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Medical Advances

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ORIGINAL ARTICLE

CONTENTS

Morphological changes in the crystallization of mixed saliva during the treatment of dentofacial anomalies with aligners

Anton Kolomiiets[1], Monika Białoszycka[2], Alisa Pachevska[1], Iryna Simonova[3], Olena Popova[1], Nadiia Drachuk[1], Tetiana Chugu[1,]

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ABSTRACT

Aim: To investigate morphological changes in the crystallization patterns of oral fluid during orthodontic treatment with aligners.

Materials and Methods: Samples of dried saliva were analyzed from young adults aged 18–25 years of both sexes in a comparison group and in two main groups: patients treated with aligners without preventive measures and patients treated with aligners with preventive care.

Results: In the main group of young adults treated with aligners three months and six months after the initiation of treatment, disturbances in saliva crystallization were observed, which were evident from both qualitative and quantitative indicators. A study of the crystallographic pattern of a dried saliva was also conducted during the use of aligners in combination with preventive agents destruction of the clear crystal structure was lesser violations were observed. After from the initiation of treatment (three and six months after the start of treatment), a reduction in the size and number of amorphous structures was observed in the peripheral (protein) zone.

Conclusions: The use of aligners in young adults induces destructive morphological changes in the crystallization of mixed saliva. Prophylactic application of a Decasan solution demonstrates a pronounced protective effect.

KEY WORDS: crystallization, saliva, aligners, preventive care

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INTRODUCTION

In recent years, saliva has become an important element of laboratory diagnostics. Researchers are continuously seeking new ways to use saliva in medical diagnostics and in monitoring treatment outcomes. The application of saliva in diagnostics is gaining increasing popularity due to the advantages it offers, not only from an economic perspective. The collection of saliva as a diagnostic material is inexpensive, non-invasive, and painless, and is also convenient for young children, older adults, and physically active individuals. Saliva can be collected at home without the involvement of medical personnel. Transportation of this material does not require special procedures, and compounds detected in saliva are generally characterized by high stability [1–4].

Saliva consists not only of secretions from the salivary glands but also of other fluid and cellular components. Water accounts for approximately 94–99.5% of its composition. The remaining fraction represents the solid component, the amount of which varies - approximately 6% under resting conditions and about 0.5% after stimulation. The main organic components of saliva include protein substances such as enzymes, serum proteins, mucins, immunoglobulins, blood group substances, kallikrein, lactoferrin, epidermal growth factor (EGF), histatin, cystatin, statherin, sialin, hormones, and vitamins A, B, C, and K. The density of saliva ranges from 1.002 to 1.012 g/mL, and its pH largely depends on the rate of secretion.

At night, when salivary secretion is slower than during the day, the pH reaches approximately 6.2–6.5; it may increase to about 8.0 due to an elevated concentration of bicarbonate ions. Salivary secretion can be stimulated by gustatory, mechanical, thermal, olfactory, visual, or psychological stimuli. Regulation of salivation occurs via the cholinergic system as well as the α- and β-fibers of the sympathetic nervous system. Basal salivary secretion averages 0.33–0.55 mL/min and varies considerably among individuals, even under standardized conditions. Following strong secretory stimulation, such as food intake, salivary flow may increase to 1.5–2.3 mL/min,

and after exposure to pharmacological agents such as pilocarpine or methacholine, it may reach up to 5.0 mL/min. The daily volume of salivary secretion depends on sleep duration, the frequency and type of meals, and the influence of emotional stimuli, averaging 1–2 liters per day. A characteristic feature of salivary secretion is its disproportionately large volume relative to the mass of salivary gland tissue and its low osmolality. Fasting saliva is hypotonic, whereas at maximal secretion it becomes isotonic with blood plasma. The volume and composition of saliva vary depending on age and sex. Relatively low salivary secretion in newborns increases with age, particularly between 3 and 5 years, reaching its first peak by the end of the first decade of life. After the age of 30, a clear tendency toward a decrease in salivary secretion is observed. Male saliva is secreted in larger volumes than female saliva and is also characterized by higher concentrations of sodium, calcium, and phosphorus. Physical exercise causes a significant increase in ion concentrations in saliva, particularly sodium [5–7].

Compounds present in saliva can be divided into two groups. The primary classification criterion is the site of formation of a given compound—either within or outside the salivary glands. Compounds synthesized in the salivary glands and classified into the first group play only a secondary role in the use of saliva as a diagnostic material. An exception within this group is secretory immunoglobulin A (sIgA), which, although lacking immunological memory, supports agglutination processes and prevents bacterial adhesion and colonization on soft tissues and teeth, acting synergistically with nonspecific defense mechanisms. Extraglandular compounds, in contrast, are formed outside the salivary glands and are transported from plasma into saliva. This transport may occur via intracellular or extracellular pathways [8–11].

Intracellular transport includes passive or specific mechanisms. Passive transport is defined as diffusion or filtration. Specific transport comprises carrier-mediated transport, energy-dependent active transport, facilitated diffusion, or pinocytosis. Extracellular transport of plasma components into saliva may occur through ultrafiltration or via disruptions of natural membranes. Compounds that enter saliva exclusively through damaged membranes include thyroxine and triiodothyronine. Thyroid hormones are examples of substances whose salivary concentrations do not reflect their systemic levels. Compounds that can be measured in saliva rather than in serum enter saliva by diffusion. Diffusion is a process dependent on three factors: molecular weight, solubility of the compound in water and/or lipids, and the degree of ionization of the compound [12–15].

Saliva not only protects the oral cavity by maintaining a buffering environment but also exhibits antibacterial and remineralizing properties, participates in taste perception, regulates water balance, and contributes to blood coagulation. Its functions also include tissue repair. Compounds that can be detected in saliva instead of serum enter saliva via diffusion [16–20].

The structures of the studied fluids are obtained through a phase transition from the liquid to the solid state by dehydration. The results of experimental studies have demonstrated that information contained in the liquid phase at the molecular level is transferred to the macroscopic level during dehydration, forming various structures that become visible to the researcher [8].

Investigations of self-organization processes in various physiological and pathological liquid media have established the primary importance of organic components present in biological fluids, even in small amounts (from 0.01 μm to 100 g/L and higher), in structure formation. The differentiation of structures into organic and inorganic components is carried out using the dehydration method, which involves applying a biological fluid onto a transparent surface in the form of droplets followed by dehydration under specific conditions. The droplet volume is determined by the ratio between the fluid's specific gravity and its surface tension forces. According to theoretical concepts, specific interacting mechanisms operate during the dehydration of biological fluids, ensuring the formation of structures within solid-phase systems and subsystems. The term facies refers to what remains of a saliva droplet after drying. A schematic representation illustrating the action of these mechanisms is provided by a sagittal-section diagram of a biological fluid droplet placed on a horizontal surface, developed by Tarasevych and Ayupova (2003) [21].

The authors noted that fluid evaporation occurs unevenly across the exposed surface of the droplet. Because the hemispherical droplet has varying thickness—greater in the central region and thinner at the periphery—the evaporation of the analyzed droplet results in a non-uniform change in solute concentration. Specifically, in the peripheral region (characterized by reduced thickness), solute concentration increases more rapidly than in the central region (with maximum thickness). During these processes, osmotic and oncotic forces interact. Since osmotic forces significantly exceed oncotic forces, salts migrate toward the center of the droplet, where the concentration of dissolved substances is lower. In contrast, in the central region, proteins and other high-molecular-weight solutes release water and shift toward the droplet periphery.

As a result, the marginal amorphous zone of the dehydrated droplet is represented by structures of organic

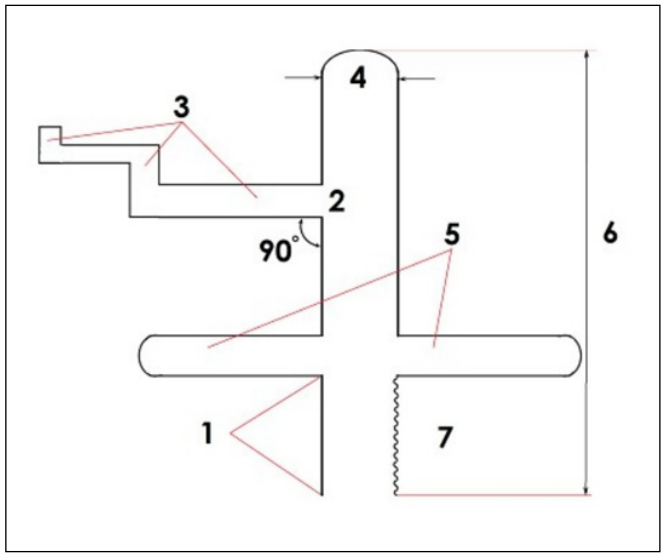


Fig. 1. Study of the crystal [21].
Marking:
Quantitative indicators:
1. Crystal length to branch point
2. Branching angle
3. Number of descendants of branches of the 1st, 2nd and other orders
4. The width of the crystal
5. Branching asymmetry
6. Crystal length
7. Number of microbranches along the length of the crystal

origin, whereas the central crystallized zone consists predominantly of salts. Thus, a highly dynamic transformation occurs, whereby unstable molecular-level structures are converted into stable macroscopic structures of the solid phase. According to the study by Annarelli et al. (2001), the central region is referred to as the zone of crystallized structures, while the peripheral region is termed the single amorphous zone [10]. The transitional zone can be observed only in studies of blood serum. The ratio between the peripheral width and the droplet diameter area is used to determine protein content in biological fluids [11]. Therefore, based on the principles of classical crystallography and early diagnosis of dental and systemic diseases, a method for crystallographic examination of one of the most accessible biological fluids—oral fluid - was proposed [12]. Oral fluid represents a highly organized and specific biological medium with unique and universal properties; it is a dynamic environment that reflects any changes occurring in the body, including pathological processes. Over recent decades, initial steps have been taken to investigate the manifestations of various diseases in the structural organization of oral fluid [13–16]. The physicochemical properties of oral fluid may be used as markers of pathological changes in the salivary glands and the oral cavity [4, 5, 8, 11, 17–21].

AIM

The aim of this study is to investigate morphological changes in the crystallization patterns of oral fluid during orthodontic treatment with aligners.

MATERIALS AND METHODS

Samples of dried saliva were analyzed from young adults aged 18–25 years of both sexes in a comparison group and in two main groups: patients treated with aligners without preventive measures and patients treated with aligners with preventive care, which consisted of using a Decasan solution in the form of rinses, applications, and irrigations twice daily (15–20 mL of solution for 30 seconds).

Oral fluid was collected using a sterile pipette in a volume of 0.2–0.3 mL from the floor of the oral cavity. Subsequently, three drops of oral fluid were applied onto microscope slides previously treated with alcohol and ether. Dehydration of the drops was carried out under standard conditions at a temperature of 22–24 °C. The micropreparations were examined using a VEGA TS 5130 MM TESCAN scanning electron microscope. Both central and peripheral zones of the oral fluid were analyzed. Fractal structures, individual crystals, and amorphous substances were evaluated. Interpretation of crystalloscopic components was performed using specialized reference tables describing the characteristics of the studied structures [21]. For quantitative assessment of microcrystals, a dedicated analysis algorithm was developed, according to which the following parameters were evaluated: length, width, and degree of curvature of the main branch; the ratio of the width of the main branch at the base and at the tip; the surface perimeter of the microcrystal; as well as the frequency and angle of dendrite deviation and the degree of symmetry of dendritic branching relative to the main axis (Fig. 1).

ETHICAL APPROVAL

The study received approval from the Institutional Bioethics Committee of the National Pirogov Memorial Medical University, Vinnytsya (protocol No. 11 of 12.01.2026), and permission to access the research was granted by University Hospital. As the study used anonymized retrospective data, informed consent was withdrawn. All procedures were in accordance with the ethical principles of the Declaration of Helsinki, which guarantees the confidentiality and anonymity of the participants.

RESULTS AND DISCUSSION

The crystallization of mixed saliva proceeded with the formation of tree-like structures. In the interpretation of

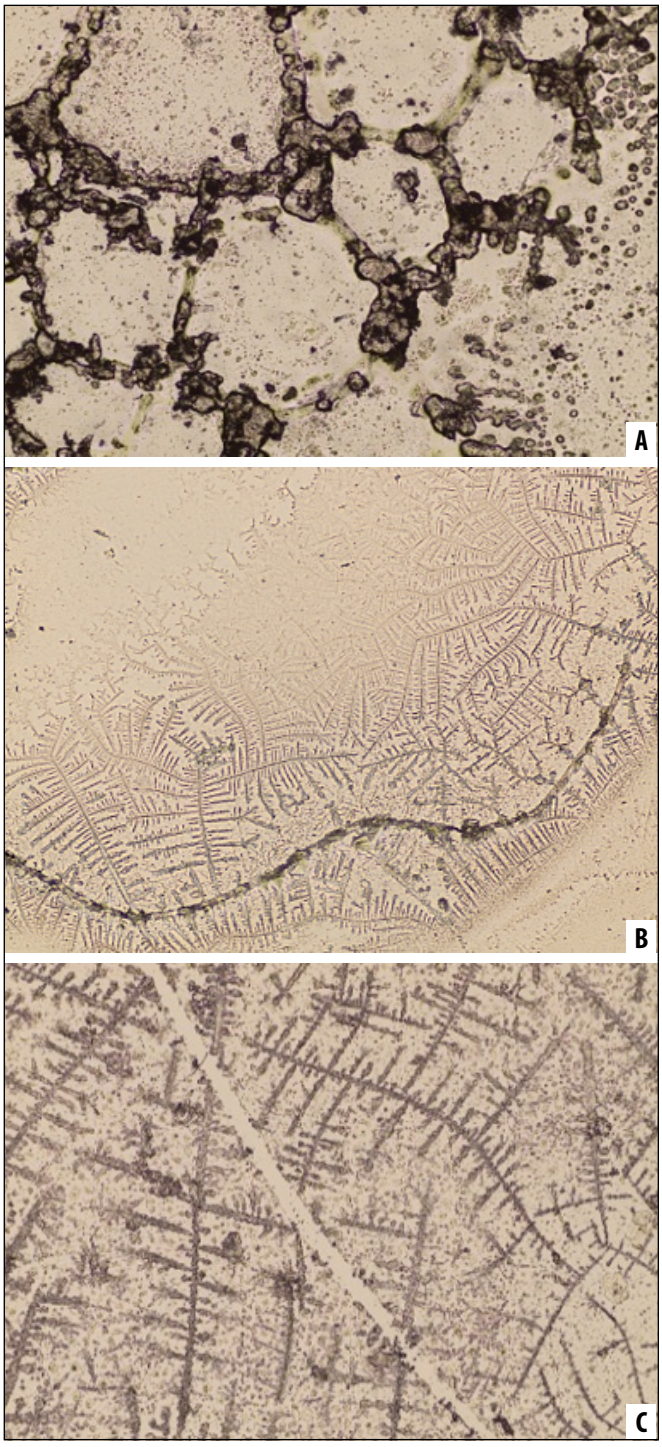


Fig. 2. Characteristics of saliva crystallization in healthy individuals. **A** - Peripheral (protein) zone. Magnification ×100. **B** - Transitional zone. Magnification ×100. **C** - Central (saline) zone. Magnification ×40
Source: Own materials

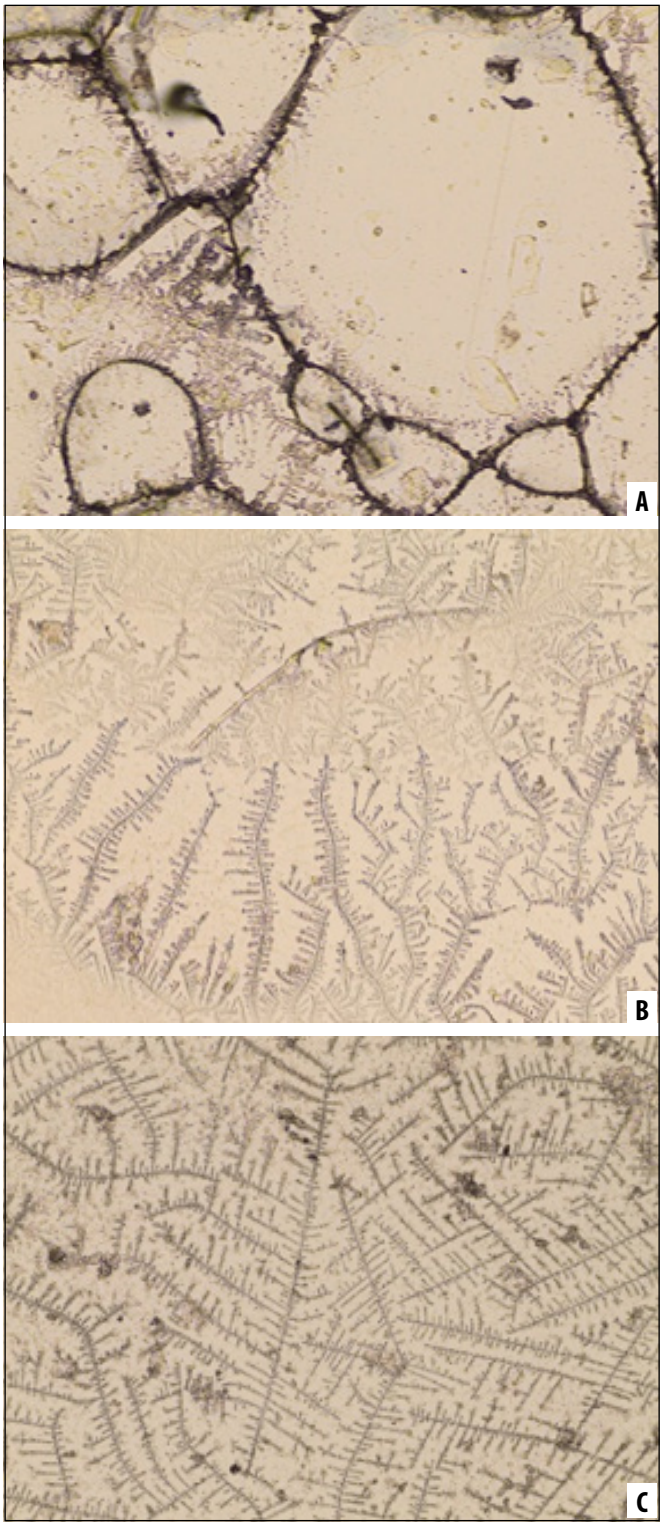


Fig. 3. Characteristics of mixed saliva crystallization at the beginning of treatment of dentofacial anomalies (DFA) with aligners. A - Peripheral (protein) zone. Magnification ×100; B –Transitional zone. Magnification ×100; C -Central (saline) zone. Magnification ×100
Source: Own materials

crystallograms, quantitative characteristics were taken into account, including crystal length up to the branching point, branching angle, the number of first-, second-, and higher-order generations, crystal width, total crystal length, and the number of microprojections along the crystal length. Qualitative features included uneven thickness within a single structure, asymmetry of branching, curvature of the main crystal trunk, crystals with indistinct contours, flat crystals, destructive-type changes, trunks without branching, and cruciform crystals.

