comparative research and standardized protocols. Preventive measures remain crucial for maintaining the health and aesthetics of the peri-implant area, particularly through patient education and strict maintenance regimens. In response, future studies should focus on improving PSTD classifications, validating novel evaluation instruments, and investigating minimally invasive regenerative methods to improve functional and aesthetic outcomes. As such, to create evidence-based recommendations that ensure the long-term viability of implant therapy, interdisciplinary collaboration among periodontists, prosthodontists, and oral surgeons will be essential.

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REVIEW ARTICLE

The use of eponymic bacterial nomenclature in medical case reports: Etymology and morphological models

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ABSTRACT

Aim: To provide healthcare professionals with relevant guidelines for the accurate utilization and comprehension of the origins of eponymic bacterial names when engaging in the genre of MCRs.

Materials and Methods: We examined the prevalence of eponymic names of bacteria in the issues of Journal of Medical Case Reports (2007 – 2025) and identified the key patterns of using bacterial nomenclature in this genre. The chosen terminological units underwent assessment through quantitative analysis, structural evaluation, etymological analysis, and contextual examination.

Conclusions: The appropriate use of bacterial nomenclature in medical case reports is paramount for the effective communication of clinical findings to colleagues across the globe. It also serves as a means to showcase one's competence and education in the field. Therefore, it becomes imperative to bring this terminological phenomenon to the attention of students, highlighting its prevalence in contemporary medical case reports.

KEYWORDS: medical terminology, eponym, bacterial nomenclature, medical case report

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INTRODUCTION

Medical eponyms are named after individuals who have made significant contributions to their discovery or understanding [1]. They are an essential component of the medical lexicon, serving as a reflection of the evolving stages of our understanding of reality, historical contexts, and the contributions of individuals to the advancement of medical science [2]. Eponyms find extensive use in the medical discourse for several reasons: they unveil the progression of medical research and practice, foster the continuity of scientific knowledge, and contribute significantly to the development of terminological competence among medical students [3]. Medical eponyms are widely used in virtually all areas of clinical and theoretical medicine, and such terms as "Alzheimer's disease", "Parkinson's disease" [4, 5], "Devic's opticomyelitis" [6], etc., clearly indicate the homage to the prominent scientists and their achievements. At the same time, while some derivations may seem quite apparent, there are numerous terms where the eponymic etymology is not immediately transparent [7]. In our previous study, we drew attention to such terms as "*Bordetella avium*" and "*Raoultella planticola*" [8], derived from the names of the Belgian immunologist and microbiologist Jules Bordet and the French bacteriologist Didier Raoult, respectively [7]. Consequently, awareness of these terms' etymology can prevent their incorrect spelling and use in the written discourse [7]. However, despite their significance, the characteristics and prevalence of eponyms in bacterial nomenclature have remained relatively unexplored, with only a few studies in this field so far [9, 10]. Therefore, our study seeks to bridge this research gap, enhancing the understanding of this terminological phenomenon among both medical professionals and linguists. Hence, the interdisciplinary nature of this research is evident.

We believe that the etymological study of eponymic bacterial names constitutes an essential component of a student's well-rounded medical education [11]. Moreover, it provides a practical tool to enhance student motivation, as delving into eponyms aids in grasping the origins of medical terms, thereby facilitating better

memory retention [12, 13]. This paper will also focus on the morphological models of eponymic bacterial nomenclature. In particular, we will emphasize the significance of mastering the key principles of Greek and Latin grammar, thereby ensuring correct spelling and punctuation when working with these terminological units.

Our prior research has comprehensively substantiated the paramount role of Greek and Latin terminologies in shaping all modern European languages, with an undeniable influence on the specialized language of medicine, particularly in English [14-18]. In the areas of modern medicine and biological sciences, the language of Latin and Greek, ancient and enduring, continues to exert its profound influence [19-21]. For instance, such bacterial names as *Kocuria rosea, Kocuria kristinae, Streptococcus mutans, Streptococcus sorbinus, Leuconostoc mesenteroides* [22, 23] or *Moraxella catarrhalis, Chlamydia pneumoniae* [24] obviously retain the traditional elements of Latin grammar in the species part of the term. Moreover, such modern terms as "leptin" [25] or "cathepsin" [26], coined in the 20th century, largely rely on the Greek lexicon. This fusion of ancient vocabulary with contemporary discoveries highlights the enduring legacy of Latin and Greek in the field of medical nomenclature. In other words, classical languages are widely used to name new phenomena in medicine and biology nowadays. In this study, we will demonstrate how the Greek and Latin naming conventions for bacterial species and genera follow a systematic structure, with genus names serving as the broader category and species names providing specific descriptors or associations. This binomial nomenclature helps maintain order and clarity in the field of microbiology and taxonomy.

We will focus on this terminological phenomenon in medical case reports (MCRs) because of the paramount importance of this genre, which proves highly effective for the global dissemination of scientific knowledge [27]. Consequently, it is essential to study the prevalence and contemporary usage of eponymic bacterial nomenclature within modern MCRs. This research endeavor aims to provide healthcare professionals with relevant guidelines for the accurate utilization and comprehension of the origins of eponymic bacterial names when engaging in the genre of MCRs.

AIM

To provide healthcare professionals with relevant guidelines for the accurate utilization and comprehension of the origins of eponymic bacterial names when engaging in the genre of MCRs.

MATERIALS AND METHODS

We examined the prevalence of eponymic names of bacteria in the issues of *Journal of Medical Case Reports* (*JMCRs*) [28], which have been published over the past 18 years, spanning from 2007 to 2025. The selection and scope of this material are driven by the journal's establishment in 2007 and its status as a prominent open-access peer-reviewed platform, ensuring effective tracking and analysis of major trends within the MCRs genre, and facilitating rapid information exchange among researchers worldwide. We selected eponymic bacterial nomenclature, using *The Latin-Ukrainian Medical Encyclopedic Dictionary* [1], *The Latin-Ukrainian Thesaurus of Clinical Terms* [29], *Bergey's Manual of Systematics of Archaea and Bacteria* [30] and the *List of Prokaryotic names with Standing in Nomenclature* database [31]. Next, we conducted an automated search on the journal's website (the "Articles" section, using the "Search articles within this journal" feature) to examine the occurrence and frequency of eponymic bacterial names in *JMCRs*. To identify the key patterns of using bacterial nomenclature in this genre, the chosen terminological units underwent assessment through quantitative analysis, structural evaluation, etymological analysis, and contextual examination. Quantitative examination allows for the collection of data to explore the numerical trends and prevalence of eponymic names of bacteria. Structural methodology, on the other hand, seeks to elucidate and dissect the constituent components and attributes of a term across various levels, encompassing aspects like morphology (features related to word construction) and syntax (the principles governing phrase formation). Structural analysis serves as a potent tool for comprehending the inherent language patterns and regularities. Etymological analysis, in turn, unveils the origins of words and their evolution through centuries. Lastly, contextual analysis involves the in-depth exploration and understanding of a term in its broader contexts, including the historical, social, and cultural ones.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

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

REVIEW AND DISCUSSION

In the first stage of our research, we analyzed eponymic bacterial names quantitatively, and identified the most common bacterial eponyms in *JMCRs*. A total of 48

Table 1. The prevalence of eponymic bacterial genera in *JMCRs* as of 2025

No.	Eponymic names of bacterial genera	Number of results in JMCRs as of 2025	Examples from JMCRs
1	*Escherichia*	198	"It has been shown that the majority of cases of EPN are caused by Escherichia coli, a finding that is consistent with the present case" (2025).
2	*Klebsiella*	124	"Systemic infections caused by Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae strains have increased in many European regions" (2019).
3	*Salmonella*	69	"This case highlights the novelty of a Salmonella enteritidis-induced septic sacroiliitis in a previously healthy adolescent of Ladino ethnicity, emphasizing the importance of considering atypical pathogens" (2025).
4	*Brucella*	65	"A variety of Brucella species, such as Brucella abortus, Brucella melitensis, and Brucella suis, have the potential to cause infectious complications affecting the kidneys" (2025).
5	*Borrelia*	50	"Although Borrelia burgdorferi-induced Lyme disease may occasionally affect balance and hearing indirectly, Lyme-related vestibular symptoms are usually part of a larger systemic picture" (2025).
6	*Neisseria*	45	"A 29-year-old Caucasian male presented with a monkeypox virus proctitis that recurred after treatment for a documented Neisseria gonorrhoeae and Chlamydia trachomatis coinfection" (2023).
7	*Bartonella*	34	"Bartonella henselae deoxyribonucleic acid was detectable in the blood samples and cardiac valve with vegetation paraffin-fixed tissue samples" (2023).
8	*Rickettsia*	34	"The result of immunofluorescence antibody testing for rickettsial immunoglobulin G was strongly positive for Rickettsia conorii with a rise in titer convalescent sera" (2018).
9	*Coxiella*	32	"We suggest that Coxiella burnetii might trigger autoimmune liver disease" (2009).
10	*Yersinia*	27	"Yersinia enterocolitica is one of the species in this genus that can cause human diseases. The other two are Yersinia pseudotuberculosis and Yersinia pestis" (2016).
11	*Nocardia*	25	"Multidrug-resistant Nocardia wallacei was cultured from a stereotactic brain biopsy and confirmed by 16S ribosomal ribonucleic acid gene sequencing" (2013).
12	*Leishmania*	25	"To the best of our knowledge, this is the first case of cutaneous leishmaniasis by Leishmania donovani in a child" (2014).
13	*Shigella*	23	"Similarly, Shogbesan et al. described a case of Shigella sonnei bacteremia in an otherwise healthy patient with a prior history of Roux-en-Y gastric bypass" (2021).
14	*Listeria*	21	"…meningitis could be caused by atypical organisms such as Listeria monocytogenes, Cryptococcus neoformans, Acanthamoeba species, or herpes simplex virus" (2021).
15	*Burkholderia*	18	"Melioidosis is a serious disease caused by the bacterium Burkholderia pseudomallei which affects humans and animals" (2023).
16	*Serratia*	18	"Serratia marcescens, a rod-shaped facultative anaerobe bacterium is a Gram-negative pathogen that can be found in respiratory and urinary tracts" (2023).
17	*Prevotella*	17	"Porphyromonas gingivalis, Tannerella forsythensis, and Prevotella intermedia are often detected in periapical biofilms" (2018).
18	*Morganella*	12	"…the regrowth of meropenem-sensitive Morganella morganii in CSF cultures" (2021).
19	*Francisella*	10	"Francisella tularensis, a facultative intracellular Gram-negative bacterium, has rarely been reported as an agent of pericarditis, generally described as a complication of tularemia sepsis" (2008).
20	*Bordetella*	10	"Bordetella bronchiseptica can be a cause of virulent pneumonia in humans with impaired immune systems" (2009).
21	*Eikenella*	8	"(…) Eikenella corrodens, and Kingella kingae group that may cause about 3% of the total endocarditis cases" (2009).
22	*Moraxella*	7	"His ICU course was further complicated by Moraxella catarrhalis pneumonia, for which he completed a course of imipenem" (2012).