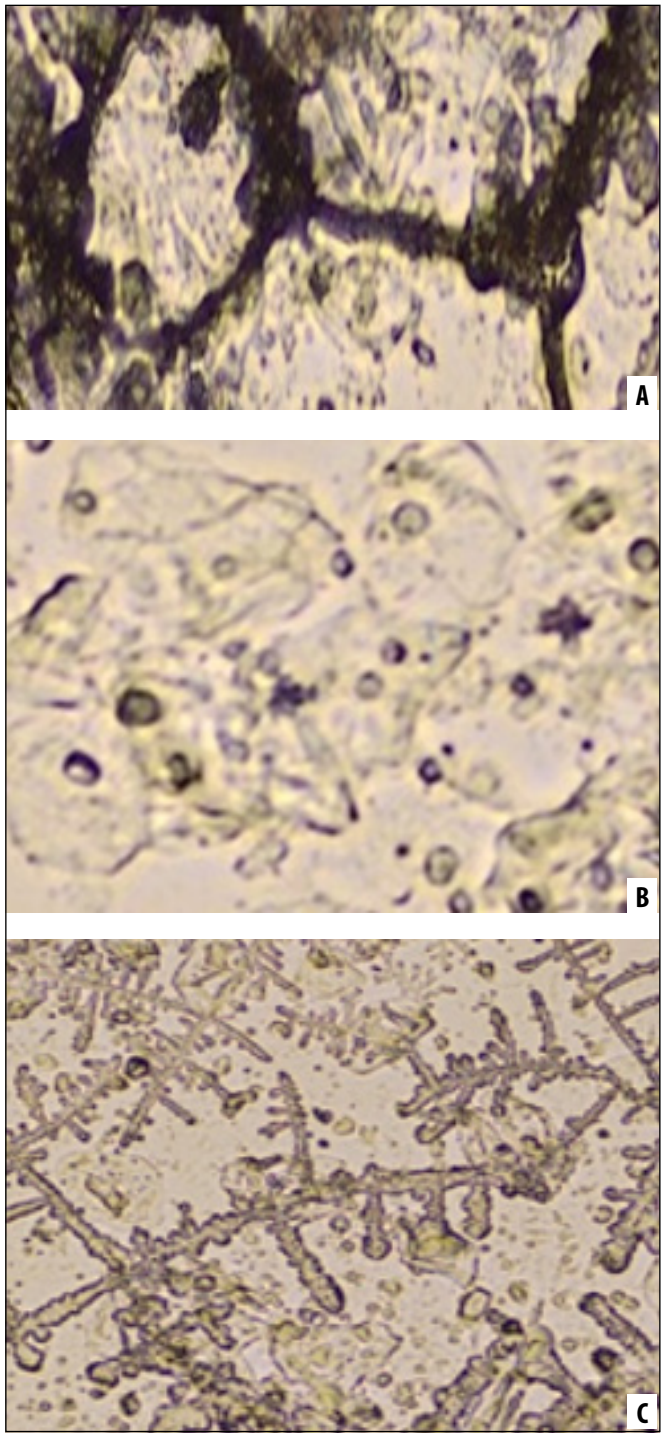


Fig. 4. Another type of crystallization at the beginning of treatment of DFA with aligners. A - Peripheral (protein) zone. Magnification ×100. B - Transitional zone. Magnification ×100. C - Central (saline) zone. Magnification ×100
Source: Own materials

The analysis of the crystallographic pattern demonstrated that, in the comparison group, crystals of uneven thickness predominated, along with structures exhibiting asymmetric branching. There were few crystallization trunks without branching and with curved main trunks. Minimal values were observed for such features as flat crystals, split crystals, unbranched trunks, and cruciform crystals.

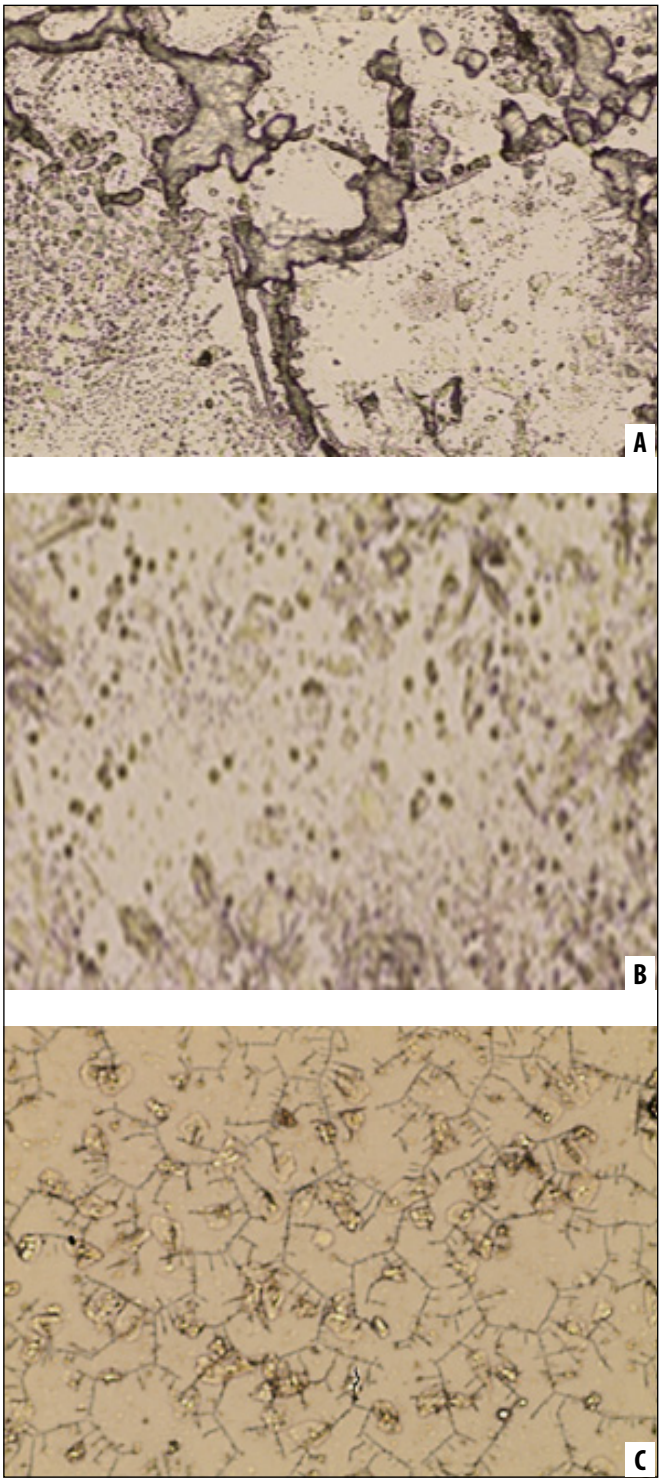


Fig. 5. Characteristics of mixed saliva crystallization during treatment of DFA with aligners after 3 months. **A** - Peripheral (protein) zone. Magnification ×100; **B** -Transitional zone. Magnification ×100; C - Central (saline) zone. Magnification ×40
Source: Own materials

In practically young adults, crystallization of saliva in the central (saline) zone was characteristically tree-like (fern-like). Large elongated crystalloprismatic structures were identified, which were properly fused with one another. In some areas, these structures were intercon-

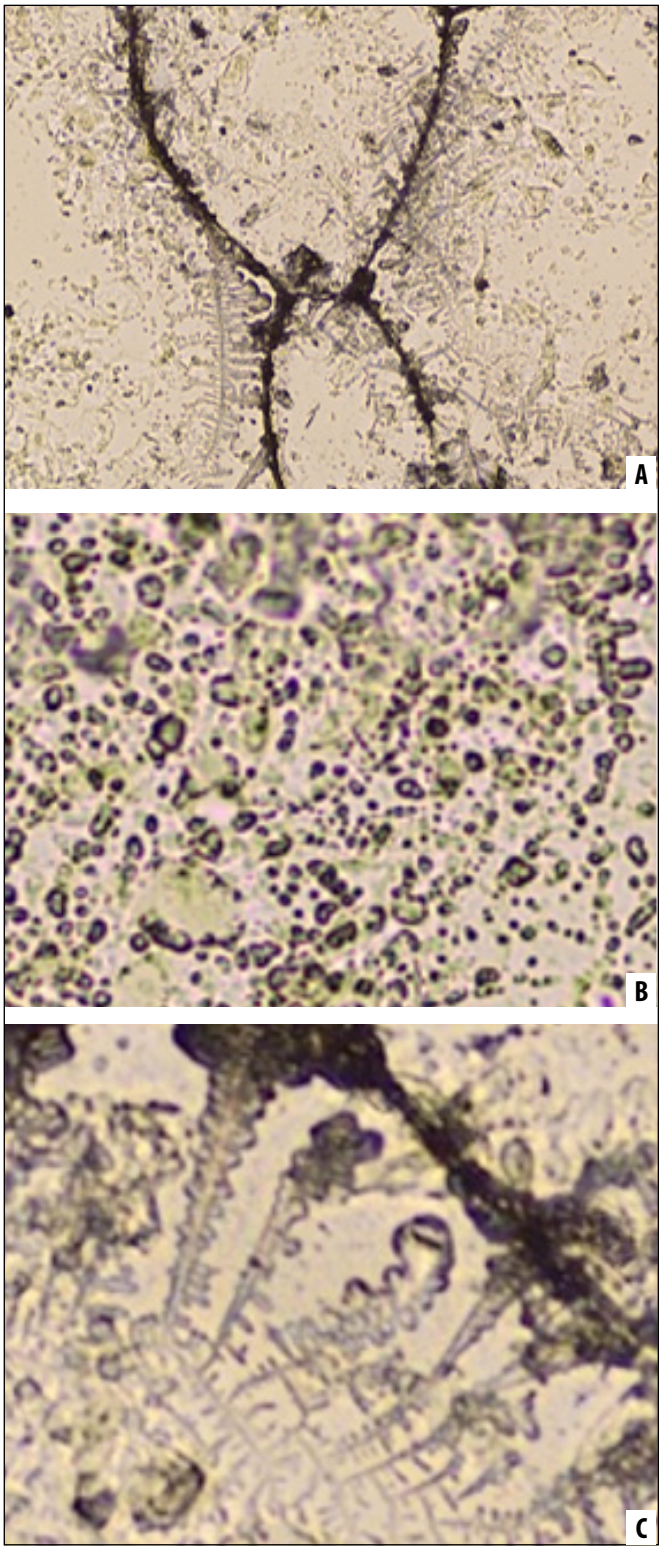


Fig. 6. The second type of crystallization in 25% of patients in group during treatment of DFA with aligners after 3 months. **A** - Peripheral (protein) zone. Magnification ×100; **B** -Transitional zone. Magnification ×100; **C-** Central (saline) zone. Magnification ×100
Source: Own materials

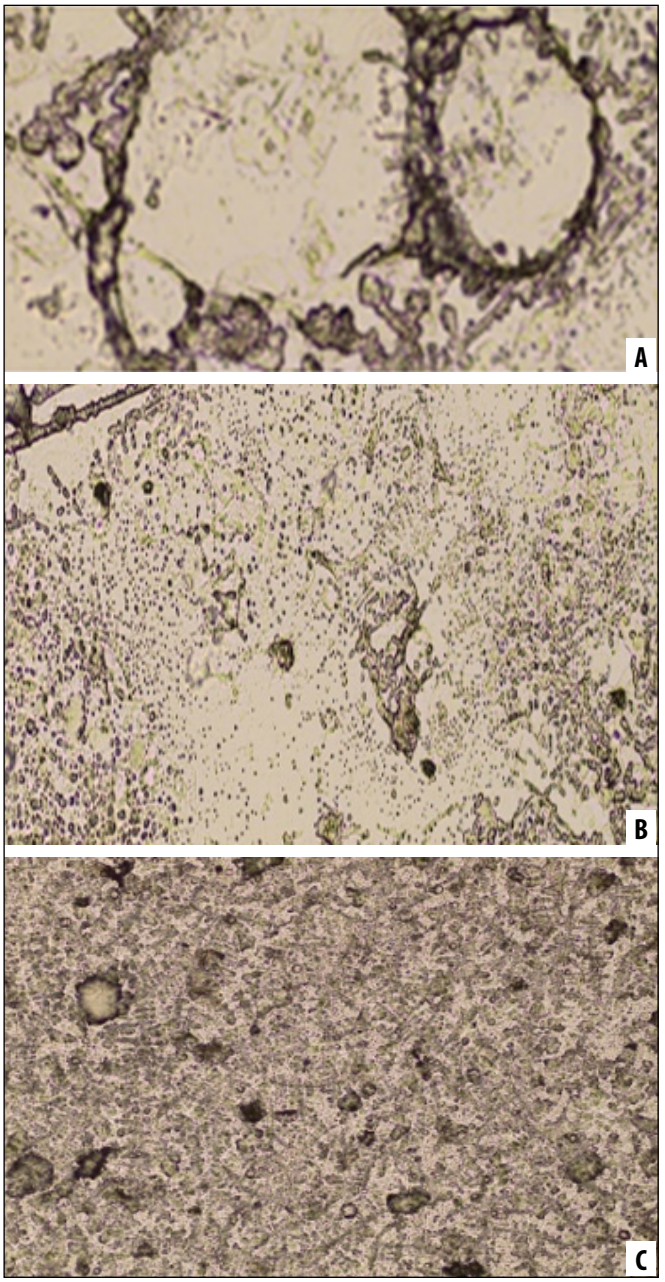


Fig. 7. Characteristics of mixed saliva crystallization during treatment of DFA with aligners 6 months after the beginning of therapy. A - Peripheral (protein) zone. Magnification ×200; B -Transitional zone. Magnification ×100. C - Central (saline) zone. Magnification ×200
Source: Own materials

nected; in the center, individual star-shaped crystals, as well as structures resembling twigs or branches, were observed. In the peripheral zone, amorphous spherical and ellipsoidal formations were noted. The transitional zone was relatively narrow (Fig. 2A-C).

The study of saliva crystallization patterns in the group of healthy young adults showed that, overall, the obtained data are consistent with the results reported by other researchers [27].

The study demonstrated that, in the main group of young adults treated with aligners, at the initial stage of treatment the crystallographic pattern was most frequently (68%) characterized by a distinct arrangement of large elongated prismatic crystals radiating from the center of the droplet

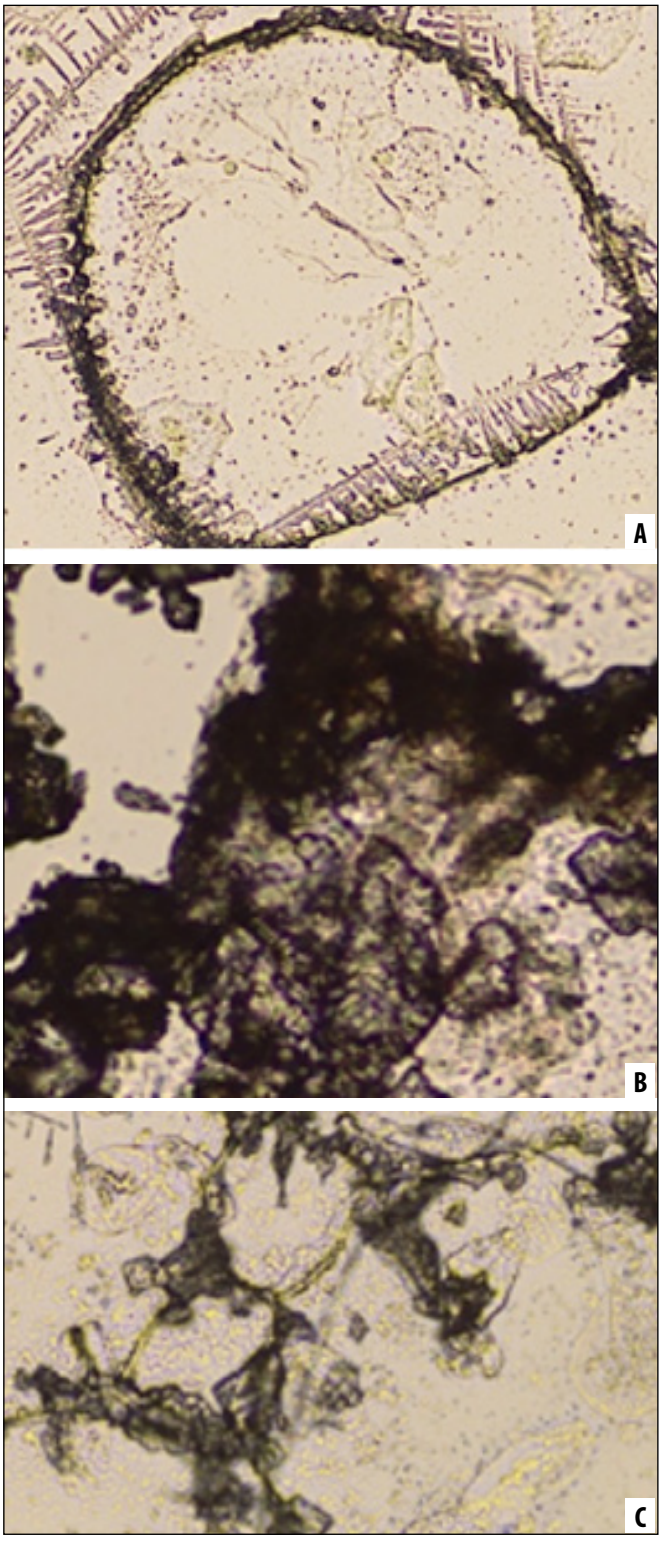


Fig. 8. Disruption of crystallization of mixed saliva according to the "coral-branch" type during treatment of DFA with aligners 6 months after the beginning of therapy. A - Peripheral (protein) zone. Magnification ×200; B -Transitional (saline) zone. Magnification ×200. C - Central (saline) zone. Magnification ×200
Source: Own materials

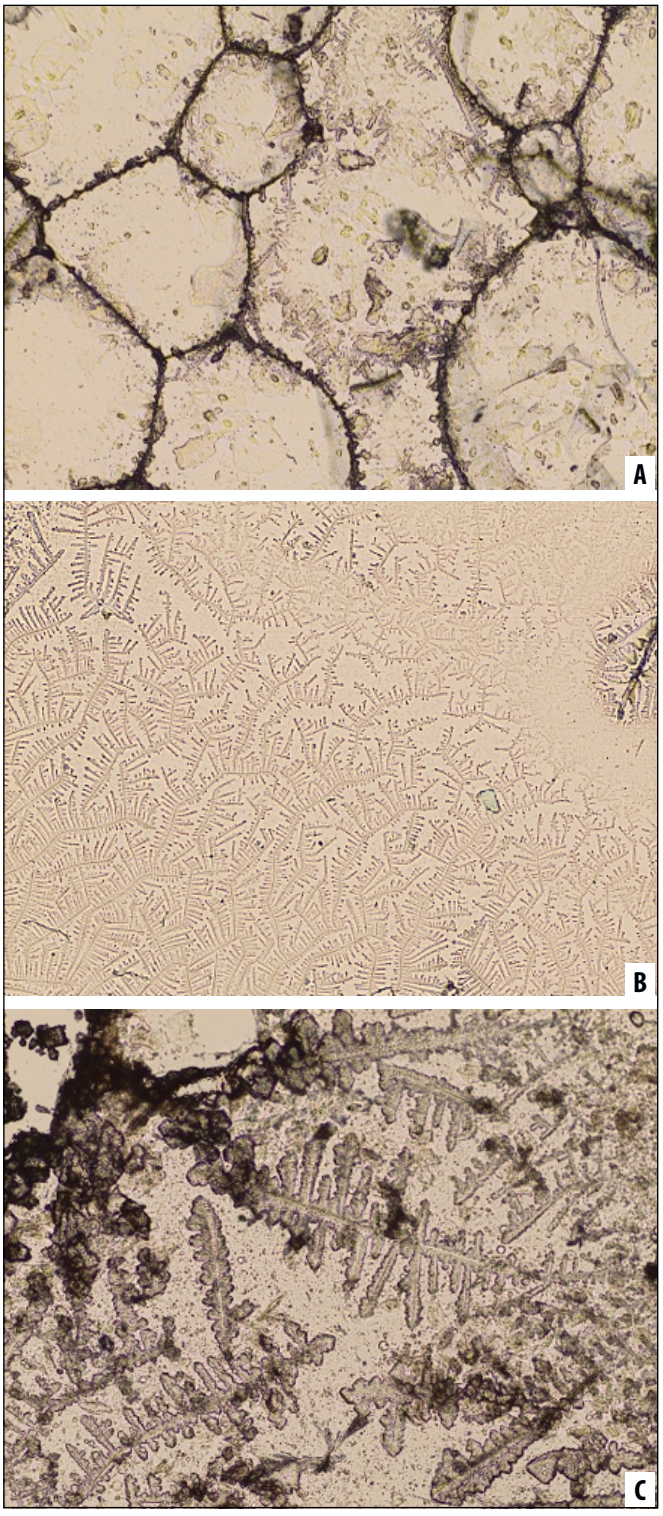


Fig. 9. Characteristics of mixed saliva crystallization during treatment of DFA with aligners at the beginning of therapy combined with the use of preventive agents. **A** - Peripheral (protein) zone. Magnification ×200; **B** -Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

and merging with one another to form so-called horsetail- or fern-like structures. The radial processes of the "fern leaves" extended through the transitional zone toward the peripheral zone. In the peripheral zone, these processes were arranged uniformly in the form of cracks. The transitional zone was wide and contained a certain number of crystals (Fig. 3A-C).

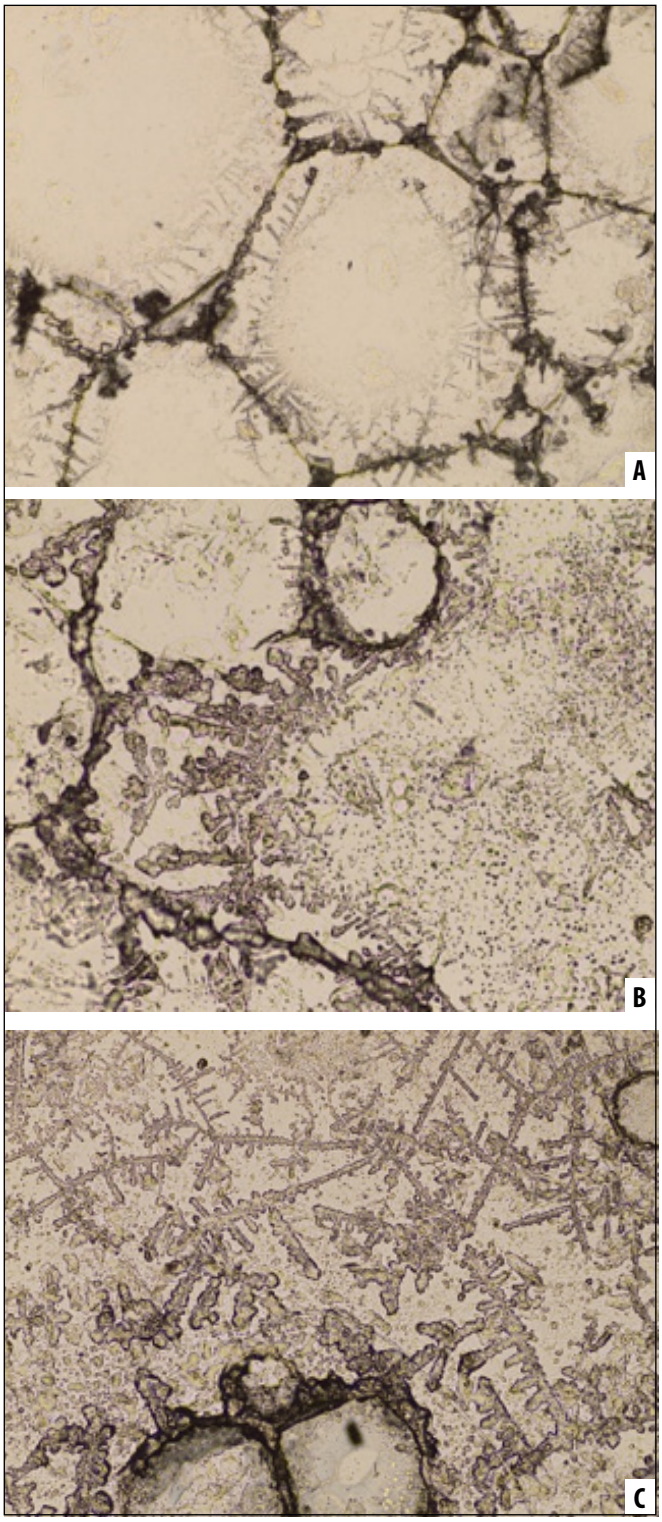


Fig. 10. Characteristics of crystal formation in mixed saliva during the treatment of DFA with aligners and the use of preventive measures after 3 months. **A** - Peripheral (protein) zone. Magnification ×200; **B** –Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

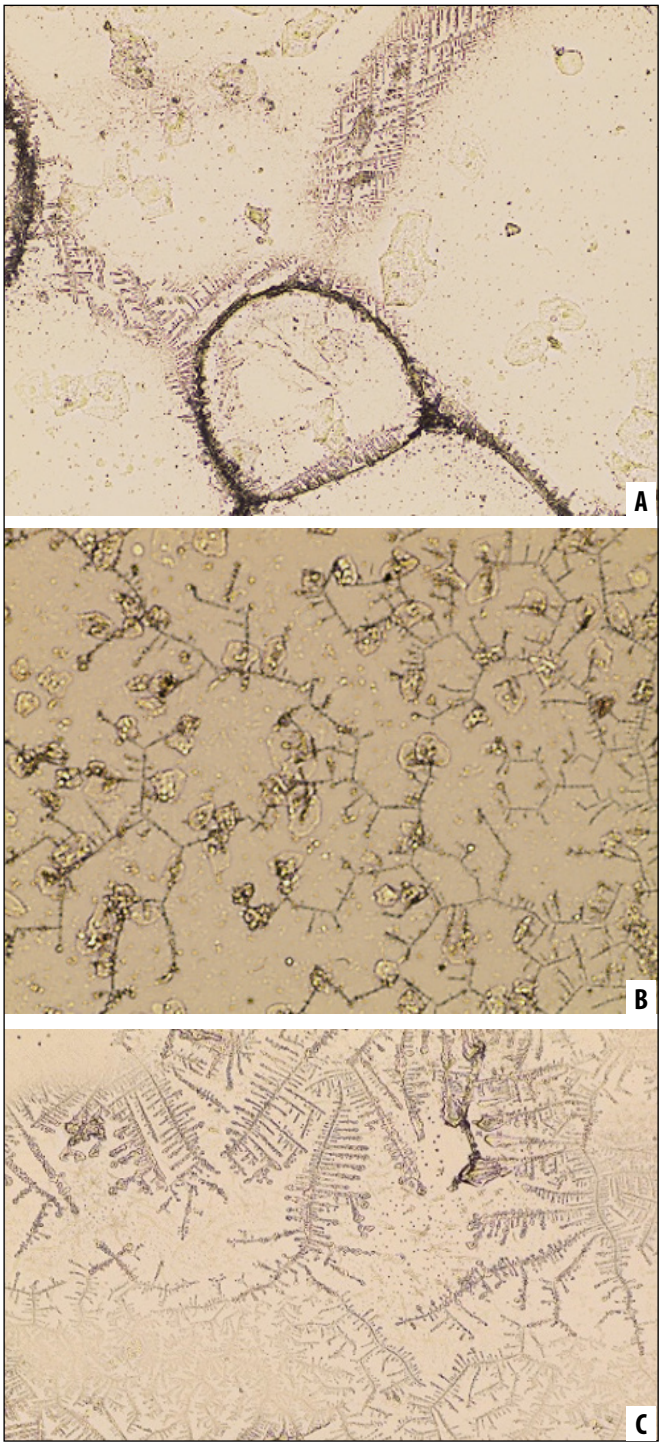


Fig. 11. Characteristics of crystallization of mixed saliva during the treatment of DFA with aligners and the use of preventive agents after 6 months. **A** - Peripheral (protein) zone. Magnification ×200; **B –**Transitional (saline) zone. Magnification ×100; **C** - Central (saline) zone. Magnification ×200
Source: Own materials

In some patients from the aligner group, isolated cruciform crystals with a smaller number of dendritic formations were observed in the central (saline) zone of the specimen. In such cases, the peripheral zone was narrowed and exhibited both radial cracks and multidirectional fine cracks. Very rarely (in 8% of patients), no saliva crystallization was observed in the central zone; instead, a large number of amorphous structures with scattered

crystal fragments and dendritic formations were present. In these cases, the peripheral (protein) zone was narrow, appearing as a strip with numerous chaotically arranged cracks and crystal-like formations (Fig. 4A-C).

Three months after the initiation of treatment, disturbances in saliva crystallization were observed, which were evident from both qualitative and quantitative indicators. In the center of the facies, crystallization still had a fern-leaf appearance; however, the leaves were smaller in size. At the periphery, cracks as well as crystal-like and amorphous formations were again observed (Fig. 5A-C).

Only 25% of patients exhibited saliva crystallization in the form of an amorphous pattern with isolated scattered 'fragments.' These 'fragments', radiating from the center of the facies, merged with one another in the peripheral zone, which was narrow and appeared as a strip (Fig. 6A-C).

Six months after the initiation of treatment, examination of dried facies revealed that, in the majority of patients (65%), there was crystal disintegration of the saliva, splitting of the crystal apex, and absence of microprocesses (Fig. 7A-C).

In 20% of patients, branching was completely absent; the processes were long and deformed, as were their apices. When branching was observed, it was limited only to the crystal apex in the form of a spike.

Occasionally, a so-called "coral branch," that is, unilateral branching, was observed. The crystallization pattern was so severely disturbed that it made quantitative assessment of saliva crystallization impossible (Fig. 8A-C).

Thus, it was established that the use of aligners leads to disturbances in mixed saliva crystallization. The peak of these disturbances occurs at the sixth month of orthodontic treatment.

A study of the crystallographic pattern of a dried saliva droplet (facies) was also conducted during the use of aligners in combination with preventive agents.

At the beginning of treatment, a distinct pattern of the dried saliva droplet was observed, with medium-length prismatic crystals extending from the center to the periphery; isolated fern-like structures were noted in the transitional zone.

The peripheral (protein) zone contained a large number of amorphous structures (Fig. 9A-C).

Three months after the start of treatment, destruction of the clear crystal structure was observed in the central (saline) zone of the facies. In the center of the droplet, individual star-shaped crystals were identified; throughout the entire area of the droplet, isometrically arranged crystalline structures of star-shaped, rounded, and irregular forms were observed (Fig. 10A-C).

After 6 months from the initiation of treatment, a reduction in the size and number of amorphous structures was observed in the peripheral (protein) zone; an expanded transitional zone with a small number of crystal-like structures; and isolated fern-like crystals in the central (salt) zone (Fig. 11A-C).

Analysis of the quantitative parameters of salivary crystallization in patients treated with aligners for DFA demonstrated that, after 3 months from the start of treatment, the crystal length decreased on average by 1.5 times, and after 6 months by 3 times in both groups. A progressive decrease in the number of branching offspring was noted (from an average of 5 at baseline to 3 at month 3 in the aligner group; and from an average of 12 at baseline to 7 at month 3 in the group receiving preventive measures; at month 6 of treatment, the values decreased to 1 and **2** branches, respectively). Crystal width decreased at months 3 and 6 of aligner treatment by 3 times, from (0.09 ± 0.003) to (0.03 ± 0.007) mm. In contrast, treatment with orthodontic appliances combined with preventive measures resulted in a 2 times reduction in crystal width at month 3, from (0.08 ± 0.002) to (0.04 ± 0.006) mm, followed by an increase at month 6 to a value 4 times higher than baseline - up to (0.32 ± 0.009) mm ($p \leq 0.05$).

At the 3rd month of aligner treatment, the crystal length decreased on average by 45% - from (0.125 ± 0.037) to (0.067 ± 0.002) mm and correspondingly from (0.40 ± 0.016) to (0.21 ± 0.001) mm. By the 6th month of treatment, this parameter was, on average, 4 times lower in both treatment groups - (0.031 ± 0.003) mm and (0.10 ± 0.001) mm, respectively ($p \leq 0.05$). The number of microbranches of the main crystal trunk at the 3rd month of treatment decreased 2.7 times in the aligner-only group and 2 times in the aligner plus prophylaxis group, while at the 6th month it decreased 4 times in both groups compared with baseline values. The branching angle, which increased 2 times at the 3rd month of treatment in the aligner-only group - from (85 ± 0.1) to (170 ± 2.1) degrees - showed almost no change (only a 5% increase) in the aligner plus prophylaxis group - from (90 ± 1.8) to (95 ± 1.8) degrees. At the 6th month, this parameter was respectively 30% and 10% lower - (60 ± 3.2) and (77 ± 2.4) degrees - compared with baseline values ($p \leq 0.05$).

CONCLUSIONS

The use of aligners for the treatment of dental anomalies in young adults aged 18–25 years induces destructive morphological changes in the crystallization of mixed saliva at 3 and 6 months after the initiation of treatment. Prophylactic application of a Decasan solution demonstrates a pronounced protective effect.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Condition of periodontal tissues in experimental animals under the model of periodontitis combined with intestinal dysbiosis

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ABSTRACT

Aim: To study the nature of clinical and morphological changes in periodontal tissues under combined modeling of periodontitis and intestinal dysbiosis in an animal experiment.

Materials and Methods: The study involved 30 rats (1 control and 2 experimental groups of 10 animals each). Histological changes in the gingiva were examined during periodontitis modeling in 10 animals over 30 days and its combination with intestinal dysbiosis in the remaining 10 animals over 30–35 days. The degree of alveolar bone ridge atrophy was assessed, and the composition of the intestinal microflora was analyzed to confirm intestinal dysbiosis.

Results: It was established that the reproduction of experimental periodontitis in animals and its subsequent combination with intestinal dysbiosis contributed to the development of a pathological process in the periodontal tissues in 100% of rats, resembling an exacerbated course of human periodontitis, with increased severity and progression. In rats with simultaneous modeling of periodontitis and intestinal dysbiosis, more pronounced pathological changes were observed—compared to those without dysbiotic lesions—in the gingival epithelium, the connective tissue of the mucous membrane, and especially in the blood vessels of the microcirculatory bed and perivascular areas.

Conclusions: Morphological studies confirmed that disturbances in the intestinal microecological system, accompanied by suppression of its saprophytic flora, aggravate the course of periodontitis, promote its generalization, and contribute to the development of abscess-forming inflammation.

KEY WORDS: periodontal disease, intestinal dysbiosis, experimental research

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INTRODUCTION

Experimental studies hold considerable significance in modern medicine [1-3]. Conducting experiments on animals allows for an objective assessment of the nature of morphological changes in body tissues during disease development. This is particularly relevant for the study of comorbid conditions, including the combination of periodontal diseases with disorders of the digestive tract.

Scientific research has demonstrated a pathophysiological interconnection between the functional elements of the digestive system and the oral cavity, due to the unity of their functions and morphological structure, as the oral cavity represents the initial segment of the digestive tract [4-6]. The relationship and mutual influence between periodontal diseases and digestive system pathologies have been noted by numerous authors [7-10].

In a limited number of studies [11-13], attention has been given to the peculiarities of periodontal disease progression when combined with intestinal dysbiosis. According to the literature [14], symptoms of dysbiosis are found in up to 80% of the healthy global population. Such a latent (compensated) stage of dysbiosis contributes to disturbances in absorption mechanisms, leading to changes in the mineral metabolism of macro- and microelements and their regulators in the blood and oral fluid, to the development of immunological imbalance, alterations in the oral microbiocenosis, and other homeostatic constants of this biotope [15].

In this context, modelling a pathological process in animals that closely resembles that in humans, and

studying the nature of periodontal tissue changes during the reproduction of a comorbid condition—specifically, the combination of periodontitis and intestinal dysbiosis—presents considerable scientific interest.

AIM

To study the nature of clinical and morphological changes in periodontal tissues during combined modelling of periodontitis and intestinal dysbiosis in an animal experiment.

MATERIALS AND METHODS

To achieve the aim, an experimental study was conducted on 30 laboratory animals — white outbred rats aged three months, with an average weight of 273±20 g (1 control and 2 experimental groups, each consisting of 10 animals).

The control group consisted of intact rats (10 animals) kept on a standard vivarium diet. Group II included 10 rats in which experimental periodontitis was induced over 30 days using the reduced masticatory function method by maintaining the animals on a special paste-like diet [16]. In Group III, after induction of periodontitis, 10 experimental animals received oral ciprofloxacin (50 mg/kg) from day 31 to day 35 to create experimental intestinal dysbiosis using the selective decolonization method [17].

Once a week, the degree of alveolar bone atrophy was visually assessed based on the periodontal tissue condition in each rat, according to the following scoring system: 0 – healthy periodontium; 1 – gingivitis (hyperemia in the frontal teeth area, slight gum swelling); 2 – periodontitis of mild to moderate severity (hyperemia, edema, bleeding of the gums in the frontal and lateral tooth regions); 3 – severe periodontitis (pronounced gingival hyperplasia, swelling, bleeding along the gingival margin, and spontaneous tooth loss). The total score for each animal was divided by the number of rats in the group.

At the end of the experiment (animals in Groups I and II – after 30 days, Group III – after 35 days), the rats were sacrificed by exsanguination. After euthanasia, the large intestine was isolated from each rat, ligated at both ends, and its contents were cultured on nutrient media to determine the qualitative and quantitative composition of the microbiota.

For isolation of pure microbial cultures, differentiation, and identification, the following media were used: meat–peptone agar, blood and serum agar, Endo, Chestovych, and Sabouraud media.

Microscopic, cultural, and biochemical methods were applied to differentiate and identify microorganisms, and their absolute values were calculated as logarithmic colony-forming units per 1 ml (lg CFU/ml).