Table 1. Cont.

23	*Kingella*	6	"Kingella kingae, though part of the normal upper respiratory tract and genitourinary tract, is increasingly being recognized as an important human pathogen" (2009).
24	*Babesia*	5	"…Babesia microti, and Borrelia burgdorferi) in addition to a Lyme antibody immunoblot test" (2023).
25	*Ehrlichia*	5	"Ehrlichia chaffeensis was first described as a human pathogen in 1986 and E. ewingii in 1996" (2008)
26	*Edwardsiella*	3	"Extraintestinal infections caused by Edwardsiella tarda in humans are rare and occur in the presence of some risk factors" (2016).
27	*Elizabethkingia*	3	"Microbiological sampling from his anterior chamber and posterior segment revealed a culture of Elizabethkingia meningoseptica" (2011).
28	*Pasteurella*	3	"We present a case of septic shock in an elderly woman due to Pasteurella multocida" (2015).
29	*Veillonella*	3	"Blood cultures revealed Veillonella parvula bacteremia, with subsequently repeated magnetic resonance imaging" (2022).
30	*Collinsella*	2	"…the relative abundances of genera Alistipes, Bifidobacterium, and Lactobacillus and the species Collinsella aerofaciens are inversely associated with IBS" (2019).
31	*Kluyvera*	2	"To the best of our knowledge, this is the first case report of a multidrug resistant Kluyvera ascorbata isolated from the blood in an adult patient with sepsis" (2010).
32	*Kocuria*	2	"Cultures of his CSF and sputum revealed the presence of Kocuria varians/rosea and Staphylococcus aureus, respectively" (2014).
33	*Ralstonia*	2	"Ralstonia mannitolilytica is an emerging opportunistic pathogen that is associated with severe disease, including septic shock" (2019).
34	*Raoultella*	2	"Raoultella Planticola is a facultative anaerobic, gram-negative, water- and soil-dwelling rod bacterium rarely reported as a cause of human disease" (2021).
35	*Tannerella*	2	"The pretreatment bacteriological examination showed that the counts of Porphyromonas gingivalis, Tannerella forsythia" (2023).
36	*Bergeyella*	1	"Bergeyella zoohelcumi" (mentioned in a table) (2017).
37	*Blautia*	1	"After FMT, Bacteroides (61.9%) increased, whereas proportions of Blautia (0.25%), ClostridiumXlVa (1.0%), Lachnospiraceae (1.6%), Fusobacterium (9.2%) were decreased" (2021).
38	*Bosea*	1	"Microimmunofluorescence serologies performed on two samples of serum drawn on day 1 and on day 8 remained negative for Bosea vestrii" (2009).
39	*Cedecea*	1	"Cedecea lapagei bacterium was discovered in 1977 but was not known to be pathogenic to humans until 2006" (2018).
40	*Erwinia*	1	"Whereas Erwinia and Tatumella organisms have not been associated with central nervous system infection, Kluyvera meningitis has been reported in a newborn" (2014).
41	*Ewingella*	1	"Here we report a case that was caused by Ewingella americana and thus required different considerations of treatment" (2014).
42	*Robinsoniella*	1	"Robinsoniella peoriensis was recently identified as a Gram-positive, spore-forming, anaerobic bacillus" (2012).
43	*Rothia*	1	"Rothia mucilaginosa is a Gram-positive bacterium occurring as a commensal in the oral cavity and upper respiratory tract" (2019).
44	*Shewanella*	1	"Shewanella putrefaciens group" (mentioned in a table) (2017).
45	*Tatumella*	1	"Indeed, P. calida is closely related to members of the Enterobacteriaceae genus Erwinia, Tatumella, Kluyvera" (2014).
46	*Tsukamurella*	1	"Serial sputum cultures grew Tsukamurella pulmonis, a rare pathogen associated with cavitary pneumonia in immunocompromised patients" (2008).
47	*Weeksella*	1	"Using API 20NE (bioMérieux, Marcy l'Etoile, France), two isolates initially identified as Weeksella virosa" (2008).
48	*Yokenella*	1	The aspirated pus grew Yokenella regensburgei, for which the patient was treated with appropriate antibiotics (2016).

Source: compiled by the authors of this study

eponymic bacterial genera were identified, showcasing their substantial presence across *JMCRs* articles, with a cumulative count of 925 instances of usage. Table 1 demonstrates the diversity of bacteria encountered in clinical practice and provides some examples from the published issues.

These findings indicate the varying prevalence of different eponymic bacterial genera in JMCRs. As shown in Table 1, *Escherichia* is the most prevalent eponymic bacterial genus, with 198 mentions in the articles. *Klebsiella* ranks second, appearing 124 times. Salmonella, Brucella, and Borrelia follow, with 69, 65, and 50 mentions, respectively. Among the analyzed eponyms, genera mentioned up to 50 times include *Neisseria* (45), *Bartonella* (34), *Rickettsia* (34), *Coxiella* (32), *Yersinia* (27), *Nocardia* (25), and *Leishmania* (25). Those cited up to 25 times include *Shigella* (23), *Listeria* (21), *Burkholderia* (18), *Serratia* (18), *Prevotella* (17), and *Morganella* (12). Less commonly represented genera include *Francisella* (10) and *Bordetella* (10), followed by *Eikenella* (8), *Moraxella* (7), *Kingella* (6), *Babesia* (5), and *Ehrlichia* (5). Several genera are mentioned only a few times, such as *Edwardsiella* (3), *Elizabethkingia* (3), *Pasteurella* (3), *Veillonella* (3), *Collinsella* (2), *Kluyvera* (2), *Kocuria* (2), *Ralstonia* (2), *Raoultella* (2), and *Tannerella* (2). Finally, a number of bacterial genera appear only once in the analyzed corpus, including *Bergeyella*, *Blautia, Bosea, Cedecea, Erwinia, Ewingella, Robinsoniella, Rothia, Shewanella, Tatumella, Tsukamurella, Weeksella*, and *Yokenella*.

In addition to bacterial genera, there are also several eponymic diseases in *JMCRs* with varying frequencies among different conditions. Hence, brucellosis stands out with 51 mentions; leishmaniasis follows with 40 cases; salmonellosis is mentioned in 10 papers; rickettsiosis is less frequently described, with 5 cases, babesiosis and listeriosis are mentioned 3 times, yersiniosis – twice, while shigellosis appears once in *JMCRs*.

In the field of microbiology and taxonomy, the naming of bacterial species and genera follows a standardized system known as binomial nomenclature, which was established by Carl Linnaeus in the 18th century. This system uses Greek and Latin to create scientific names for organisms. These names typically consist of two parts: the genus name (capitalized and italicized) and the species epithet (italicized).

Genus name is the first part of the term, a noun in the nominative singular form. It is always written with an initial capital letter. It is the broader category that groups related species together based on shared characteristics. For example, in the case of *"Escherichia coli," "Escherichia"* is the genus name. These names are often chosen to honor a scientist, describe a characteristic, or have historical significance.

Species name is the second part of the term, an adjective in the nominative singular form that agrees in gender with the genus name. It is always written in lowercase and is typically an adjective that describes a characteristic of the organism or its habitat. For instance, in *"Escherichia coli," "coli"* refers to the organism's habitat, the colon, and in *"Klebsiella pneumoniae", "pneumoniae"* indicates its association with lung infections. It is necessary to point out that species names can also honor individuals, describe physical features, or have other meaningful associations, e.g. *Borrelia burgdorferi* honors Dr. Willy Burgdorfer, who discovered it.

Subspecies and strain names: in some cases, there may be further subdivisions within a species, such as subspecies or strains. These names follow the genus and species names and can also be descriptive or honor individuals. For instance, *"Escherichia coli O157:H7"* denotes a specific strain with particular characteristics.

The use of Greek and Latin in scientific names allows for a universal and standardized way of referring to organisms. It ensures clarity and consistency in taxonomy and helps scientists communicate effectively across languages and regions [16]. Additionally, the use of descriptive terms in species names often provides insights into the characteristics, habitat, or historical context of the organism, which can be valuable information for researchers and clinicians. Hence, the Greek and Latin naming conventions for bacterial species and genera follow a systematic structure, with genus names serving as the broader category and species names providing specific descriptors or associations.

Therefore, remembering and adhering to Latin grammar rules when spelling and forming scientific names in microbiology and taxonomy is of utmost importance for several reasons. First, it ensures clarity and precision, which are essential for effective communication among scientists, researchers, and clinicians worldwide. Next, it provides grounds for universal understanding: Latin is a universal language in science, and using it in scientific names allows researchers from different linguistic backgrounds to understand and communicate effectively. It transcends language barriers, ensuring that the same name is used and understood globally. This universal understanding is crucial for collaboration and the exchange of scientific knowledge. Furthermore, the rules of Latin grammar help maintain consistency in taxonomy, which is vital for organizing and categorizing the vast diversity of life on Earth. Correctly formed names facilitate the grouping of related organisms into genera and species, ensuring that these classifications accurately reflect evolutionary relationships and shared characteristics. Another essential aspect is respect for tradition, as Latin has a long tradition of use in scientific

nomenclature, dating back to the time of Carl Linnaeus. Respecting this tradition by adhering to Latin grammar rules pays homage to the history and foundations of taxonomy and microbiology. It can also help in preventing ambiguity and misinterpretation. Deviations from Latin grammar rules can lead to confusion and hinder the accurate identification of organisms, potentially affecting research outcomes or clinical diagnoses.

In particular, highlighting specific Latin grammar rules, such as the use of the genitive singular forms for nouns and the importance of gender agreement in adjectives, is crucial when forming scientific names for bacterial genera and species. For instance, in Latin grammar, the genitive singular form "ae" is used for feminine nouns, which is often observed in the species part of the binomial name. In addition, Latin adjectives must agree in gender, number, and case with the nouns they modify. This means that when forming a scientific name, the adjectives used to describe the characteristics of the species or genus must match the gender of the genus name. For example, if the genus name is feminine, adjectives used to describe a species within that genus should also be in the feminine form.

By remembering these Latin grammar rules, scientists and researchers in the field of microbiology and taxonomy can ensure the accurate formation of scientific names. This adherence to proper grammar not only maintains clarity and precision but also upholds the tradition and integrity of scientific nomenclature. It is essential for effective communication, both within the scientific community and among those who rely on accurate scientific names for research, diagnosis, and classification.

In this educational context, we believe that providing basic biographical information in the classroom holds substantial educational value. This historical context not only provides insight into the contributions of these scientists but also helps researchers and students connect with the history of microbiology. It highlights how the work of these individuals laid the foundation for our current understanding of bacteria and infectious diseases. This approach embeds the achievements of scientists in a meaningful historical context, enabling medical students to better comprehend the historical backdrop. They can compare similar innovations or discoveries in their own country and internationally, thus gaining a deeper understanding of the evolution of medical science over time. All these elements collectively contribute to the improved memorizing and accurate usage of eponyms in students' future professional endeavors.

Table 2 demonstrates our approach to presenting the etymological aspects of eponymic bacterial nomenclature to medical students in the classroom.

Table 2 provides several insights into the nature of some eponymic bacterial nomenclature. Our study demonstrated that eponyms are effective means of knowledge continuity: the tradition of naming bacterial genera and species after notable bacteriologists and scientists who have made significant contributions to microbiology ensures that the legacy and work of these individuals continue to be recognized and remembered in the field. As one can observe, the majority of eponymic bacterial genera stem from the last names of scientists, with several exceptions, e.g., *Erwinia,* which derives from the researcher's first name. The detected terms are formed by adding the suffixes "-ia", "-ella", "-iella", and "-a" to the stem. An interesting case is also the eponym *Elizabethkingia,* which contains both the first name and last name of the researcher. Moreover, the name of Elizabeth Osborne King is reflected in two eponyms in the frame of our research material: *Kingella* and *Elizabethkingia*. It is also necessary to point out that some eponyms, such as *Borrelia burgdorferi* and *Rickettsia slovaca*, are named after geographic locations where the organisms were first isolated. This practice provides information about the geographical distribution of bacterial species.

Another interesting finding is the revealed scientific contribution in bacteriology by country, which can also be used in the classroom to increase students' intercultural competence and readiness to work in the international professional setting. Table 2 reflects contributions from scientists of various nationalities, emphasizing that medical research is a global effort, and eponyms bridge cultural and geographic boundaries. The studied bacterial genera were named after doctors from various countries, including the United States, the United Kingdom, France, Germany, Italy, Argentina and Peru, Switzerland, Japan, Slovakia, Romania, and others. The majority of the scientists mentioned are affiliated with the United States, with numerous bacterial species named after American microbiologists or associated with U.S. research institutions. This analysis highlights how eponyms play a valuable role in the continuity of knowledge and recognition of scientific contributions in bacteriology. It also underscores the global nature of scientific research, with scientists from various countries contributing to our understanding of bacterial diversity and classification.

Hence, eponyms in bacterial nomenclature serve an educational purpose by allowing students and researchers to learn about the scientists and their contributions to the field. This historical and contextual information enhances the understanding of bacteriology as a science. Through eponyms, the legacies of

Table 2. Etymological features of some eponymic bacterial nomenclature in JMCRs

No.	Eponymic names of bacterial genera	Reference information
1	*Babesia*	Named after Victor Babeș (1854-1926), a Romanian physician and bacteriologist. He co-authored one of the earliest bacteriology treatises in 1885.
2	*Bartonella*	Alberto Barton (1870-1950) was a microbiologist originally from Argentina but later affiliated with Peru. Cf. Bartonella henselae, of Hensel, named to pay tribute to Diane M. Hensel, who was responsible for isolating a significant number of the original strains found in bacteremic patients.
3	*Bergeyella*	Named after the American bacteriologist David H. Bergey (1860-1937), known for their contributions to taxonomy.
4	*Blautia*	Named in recognition of Michael Blaut, a German microbiologist renowned for his work in gastrointestinal microbiology.
5	*Bordetella*	Jules Bordet (1870–1961), along with Octave Gengou (1875-1957), isolated the organism responsible for pertussis.
6	*Borrelia*	Named after Amedée Borrel (1867-1936), a French bacteriologist. Cf. Borrelia burgdorferi is named after Willy Burgdorfer.
7	*Bosea*	Honoring Sir Jagadish Chandra Bose (1858-1937), founder of the Bose Institute where the type species Bosea thiooxidans was isolated.
8	*Brucella*	Named after Sir David Bruce (1855–1931), who first identified the organism causing undulant (Malta) fever. Cf. Brucella melitensis, associated with the Island of Malta (Melita), where it was initially isolated.
9	*Burkholderia*	Named after Walter Hagemeyer Burkholder (1891-1983), the American bacteriologist.
10	*Cedecea*	Named after the abbreviation CDC by Patrick A. D. Grimont and Francine Grimont at the Centers for Disease Control, Atlanta, Georgia.
11	*Collinsella*	Honoring Matthew D. Collins, an English microbiologist known for his contributions to microbial taxonomy and phylogeny.
12	*Coxiella*	Named after Harold R. Cox (1907-1986), who first isolated this organism in the United States. Cf. Coxiella burnetii is named after Frank MacFarlane Burnet, the first researcher to investigate the characteristics of this organism.
13	*Edwardsiella*	Named after American bacteriologist Philip Rarick Edwards (1901-1966).
14	*Ehrlichia*	Named after Paul Ehrlich (1854-1915), a German physician and scientist who made significant contributions to immunology and chemotherapy. Cf. Ehrlichia chaffeensis, associated with Fort Chaffee in western Arkansas, where the patient from whom the initial isolate was prepared was identified. Ehrlichia ewingii is named in recognition of Sidney A. Ewing for his groundbreaking work with this agent.
15	*Eikenella*	Named after M. Eiken, a Scandinavian biologist who first classified the type species.

Source: compiled by the authors of this study

pioneering scientists are preserved, ensuring that their names and achievements are remembered and celebrated within the scientific community. The analysis of the data from the table reflects the diversity of bacterial genera, the global nature of scientific research, and the historical and contemporary significance of scientists' work. Eponyms play a multifaceted role in recognizing and honoring these contributions while facilitating communication and knowledge continuity in the field of microbiology.

Our findings demonstrated the prevalence and significance of eponymic bacterial nomenclature within the corpus of *JMCRs*. The etymological origins of these names were revealed, shedding light on the historical figures whose contributions led to their immortalization in the world of microbiology. Utilizing the information found in Table 2 within the classroom setting can significantly enhance the processes of understanding and memorizing eponymic bacterial nomenclature. This, in turn, has the potential to boost students' motivation to learn, as it places terms within a meaningful context [12] and contributes to the development of their terminological competence – a fundamental skill in medical education [11].

Furthermore, this study revealed common errors in the spelling of certain bacterial names, emphasizing the need for precision and accuracy in medical terminology. Spelling errors in bacterial names can occur due to various reasons, including typographical mistakes or lack of familiarity with Latin grammar rules. Some common errors in spelling bacterial names include typos and misspellings; incorrect capitalization; incorrect gen-

der agreement; transposition of letters. To avoid these common errors, it is essential to refer to authoritative sources, double-check spellings, and ensure adherence to Latin grammar rules when using or reporting bacterial names in scientific writing.

For instance, in *JMCRs,* we detected a misspelling of bacterial genus *Ehrlichia:* "Infectious and autoimmune etiologies were investigated with complete blood count, C-reactive protein (CRP), erythrocyte sedimentation rate, antinuclear antibodies (ANA), blood culture, and serum analysis for antibodies directed against *Treponema pallidum*, hepatitis C, and tick borne diseases (*Erlichia chaffeensis, Anaplasma, Babesia microti,* and *Borrelia burgdorferi*) in addition to a Lyme antibody immunoblot test" (2023) [28]. In addition, we found a non-italicized spelling of bacterial genera in an article from *JMCRs:* "Further serum antibody tests were ordered for Listeria monocytogenes, Borrelia burgdorferi, Mycoplasma pneumoniae (MP) and Ureaplasma ureolyticum" (2021) [28].

Our study did not find a significant number of errors in the use of eponymic bacterial names in *JMCRs*. However, we found numerous cases across other journals, e.g., in the PubMed database, for instance, *Neiseria gonorrhoeae* (missing "s") [33]; *Erlichia chaffeensis* (missing "h") [34]; *Weaksella virosa* ("a" instead of "e") [35]; *Fransicella tularensis* (transposition of letters "c" and "s") [36]; *Burkholdria* (missing "e") [37] and others. Therefore, it is obvious that this terminological phenomenon remains a crucial aspect that demands meticulous attention in medical education in order to minimize and prevent potential errors in medical discourse.

To address these challenges, we developed several guidelines and recommendations aimed at facilitating the correct utilization of eponymic bacterial nomenclature by medical professionals. In such a manner, we advocate the importance of a comprehensive understanding of a term's etymology as a powerful tool for preventing and reducing errors in medical case reports.

1. Italicizing bacterial genera: Thines et al. (2020) argue that italicizing taxonomic ranks, including genus and species names, aids in their rapid recognition and differentiation from informal names [32].

2. Initial use: The first time a bacterium is mentioned in a research article, it is written in full. This provides readers with the full taxonomic information.

3. Uppercase, lowercase and punctuation: All subsequent mentions in the text should be abbreviated according to the following capitalization pattern: "*E. coli*".

Gaining insight into the origins of eponyms is equally crucial. Familiarity with the background and historical context of eponymous terms can assist medical students and researchers in using them accurately. We endorse the integration of eponyms exploration as a fundamental aspect of medical education and ongoing professional development for doctors, with the aim of reducing and mitigating errors in medical discourse. Achieving this goal may involve the development of informative dictionaries and other reference materials dedicated to medical eponyms, offering comprehensive background information about the individuals whose names have been immortalized in these terms.