After extraction of the jaw bone tissue, the nature of the dystrophic process in periodontal tissues was evaluated using biometric methods by determining the linear dimensions of molar root exposure with a binocular loupe equipped with an eyepiece scale (graduation value 0.05 mm).

The degree of relative exposure of the molar roots (K) was calculated using the formula and expressed as a percentage: $\hat{E} = \frac{l \times 100}{l}$,

where M – distance from the edge of the dental alveolus to the cusp tip of the tooth, and l – distance from the edge of the dental alveolus to the anatomical neck of the tooth. Intact rats served as the control.

For histological examination of the gingival structures, sections 5 μm thick were prepared and stained with hematoxylin and eosin, Van Gieson's stain, and toluidine blue. Microscopic analysis was performed using a light microscope with objectives ×4, ×10, ×40 and an eyepiece ×10. Photographs were taken with a digital camera.

The reliability of the results was evaluated using Student's t-test. Statistical processing of the obtained data was performed according to the recommendations [18].

ETHICS

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

FRAMEWORK

The study was carried out within the framework of the scientific topic "*Individualization of approaches to dental treatment within the structure of a comprehensive model for predicting therapeutic outcomes*" state registration number 0123U104050 of the state university "Uzhhorod National University".

RESULTS

At the beginning of the study, clinical observation of the animals' general condition, appearance, behavior, and reactions revealed no changes that would prevent the creation of the experimental model of periodontitis and its combination with intestinal dysbiosis.

The anatomical and histological structure of the soft periodontal tissues (gingiva and periodontium) of intact rats did not differ from those described in the literature [19] (Fig. 1).

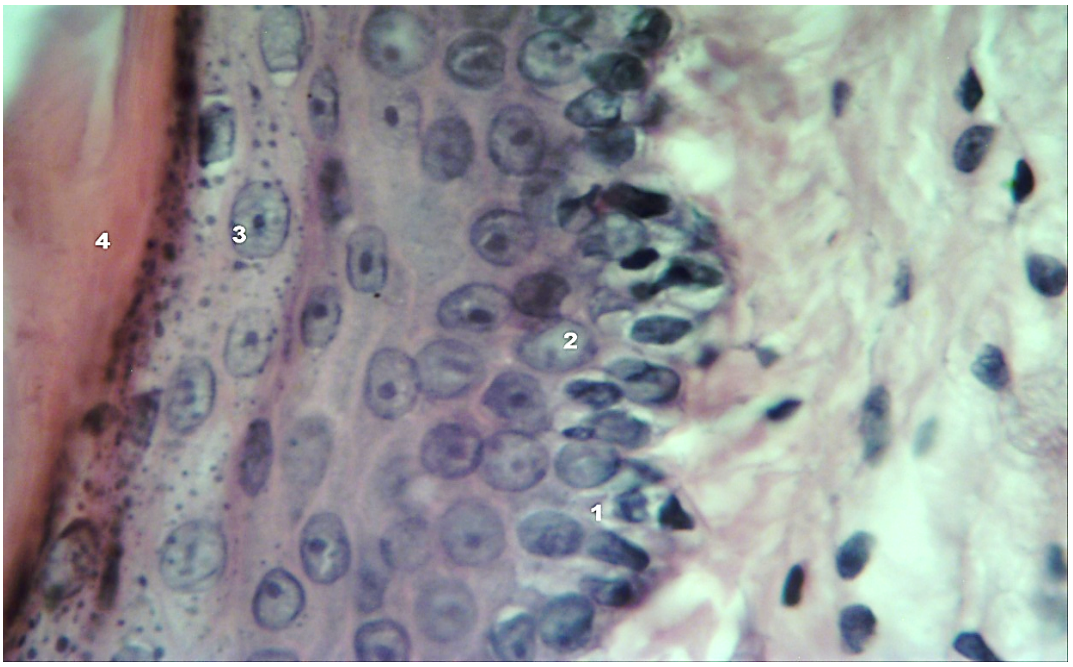


Fig. 1. Gingiva of intact rat No. 5, Group I. Hematoxylin and eosin staining. Magnification: ×400. 1 – basal layer epithelial cells; 2 – prickle layer epithelial cells; 3 – granular layer epithelial cells; 4 – keratinized layer
Picture taken by the authors

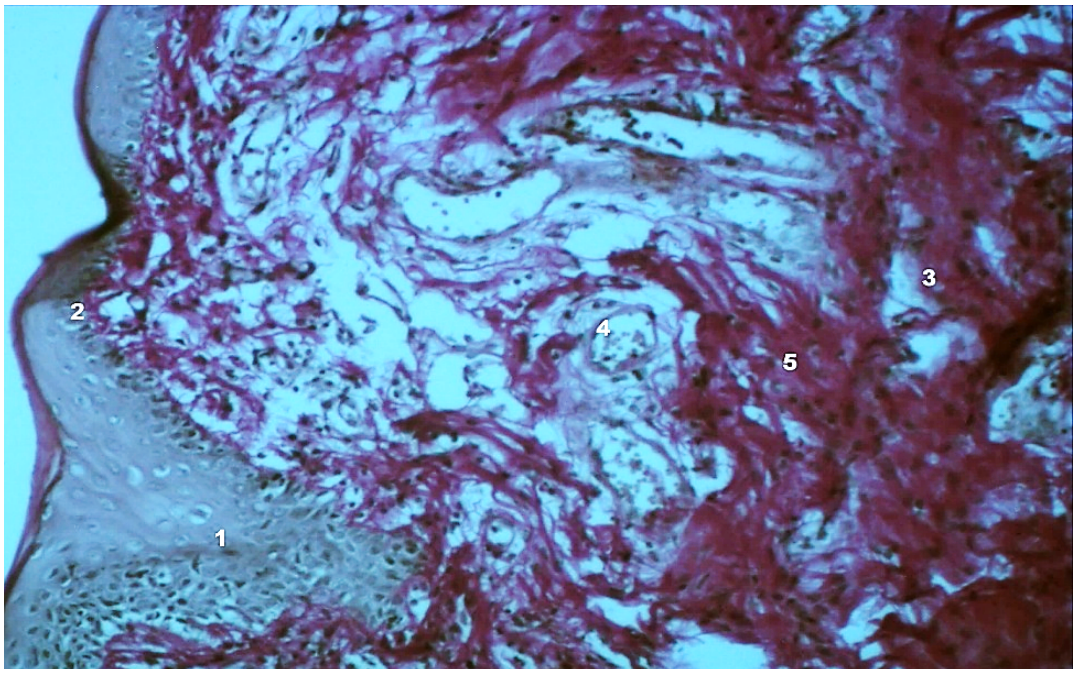


Fig. 2. Gingiva of rat No. 1, Group II, day 30 of the experiment. Diagnosis: experimental periodontitis. Destruction of collagen fibers and leukocytic infiltration observed in the basal membrane of the epithelial lining of the oral mucosa. Hematoxylin and eosin staining. Magnification: ×400. 1 – basal layer epithelial cells; 2 – prickle layer epithelial cells; 3 – basal membrane; 4 – leukocytic infiltration
Picture taken by the authors

When modeling periodontitis, after one week of the experiment, 2 out of 10 animals (20%) showed hyperemia and swelling of the marginal gingiva. The gingival mucosa of the remaining rats in Group II was pale pink, firm on palpation, non-bleeding, with no pathological pockets, and the teeth remained immobile. The periodontal tissue condition score was 0.2 points.

After two weeks, 7 animals (70%) in Group II developed gingival hyperemia, edema, and bleeding; however, the tooth–gingival attachment remained intact and the teeth were not mobile. The clinical evaluation score of the periodontal condition was 0.8 points.

After three weeks of the experimental study, 3 animals (30%) in this group exhibited gingival hyperemia, swelling, bleeding, loss of tooth–gingival attachment, and tooth mobility. In 5 animals (50%), the clinical condition of the gums corresponded to gingivitis. The total periodontal tissue condition score was 1.1 points.

After 30 days, the periodontal tissue condition in experimental rats of Group II was as follows: in 4 animals (40%) – severe periodontitis (marked gingival hyperemia, swelling, bleeding, presence of periodontal pockets, and tooth mobility); in 4 animals (40%) – moderate to mild periodontitis (gingival hyperemia, swelling,

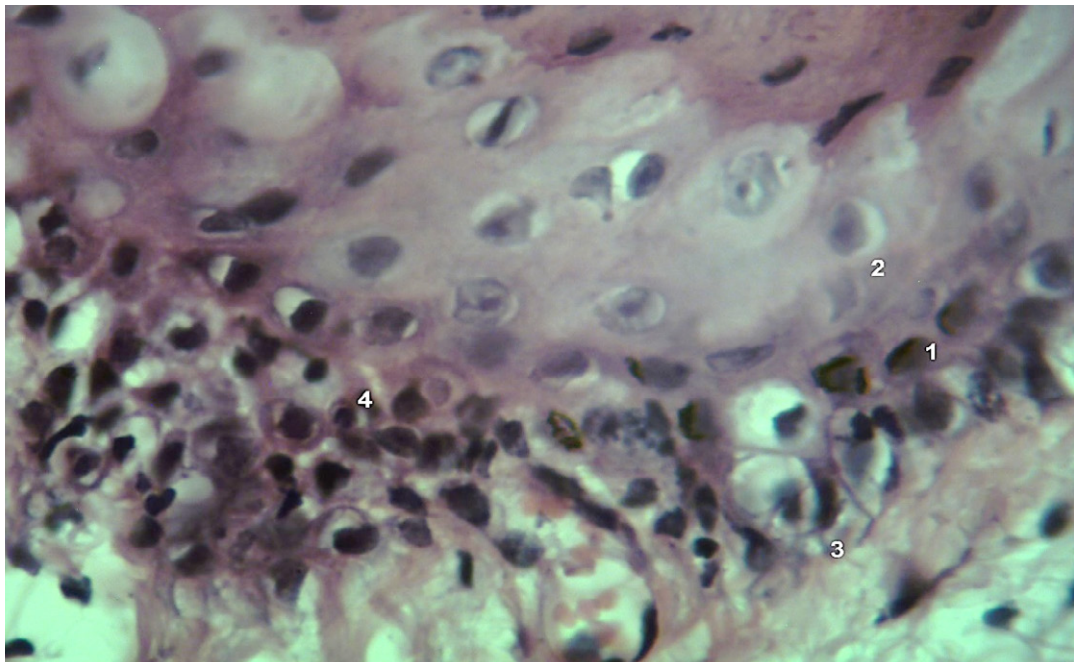


Fig. 3. Gingiva of rat No. 3, Group II, day 30 of the experiment. Diagnosis: experimental periodontitis. Destruction of collagen fibers observed in the papillary layer of the oral mucosa. Van Gieson staining. Magnification: ×100. 1 – stratified keratinized squamous epithelium; 2 – papillary layer of the oral mucosa; 3 – reticular layer of the oral mucosa; 4 – blood vessels; 5 – collagen fibers
Picture taken by the authors

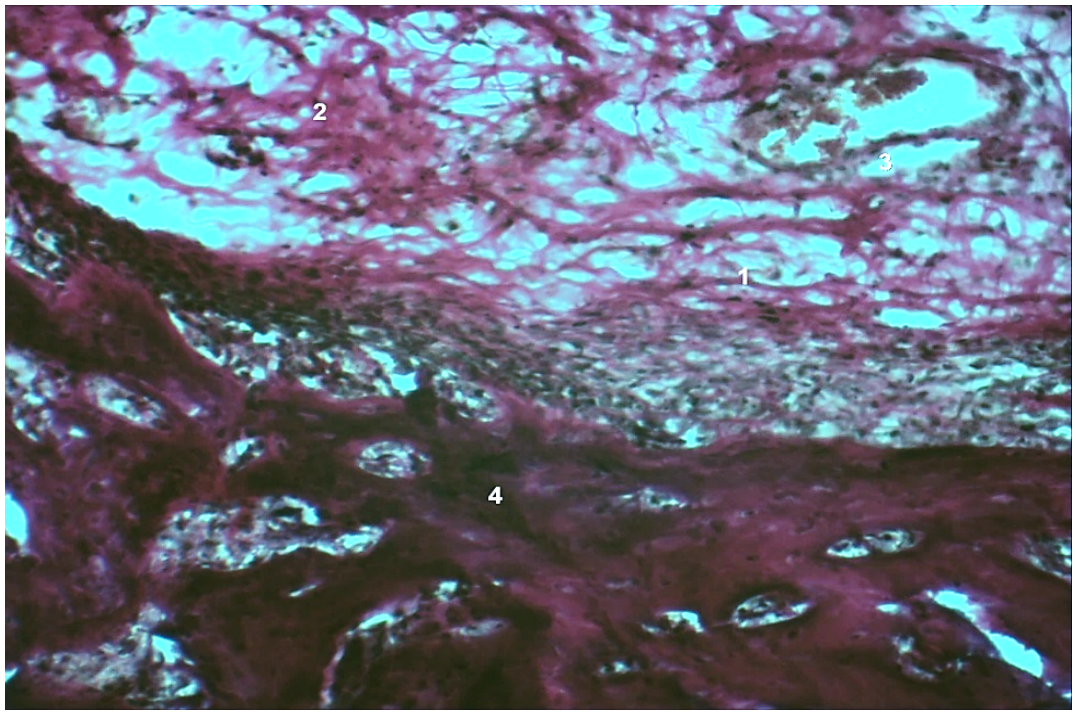


Fig. 4. Gingiva of rat No. 6, Group II, day 30 of the experiment. Diagnosis: experimental periodontitis. Vascular resorption of bone tissue observed. Van Gieson staining. Magnification: ×100. 1 – reticular layer of the oral mucosa; 2 – collagen fibers; 3 – perivascular interstitial edema; 4 – bone tissue
Picture taken by the authors

bleeding, destruction of the tooth–gingival attachment, and shallow periodontal pockets detected by probing); in 2 animals (20%) – gingivitis (slightly pronounced hyperemia and swelling of the marginal gingiva).

The average periodontal tissue condition score was 2.2 points. The degree of dystrophy of the alveolar process of the jaws was 36.8±0.33% (compared with 28.3±0.21% in the control group), $p < 0.001$.

On the 30th day of the experiment, morphological examination revealed changes in the epithelial layer and connective tissue of the gingiva. The epithelial layer was uneven in thickness — thinned in some areas and thickened in others. Compared with rats of the intact group, the keratinized layer was thicker and showed detachment over large portions of the epithelial surface. Nucleoli were often absent in the nuclei of prickle-cell layer cells, indicating a decrease in their functional activity. In the basal layer, intercellular spaces were widened, and the cells appeared vacuolated.

In some areas, the boundary between the epithelium and connective tissue was flattened. Collagen fibers of the basal membrane were loosened, and among them

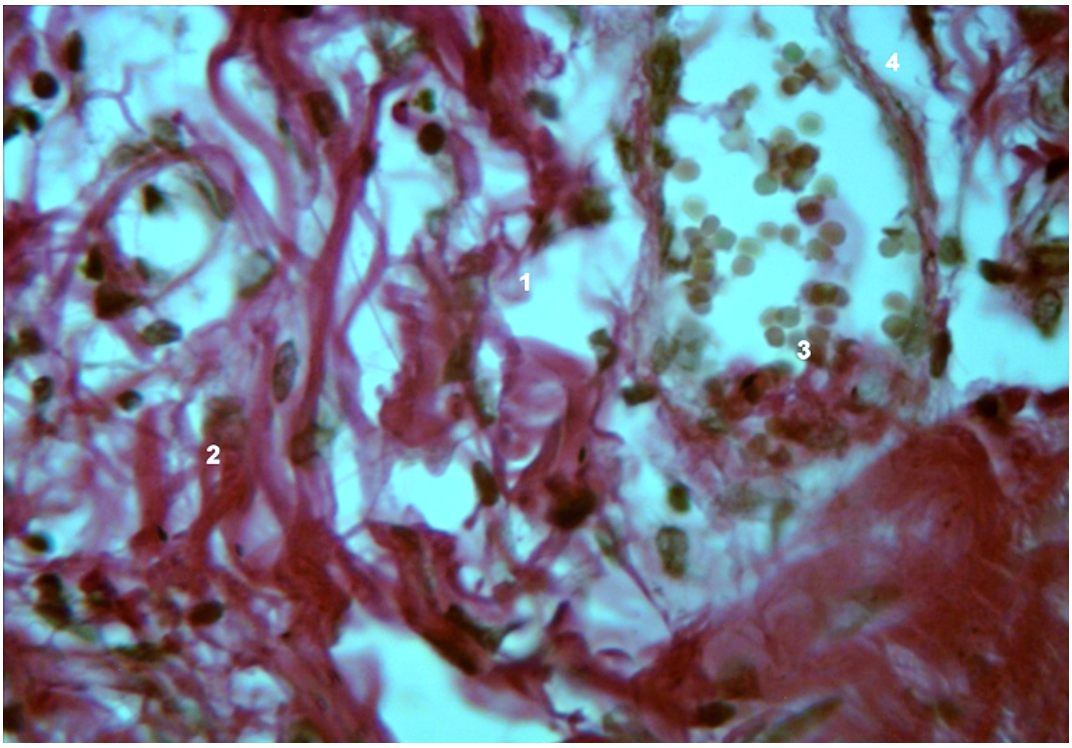


Fig. 5. Gingiva of rat No. 4, Group II, day 30 of the experiment. Diagnosis: Experimental periodontitis. Dilated vascular lumens, leukocyte stasis, and marginal accumulation in venules. Staining: Van Gieson method. Magnification: ×400. 1 – reticular layer; 2 – collagen fibers; 3- leukocyte stasis and marginal accumulation in venules; 4 – perivascular interstitial edema
Picture taken by the authors

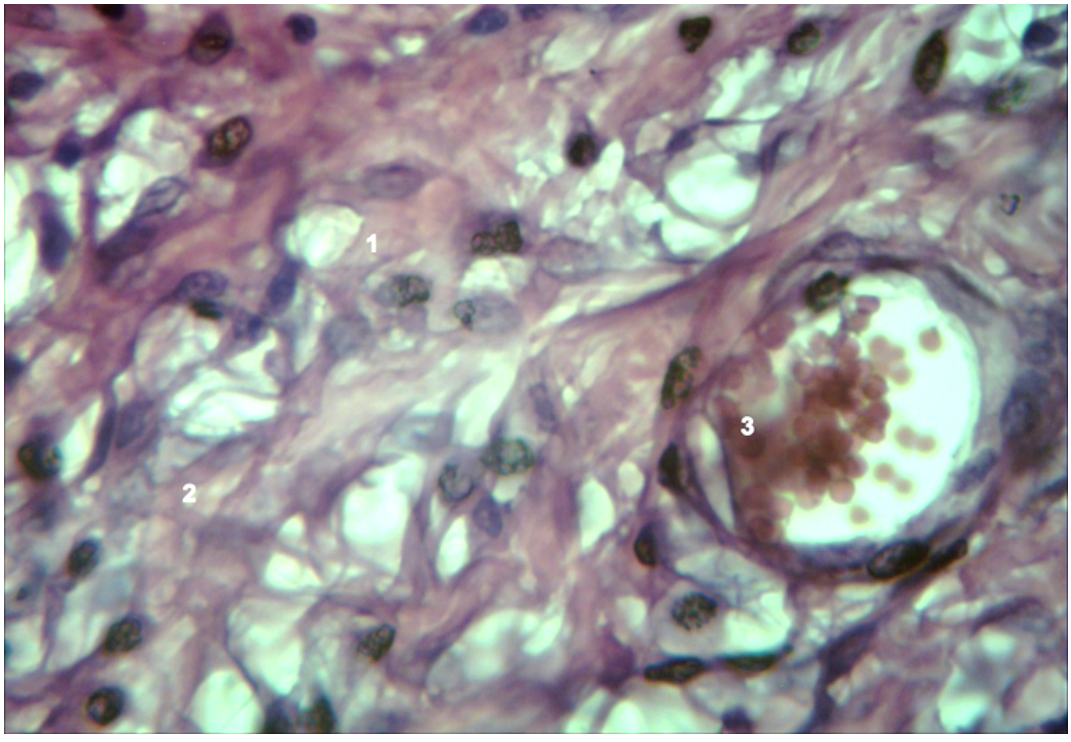


Fig. 6. Gingiva of rat No. 7, Group II, day 30 of the experiment. Diagnosis: Experimental periodontitis. Presence of mural thrombi in arterioles. Staining: Hematoxylin and eosin. Magnification: ×400. 1 – reticular layer; 2 – collagen fibers; 3 – mural thrombi in arterioles
Picture taken by the authors

were lymphocytes, histiocytes, and neutrophilic leukocytes. In certain regions, the collagen fibers appeared disorganized (Fig. 2).

In the papillary layer of the gingival connective tissue, areas of edema, destruction, and disorganization of collagen fibers were detected; the fiber bundles were separated and loosened. The cytoplasm of fibroblasts and fibrocytes appeared pale and swollen, and their nuclei were pyknotic and often showed destructive changes. Blood vessels were engorged, with dilated lumina (Fig. 3).

In the deep regions of the reticular layer, leukocytic infiltrates were observed. Changes were also found in the bone tissue of the alveolar processes of the jaws: lacunar resorption, dilation of the central vascular channels of osteons filled with leukocytes, and proliferation

Table 1. Results of the study of intestinal microbiocenosis in experimental animals, lg CFU/ml

Microorganisms	Groups of examined animals		
	Group I (control) n=10	Group II (with simulated periodontitis) n=10	Group III (with simulated periodontitis and intestinal dysbiosis) n=10
Total bacteria count	9.5±0.11	9.6±0.08 p>0.05	9.8±0.36 p>0.05
E.coli	6.2±0.08	6.3±0.10 p>0.05	6.0±0.09 p>0.05
Pseudomonas aeruginosa	1.0±0.05	1.2±0.08 p>0.05	1.9±0.06 p<0.001
Enterococus faecalis	1.0±0.11	1.2±0.09 p>0.05	1.9±0.07 p<0.001
Staphylococcus aureus	2.5±0.23	2.6±0.10 p>0.05	3.7±0.12 p<0.001
Fungi Candida	1.9±0.04	2.0±0.06 p>0.05	3.0±0.14 p<0.001
Bifidobacterium	3.1±0.18	2.8±0.18 p>0.05	1.0±0.04 p<0.001
Lactobacillus	4.0±0.15	3.7±0.10 p>0.05	1.4±0.04 p<0.001
Unidentified microorganisms	+	+	+

Note: p – is the significance of the difference in indicators between the experimental groups of animals (II and III) and the control group
Source: compiled by the authors of this study

of loose connective tissue around them. Osteoclasts were located along the edge of the bone lamella, while osteocytes showed signs of edema and cytoplasmic clearing (Fig. 4).

Significant alterations of blood capillaries, arterioles, and venules were noted. The walls of some capillaries were discontinuous. In certain regions, desquamation and swelling of endothelial cells were detected. The basal membrane in the capillary walls appeared loosened, and diapedetic hemorrhages were found around some blood capillaries.

The venules were dilated and engorged. Their lumina contained numerous leukocytes (both granular and agranular), exhibiting stasis, margination, and diapedesis through the vessel walls. Occasionally, mural thrombi were observed. The integrity of most venular walls was disrupted, with pronounced interstitial edema and perivascular hemorrhages. The perivascular spaces contained increased numbers of tissue basophils and leukocytes (Fig. 5).

The walls of the arterioles were thickened, with proliferation of collagen fibers in the adventitial layer and hyperplasia and hypertrophy of smooth muscle cells in the middle layer. The lumina of these vessels were narrowed. In some arterioles, mural thrombi almost completely occluded the vessel lumina (Fig. 6). The lumina of lymphatic vessels were dilated and filled with lymph.

On the 35th day of the experiment, when reproducing the model of experimental periodontitis in combination with intestinal dysbiosis, 100% of the rats exhibited gingival hyperemia with a bluish tint, swelling of the gingival papillae, the presence of periodontal pockets containing serous-purulent exudate, and tooth mobility, which were clinically assessed as periodontitis. In one rat, a periodontal abscess was diagnosed, indicating an aggressive nature of the pathological process in the periodontal tissues of this animal.

To confirm intestinal dysbiosis in Group III rats, the state of the colonic microbiota was compared with that of Groups I and II after completion of the experiment (Table 1). The obtained data demonstrated a generally stable species structure of the colonic microbiota, which did not change significantly during the modeling of periodontitis and intestinal dysbiosis. It was represented by monocultures and associations of conditionally pathogenic gram-negative bacteria of the family *Enterobacteriaceae* (*Escherichia coli*, *Enterococcus*), pathogenic microorganisms of the genus *Pseudomonas* (*Pseudomonas. aeruginosa*), as well as individual species of coccal (*Staphylococcus aureus*) and fungal (*Candida*) flora, and anaerobic antagonistically active bifidobacteria and lactobacilli, which suppress the growth and reproduction of pathogenic and conditionally pathogenic enteroba cteria.

The total microbial load in both experimental groups did not differ significantly from that of the control group

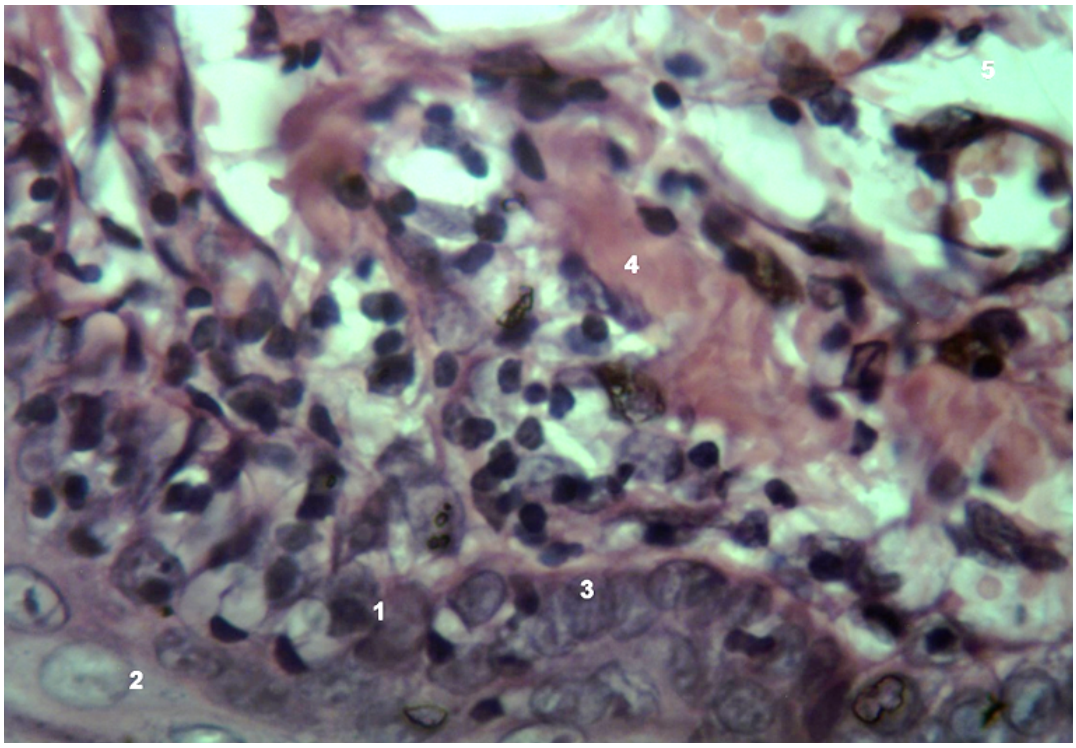


Fig. 7. Gingiva of rat No. 5, Group III, day 35 of the experiment. Diagnosis: Experimental periodontitis combined with intestinal dysbiosis. Destruction of collagen fibers and leukocyte infiltration within the basal membrane of the epithelial lining of the mucosa. Staining: Hematoxylin and eosin. Magnification: ×400. 1 – basal layer epithelial cells; 2 – prickle layer epithelial cells; 3 – basal membrane; 4 – collagen fibers; 5 – perivascular interstitial edema
Picture taken by the authors

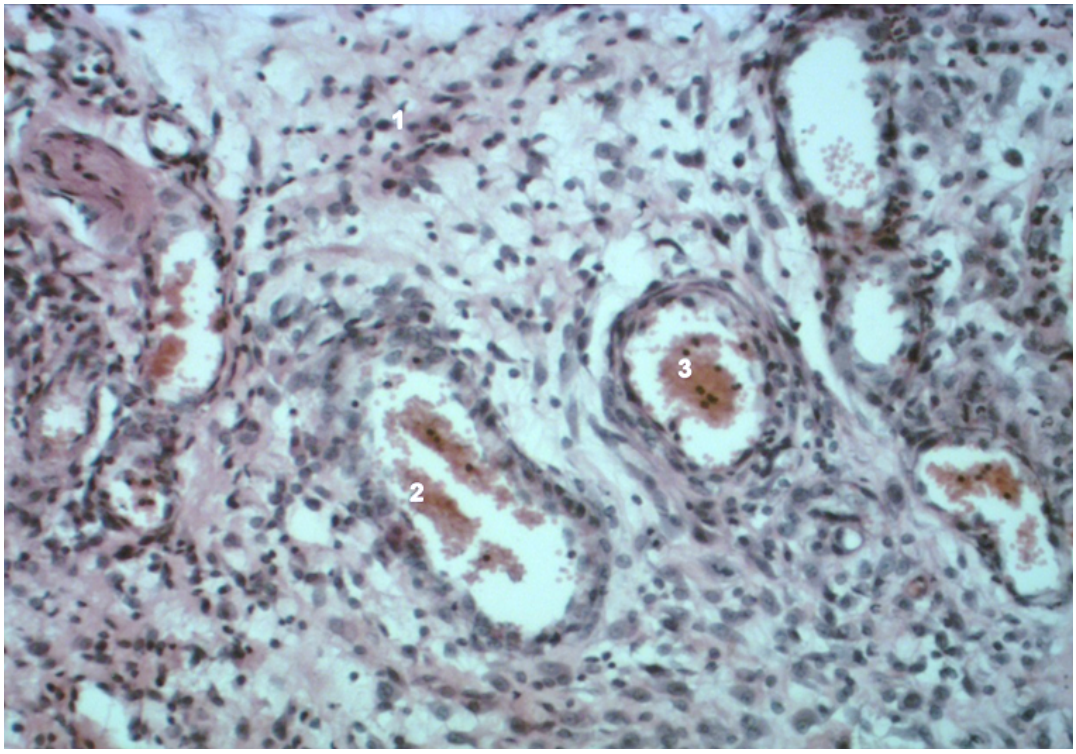


Fig. 8. Gingiva of rat No. 7, Group III, day 35 of the experiment. Diagnosis: Experimental periodontitis combined with intestinal dysbiosis. Diffuse infiltration by polymorphonuclear leukocytes. Mural thrombi in venules and arterioles. Staining: Hematoxylin and eosin. Magnification: ×100. 1 – reticular layer of the mucosa; 2 – mural thrombi in venules; 3 – mural thrombi in arterioles; 4 – infiltration by polymorphonuclear leukocytes
Picture taken by the authors

($p > 0.05$). However, keeping the experimental animals on a paste-like diet led to a slight decrease in the colonization of the large intestine by indigenous probiotic microflora (*Bifidobacterium* and *Lactobacillus*). Administration of ciprofloxacin to Group III rats, against the background of the induced experimental model of periodontitis, resulted in a significant decrease in the autochthonous intestinal microflora responsible for the colonization resistance of the gastrointestinal tract (bifidobacteria and lactobacilli), accompanied by a corresponding increase in the number of pathogenic and conditionally pathogenic microorganisms, confirming the development of dysbiosis in this biotope.

Using the biometric method, a higher intensity of the dystrophic process in the bone tissue of the periodontium was established during the combined modeling of periodontitis and intestinal dysbiosis, amounting to 41.1±0.61%, with a highly significant difference compared to Groups I and II ($p < 0.001$).

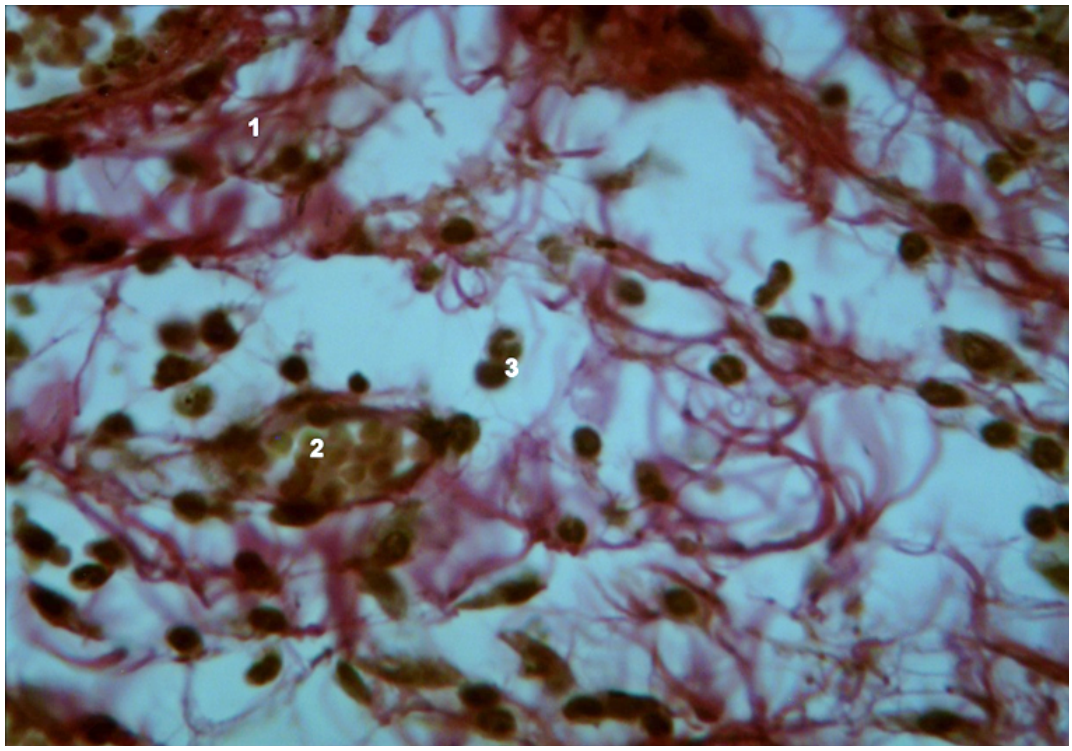


Fig.9. Gingiva of rat No. 9, Group III, day 35 of the experiment. Diagnosis: Experimental periodontitis combined with intestinal dysbiosis. Mural thrombi in venules. Pronounced interstitial edema in the reticular layer of the mucosa. Staining: Van Gieson method. Magnification: ×400. 1 – collagen fibers; 2 – thrombi in venules; 3 – infiltration by polymorphonuclear leukocytes
Picture taken by the authors

Morphological examination in Group III animals revealed more pronounced structural changes in the gingiva compared to Group II. In certain areas of the epithelial covering, disorganization and cellular damage in the form of necrosis were observed. In some regions, the keratinized, granular, and prickle-cell layers of the epithelium were absent. Individual basal layer cells appeared deformed and vacuolated, with widened intercellular spaces filled with lymphocytes and neutrophilic granulocytes. The basement membrane was discontinuous; its collagen fibers were loosened and disorganized, and lymphocytes, histiocytes, and neutrophilic leukocytes were present among them (Fig. 7).

In the connective tissue of the papillary layer of the gingival mucosa, fibroblast proliferation was observed in the region of the gingival papillae. Signs of edema in this layer and hypertrophy of collagen fibers were more pronounced than in rats of Group II. In the microcirculatory bed of the papillary layer, blood vessel lumina were dilated and engorged. The integrity of the capillary walls was disrupted, the endothelial lining was discontinuous, and the basement membrane appeared loosened. Numerous hemorrhages were found around the capillaries. In addition, polymorphonuclear leukocyte infiltrates were present in the papillary layer. The lymphatic vessels were dilated and filled with lymph.

In the reticular layer, edema of the connective tissue was more pronounced than in the papillary layer. The lumina of the microcirculatory vessels were markedly dilated and hyperemic (Fig. 8). Some blood vessels in this layer exhibited irregular blood filling.

The walls of the arterioles were thickened, and their lumina were narrowed. The endothelial lining was discontinuous, with areas showing both desquamation and regeneration. In the middle layer of the arterioles, hypertrophy of smooth muscle cells was observed.

The capillary lumina were also dilated and engorged; the endothelial lining was discontinuous, and the basement membrane was loosened. In some areas, desquamation of endothelial cells was detected. The perivascular connective tissue was loosened, with histiolymphocytic infiltration visible in many microscopic fields, and isolated hemorrhages found around capillaries.

The venules were dilated and markedly engorged. In some cases, mural thrombi were detected (Fig. 9). The endothelial lining of the venules was discontinuous, with frequent foci of endothelial desquamation. Numerous areas of leukocyte stasis, margination, and diapedesis through the venular walls were observed. Compared with the previous group, there was a more pronounced interstitial edema of the gingival reticular layer and diffuse inflammatory infiltration with neutrophilic leukocytes. In some regions, lymphocytes and histiocytes were also found. The lumina of lymphatic vessels in this layer were dilated and filled with lymph.

DISCUSSION

Thus, the results of this study demonstrated the expediency of conducting experimental research on animals to gain a more detailed understanding of the course of periodontal tissue diseases when combined

with other lesions of the digestive tract, particularly intestinal dysbiosis.

The widespread prevalence of dysbiotic conditions observed in all open cavities of the human body (oral, nasal, intestinal, etc.) makes it necessary to study this issue comprehensively from the standpoint of comorbidity. Meanwhile, both domestic and international scientific studies have focused on many aspects of dysbiosis as an independent process in dental diseases (such as dental caries, periodontal and oral mucosal diseases) and in the intestine [20-22]. However, the question of the negative impact of intestinal microflora imbalance and the replacement of its ecological niches on the course of periodontal diseases—frequently encountered in clinical practice—remains insufficiently addressed in the literature, and morphological studies in this context are virtually absent.