By following these guidelines, we ensure consistency and precision in our scientific writing, facilitating effective communication in the realm of microbiology and beyond. Accurate representation of bacterial genera is essential in maintaining the integrity of scientific literature and promoting clarity in research publications.

Thus, the appropriate application of bacterial nomenclature in medical case reports plays a pivotal role in effectively communicating clinical findings to a global audience of healthcare professionals. It not only reflects the competence and education of medical practitioners but also contributes to the dissemination of scientific knowledge. As such, it is imperative to introduce students to the prevalence of eponymic bacterial nomenclature in contemporary medical case reports, equipping them with the knowledge and skills needed for their accurate usage while appreciating the historical context. Incorporating the study of these terms into medical education and ongoing professional development is essential to foster interdisciplinary growth in modern medical practice.

CONCLUSIONS

In the constantly evolving landscape of medical literature and scientific communication, the accurate use of bacterial nomenclature in medical case reports stands as a keystone for effective knowledge dissemination. MCRs reports serve as vital media through which clinicians and researchers share their insights, experiences, and findings with peers and colleagues worldwide. It is not merely a matter of lexical correctness but a demonstration of one's competence and excellence within the medical field. This emphasizes the significance of instilling an understanding of this terminological phenomenon. The awareness and comprehension of eponymic bacterial nomenclature, encompassing its historical underpinnings and intricacies, are essential tools for students in their medical education. Equipped with this knowledge, they are better prepared to navigate the multifaceted world of medical terminology and use these terms accurately and confidently. Such familiarity empowers them not only to employ these terms correctly but also to appreciate the context and significance of their origins.

Thus, the study of this unique category of terms should form an integral and indispensable component of medical education and ongoing professional development for healthcare practitioners.

Embracing this holistic approach to medical education and terminology enhances the learning experience, fostering an interdisciplinary and synergistic outlook among future healthcare specialists. These individuals, armed not only with clinical expertise but also a profound understanding of the linguistic and historical facets of their field, will be able to make substantial contributions to the ever-advancing realm of medical science. Through this comprehensive growth, they can bridge the gap between the medical past and future, ensuring the continued progress and excellence of the healthcare profession on a global scale.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

Exploring the potential and challenges of artificial intelligence (AI) for intervention and prevention of suicidality to achieve SGDs: A thematic review

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ABSTRACT

Aim: To explore the potential and challenges of Artificial Intelligence for the intervention and Prevention of Suicidality to achieve the SDGs.

Materials and Methods: A thematic review was conducted whereby the databases included a) EBSCO *host,* b) Sociological Abstracts, c) Embase, and d) SCOPUS. A total of 10 papers (published since 2015-2026) were sampled and analysed by using Braun and Clark's reflexive thematic analysis.

Conclusions: AI can be used effectively to identify, intervene and prevent suicidality. Ethical considerations are important, and appropriate utilisation can help achieve SGDs relevant to mental health.

KEYWORDS: Suicidality, Suicide, Artificial Intelligence, Identification, Prevention, SGDs

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INTRODUCTION

Suicidality is a key health, social, and psychological phenomenon globally [1]. Younger populations are at increased risk of developing suicidality, leading to one of the leading causes of death among individuals aged between 16 and 30 years [2]. Suicidality has been widely researched across various contexts, including age-related differences, economic domains, cultural explanations, social factors, health-related domains, religious dimensions, and health indicators [3]. The dimensions are explored in the context of contributing to suicidality as risk factors, as well as in management and prevention.

Artificial Intelligence (AI) is a revolution of the current decade [4]. It is part of routine life, healthcare, education, and sports, and, in short, it impacts each sector of life. AI development and utilisation are expanding in daily life. The healthcare sector is a significant beneficiary of AI technology [5]. AI is revolutionising healthcare by assisting with diagnosis, treatment, and personalised recommendations to improve health indicators. Therefore, research shows that AI is significant for achieving health-related SGDs [6].

With the power of revolution in general and in healthcare, will AI be powerful enough to be utilised for identifying suicidality patterns, and will treatment and prevention remain key questions? Keeping this question in view, this work provides a prospective view of the utilisation of AI for the management of suicidality, with a focus on potential and challenges, which is the rationale for this study. To date, there are few studies on the theme of AI and suicidality. Stokel-Walker explored AI-driven suicidality. The study provides grounds for the importance of AI in relation to suicide and related behaviours [7]. On the other hand, AI has the potential to identify suicide-related behaviours. Parsapoor Mah Parsa and colleagues examined the importance and potential role of Machine Learning models and algorithms in analysing text to identify suicidal behaviours [8]. Social media content, including writing, posting, and interactions with other users, supports multimodal behavioural analysis, including mental status assessment. Mental status is important in connection with suicidal

Table 1. Sampled Papers

S. No	Authors	Title	Methodology	Major Findings
1	Liu et al. (2025)	Proactive suicide prevention online (PSPO): machine identification and crisis management for Chinese social media users with suicidal thoughts and behaviours	microblog group online; best performing model	The PSPO model is suitable for identifying populations that are at risk of suicide. When followed up with proactive crisis management, it may be a useful supplement to existing prevention programs because it has the potential to increase the accessibility of anti-suicide information to people with suicidal thoughts and behaviours, but a low motivation to seek help.
2	Lekkas et al. (2021)	Predicting acute suicidal ideation on Instagram using ensemble machine learning models	Ensemble machine learning model	Online Social Networking Data (SN) can provide insight into suicidal behaviour due to its rich data stream.
3	Ramírez-Cifuentes et al. (2020)	Detection of suicidal ideation on social media: multimodal, relational, and behavioral analysis	Experimental analysis with 2 control groups	Social media content, such as writing, posting, and interactions with other users, supports multimodal behavioural analysis, including mental status assessment.
4	Hopkins et al. (2024)	Technology in Suicide Prevention: Fears and Functionality for Crisis Supporters, Human Behaviour and Emerging Technologies	Thematic Survey design	AI technologies can be effective only if they are perceived as useful by the users.
5	Lejeune et al. (2022)	Artificial intelligence and suicide prevention: a systematic review	Systematic review	AI has significant potential for the identification of the risk of suicide among vulnerable individuals; however, ethical issues are also important to be considered.
6	Biscaia Fernández et al. (2026)	La inteligencia artificial en la prevención de conductas suicidas: aspectos técnicos y consideraciones ético-legales	Analysis of social networking environment	Social networking can be analysed as an important source for identifying suicidality using machine learning (AI).
7	Mondragón-Barrios et al. (2026)	Ethical challenges of using artificial intelligence in suicide prevention: a literature review	Literature Review (A non-systematic review)	The sensitive and confidential nature of data regarding suicide is posing a major threat to the extensive utilisation of AI for suicide prevention.
8	Stokel-Walker (2025)	AI-driven psychosis and suicide are on the rise, but what happens if we turn the chatbots off?	Investigative commentary with narrative case study	Chatbots seem to help individuals with mental health issues when they are alone; however, it is harmful in the long term, including developing suicidality.
9	Parsapoor et al. (2023)	Suicide risk detection using artificial intelligence: the promise of creating a benchmark dataset for research on the detection of suicide risk	Benchmark data set	A vocal and textual dataset can identify suicidal ideation. At this point in time, standard dataset based in logical AI are not available
10	Mokheleli et al. (2026)	Artificial intelligence in suicide risk assessment: a systematic literature review	Systematic Review	AI cannot replace traditional methods of intervening in suicidality; however, it can be a useful tool. Ethical considerations are very important when using AI for the identification and intervention of suicidality.

Source: compiled by the authors based on [7, 8, 9, 10, 17, 19, 21, 22, 28, 30]

ideation [9]. Hopkins and colleagues found that there are motivations for utilising Machine Learning tools (AI) for preventing suicides; however, attitudes towards AI can also be a barrier [10].

Considering the above arguments, this study extends the debate by situating it within theoretical explanations and the Sustainable Development Goals (SDGs) to improve mental health. Improving mental health is an important way to intervene and prevent suicidality. In this connection, this research aims to understand the link between existing theoretical explanations and AI utilisation for suicidality; the potential and challenges of utilising AI in the identification and management of suicidality; and the importance of AI utilisation in achieving the SDGs.

AIM

The aim of this study is to explore the potential and challenges of Artificial Intelligence for the intervention and Prevention of Suicidality to achieve the SDGs. The specific objectives are:

- To explore the potential of AI and challenges to utilisation in the identification and management of suicidality.
- To develop an understanding of AI utilisation as per available theoretical frameworks
- To align the effective utilisation of AI for identifying and managing suicidality for achieving SDGs regarding mental health and well-being
- To extract the findings regarding the status of AI and its utilisation regarding suicidality

MATERIALS AND METHODS

This study utilised the available body of knowledge regarding AI and its role in identifying and managing suicidality. For this purpose, a systematic review was conducted whereby the databases included a) EBSCO *host,* b) Sociological Abstracts, c) Embase, and d) SCOPUS. The keywords included

1) Suicide AND Artificial Intelligence
2) Artificial Intelligence AND Suicide Prevention
3) Artificial Intelligence AND Suicide Prevention AND Sustainable Development Goals
4) Artificial Intelligence AND Suicide Prevention AND Sustainable Development Goals AND Mental Well-being

The search strategy was limited to peer-reviewed literature only. In addition, the latest research was considered, i.e., research published since 2015. In the last decade, AI has brought about a revolution, which is a core dimension of this study. Further, a total of 10 research papers are sampled for this study. The sample size is justified according to Malterud and colleagues' recommendations for determining sample size in qualitative research [11]. This sample size technique is based on the philosophy that sampled papers must be best aligned with the objectives of the studies so that they provide an in-depth understanding for answering the research question. The details of the sampled papers are presented in table 1.

Bias was reduced when sampling research papers by reviewing them alongside 2 social science experts from academia. After cross-checking the abstract and a pool of selected papers, 11 papers were sampled. The 2 experts were provided with purposeful criteria, including a) the paper must be relevant to suicidality, b) AI-based intervention for mental health and suicide, and c) papers on AI with a specific focus on mental health and SGDs. The exclusion criteria included a) papers regarding the general context of mental health and AI; b) papers explaining AI in a general context. Finally, Braun and Clark's reflexive thematic analysis has been used to analyze the information from the sampled paper. A six-step mechanism was used to analyse the data [12].