In this study, our results confirmed previous experimental findings regarding changes in periodontal tissues during periodontitis [23, 24] and alterations in intestinal microbiota during dysbiosis [25]. At the same time, we performed a comparative assessment of these parameters under conditions of consecutive modeling of periodontitis followed by intestinal dysbiosis.

It was established that disturbances in the microecological system of the intestine—even subclinical ones—accompanied by suppression of its saprophytic flora, complicate the course of periodontitis, promote its generalization, and contribute to the development of aggressive inflammatory manifestations in the gingiva, including abscess formation. This was supported by morphological findings: simultaneous modeling of periodontitis and intestinal dysbiosis in rats revealed more pronounced pathological changes than in those without dysbiotic disorders. These included alterations in the gingival epithelium, connective tissue of the papillary and reticular layers of the mucosa, and especially in the vessels of the microcirculatory bed and perivascular areas. In our opinion, the observed increase in the number and dilation of blood and lymphatic capillaries may represent a compensatory mechanism, while a slight increase in lymphocytes should be regarded as an adaptive response.

CONCLUSIONS

It was established that the induction of experimental periodontitis in animals using the reduced masticatory function model, followed by its combination with intestinal dysbiosis via selective decolonization, led to the development of a pathological process in the periodontal tissues in 100% of rats. This process resembled the exacerbated course of human periodontitis, with increased severity and progression. These findings indicate the high adequacy of this model for studying aggravating factors in the course of inflammatory-dystrophic lesions of human periodontal tissues.

It was found that modeling intestinal dysbiosis against the background of experimental periodontitis results in disruption of the colonic microbiome: a statistically significant decrease in bifidobacteria and lactobacilli, along with pronounced contamination by pathogenic and opportunistic enterobacteria and Candida fungi. This leads to a marked deficiency of autochthonous bacteria, impairing the colonization resistance of the intestinal mucosa and weakening the nonspecific anti-infective defense of the gastrointestinal tract, thereby intensifying the negative impact on periodontal tissues.

Assessment of the degree of dystrophic changes in the periodontium of experimental animals using biometric analysis revealed a higher intensity of alveolar process degeneration under sequential modeling of periodontitis and intestinal dysbiosis. This indicates early generalization of the pathological process in periodontal tissues and its progression under the influence of dysbiotic disturbances in the gastrointestinal tract.

In experimental animals with combined modeling of periodontitis and intestinal dysbiosis, compared to those without dysbiotic disorders, more pronounced pathological changes were observed in the gingival epithelium (intercellular space widening, vacuolar degeneration and necrosis of epithelial cells, focal absence of the keratinized, granular, and prickle layers, loosening and disorganization of collagen fibers in the basal membrane) and in the connective tissue (edema of the papillary and reticular layers, leukocytic infiltration, fibroblast hypertrophy and hyperplasia, signs of edema, disorganization and destruction of collagen fibers, their delamination and loosening). Vascular changes in the microcirculatory bed included: in arterioles – thickened walls, narrowed lumens, discontinuous endothelial lining with areas of desquamation and regeneration, hypertrophy of smooth muscle cells in the tunica media; in capillaries – dilated and congested lumens, discontinuous endothelial lining, desquamation of endothelial cells, histio-lymphocytic infiltration in perivascular connective tissue, and occasional hemorrhages around capillaries; in venules – markedly dilated and congested lumens, mural thrombi, multiple zones of leukocyte stasis, marginal positioning, and diapedesis. Lymphatic vessel lumens were dilated and filled with lymph. These findings confirm the development of severe complications in the clinical course of experimental periodontitis when combined with intestinal dysbiosis, up to its most aggressive manifestation: periodontal abscess formation.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Himdani dual (heavy/light body) impression technique *(in vitro)* cross-sectional comparative study

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ABSTRACT

Aim: To evaluate the accuracy of the modified Himdani one-step impression technique compared with the conventional two-step technique.

Detailed objectives: To measure dimensional changes in each impression type;

To compare the effect of partial putty setting on the final impression accuracy;

To determine whether the scanning process influences dimensional measurements.

Materials and Methods: An *in vitro* design using 20 casts was employed in this study, comprising 10 casts from each of the two impression techniques. The two-step and modified single-step impression techniques were used in groups A and B, respectively. A putty was used for 1 minute of polymerization relined with light body on and around the teeth inside the putty impression. The putty was then re-seated until both were fully seated before removal. Each cast virtual model was superimposed on the arch virtual model using Exocad Dental DB software for matching. The buccal, lingual, mesial, distal, and occlusal aspects of both teeth 24 and 26, as well as the interabutment distance between the distal surface of tooth 24 and the mesial surface of tooth 26, were measured for each sample, revealing the maximum discrepancy in these nine areas.

Results: The modified one-step technique demonstrated significantly lower dimensional discrepancies across all measured surfaces compared with the two-step technique ($p<0.001$).

Conclusions: The modified technique demonstrated numerous advantages over the conventional technique, with greater accuracy in capturing dental impressions for dental prostheses. It was also simple and easy to apply in dentistry, which can replace the traditional impression technique.

KEY WORDS: conservative therapy, prosthesis fitting, dental materials, dental equipment, clinical-laboratory technique, impression accuracy, digital scanning

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INTRODUCTION

Restorative dentistry encompasses a broad range of clinical procedures, from simple conservative restorations to complex fixed prosthodontics treatment for tooth replacement [1-3]. One of the critical factors influencing the long-term success of restorations is the accurate reproduction of surface details of the prepared abutment teeth and the precise measurement of the distance between these teeth [4, 5].

Accurate registration can be achieved by meticulous impression taking. Impression procedure should consider the use of suitable impression material(s) [6, 7] and careful impression technique [8, 9]. Among elastomeric impression materials, addition silicone (polyvinyl siloxane) has been shown to exhibit superior accuracy, dimensional stability, and elastic recovery [10-12].

Two widely used procedures - one-step and two-step techniques. Both utilize heavy -body/light-body materials - are widely used in clinical practice. Each method, however, presents certain limitations. The one-step technique may result in inadequate wash space and localized pressure, while the two-step technique may produce discrepancies due to putty polymerization shrinkage and improper relief control [13, 14]. Despite multiple attempts to modify these techniques, the literature - particularly from Iraq remains limited regard-

ing clinically applicable improvements that enhance impression accuracy.

AIM

Therefore, this study introduces and evaluates a modified one-step (Hamdani) impression technique. This evaluation aims to determine whether this technique can overcome the shortcomings associated with the conventional two-step method. The dimensional accuracy of this modified technique to be assessed and compared with the standard two-step approach for fixed dental prosthesis construction.

Detailed objectives:

1. To measure dimensional changes in each impression type;
2. To compare the effect of partial putty setting on the final impression accuracy;
3. To determine whether the scanning process influences dimensional measurements.

MATERIALS AND METHODS

This technique was suggested to reduce the need for a dental assistant during impression preparation, maximize the benefit of the setting time for the putty material, and, most importantly, save chair-time procedure (only material setting time). To test the author's hypothesis regarding its accuracy compared to the standard two-step technique, an in vitro, cross-sectional study design was employed as follows.

An upper arch model with plastic teeth (Prosthetic Restoration Jaw Model, Nissin, Kyoto, Japan) was used in this study (Fig. 1). Tooth 25 (maxillary left second molar) was removed, and its socket was blocked with flowable composite to simulate the extracted tooth area. Teeth 24 (maxillary left first premolar) and 26 tooth (maxillary left first molar) were prepared according to the guidelines to receive a three-unit fixed zirconia bridge.

Two impression techniques were used with the addition of silicon impression material to make ten impressions each group(n=10). The first technique was a two-step impression technique, in which the impression was made with putty that was mixed according to manufacturer's instructions and then loaded into the stock tray and seated onto the plastic model until it was fully set, then it was removed. The impression material was mixed according to the manufacturer's instructions. Plastic wrap was used as a spacer to create space for the light body. The light body was mixed with an automixing tip and injected around and over all teeth, including inside the set putty. Then it was re-seated on the model and waited until the set of light body was removed and poured into the stone cast. The second technique was a modified version of the single-step impression technique proposed by Dr. K. A. This technique involved using putty that was mixed and loaded into the tray, which was seated onto the plastic model and waiting for 1 minute (60 seconds) after the start of mixing to allow for partial setting and initial polymerization before removal to avoid permanent distortion. The putty was then removed with single vertical movement, and light body was immediately injected on and around the teeth, as well as inside the putty impression using a tray designed to ensure accurate reseating for the wash material. The piece was re-seated until both the putty and light body were fully set, after which it was removed.

All impressions were made using vinyl polysiloxane putty (Elite HD+ Putty Soft, Zhermack, Italy) and light body (Elite HD+ Light Body, Zhermack, Italy), and then poured using dental stone (Elite Rock, Zhermack, Italy). The setting time of impression materials recommended by the manufacturer was doubled to ensure proper setting at room temperature (23 ± 1°C) rather than 37°C as previously [18]. All impressions were kept at room temperature for 1 hour before pouring. Impressions were poured with IV dental stone (Elite Rock, Zhermack, Italy), which was mixed with a 20ml/100g water/powder ratio, and the cast was removed after 45 minutes according to the manufacturer's instructions and stored for 24 hours prior to digital scanning to ensure complete setting of the dental stone.

A total of 20 stone casts were obtained, 10 from each impression technique (n = 10). In Group A, the two-step impression technique was used. In Group B, the modified single-step impression technique was used.

An intraoral scanner (Trios 4, 3shape, Copenhagen, Denmark) was used to register a virtual model for the arch model and all the stone casts obtained from both impression techniques. All scans were performed by a single operator using identical scanning parameters to eliminate operator-related variability.

Each virtual cast model was superimposed onto the virtual master model using Exocad Dental DB software (version 3.0, Exocad GmbH, Germany) utilizing a global best-fit alignment based on an iterative closest point (ICP) algorithm [20].

Nine linear discrepancy measurements were recorded for each sample at the buccal, lingual, mesial, distal, and occlusal surfaces of teeth 24 and 26, as well as the inter-abutment distance between the distal surface of tooth 24 and the mesial surface of tooth 26.

The maximum discrepancy was defined as the largest point-to-point deviation detected among these nine measurement sites (Fig. 2).

To check the accuracy of the intraoral scanner used in this study and to negate any technical

Table 1. Maximum linear discrepancy (mm) for each measured surface of the prepared premolar and molar teeth obtained using the two impression techniques (n = 10 per group)

Surface & distance measurements	Technique	Minimum	Maximum	Mean	Std. Deviation	P value
Molar buccal surface	Modified one-step	0.049	0.461	0.1813	0.120147	0.043
Molar buccal surface	Two-step	0.113	0.859	0.3284	0.208612	
Molar lingual surface	Modified one-step	0.01	0.59	0.2526	0.171876	0.075
Molar lingual surface	Two-step	0.115	0.743	0.4414	0.199725	
Molar mesial surface	Modified one-step	0.021	0.09	0.0584	0.0255	0.143
Molar mesial surface	Two-step	0.026	0.589	0.1383	0.165189	
Molar distal surface	Modified one-step	0.031	0.23	0.1165	0.060612	0.190
Molar distal surface	Two-step	0.043	0.506	0.2062	0.15932	
Molar Occlusal surface	Modified one-step	0.027	0.323	0.1664	0.084217	0.089
Molar Occlusal surface	Two-step	0.032	1.043	0.3247	0.294915	
Premolar buccal surface	Modified one-step	0.029	0.244	0.1139	0.070622	0.063
Premolar buccal surface	Two-step	0.051	0.696	0.2429	0.194652	
Premolar lingual surface	Modified one-step	0.03	0.265	0.0804	0.069639	0.003
Premolar lingual surface	Two-step	0.05	0.513	0.232	0.153462	
Premolar mesial surface	Modified one-step	0.023	0.082	0.0429	0.019986	0.004
Premolar mesial surface	Two-step	0.036	0.347	0.1353	0.093539	
Premolar distal surface	Modified one-step	0.032	0.193	0.0861	0.051634	0.015
Premolar distal surface	Two-step	0.048	0.359	0.194	0.113426	
Premolar occlusal surface	Modified one-step	0.056	0.266	0.1156	0.06167	0.089
Premolar occlusal surface	Two-step	0.055	0.816	0.2211	0.21967	
Distance between abutments surface	Modified one-step	0.001	0.164	0.0636	0.050882	0.105
Distance between abutments surface	Two-step	0.006	0.292	0.1306	0.099376	

Notes: Maximum discrepancy was defined as the largest point-to-point linear deviation measured at each specified surface following global best-fit superimposition

Source: Compiled by the authors of this study

errors that might interfere with the results of this study, a second scan was made of the arch model after all scans of the casts. The virtual model obtained from the second scan was superimposed on the first scan. A Minimal deviation was observed between the two dataset was observed between these virtual models, indicating that the scanner did not affect the results (Fig. 3).

RESULTS

Table 1 provides the measurement details in millimeters for the maximum discrepancy in both techniques for each included surface in the measured teeth for the 10 samples per group. For the conventional two-step technique, the mean maximum discrepancy ranges from 0.10 to 0.40 mm The least discrepancy was found in the distance between the two prepared molar and premolar teeth, whereas the highest discrepancy was found in the lingual surface of the prepared molar tooth, almost 0.5 mm. Apart from the mesial molar surface, all premolar discrepancies in measurement were found to be less in the prepared molar tooth. In terms of surfaces, mesial surfaces in both prepared molar and premolar teeth were found with the least discrepancies.

On the other hand, the mean discrepancy for the measured surfaces in the modified single-step impression technique ranges from 0.06 to 0.59 mm. Similar to the conventional two-step impression technique, the highest measured discrepancy was found in the prepared lingual molar tooth surface. This was the same for the lowest recorded discrepancy. The distance between the prepared premolar and molar teeth was the least in the distance between two opposing prepared molar and premolar surfaces. Similarly, the two mesial surfaces of both prepared teeth showed the least discrepancy in terms of the prepared tooth surfaces.

The statistical difference in the level of discrepancy between the 10 samples for both techniques was found on the buccal molar surface, lingual molar surface, mesial premolar surface, and distal premolar surface. The difference between the two impression techniques was found to be highly significant on the lingual and mesial premolar surfaces.



Fig. 1. Maxillary arch model used for impression registration
Source: Own materials

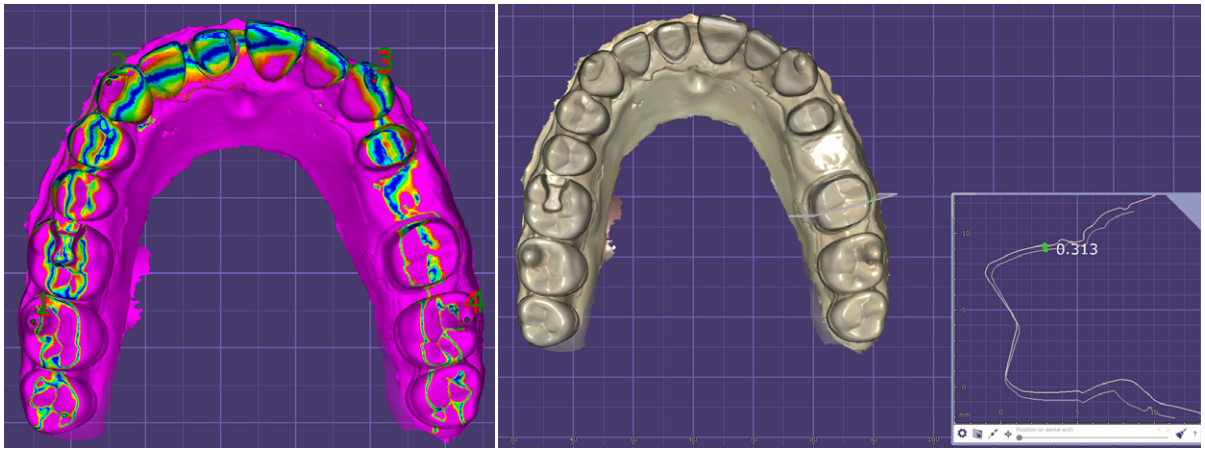


Fig. 2. Three-dimensional superimposition of a representative stone cast onto the virtual master model using global best-fit alignment. Color-coded deviation maps illustrate point-to-point linear discrepancies (mm) at the buccal, lingual, mesial, distal, and occlusal surfaces of the prepared premolar and molar teeth, as well as the inter-abutment distance
Source: Own materials

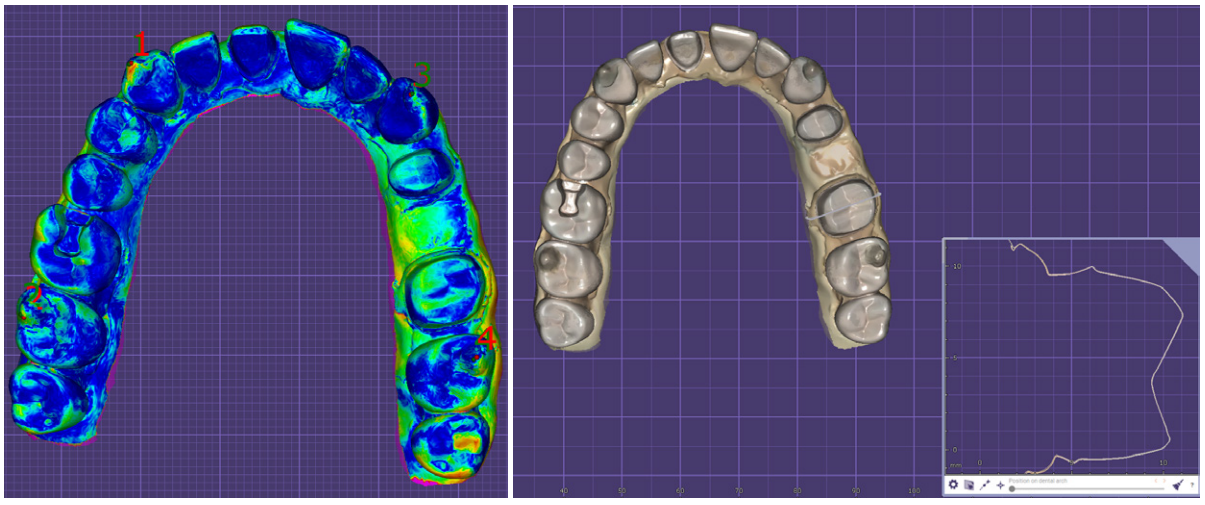


Fig. 3. Superimposition of two independent scans of the master model obtained using the same intraoral scanner and scanning protocol. Global best-fit alignment was performed using an iterative closest point (ICP) algorithm. The color-coded deviation map demonstrates minimal point-to-point discrepancies (mm), indicating high scanner repeatability
Source: Own materials

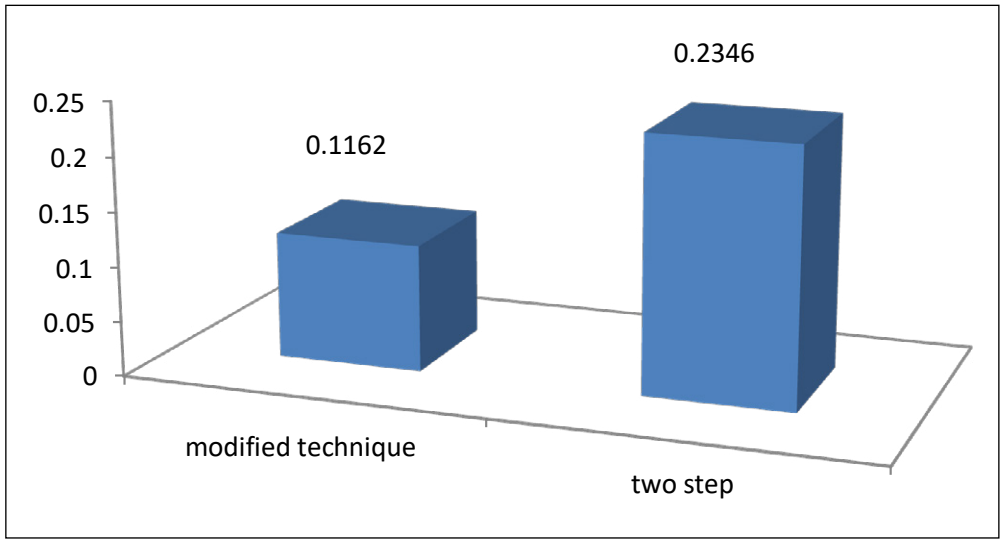


Fig. 4. Comparison of the mean maximum discrepancy (mm) between the modified one-step and conventional two-step VPS impression techniques across all measured premolar and molar surfaces (n = 10 per group). Error bars represent standard deviation. A statistically significant difference was observed between the two techniques ($p < 0.001$)

The overall difference between the modified single-step and two-step techniques is shown in Figure 4. The mean discrepancy degree in the modified one-step technique for the 10 samples, involving all molar and premolar surfaces, is less than that of the conventional two-step technique. This difference has been statistically significant ($P < 0.001$).

DISCUSSION

The current in-vitro study evaluated the accuracy of applying the two-step and modified one-step putty/light body impression techniques. Both of these techniques require two-handed work in a clinical setting. This may justify the comparison of the modified one-step technique with the two-step technique. The results showed a higher accuracy for the modified one-step impression technique. A several explanations may account for this improved technique:

First, in the modified one-step technique, the putty impression acts as an accurate special tray, which leads to equal pressure throughout all parts of the impression, especially for the critical details in the sulcus area, resulting in sound diffusion of light body material. In contrast, with the two-step technique, the thickness of the light body cannot be control, as the plastic wrap can fold and create uneven spaces necessary for the diffusion of the light material. This uncontrolled gap results in unequal and/or insufficient pressure, which is necessary for the diffusion of light materials. These tiny, important details are still behind the lower inaccuracy of the two-step impression technique [1].

Second, in the modified one-step method, the light body was injected prior to the whole setting of the putty. This procedure will enhance the adhesion of both components and reduce the likelihood of sloughing of the light body from the putty, which is not uncommon in the two-step technique, especially if the putty was contaminated before the application of the light body.

Third, another possible cause is that in the two-step technique, the putty was re-seated after it was fully set. This may prevent or interfere with the full seating of putty as it gains some hardness, especially in interdentally and undercut areas, reducing the pressure needed for light body spread. On the other hand, in the modified one-step technique, the putty remained unset and possessed adequate softness, allowing it to be re-seated appropriately.

In previous studies, comparisons between two-step and one-step techniques have been conducted, although some studies reported no difference in accuracy [15-23]. Other studies, however, concluded that two-step techniques were more accurate than one-step techniques [16-19]. Most of the dimensional changes occur due to polymerization shrinkage of the impression material, which is particularly pronounced in the putty step, as it has a greater mass than the light body. That is why the two-step technique showed higher accuracy, as the putty was allowed to be set prior to the light body, which compensates for most of the shrinkage that occurred. While in one step, there is no chance for the light body to compensate for putty shrinkage as it is both used simultaneously, besides that, there is the possibility that some details will be registered by putty only as the light body might be washed

out. However, the results of the current study showed that the suggested (modified one-step technique) provides more accurate results than the two-step technique.

To the best of the authors' knowledge, this is the first study to examine the dimensional accuracy of modified one-step technique. The modified one-step technique allowed for a partial setting of putty with concomitant shrinkage before light body application, compensating for sufficient shrinkage. Rocking and removal of putty while

it is still soft will create a space for a light body, reducing the possibility of being washed out during re-seating. In addition, re-seating still soft putty could result in better adaptability than fully set putty.

LIMITATIONS

This study was conducted in vitro and therefore does not account for intraoral variables such as saliva, blood, tissue movement, patient cooperation, or temperature variations, all of which may influence clinical accuracy. Future clinical studies are needed to validate these findings in real patient settings.

CONCLUSIONS

Within the limitation of this in-vitro study, the modified one-step putty/light body impression technique was presented in this study. It demonstrates several advantages over the conventional two-step impression technique and shows lower maximum dimensional discrepancy in capturing dental impressions. The modified one-step approach may offer a feasible alternative impression technique under managed conditions. However, clinical (*in-vivo*) studies are required to confirm these findings and to impact influence of intraoral factors on impression precision before routine clinical application can be recommended.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Combined treatment of intestinal dysbiosis in patients with chronic Hepatitis C on the background of obesity

Mariya A. Derbak, Vira V. Vorobets, Yevheniia E. Dankanych, Andrii D. Sitkar, Olesya M. Horlenko, Anatolija A. Krasnova, Myroslava M. Bletskan
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ABSTRACT

Aim: To study the effectiveness of ursodeoxycholic acid use in the combined treatment of intestinal dysbiosis in patients with chronic Hepatitis C associated with obesity.

Materials and Methods: 84 patients with CHC were examined. All patients underwent a microbiological study of stool, the levels of cytokine profile indicators were determined and the psychological status and the quality of life were assessed. Two groups were formed: 1 gr (n=40) – received LA-5 and BB-12 1 gtt. x TID and 2 gr (n=44) - LA-5 + BB-12 + UDCA 500 mg qhs for 1 month.

Results: As a result of simultaneous administration of LA-5 + BB-12 + UDCA in the above-mentioned doses, in 93.2% of patients with CHC an normalization of the act of defecation and the disappearance of symptoms of intestinal dysbiosis were found. In patients taking only LA-5 and BB-12, the above-mentioned positive changes were observed in 62.5%.

Conclusions: It was found that the additional prescription of UDCA in combination with a probiotic not only contributes to the restoration of colon microbiocenosis, but also improves the course of CHC, and increases the quality of life of patients.

KEY WORDS: dysbiosis, chronic Hepatitis C, obesity, Ursodeoxycholic acid, probiotics

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INTRODUCTION

According to WHO data, approximately 71 million people are affected from chronic hepatitis C (CHC), and 350,000–399,000 die each year due to liver damage and complications caused by the hepatitis C virus (HCV) [1]. Every year, 1.75 million new cases of viral hepatitis C are registered, of which 70–85% progress to CHC [2]. Direct-acting antiviral agents (DAAs) have made a breakthrough in the treatment of CHC, leading to sustained virological response (SVR) in more than 95% of patients [3] and reducing the degree of liver fibrosis. However, in some patients, after successful eradication of HCV with DAAs, progression of liver fibrosis and/or development of hepatocellular carcinoma (HCC) may still occur [4].

The rate of fibrosis progression is associated with various factors, including alcoholic and non-alcoholic fatty liver disease, co-infection with hepatitis B virus and HIV, as well as obesity and intestinal dysbiosis (ID). In the case of prolonged intestinal ID, the risk of developing metabolic liver diseases such as non-alcoholic fatty liver disease (NAFLD), cholestasis, and dyskinetic biliary tract disorders increases due to inflammatory and immune responses both at the local and systemic levels [5, 6]. People with obesity often show reduced diversity of gut microbiota and an altered balance of major bacterial groups: the number of Bacteroidetes decreases, while the proportion of Firmicutes increases. Any imbalance leads to a reduction in protective mechanisms, increased intestinal wall permeability, and the initiation of systemic inflammation. Studies have shown that the structure of the gut microbiome in obese individuals in Ukraine is characterized by a significantly higher content of Firmicutes and a lower content of Bacteroidetes compared to individuals with normal or reduced body weight [7].

It is well known that prebiotics, probiotic preparations, and herbal products that stimulate the growth of normal microflora are used to correct dysbiotic changes [8, 9]. One such probiotic is a capsule containing at least 1×10^9 colony-forming units (CFU) of *Lactobacillus acidophilus* (LA-5) and 1×10^9 CFU of *Bifidobacterium an-*

imalis subsp. lactis BB-12. Both bacteria are components of the normal human gut microbiota and are resistant to gastric acid and bile, which increases their survival rate as they pass through the stomach and duodenum. They have GRAS status (Generally Recognized As Safe) [10].

Although probiotics and prebiotics currently play a key role in the treatment of intestinal dysbiosis and the prevention of NAFLD [11, 12], contradictory results are often observed in individuals with obesity. Therefore, a more meticulous experimental design, improved quality of clinical studies, and confirmation of therapeutic effects are necessary [13].

Ursodeoxycholic acid (UDCA) is a therapeutic bile acid used not only as a hepatoprotective agent in CHC but is also prescribed for metabolic disorders (metabolic syndrome, obesity, NAFLD) [14, 15], given its broad spectrum of therapeutic activity [16].

The results of Pearson T. et al. (2019) indicate a close relationship between the gut microbiome and the composition of bile acids, which changes under the influence of UDCA in patients with colon polyps [17]. An excessive growth of *Faecalibacterium prausnitzii* was observed in the group of patients receiving UDCA, and an inverse relationship was found between *F. prausnitzii* and *Ruminococcus gnavus*. In animal models, intestinal inflammation was shown to decrease through the regulation of macrophage polarization, involvement of FXR, and suppression of NF-kB activation during UDCA treatment, which occurs due to changes in bile acid metabolism associated with dysbiosis [18]. Changes in the gut microbial profile have also been demonstrated in experimental animal models of NAFLD following intragastric administration of UDCA. UDCA treatment significantly reduced liver inflammation in mice with NASH and partially restored intestinal microbiota dysbiosis [19].

Thus, experimental studies using UDCA, considering its multifaceted properties, allow for the expansion of its therapeutic potential beyond its well-known effects and support its use as part of combined treatment of intestinal dysbiosis in patients with CHC associated with obesity.

AIM

The aim of the research was to study the effectiveness of ursodeoxycholic acid use in the combined treatment of intestinal dysbiosis in patients with chronic hepatitis C associated with obesity.

MATERIALS AND METHODS

A total of 84 patients with chronic hepatitis C (CHC) on the background of obesity and intestinal dysbiosis (ID) were under observation. Among them, 41.7% (35) were men and 58.3% (49) were women. The average age of the patients was 56.7 ± 1.3 years.

The study was conducted at the clinical base of the Department of Faculty Therapy, Medical Faculty, Uzhhorod National University, during the period 2023–2024. The scientific research was carried out within the framework of the initiative theme of the Department of Faculty Therapy of the State University "UzhNU": «Combined pathology and correction of disorders of homeostasis of residents of the Carpathian region, taking into account the effect of adverse factors», state registration number 0121U110808. The research was carried out with informed consent from the patients, and the methodology complied with the Declaration of Helsinki (1964–2016), the Council of Europe Convention on Human Rights and Biomedicine (1997), the International Code of Medical Ethics (1983), and relevant laws of Ukraine. The study was approved by the local bioethics committee (Protocol No.7/3 dated 16.03.2023) of Uzhhorod National University. All patients provided signed consent for the collection of personal data for the database, for the use of blood and stool samples for research purposes, and for participation in the study. The diagnosis of CHC was established according to the International Classification of Diseases, 10th Revision (ICD-10), and confirmed by the detection of total anti-HCV IgG antibodies using the ELISA method, as well as by detection of HCV RNA in the blood using the polymerase chain reaction (PCR). The classification of chronic hepatitis proposed at the International Congress of Gastroenterologists (Los Angeles, 1994) was also used. Markers of hepatitis B and C were determined using ELISA, followed by detection of HCV RNA, genotype, and viral load using PCR.

The degree of liver fibrosis and steatosis was determined using the non-invasive diagnostic method FibroMax (BioPredictive, Paris) and liver elastography (FibroScan-502 F01261, M 7 70129 probe, France, Regional Clinical Infectious Diseases Hospital, Uzhhorod). Abdominal ultrasound (US) was performed for all patients using a Philips HDI-1500 device with a convex probe operating at a frequency of 3.5 MHz. General clinical, biochemical, serological, and molecular-genetic tests were performed in certified laboratories of the Regional Clinical Infectious Diseases Hospital (Uzhhorod) and in commercial laboratories ("Dila" and "Synevo"). The functional state of the liver was assessed by measuring the activity levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), conjugated bilirubin, and gamma-glutamyl transpeptidase (GGT). The degree of activity of the pathological process was evaluated

based on ALT elevation levels according to the international classification of liver diseases (Los Angeles, 1994). The blood lipid profile was determined, including total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TG). The trophological status of the patients was assessed using standard anthropometric indicators. Obesity was determined based on the Quetelet index or body mass index (BMI).

The state of the intestinal microbiocenosis was assessed by microbiological analysis of fecal samples. To detect colonic dysbiosis, a quantitative analysis of microorganisms grown on nutrient media (agar, Sabouraud, Endo, and 5% blood agar) was performed, recalculating the results per 1 g of feces while accounting for the amount of inoculated material and its dilution. Identification of bacterial cultures was carried out using biochemical tests and the Enterotest system. All patients had their cytokine profile levels measured, and psychological status and quality of life were assessed.

Depending on the prescribed treatment, patients were divided into two subgroups: Subgroup 1a (n=40): Patients with CHC, obesity, and colonic dysbiosis who received a probiotic containing *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium animalis* subsp. lactis (BB-12), 1 capsule three times a day. Subgroup 1b (n=44): Patients who, in addition to the probiotic (LA-5 and BB-12), received UDCA 500 mg at night for 1 month. The treatment lasted for 1 month.

The two-sided Fisher's exact test was used with the program Statistica 8.0 for Windows. Differences were considered statistically significant at $p < 0.05$. Statistical analysis of the obtained results was performed using Jamovi software.

FRAMEWORK

The scientific research was carried out within the framework of the initiative theme of the Department of Faculty Therapy of the State University "UzhNU": «Combined pathology and correction of disorders of homeostasis of residents of the Carpathian region, taking into account the effect of adverse factors», state registration number 0121U110808.

RESULTS

Combined therapy on the background of standard treatment positively affected the quantitative and qualitative composition of the colonic microflora in patients with CHC, especially in patients of subgroup 1b, who received UDCA in addition to the probiotic. Follow-up microbiological analysis of fecal samples showed a significant increase in the number of bifidobacteria and lactobacilli, which was accompanied by a decrease in aggressive pathogenic flora. The most pronounced positive dynamics were observed in subgroup 1b: in 100.0% of patients, the number of *Bifidobacterium* increased to 8.07 ± 0.05 lg CFU/g, whereas in subgroup 1a patients, an increase to 7.21 ± 0.06 lg CFU/g was detected in only 87.5% ($p < 0.05$).

Similarly, in 100.0% of subgroup 1b patients, *Lactobacillus* increased to 6.11 ± 0.08 lg CFU/g, while in subgroup 1a, the increase was only up to 5.02 ± 0.06 lg CFU/g ($p < 0.05$) in 80.0% of cases. A significant increase in *E. coli* with normal enzymatic activity up to 7.05 ± 0.07 lg CFU/g was observed in 90.9% of patients, compared to only 70.0% in subgroup 1a, where levels rose to 6.33 ± 0.09 lg CFU/g ($p < 0.05$).

Elimination of intestinal microbiocenosis imbalance was accompanied by a reduction in pathogenic and opportunistic microflora. The frequency of *E. coli* with hemolytic properties decreased 2.0-fold ($p < 0.05$) to 2.23 ± 0.05 lg CFU/g ($p < 0.01$) in subgroup 1b, compared to a 1.3-fold decrease in subgroup 1a. *Enterobacter* was detected in 6.8% of subgroup 1b patients at 1.64 ± 0.06 lg CFU/g, compared to 2.67 ± 0.08 lg CFU/g in 17.5% of subgroup 1a patients ($p < 0.01$). The *Citrobacter* count decreased to 1.25 ± 0.11 lg CFU/g in subgroup 1b, while higher concentrations (1.98 ± 0.09 lg CFU/g) were found in subgroup 1a patients.

After treatment, *Klebsiella* levels were 2.37 ± 0.05 lg CFU/g in subgroup 1a and significantly lower at 1.34 ± 0.03 lg CFU/g in subgroup 1b ($p < 0.05$). Staphylococcus aureus decreased 2.2-fold in subgroup 1b, to 2.88 ± 0.04 lg CFU/g, compared to a 1.3-fold reduction to 3.73 ± 0.07 lg CFU/g in subgroup 1a ($p < 0.05$).

Clostridium was isolated in 18.2% of subgroup 1b patients at 2.05 ± 0.08 lg CFU/g during follow-up, compared to 46.5% before treatment at 5.17 ± 0.11 lg CFU/g ($p < 0.01$). *Proteus* was detected in 11.4% of subgroup 1b patients at 1.14 ± 0.05 lg CFU/g, whereas in subgroup 1a, it was found in 20.0% of patients at 2.26 ± 0.03 lg CFU/g ($p < 0.05$). After the course of treatment, Candida was detected in only one patient (2.3%) in subgroup 1b whereas in subgroup 1a, it was isolated again in six patients (15.0%) ($p < 0.01$) (Table 1**).**

Thus, combination therapy using the probiotic LA-5 and BB-12 together with UDCA is a more effective method for normalizing the quantitative and qualitative composition of colonic microflora in CHC patients with obesity.