REVIEW AND DISCUSSION

THEME ONE: THE POWER OF AI IN THE IDENTIFICATION OF HEALTH-RELATED INDICATORS

There is a substantial body of research on the role of AI in health assessment. In this context, AI is used to assess patterns of physical activity, such as steps, SP2 levels, and heart rate. Currently, advanced AI gadgets can even assess heart rhythms [13]. There is a need to expand AI technology for assessing suicidality. The proposed innovation in this regard is to integrate AI technologies that can correlate stress with agitation, changes in activity, concentration patterns, and even speech. For example, a Samsung watch noting higher stress levels, leading to sudden changes in activity and speech tone, can be assessed as a warning sign. However, how to integrate it remains a key question for AI developers. The work by Al Abdi and colleagues is relevant, as it illustrates AI's capability in assessing stress levels [14]. Further, digital phenotyping is a useful way to understand time-to-time variation in human behaviour. Wearables have the capability to understand the patterns of variation in human behavior, where sleep disorders linked with suicidal ideation among marines have been detected through digital phenotyping [15] (Table 2).

THEME TWO: CORRELATION OF ACTIONS AS PRECURSORS OF SUICIDALITY

Research shows that suicidality results from multifaceted factors. There are certain variables which are directly correlated with suicidality, including depression, impulsivity and stressful life events [1]. In addition, there are certain factors which play mediating and moderating roles, such as familial negation, moderating the role between depression and suicidality. Loneliness can mediate the relationship between depression and suicidality. AI can be utilized to understand patterns of social interaction (for example, within a family), as well as stress and loneliness. Improved AI integration can assist mental health services in identifying precursors to suicidality [16]. Another example in this regard is noticing incidents of impulsive behavior, such as several aggressive statements. These incidents can be analyzed

Table 2. Braun and Clark (2019) Thematic Table

First Order Codes	Second Degree Concepts	Overreaching Themes
• Advanced AI gadgets can even assess heart rhythms[13] • digital phenotyping is a useful way to understand time-to-time variation in human behaviou [15]	Assessment Variation Identification gadgets Stress level	Theme One: The Power of AI in the Identification of Health-Related Indicators
• AI can be utilised to understand the patterns of social interaction[16] • AI have the capability to predict suicidality among individuals with mental health issues [18] • noticing incidents of impulsive behaviour, such as several aggressive statements[17]	Patterns Capability Physiological Mental Predict	Theme Two: Correlation of Actions as Precursors of Suicidality
• AI models can analyse phone text and social media activity to predict suicidal behaviours [19] • help-seeking agencies remain the most important stakeholder [20] • AI has the capacity to be used for personalised care for individuals with suicidal ideation and increased risk of suicide attempts [21]	Text Social media communication Risk Agencies Care	Theme Three: The Link between Source and Healthcare Services Sub-theme One: Help Seeking Agencies, AI detection and Suicidality Sub-theme Two: Personalised care
• Taking records from routine activities of individuals poses a significant challenge [22] • AI-based assessments are not error-free [24] • Individuals with suicidal ideation can be targeted for medical business purposes [23]	Records Errors Business purpose Ethics Targeted	Theme Four: Ethical Considerations Sub-theme One: Biases and Errors
• Targets such as 3.4, 3.5 and 3.8 are explicitly about mental health. • AI can be effectively utilised for sustainable mental well-being [25] • AI is used for personalised programmes for physical and mental health and plays a crucial role in diagnosis and treatment [26] • The developing world is lagging far behind the developed world in terms of technological advancement [27] • AI cannot replace the traditional methods of intervening suicidality [28] • AI technologies are gaining momentum to be used as assistance tools for mental health professionals [29]	Personalized	Theme Five: AI, SGDs and Mental Well-being Sub-theme One: Intersectionality, AI and Suicidality Sub-theme Two: prioritizing using AI as tool in assistance instead of thinking it as replacement

Source: compiled by the authors based on [13, 15-17, 19-29]

in a broader context in routine life to assess the risk of suicidality [17]. Further, AI have the capability to predict suicidality among individuals with physiological mental health issues, i.e., degenerative disorders. Wearables can detect variations in heart rate and muscle movement, as well as variations in the pattern of speech [18].

THEME THREE: THE LINK BETWEEN SOURCE AND HEALTHCARE SERVICES

It is important to understand that if an AI-based source detects a risk of suicidality, what possible pathways for help exist? The most important intervention will be the link between AI-based programs on phones, smart-watches, or other devices and help-seeking services. AI models can analyse phone text and social media activity to predict suicidal behaviours, and machine learning models are particularly useful in this context [19]. Instagram has been already tested for mechanism to identify acute suicidal ideation among users [30].

SUB-THEME ONE: HELP SEEKING AGENCIES, AI DETECTION AND SUICIDALITY

One of the key questions is the reporting mechanism of AI-based detection of precursors of suicidality. Should it

be conveyed to family members, or should it warn the individual? However, considering the broader literature, help-seeking agencies remain the most important stakeholders in this regard. In England, Samaritans is an important agency to intervene and prevent the act of suicide [20]. There is a need to link AI-based facilities to help agencies identify and prevent suicidality.

SUB-THEME TWO: PERSONALISED CARE

Biscaia and colleagues explored the role of AI in personalised care. AI has the capacity to be used for personalised care for individuals with suicidal ideation and increased risk of suicide attempts. AI can reduce costs for such individuals by offering breathing exercises to help relax when devices detect stress. In addition, AI devices can help mental health professionals by providing real-time data when a vulnerable user consents [21].

THEME FOUR: ETHICAL CONSIDERATIONS

In the context of suicidality and integration of AI to tools, ethical concerns pose a major challenge. Collecting records of individuals' routine activities poses a significant challenge. Consent and voluntary participation of high-risk individuals can provide solutions. For example, individuals with diagnosed minor and major depression, impulsivity, family history of suicidality, experience of active stressful life events (i.e., death of a sibling, partner or parent, divorce, unemployment, etc.) can be sampled. This technique also shows that high-risk individuals can be assessed effectively to prevent the act of suicide, and at the same time, with minimal records of precursors and routine life. Probably the most important aspect is storage and appropriate use of information [22]. It is a fact that AI is used for advertising purposes as well, i.e., targeted advertising by assessing users' social media activity. In such a case, individuals with suicidal ideation can be targeted for medical business purposes by suggesting antidepressants or counselling services. This may interfere with personal life and may even lead to further harm. The argument about targeted advertisement leading to further harm for individuals with suicidal ideation [23].

SUB-THEME ONE: BIASES AND ERRORS

AI-based assessments are not error-free. AI can misinterpret normal behaviours, leading to misdiagnosis or wrong diagnosis. In addition, AI-related biases and errors can impose economic costs on individuals and burden the healthcare system [24].

THEME FIVE: AI, SGDS AND MENTAL WELL-BEING

SDG 3 is about health and well-being, where mental health is a significant focus. A multifaceted approach is considered a pathway to ensure better mental health. Targets such as 3.4, 3.5 and 3.8 are explicitly about mental health. AI can be effectively utilised for sustainable mental well-being [25]. AI is pivotal to the adoption of personalised mental health programs, where individualised considerations are crucial. AI is used for personalised programmes for physical and mental health and plays a crucial role in diagnosis and treatment. Similarly, AI's utilisation for the diagnosis of mental health problems is increasingly evident; however, it is still lagging when its utilisation regarding physical health is considered [26].

SUB-THEME ONE: INTERSECTIONALITY, AI AND SUICIDALITY

The notion of intersectionality is applicable globally in the use of AI for mental health and well-being. The developing world is far behind the developed world in technological advancement, including AI. Discrimination and distress predict suicidality. In the context of intersectionality, a wide range of demographic factors contribute to suicidality, including age, gender, race, and ethnicity. These indicators affect life in unique ways in the developing world; for example, gendered norms in traditional societies and the gendered status of women in poor societies have distinct patterns. These indicators affect the use of AI in the developing world differently, with intersectional indicators intensified [27].

SUB-THEME TWO: PRIORITISING THE USE OF AI AS AN ASSISTIVE TOOL RATHER THAN AS A REPLACEMENT

Careful consideration is needed when utilising AI for suicidality. For example, it should be used as a tool for suicide risk assessment. AI cannot replace traditional methods of intervening in suicidality; however, it can be a useful tool [28]. Current evidence shows that if AI is used as a tool to help medical and psychological professionals understand patterns of suicidal behaviours, it would be more beneficial than replacing them. AI technologies are gaining momentum for integration to assist professionals in managing mental health issues [29].

There are two major domains of AI and suicidality: first, AI-driven suicidality; and second, the utilisation of AI for suicidality. This work focuses on the utilisation of AI for intervention and prevention of suicidality. AI has the potential to identify suicide-related behaviours [8]. Advanced AI devices can even assess heart rhythms [13], underscoring

the usefulness of AI. Most importantly, digital phenotyping is a useful way to understand time-to-time variation in human behaviour [15]. AI can be utilised to understand patterns of social interaction. Patterns of social interaction can provide hints for identifying suicidality, such as chat patterns, Google searches, and even physical interactions [16].

Recent research shows that AI can predict suicidality in individuals, for example, physiological and mental health issues can be identified using AI tools [18]. AI can detect incidents of impulsive behaviour, such as aggressive statements [17]. Another example is searching for methods on Google, where AI integration with phone technology can play a pivotal role in detecting suicidal behaviours. In addition, AI models can analyse phone text and social media activity to predict suicidal behaviours [19]. This is useful for reporting behaviours to help-seeking organisations and even family and friends, enabling effective intervention.

AI has the capacity to support personalised care for individuals with suicidal ideation and an increased risk of suicide attempts [21]. AI cannot replace traditional methods of intervening in suicidality; however, it is a tool that can assist professionals in the better management and care of individuals with suicidal ideation [28].

Ethical dilemmas are a pivotal consideration when it comes to the use of AI tools for suicide risk assessment, intervention and prevention. Collecting records from individuals' routine activities poses a significant challenge [22]. In addition, AI-based assessments are not error-free [24]. For example, AI tools can detect aggression and impulsivity, which are associated with suicidality, but they are not always leading factors.

The Sustainable Development Goals (SDGs) include health as an important element. Targets such as 3.4, 3.5 and 3.8 are explicitly about mental health. AI can be effectively utilised for sustainable mental well-being [25]. AI is used to deliver personalised programmes for physical and mental health and plays a crucial role in diagnosis and treatment [26]. However, inequalities are barriers to the effective use of AI in the diagnosis and treatment of suicidality when an intersectional perspective is applied. The developing world is far behind the developed world in technological advancement [27, 31]. Finally, it is concluded that AI cannot replace traditional methods of intervening in suicidality; however, it can be used as a useful tool [28]. Therefore, AI and human collaboration are suggested to achieve the SDGs, specifically 3.4, 3.5, and 3.8.

THEORETICAL FRAMEWORK

There are many potential theories and perspectives that can serve as a guiding framework for AI utilisation to prevent, identify, and manage suicidality. The Technology Acceptance Model (TAM) is the most important. Hopkins and colleagues explored the importance of attitude towards Machine Learning (ML) technology in preventing suicides. Beliefs about technology play a pivotal role in help-seeking behaviours supported by ML technologies [10]. So far, evidence shows mixed opinions about the effectiveness of ML technologies in preventing suicides. The belief that AI can assist humans in improving mental health, thereby indirectly reducing suicidality, is important. Extending human-AI collaboration to achieve the SDGs, specifically 3.4, 3.5, and 3.8, can be guided by the Technology Acceptance Model (TAM).

Further, the identification of suicidal ideation with the Integrated Motivational-Volitional Model (IMV), 3-Step Theory, and Fluid Vulnerability Theory (FVT). This is a very useful juxtaposition of AI-based identification, particularly in terms of its effectiveness. For example, AI and ML models can detect hopelessness and the capability to attempt suicide from text (i.e., chat) and voice notes. Ethical integration of AI into devices can help identify suicidality by analysing web searches, chat, and social media content [8].

CONCLUSIONS

This study aimed to explore the potential and challenges of Artificial Intelligence for the intervention and prevention of suicidality to achieve the SDGs. Methodologically, this study is a thematic review based on Braun and Clark's reflexive thematic analysis. A total of 11 studies were sampled using Malterud and colleagues' information power mechanism [11]. A total of five major themes were extracted, namely, the Power of AI in identification of health-related indicators, Correlation of Actions as Precursors of Suicidality, the Link between Source and Healthcare Services, Ethical Considerations and AI, and SGDs and Mental Well-being. It is concluded that there are two major domains of AI and suicidality: first, AI-driven suicidality; and second, utilisation of AI for suicidality. AI has the potential to identify suicide-related behaviours. For example, AI-enabled devices and AI integration into phone technology are useful tools. Digital phenotyping is a useful way to understand time-to-time variation in human behaviour, which can help in the identification of suicidal behaviours. In addition, physiological and mental health issues (e.g., impulsive behaviours) can be identified using AI tools. AI models can analyse phone text and social media activity to predict suicidal behaviours. Most importantly, AI has the capacity to be used for personalised care for individuals with suicidal ideation and increased risk of suicide attempts.

However, ethical dilemmas are always present when using AI for the intervention and prevention of suicidality. Two key areas of concern are the use of records from individuals' routine activities and potential errors in assessment. A useful approach to tackling these dilemmas is to view AI as an assisting tool rather than a replacement for traditional methods of intervening in suicidality. AI technologies are increasingly being used as assistive tools for mental health professionals. It is also concluded that AI can contribute to achieving the SDGs relevant to health and well-being by assisting in suicide-related intervention and prevention. However, intersectionality and inequalities are relevant, as the developing world is lagging in the application of technology in the healthcare sector.

SUGGESTIONS FOR FUTURE RESEARCH

Phenomenological studies, specifically those with an experimental basis, are required to understand the experiences of those who allow AI devices to record their tendencies towards suicidal behaviour. This will enable researchers to better equip AI tools and integrate the technology to assist in managing suicidality. Further, it is suggested that large-scale surveys can provide a comparative analysis of AI integration and use by professionals in the developing and developed worlds, which is important for achieving SDGs. There is a need to research the ethical aspects of AI and human collaboration to understand, identify, and prevent suicidality. There is also a need for theoretical debate, supported by empirical research, regarding suicidality and AI utilisation.

STUDY LIMITATIONS

This research is a review-based paper and does not provide firsthand insight into the experiences of individuals with suicidality undergoing AI-based interventions, nor into the experiences of professionals involved in assessing suicidality with assistance from AI tools.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

Anti-inflammatory therapy in patients with coronary artery disease: A new era in the treatment of atherosclerosis or false hopes?

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ABSTRACT

Aim: To investigate the potential use of anti-inflammatory drugs in patients with acute myocardial infarction (MI).

Materials and Methods: During December 2025, a comprehensive search was conducted in the scientometric databases PubMed, Scopus, Web of Science and Google Scholar for data from peer-reviewed English-language studies on the use of anti-inflammatory drugs in patients with CAD, published over the past 10 years. The search used the following terms: anti-inflammatory drugs, MI, acute coronary syndrome, chronic coronary syndrome, CVE, CAD, atherosclerosis. Preference was given to the most relevant sources of information, results of randomized placebo-controlled trials, international recommendations, meta-analyses, and more recent literary sources. A total of 94 literary sources were analyzed.

Conclusions: Measuring levels of inflammatory markers and using anti-inflammatory agents, particularly colchicine, in addition to baseline therapy in patients with acute MI to prevent recurrent index events is justified and promising.

KEYWORDS: atherosclerosis, cardiovascular mortality, anti-inflammatory therapy, myocardial infarction, cardiovascular events

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INTRODUCTION

Atherosclerosis is a complex and multifactorial process. In addition to the «classic» factors of its onset and progression, such as dyslipidemia, hypertension, diabetes mellitus, and smoking, the scientific community is actively discussing the importance of inflammation [1].

The basis for this was the results of a 30-year observation of Redker P. and co-authors on 12,530 women. Traditionally, it was believed that patients without "classical" risk factors or after their correction have a relatively low risk of cardiovascular events (CVE). However, it turned out that with high levels of systemic inflammation, in particular highly sensitive C-reactive protein (hsCRP) >3 mg/L, the risk of recurrent CVE remains disproportionately high. Thus, the risk of CAD in this cohort increased by 77%, stroke - by 39%, and major adverse cardiac events (MACE) - by 52%, compared to a group of patients without traditional risk factors and with low hsCRP levels [2].

hsCRP is one of the most indicative pro-inflammatory markers, the increase of which is closely correlated with the risk of MACE. Its level is recommended to be determined in all patients with CAD [3, 4].

In addition to laboratory markers for personalized determination of the degree of inflammation in the coronary arteries and stratification of cardiovascular risks, the Fat Attenuation Index (FAI Score) scale can be used, based on CT coronary angiography data with assessment of perivascular fat. This method has high prognostic value, however, it requires a high-resolution multispiral tomograph and special software [5].

Considering the above, assessing the level of systemic inflammation and correcting it in patients with obstructive forms of coronary artery atherosclerosis is no less important than treating dyslipidemia [4].

AIM

To investigate the potential use of anti-inflammatory drugs in patients with acute myocardial infarction (MI).

MATERIALS AND METHODS

During December 2025, a comprehensive search was conducted in the scientometric databases PubMed, Scopus, Web of Science and Google Scholar for data

from peer-reviewed English-language studies on the use of anti-inflammatory drugs in patients with CAD, published over the past 10 years.

The search used the following terms: anti-inflammatory drugs, MI, acute coronary syndrome, chronic coronary syndrome, CVE, CAD, atherosclerosis.

Preference was given to the most relevant sources of information, results of randomized placebo-controlled trials, international recommendations, meta-analyses, and more recent literary sources. A total of 94 literary sources were analyzed.

ETHICS

All sources used in this literature review are publicly available.

AI STATEMENT

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

REVIEW AND DISCUSSION

Statins are currently the cornerstone of pathogenetic therapy for atherosclerosis, not only due to their lipid-lowering effect, but also due to the whole spectrum of pleiotropic effects, including anti-inflammatory. Reducing the systemic inflammatory response in the vascular walls makes it possible to stabilize and slow the progression of atherosclerosis [3, 4, 6].

A rather active search for agents with anti-inflammatory effects that could be used to treat atherosclerosis is being carried out among drugs of the rheumatological group, since hyperreactivity and aseptic inflammation also play a key role in the pathogenesis of rheumatic diseases [7].

In particular, the CANTOS trial tested three doses of the monoclonal antibody to interleukin-1β, canakinumab, against placebo in more than 10,000 patients with a history of MI and plasma CRP level ≥2 mg/L. Only the highest dose of the drug (300 mg every 3 months) had a significant positive effect on reducing plasma interleukin-6 and CRP levels, as well as improving the composite endpoint of cardiovascular mortality (CVM), nonfatal MI, and nonfatal stroke. Despite the positive results, further study of the drug in this indication was stopped due to the risk of fatal infections and high cost [8].

The CIRT study focused on the possibility of using low doses of methotrexate (15-20 mg once a week) for secondary prevention of CVE in patients with type 2 diabetes mellitus or metabolic syndrome and a history of MI. The study included nearly 5,000 participants. However, since the announced endpoints (reduction in CVM, risk of non-fatal MI, non-fatal stroke, or need for urgent revascularization due to unstable angina (UA)) were not achieved, it was terminated early [9].

The theory that atherosclerosis is a crystallopathy and that it is the crystallization of free cholesterol in atherosclerotic plaques that causes crystal-induced inflammation, similar to the pathophysiological mechanisms of gout, has proven to be quite promising. It has been suggested that antigout drugs, in particular colchicine, may be used in the treatment of CAD. The mechanism of action of the drug is to block the NLRP3 inflammasome, which interrupts the inflammatory cascade IL-1β→IL-6→CRP, thus inhibiting the key processes underlying atherosclerosis [7].

One of the first scientific tests of this theory was the Australian LoDoCo study (2012). It aimed to evaluate the effect of low-dose colchicine (0.5 mg/day) on the rate of CVE in patients with stable forms of CAD. The result was positive, but the study had a small cohort (only 532 patients) and was not placebo-controlled [10].

The next research that initiated the study of colchicine in patients with obstructive forms of CAD was COLCOT. It suggested that patients with acute forms of CAD have a very high level of systemic inflammation and the use of a drug with anti-inflammatory effect may contribute to the stabilization of the condition and be a means of secondary prevention of recurrent CVE. The study included 4745 patients with a recent MI (less than 30 days) regardless of the level of CRP [10, 11].

During 2.3 years of follow-up, colchicine had a significant positive effect on both the composite primary endpoint (CVM, resuscitated cardiac arrest, nonfatal MI, nonfatal stroke, urgent revascularization due to UA) compared with placebo (5.5 vs. 7.1%, respectively, $p=0.02$), and on each indicator separately. Analysis in subgroups based on the timing of colchicine administration showed that the best results were achieved in patients who received colchicine within 3 days of the index event, compared with groups receiving it between 4 and 7 days and after 8 days [10, 11].

The difference in the frequency of adverse drug reactions, such as diarrhea, between the comparison groups was statistically insignificant (9.7% vs. 8.9%, $p>0.05$). No cases of serious infectious complications or myopathy (although 99% of patients were taking statins) were recorded [10, 11].

The joint Australian-Dutch LoDoCo2 study, which included 5,522 patients with stable forms of CAD, was more thoroughly organized. It should be noted that 84% of the patients included in the study had a history of MI. During 2.4 years of follow-up, the group of

patients who took colchicine at a dose of 0.5 mg/day showed a significantly positive effect on the primary composite endpoint (CVM, spontaneous MI or stroke, urgent revascularization due to ischemia) compared to the control group (6.8% vs. 9.6%, respectively, p<0.001), as well as on secondary endpoints (CVM and non-fatal MI or stroke) (4.2% vs. 5.7%, respectively, p=0.007) [12].

A meta-analysis (12,869 patients) was initiated to summarize the results of studies on the use of colchicine for the prevention of MACE in patients with CAD, which confirmed that low-dose colchicine in this cohort significantly reduced the risk of MI, stroke, and the need for urgent revascularization due to UA. There was no significant difference in the rate of death, death from any cause, or adverse drug reactions from the gastrointestinal tract compared with placebo [13; 14].

The accumulated scientific and clinical data allowed colchicine to be included in the European and American guidelines for the treatment of acute and chronic coronary syndromes as an additional means of secondary prevention of recurrent CVE. And the sufficient safety profile of this agent for use in wide clinical practice is confirmed by the approval of the FDA ((Food and Drug Administration, USA) [3, 4, 6].

However, recent studies have cast doubt on the possibility of using colchicine as a secondary prevention agent for cardiovascular and cerebrovascular events in patients with CAD.

In particular, in the CLEAR SYNERGY study, which included 7062 patients with MI after primary percutaneous coronary intervention, during 3 years of follow-up, a positive effect on the primary composite endpoint (CVM, recurrent IM or stroke, and the need for urgent revascularization due to ischemia) was not achieved with colchicine compared to placebo (9.1% vs. 9.3%, respectively, p=0.93) [15, 16].

The CONVINCE and CHANCE-3 trials did not provide convincing evidence that low-dose colchicine can reduce the risk of recurrent strokes or transient ischemic attacks in patients with acute non-cardioembolic stroke [17, 18].

To resolve the discrepancy between the results of the studies and to make a final assessment of the advisability of using colchicine in patients with CAD as a means of secondary prevention, a new retrospective meta-analysis of existing randomized placebo-controlled studies was initiated, regardless of when they were conducted, the language of publication of the results, and the ethnicity of the patients included in the studies (31,397 cases). The results showed that colchicine at a dose of 0.5 mg/day for a treatment duration of ≥ 6 months significantly reduces the risk of MACE (by 30%) in both acute atherothrombotic and all cardiovascular diseases in representatives of different ethnic groups. There was no increase in all-cause mortality with colchicine treatment, as reported in some studies [19].

Colchicine significantly reduces the risk of CAD destabilization and the need for urgent revascularization, but the reduction in the risk of non-cardioembolic ischemic stroke while taking the drug is borderline statistically significant [19].

Serious adverse drug reactions affecting the gastrointestinal tract, including gastrointestinal bleeding and hospitalization due to these complications, did not differ significantly between the colchicine groups and the comparison group. The incidence of adverse drug reactions not related to the gastrointestinal tract did not differ between the colchicine and control groups [19].

Patients with MI against a background of non-obstructive forms of CAD (MINOCA) are quite difficult to treat. The frequency of these forms can reach 5 to 15% of all cases of acute coronary syndrome. Inflammation also plays an important role in the development of this syndrome [20].

A recent retrospective cohort study involving 42,167 patients with MINOCA showed a positive effect of colchicine on the primary endpoint (recurrent MI, all-cause mortality, CVE, and all-cause hospitalization) compared with placebo (49% vs. 55.1% respectively, p<0.001), and secondary endpoints - recurrent MI and all-cause mortality were significantly lower in the colchicine group (29.6% vs. 33.5% and 1.9% vs. 3.9%, respectively, p<0.001). The incidence of heart failure and hospitalizations for any reason tended to decrease with colchicine, but did not reach statistical significance. No differences in the incidence of cerebrovascular events were observed between the comparison groups [20].

To sum up the above, it should be noted that the assessment of systemic inflammation markers, in particular hsCRP, in patients with CAD is no less important than the assessment of the lipid profile. A comprehensive assessment of clinical status allows for personalised pathogenetic therapy in this category of patients and achieves the most positive impact on prognosis.

The use of anti-inflammatory agents is an important component of prognosis-modifying therapy, especially in clinically complex patients who have recurrent index events on background therapy.

Currently, colchicine appears to be the most promising anti-inflammatory drug for patients with CAD. Its effectiveness in reducing the risk of recurrent CVE in patients with acute and chronic coronary syndromes and its adequate safety profile have been proven in a large number of randomised placebo-controlled studies.

Of course, anti-inflammatory drugs are not an alternative to basic prognosis-modifying therapy. And at present, there are still many unresolved issues regarding the prospects for the use of this group of drugs in broad clinical

practice. In particular, questions remain unanswered regarding the timing of their administration in patients with acute coronary syndrome from the onset of the index event, the duration and long-term effects of such treatment (in existing studies, the observation period for patients did not exceed 3 years), markers for monitoring its efficacy and safety, the advantages of such therapy in patients with multivessel coronary artery disease, high levels of systemic inflammation, and concomitant diseases that are also characterized by general hyperreactivity (diabetes mellitus, gout, and other rheumatic nosologies) as opposed to patients without such clinical characteristics.

It is also unclear whether anti-inflammatory therapy can affect the frequency of cardiovascular events associated with restenosis of implanted stent systems, since there is also an inflammatory component in the pathogenesis of this process.

CONCLUSIONS

1. Measuring levels of inflammatory markers, particularly hsCRP, is an essential component of a comprehensive clinical assessment of patients with CAD and an important predictor of recurrent adverse cardiovascular events.

2. Anti-inflammatory agents, when added to standard CAD therapy, help reduce the risk of recurrent major adverse cardiovascular events.

3. Colchicine is an anti-inflammatory agent with the most well-established prognosis-modifying effect in patients with CAD and a favorable safety profile.

4. Long-term outcomes of anti-inflammatory therapy, the timing of its initiation following an index event, benefits in specific subpopulations of CAD patients, and the impact on stent restenosis remain subjects for further study.

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REVIEW ARTICLE

Exercise-related dysglycemia in type 1 diabetes: Endocrine counterregulation, exercise modality, risk modifiers and technology – supported prevention

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ABSTRACT

Aim: To summarise the current state of knowledge regarding how physical activity may stimulate counter-regulatory responses in type 1 diabetics and which clinical features can enhance the risk of either hyper - or hypoglycaemia.

Materials and Methods: A targeted and evidence-based summary of articles published in PubMed from 2022 to 2026, regarding the physiological responses to exercise, continuous glucose monitoring (CGM) systems, automated insulin delivery (AID) systems in regular physical activity among type 1 diabetics.

Results: Type 1 diabetics experience a delayed reduction in the amount of insulin that can be reduced at the onset of exercise. Their body's production of glucagon and sympathetic-adrenal responses tend to be lower than normal. There is an increased risk for developing hypoglycemia due to this imbalance. The most significant factors that affect dysglycemic responses during physical activity include: how much "insulin on board" is present, when an individual's last meal bolus was taken, the amount of glucose within the bloodstream at the onset of physical activity and its trend and intensity of physical activity being performed.

Conclusions: Type 1 diabetes exercise management should be tailored to the individual and the nature of the exercise, as well as the patient's level of insulin exposure at that time and the dynamic blood glucose levels. The use of technology is most successful when it is used in conjunction with formal educational programs for patients and when a pre-exercise plan is put into place by health care providers.

KEYWORDS: type 1 diabetes, hypoglycemia, hyperglycemia, continuous glucose monitoring, insulin infusion systems

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INTRODUCTION

Many people who have type 1 diabetes have stated that taking part in some form of physical exercise can produce several very good changes within their body (i.e. better heart/lung functions and more sensitive to insulin). As a result of these positive physiological changes that occur from participating in physical exercise, many individuals who suffer from diabetes state that the overall quality of their lives improve. However, although many individuals suffering from diabetes have made great improvements in their physiological well-being through exercising regularly, most still fail to continue doing so on a consistent basis. The main reasons why most diabetics will not take up a "healthy" lifestyle of activity is because they fear developing low blood glucose or what is medically referred to as hypoglycemia [1-4]. Patients with type 1 diabetes require exogenous insulin to manage their blood glucose levels while engaging in physical activities. Unlike other individuals who naturally secrete insulin, patients with type 1 diabetes lack the ability to self-regulate their own body's response to their own metabolic systems [1, 4-6].

AIM

The main purpose of this investigation was to examine whether there has been recent research into how people with type 1 diabetes respond with their endocrine system during physical activity, investigate the changes that occur in blood glucose levels resulting from a variety of commonly practiced forms of physical activity and to present information regarding how glucose monitors and insulin pumps may be used as preventive measures against low blood glucose (hypoglycemic episodes).

MATERIALS AND METHODS

This review was designed as a focused narrative review. A search of the PubMed database for all available ar-

ticles published in the period from 2022 to 2026 that included the use of the following search terms: "type 1 diabetes", "exercise", "hypoglycemia", "hyperglycemia", "counterregulatory response", "continuous glucose monitoring" and "automated insulin delivery" was performed. Additionally, a review of reference lists for relevant articles and current professional guidelines and recommendations were reviewed. International guidelines, systematic reviews, randomized controlled trials, observational studies were given priority when selecting articles. Articles were selected if at least one of the following areas were being discussed: endocrine response to hypoglycemia, glycemic effects by type of exercise, risk modifiers, pre-exercising safety, diabetes technology use during exercise, behavioral barriers to physical activity.

The authors used AI-assisted resources to improve writing clarity, grammar and formatting, as well as assist with a review process. All remaining authorship responsibilities included interpretation of data, source selection, synthesis of all study results and final approval of the written manuscript.

REVIEW

COUNTERREGULATION AND SUSCEPTIBILITY TO EXERCISE-RELATED HYPOGLYCEMIA

The effects of physical activity on non-diabetic people is that their circulating insulin levels decrease while their circulating glucagon, catecholamine (epinephrine/adrenaline), corticosteroid (cortisol) and growth hormone increase. Each of these hormones can cause an elevation in blood glucose by releasing it from the liver, thus they serve as a protective response to prevent dangerously low blood glucose. However, patients with type 1 diabetesbexperience a disruption in their body's physiological responses to low blood glucose during periods of physical activity. Therefore, T1D patients' ability to physiologically decrease their exogenous administration of insulin is less than their bodies' natural rate of reduction of endogenous insulin production. Furthermore, the glucagon and other symptoms associated with low glucose levels are often diminished in long-standing T1D patients who have been afflicted with the disease for longer than 5 years, particularly those experiencing repeated episodes of hypoglycemia [1,8,9]. A systematic review of stepped hypoglycemic clamp studies showed that counterregulatory hormone and symptom thresholds occur at lower glucose concentrations in people with type 1 diabetes than in individuals without diabetes [8]. The Hypo-RESOLVE clamp study further confirmed reduced glucagon and epinephrine responses and weaker symptom perception in type 1 diabetes [9].

EXERCISE MODALITY AND ACUTE GLYCEMIC RESPONSES

Exercise modality was identified as an important determinant of acute glycemic response. In the T1DEXI study, adults with type 1 diabetes experienced the greatest glucose declines during aerobic exercise, followed by interval exercise, whereas resistance exercise was associated with the smallest decreases [5]. A meta-analysis similarly found that high-intensity interval exercise leads to more heterogeneous glucose responses than continuous aerobic exercise and is not uniformly protective against hypoglycemia [7]. A recent synthesis of postprandial exercise management found that prandial state may influence glycemic response more strongly than exercise intensity alone [12, 16]. Even interval-based exercise may therefore be followed by a substantial glucose decline when a recent meal bolus remains active.

KEY MODIFIERS OF EXERCISE-RELATED DYSGLYCEMIA

Among the clinical variables identified, insulin on board was the strongest predictor of exercise-induced hypoglycemia. Analyses from T1DEXI and risk-prediction models showed that residual bolus insulin, recent glucose decline and recent time below range increase the likelihood of hypoglycemia during exercise [6, 25, 26]. Meal timing was also consistently associated with glycemic outcomes. Exercise performed within 2 hours of a meal bolus was linked to greater hypoglycemia risk, especially during aerobic activity [12-16]. Pre-exercise glucose level alone was not sufficient for risk assessment, glucose trend and surrounding clinical context were also relevant. The effect of time of day was less consistent. Some smaller studies suggested a smaller acute glucose drop during morning exercise, whereas larger datasets indicated that time of day had less predictive value than insulin exposure, exercise type and pre-exercise glucose dynamics [14, 24, 27]. Additional factors such as habitual fitness and selected patient characteristics may contribute to interindividual variability [24].

HYPERGLYCEMIA, KETONES AND PRE-EXERCISE SAFETY

Exercise did not have a uniform lowering effect on blood glucose levels. Because the short intense or

psychologic nature of the exercise may cause an initial increase in glucose due to increased catecholamine release which results in an excessive amount of glucose being produced by the liver compared to the peripheral tissues that can take it up from the blood [7, 12]. On the other hand, when there is some level of relative insulin deficiency during exercise, this may lead to worsening hyperglycemia with the promotion of ketosis rather than improvement of glycemic control [1,4]. The 2025 EASD/ISPAD position statement recommended postponing exercise when hyperglycemia is accompanied by elevated ketones or suspected pump failure [4]. Therefore, mild hyperglycemia without ketoacidosis was not considered an absolute contraindication for exercise as long as the individual had sufficient insulin delivery and were otherwise metabolically stable.

TECHNOLOGY-SUPPORTED PREVENTION

Recent studies have shown that using the existing forms of technology related to diabetes can provide a safer environment to engage in physical activity by those with diabetes. The American Diabetes Association (ADA) states as part of their 2026 Standards of Care that Continuous Glucose Monitoring (CGM) systems should be used by every individual diagnosed with diabetes as soon as they are prescribed insulin. Also, the ADA stated Automated Insulin Delivery (AID) systems as their preferred form of insulin delivery for almost all people with type 1 diabetes [2]. CGMs provides users with real time glucose trends, predictive alert notifications and increases the likelihood of detecting asymptomatic and nocturnal hypoglycemic events occurring while exercising in comparison to capillary glucose testing [2, 10]. At the same time, limitations were reported. Sensor lag may become more relevant during rapid glucose changes and factors such as sweating, heat and signal interruption may reduce measurement accuracy [4, 11]. AIDs improves time in range and reduces treatment burden, but does not replicate physiological counterregulation [4, 11]. In the PACE trial a predictive hypoglycemia alert reduced exposure to clinically significant hypoglycemia overall and within 24 hours after exercise [10]. A 2025 trial examining the timing of a temporary elevated glucose target before exercise found favorable glycemic outcomes regardless of target timing and did not demonstrate a significant difference in time below range in a well controlled cohort [14].

EDUCATION, ADHERENCE AND FUTURE-ORIENTED FINDINGS

Fear of hypoglycemia remained the main perceived barrier to physical activity despite the increasing availability of diabetes technologies [3, 18, 23]. A systematic review and meta-analysis showed that diabetes technologies reduce fear of hypoglycemia, although the effect size was small to moderate [18]. Educational and behavioral interventions were also shown to improve exercise readiness and reduce fear of hypoglycemia [19, 20]. Predictive models based on CGM have shown promise in forecasting exercise-related glycemic events and may contribute to practical decision-support tools in the future [22].

DISCUSSION

The findings of this review indicate that exercise-related dysglycemia in type 1 diabetes results from the interaction between impaired endocrine counterregulation, persistent exogenous insulin action and the specific metabolic demands of different exercise modalities. The reduced glucagon and sympatho-adrenal responses observed in type 1 diabetes help explain why many individuals remain vulnerable to hypoglycemia during and after exercise, even when they are aware of the potential risk.

The reviewed evidence also suggests that exercise modality alone is not sufficient to predict glycemic response. Although aerobic exercise is generally associated with the largest glucose decline, postprandial status and insulin on board appear to modify glycemic response more strongly than exercise intensity in many real-world situations. This is clinically important because it shifts attention from simplistic exercise classification toward individualized metabolic context.

Among all modifiers, insulin exposure appears to have the greatest practical relevance. A residual bolus of insulin, current downward trending glucose levels and most recently spent in hypoglycemic zone (time below range) are all individually predictive of increased likelihood of subsequent hypoglycemia. Therefore, preventing hypoglycemia is best achieved through adjusting the amount of basal insulin as an anticipatory measure for potential falls in blood glucose rather than solely providing corrective amounts of carbohydrates after the glucose level begins falling. Additionally, assessing a patient's blood glucose before exercise should be based upon more than just a single static value.

The review also highlights that hyperglycemia before exercise should not be interpreted uniformly. Although mild hyperglycemia with sufficient available insulin is likely to be tolerated. However, exercising while being relatively insulin deficient will likely increase the risk for ketosis and worsening metabolic instability. Therefore, it is reasonable that an individual's exercise should undergo a more detailed safety evaluation prior to

engaging in physical activity, particularly in individuals using pumps.

Although new technology that helps people living with diabetes safely participate in physical activities has been developed to help make diabetes-related risks less likely by allowing individuals to see how high or low their blood glucose levels are at a given time, warn individuals of impending low blood glucose episodes, allow individuals to alter their insulin dosing, etc. The new technology does not remove all of the physiological risks associated with hypoglycemia caused by physical activity. New technology can only be as good as the information, education and experience the individual receives through structured educational programs and the collaborative development of personal plans and decisions based upon the individual's specific needs. This reality is evident because despite the increased availability of advanced technology, many people still fear having a hypoglycemic episode.

The collective evidence suggests a personalized approach to managing type 1 diabetes during and after each specific type, duration, intensity and frequency of exercise. The plan for management will need to account for all of the above factors as well as what has worked previously for this particular individual. The most effective strategy for protecting against hyperglycemia or hypoglycemia would appear to include a structured regimen of adjusting insulin doses, consuming carbohydrates to stabilize blood glucose levels, utilizing CGMs in real time before and after exercising and performing regular surveillance on blood glucose levels after each exercise session.

Future studies need to concentrate a great deal more on real life exercise as opposed to strictly regulated lab-based methods. Additional data is required on uncontrolled (or unplanned) physical activities, varying meal intake, distinctions in patient subgroups and whether or not predictive decision support systems will be useful over time.

CONCLUSIONS

How often hypoglycemia occurs in an athlete using exogenous insulin for their diabetes prior to participating in a physical activity depends on several variables. For example, one variable is the amount of insulin in the athlete's system when they have a hypoglycemic episode ("insulin on board"); another variable is if the athlete ate something previously to prevent their blood glucose from dropping during the activity; yet another variable is what kind of activity the athlete is doing compared to continuous aerobic activity and (if applicable) the athlete's response to a hypoglycemic episode through consumption of carbohydrates.

The use of modern technologies for managing type 1 diabetes may potentially increase safety and enable individualized approaches to managing type 1 diabetes. However, the potential of the technological tools will be optimized with pre-planning, the development of an individualized carbohydrate plan, timely modification of the insulin regimen and instruction as to how to monitor and manage blood glucose levels during physical activity.

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