After the treatment, all patients showed improved well-being. As a result of simultaneous administration of LA-5 and BB-12 with UDCA in the above doses in CHC patients with obesity, there was an increase in the num-

Table 1. Evaluation of the dynamics of quantitative and qualitative composition of colonic microflora in patients with CHC during treatment

Indicator	Examined patients		
	Before treatment	After treatment	
		Subgroup 1a (n=40), abs./%	Subgroup 1b (n=44), abs./%
Bifidobacterium:			
frequency(%)	52 / 61.9 %	35 / 87.5 %*	44 / 100.0 %**+
lgCFU/g	5.07±0.12	7.21±0.06*	8.07±0.05**+
Lactobacillus:			
frequency(%)	45 / 53.6 %	32 / 80.0 %*	44 / 100.0 %**+
lgCFU/g	3.71±0.08	5.02±0.06*	6.11±0.08**+
***E. coli* with normal enzymatic properties:**			
frequency(%)	40 / 47.6 %	28 / 70.0 %*	40 / 90.9 %**+
lgCFU/g	4.67±0.12	6.33±0.09*	7.05±0.07**
***E. coli* (hemolytic form) before treatment**			
frequency(%)	19 / 22.9 %;	7 / 17.5 %	5 / 11.4 %*
lgCFU/g	5.07±0.21	3.75±0.07*	2.23±0.05**+
Enterobacter:			
frequency(%)	17 / 20.2 %	7 / 17.5 %	3 / 6.8 %**++
lgCFU/g	3.46±0.17	2.67±0.08*	1.64±0.06**+
Citrobacter:			
frequency(%)	34 / 40.5 %	9 / 22.5 %*	5 / 11.4 %**+
lgCFU/g	2.94±0.12	1.98±0.09*	1.25±0.11**
Klebsiella:			
frequency(%)	24 / 28.6 %	8 / 20.0 %	4 / 9.1 %**++
lgCFU/g	(3.86±0.15)	2.37±0.05*	1.34±0.03**+
Staphylococcus:			
frequency(%)	21 / 25.0 %	6 / 15.0 %*	4 / 11.4 %**
lgCFU/g	4.96±0.08	3.73±0.07*	2.88±0.04**+
Clostridium:			
frequency(%)	39 / 46.5 %	10 / 25.0 %*	8 / 18.2 %**
lgCFU/g	5.17±0.11	3.44±0.08*	2.05±0.08**+
Proteus:			
frequency(%)	28 / 33.3 %	8 /20.0 %*	5 / 11.4 %**+
lgCFU/g	3.15±0.14	2.26±0.03*	1.14±0.05**+
Candida:			
frequency(%)	24 / 28.6 %;	6 / 15.0 %*	1 / 2.3 %**++
lgCFU/g	4.20±0.17	2.03±0.06**	1.48±0.07***+

Note: Differences between indicators before and after treatment are significant: * - $p < 0.05$; * * - $p < 0.01$; * * * - $p < 0.01$. Differences between indicators in patients of subgroups 1a and 1b after treatment are significant: + - $p < 0.05$; ++ - $p < 0.01$

Source: compiled by the authors of this study

ber of bifidobacteria, lactobacilli, and *E. coli* with normal activity, and a decrease in the number of hemolytic microorganisms, *Proteus, Staphylococcus*, and yeast-like fungi. This was accompanied by normalization of bowel movements and disappearance of intestinal dysbiosis symptoms. Elimination of dysbiosis symptoms, including constipation and flatulence, contributed to positive dynamics in the clinical course of CHC. Thus, the number of patients complaining of pain, heaviness in the right hypochondrium, flatulence, weakness, and rapid fatigue significantly decreased, with a reliable predominance in the second group.

Analysis of the obtained data indicates a 3.7-fold reduction ($p < 0.01$) in manifestations of astheno-vegetative syndrome in patients of subgroup 1b, whereas in subgroup 1a patients, signs of rapid fatigability, irri-

Table 2. Dynamics of dyspeptic syndrome symptoms in CHC patients during treatment

Clinical signs	Examined patients		
	Before treatment (n=84)	After treatment	
		1a subgroup (n=40)	1b subgroup (n=44)
Manifestations of biliary dyspepsia:	52 / 61.9 %	18 / 45.0%*	7 / 15.9 %***++
- Nausea	29 / 34.5 %	12 / 30.0 %	2 / 4.5 %**++
-Vomiting	6 / 7.1 %	2 / 5.0 %	0
- Bitter belching	23 / 27.4 %	7 / 17.5 %	1 / 2.3 %***++
- Bitterness in mouth	19 / 22.6 %	6 / 15.0 %	1 / 2.3 %**++
- Decreased appetite	23 / 27.4 %	6 / 15.0 %*	3 / 6.8 %**+
Manifestations of intestinal dyspepsia:			
Complains of impaired defecation			
Constipation	58 / 69.0 %	11 / 27.5 %**	2 / 4.5 %***++
Diarrhea	26 / 31.0 %	4 / 10.0 %**	1 / 2.3 %***+
Flatulence	32 / 38.1 %	6 / 15.0 %**	1 / 2.3 %***++
Intestinal dysfunction	55 / 65.5%	-	0

Note: Differences between indicators before and after treatment are significant: * – $p<0.05$; * * – $p<0.01$; * * * – $p<0.01$. Differences between subgroups 1a and 1b after treatment are significant: + – $p<0.05$; ++ – $p<0.01$

Source: compiled by the authors of this study

Table 3. Dynamics of biochemical blood parameters in patients with CHC and colonic dysbiosis during combined treatment

Parameter	Examined patients		
	Before treatment (n=84)	After treatment	
		1a subgroup (n=40)	1b subgroup (n=44)
Total bilirubin, μmol/L	37.8±3.4	30.4±2.1	17.6±1.8*+
ALT, U/L	118.4±10.5	82.2±4.4**	43.2±3.5*+
AST, U/L	75.3±6.2	62.4±2.6	32.5±1.5*+
ALP, U/L	126.5±18.4	96.0±11.2**	52.4±2.6*++
GGT, U/L	77.6±8.4	60.3±2.0	34.8±2.4*

Note: Differences between indicators before and after treatment are significant: * – $p < 0.01$; * * – $p < 0.05$. Differences between 1a and 1b subgroups after treatment are significant: + – $p < 0.01$

Source: compiled by the authors of this study

tability, and general weakness decreased only 1.5-fold ($p < 0.01$). As a manifestation of biliary dyspepsia, skin itching in subgroup 1b patients decreased 3.4-fold ($p < 0.01$), while in patients who did not receive UDCA, it decreased only 1.4-fold ($p < 0.05$). Pain in the right hypochondrium decreased to 6.8% in subgroup 1b patients during follow-up clinical examination, while in subgroup 1a patients after treatment, 20.0% still reported this complaint.

As indicated by the results, in patients of subgroup 1a, manifestations of biliary dyspepsia decreased by only 16.9% ($p<0.05$), whereas in subgroup 1b, biliary dyspepsia decreased by 46.0% after treatment ($p<0.001$). Accordingly, in subgroup 1b, symptoms of biliary dyspepsia such as nausea, bitter belching, and bitterness in the mouth decreased by 30.0% ($p<0.01$), 25.1% ($p<0.01$), and 20.3% ($p<0.01$), respectively. In patients of subgroup 1a, who did not additionally receive UDCA as part of the treatment, the above symptoms decreased upon re-evaluation by only 4.5%, 9.9%, and 7.6%, with no statistically significant differences detected during treatment (Table 2).

The most objective criterion for determining the clinical effectiveness of the prescribed therapeutic complex in patients with CHC and colonic dysbiosis is the assessment of changes in intestinal dyspepsia. Constipation, which dominated the clinical picture, decreased by 15.3 times in subgroup 1b ($p<0.001$) and only by 2.5 times in subgroup 1a ($p<0.01$). Diarrhea, an atypical sign of colonic dysbiosis in CHC patients, decreased by 13.5 times in subgroup 1b ($p<0.001$) and only by 3.1 times in subgroup 1a. Flatulence, which frequently troubled patients with CHC and colonic dysbiosis before treatment, decreased by 16.5 times

Table 4. Dynamics of lipid metabolism indicators in serum of patients with CHC and colonic dysbiosis during combined treatment

Parameter	Examined patients		
	Before treatment (n=84)	After treatment	
		1a subgroup (n=40)	1b subgroup (n=44)
TC, mmol/L	5.89±0.11	5.62±0.07	4.78±0.08*+
TG, mmol/L	2.34±0.09	2.21±0.04	1.81±0.06*+
LDL-C, mmol/L	3.46±0.07	3.15±0.05	2.45±0.08*+
VLDL-C, mmol/L	1.42±0.06	1.29±0.05	0.90±0.04*+
HDL-C, mmol/L	1.07±0.08	1.11±0.06	1.53±0.05*+
ApoA1, g/L	0.78±0.07	0.84±0.05	1.02±0.06*
ApoB, g/L	2.08±0.09	1.97±0.07	1.59±0.08*+

Note: Differences before and after treatment are significant: * – $p < 0.05$; differences between 1a and 1b after treatment are significant: + – $p < 0.05$
Source: compiled by the authors of this study

during repeated assessment with probiotic and UDCA administration, whereas in subgroup 1a – only by 2.5 times. The prescribed treatment complex (LA-5 and BB-12 combined with UDCA) had a positive effect on intestinal dysfunction, which was completely absent in subgroup 1b by the end of therapy.

Thus, the combination of LA-5 and BB-12 with UDCA is a more effective method for correcting both microbiological and clinical manifestations of colonic dysbiosis in CHC patients.

An evaluation of blood biochemical parameters (indicators of cytolytic and cholestatic syndromes present in CHC patients with colonic dysbiosis before treatment) was conducted during differentiated combination therapy – Table 3.

According to the obtained data, baseline therapy combined with a probiotic preparation containing LA-5 and BB-12 did not significantly affect laboratory indicators of cytolytic and cholestatic syndromes, whereas the additional administration of UDCA led to positive changes in repeated biochemical blood testing. In patients of subgroup 1b, total bilirubin decreased by 30.2 ± 1.6 μmol/L ($p < 0.01$), whereas in subgroup 1a only by 7.4 ± 1.3 U/L ($p > 0.05$); ALP decreased by 74.1 ± 15.8 U/L ($p < 0.01$), while in subgroup 1a by only 30.5 ± 7.2 U/L ($p < 0.05$); and GGT decreased by 42.8 ± 6.0 U/L ($p < 0.01$), compared to 17.3 ± 6.4 U/L in subgroup 1a ($p > 0.05$). A pronounced reduction in cytolytic enzyme activity in serum was observed predominantly in patients with CHC and dysbiosis who received LA-5 and BB-12 combined with UDCA. In subgroup 1b, ALT activity decreased by 75.2 ± 7.0 U/L ($p < 0.01$), whereas in subgroup 1a only by 36.2 ± 6.1 U/L ($p < 0.05$). AST activity also significantly decreased only in subgroup 1b by 42.8 ± 4.7 U/L ($p < 0.01$), compared to 12.9 ± 3.6 U/L in subgroup 1a ($p > 0.05$).

Thus, the combination of the probiotic LA-5 and BB-12 with UDCA is an effective method not only for correcting dysbiotic changes in the colon but also for reducing and normalizing manifestations of cytolytic and cholestatic syndromes in patients with CHC and colonic dysbiosis.

The administration of LA-5 and BB-12 with UDCA resulted in a significant decrease in total cholesterol (TC) in subgroup 1b by 0.84 ± 0.01 mmol/L ($p < 0.05$), whereas in subgroup 1a only by 0.84 ± 0.01 mmol/L ($p > 0.05$). Triglycerides (TG) in serum also significantly decreased in subgroup 1b by 0.53 ± 0.02 mmol/L ($p < 0.05$), compared to 0.13 ± 0.05 mmol/L in subgroup 1a ($p > 0.05$). LDL-C and VLDL-C in subgroup 1b significantly decreased by 1.01 ± 0.01 mmol/L and 0.52 ± 0.02 mmol/L ($p < 0.05$), while in subgroup 1a by only 0.31 ± 0.02 mmol/L and 0.13 ± 0.01 mmol/L, respectively ($p > 0.05$). The reduction in LDL-C and VLDL-C was accompanied by an increase in HDL-C in patients with CHC and colonic dysbiosis. A significant improvement in HDL-C was observed in subgroup 1b, with an increase of 0.46 ± 0.03 mmol/L ($p < 0.05$), compared to only 0.04 ± 0.02 mmol/L in subgroup 1a ($p > 0.05$) (Table 4).

The SteatoTest index significantly decreased after treatment by 1.4 times ($p < 0.05$) in subgroup 1b, whereas no positive dynamics were observed in subgroup 1a. The AshTest result, indicating probable NAFLD development, remained unchanged in subgroup 1a, while a slight decrease (1.3 times, $p < 0.05$) was observed in subgroup 1b.

At the same time, although liver fibrosis (FibroTest and elastography) showed a trend toward improvement, no significant dynamics were established in either group. Thus, treatment of liver function and colonic dysbiosis in CHC patients is a stage aimed at improving clinical symptoms, dysbiosis severity, and biochemical indicators to prevent the addition of a "new" factor in CHC with obesity – NAFLD. However, the main stage of treatment is antiviral therapy aimed at complete elimination of hepatitis C virus.

Therefore, in treating CHC patients with colonic dysbiosis, correction should target not only the microbial profile but also liver function, using drug combinations with multifactorial effects, including reduction of hepatic steatosis to prevent and/or halt NAFLD progression in CHC.

When assessing quality of life (QoL) after treatment, improvement trends were observed in both groups, but significantly more pronounced in subgroup 1b. Pain intensity, general health, vitality, and social functioning scores increased 1.5-fold ($p < 0.01$) in subgroup 1b, while in subgroup 1a, general health and vitality improved 1.2-fold ($p < 0.05$), and pain intensity and social functioning only 1.1-fold. Significant 1.4-fold ($p < 0.01$) increases were also observed in role functioning and physical functioning in subgroup 1b, whereas in subgroup 1a, these QoL indicators improved only 1.2-fold ($p < 0.05$). Role functioning and mental health also significantly improved on the proposed treatment in subgroup 1b.

Integrated indicators of both physical functioning and mental health improved more substantially in subgroup 1b, i.e., with combined use of LA-5 and BB-12 and UDCA, by 1.4 and 1.5 times, respectively ($p < 0.01$).

Here is the professional English translation, with no added words or interpretation:

An assessment was conducted of the dynamics of the Spielberger–Khanin self-assessment anxiety scale indicators in patients with chronic hepatitis C (CHC) and colonic dysbiosis during combined therapy.

The number of patients who showed no signs of anxiety according to the Spielberger–Khanin self-assessment scale in subgroup 1b after treatment increased by 8.1 times ($p < 0.01$), whereas in subgroup 1a – only by 2.1 times ($p < 0.05$). Accordingly, this was accompanied by a decrease in the number of individuals with situational anxiety by 7.2 times ($p < 0.01$) in subgroup 1b patients compared to 1.2 times in subgroup 1a patients.

Thus, the prescribed treatment aimed at reducing the severity of colonic dysbiosis and improving liver function in patients with CHC using LA-5 and BB-12 and UDCA is an effective method not only for normalizing the quantitative and qualitative composition of the colonic microflora and liver function, but also for correcting quality of life indicators in these patients, which arises due to the reduction of clinical symptom severity.

DISCUSSION

The effectiveness of combined therapy using the probiotic LA-5 and BB-12 in combination with UDCA is due to the fact that LA-5 and BB-12 inhibit the growth of pathogenic bacteria, leading to a decrease in pH in the intestinal tract (due to the ability of LA-5 to produce lactic acid, and BB-12 to produce, in addition to lactic acid, acetic and succinic acids). The combination of LA-5 and BB-12 promotes the production of metabolites that are toxic to pathogenic bacteria (production of hydrogen peroxide). Additionally, LA-5 secretes acidocin, a broad-spectrum bacteriocin that inhibits the growth of bacteria and fungi [20]. *Lactobacillus acidophilus* and *Bifidobacterium animalis subsp*. lactis compete with pathogenic bacteria for nutrients and occupy adhesive receptors, thereby inhibiting the colonization of other potentially pathogenic microorganisms. The results obtained by us are consistent with the data obtained by Egyptian researchers led by Allam NG, who demonstrated the antibacterial activity of lactobacilli and bifidobacteria against pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *enterobacteria, Klebsiella*. [21]. The positive impact of lactobacilli and bifidobacteria in the treatment and prevention of disorders associated with obesity has also been proven by Kyiv scientists [22].

On the other hand, it is known that UDCA, in addition to its positive effect on liver function and the biliary system, also exerts systemic effects, including on the gut microbiome. Such studies were conducted in patients with primary biliary cirrhosis (Tang R. et al., 2018), demonstrating normalization of the intestinal microbiome after UDCA administration in these patients [23].

Thus, the complementary effects of LA-5 and BB-12 and UDCA effectively reduce the severity of colonic dysbiosis in patients with CHC and obesity, which, in turn, leads to a reduction or complete disappearance of clinical manifestations of dysbiosis.

CONCLUSIONS

Combined therapy using LA-5 and BB-12 and UDCA is pathogenetically justified and leads not only to the correction of intestinal microbiocenosis disorders, but also to a reduction in the activity of cytolytic and cholestatic syndromes, a trend toward normalization of the blood lipid profile, and improvement of quality of life and emotional status in these patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Non-invasive assessment of liver steatosis in children: The role of steatometry and metabolic markers

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ABSTRACT

Aim: The aim of this study was to assess the prevalence and staging of steatotic liver disease among overweight adolescents using non-invasive diagnostic methods, as well as to analyse metabolic correlations and the effectiveness of lifestyle interventions.

Materials and Methods: The study included 42 overweight children aged 12-18. Disease was diagnosed using ultrasound steatometry, shear wave elastography and biochemical indicators. Correlation analysis was performed. A six-month lifestyle modification programme was implemented, including dietary modification, physical activity and psychological support. Changes in the degree of steatosis, metabolic parameters were monitored before and after.

Results: Metabolic dysfunction–associated steatotic liver disease was detected in 88% of participants, of which 52.4% were in stage S1, 35.7% in stage S2, and 11.9% in stage S3. No fibrosis was detected in any patient. Stage S1 steatosis was asymptomatic, while stages S2 and S3 were associated with metabolic disorders, insulin resistance and micronutrient deficiencies. After the intervention, there was a significant reduction in hepatic fat accumulation, with the attenuation coefficient decreasing by 40%. Patients with stage S3 showed improvement in triglyceride levels, insulin sensitivity, and normalisation of liver enzymes.

Conclusions: Steatometry is an effective non-invasive method for diagnosing early-stage metabolic dysfunction–associated steatotic liver disease. Lifestyle interventions, including dietary correction, physical activity, and psychological support, significantly improve liver condition and metabolic parameters in adolescents. Early detection and treatment are crucial for preventing disease progression and related metabolic complications in adulthood.

KEY WORDS: steatometry, children, liver steatosis

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INTRODUCTION

Steatotic liver disease (SLD) and metabolic dysfunction–associated steatotic liver disease (MASLD) have become the most common chronic liver disease among children and adolescents [1]. The new nomenclature reflects the close relationship between the disease and metabolic dysfunction, obesity, and insulin resistance. The prevalence of MASLD in the paediatric population is increasing in parallel with the global obesity epidemic, affecting up to 40% of obese children [2].

Unhealthy diet, sedentary lifestyle, and genetic predisposition are the provocative factors in the complex and multifactorial pathogenesis of MASLD in children [3]. According to recent studies, children may have a more aggressive form of the disease than adults, with a higher likelihood of developing liver fibrosis and metabolic dysfunction-associated steatohepatitis (MASH) [4]. Routine screening of high-risk paediatric groups, especially those with obesity, type 2 diabetes or a family history of metabolic disorders, is important for early detection and treatment, as MASLD is often asymptomatic in its early stages [5].

Lifestyle modifications, including weight loss, optimisation of diet and eating habits, and increased physical activity, are the basis for the treatment of MASLD in children [6]. Recent clinical studies have investigated pharmacological treatments, such as glucagon-like peptide-1 receptor agonists (GLP-1RAs), which have shown promise in treating obesity and related liver problems [7]. Despite these advances, lifestyle modification remains the cornerstone of therapy.

Given the trend of MASLD spreading among the paediatric population, there is an urgent need to raise awareness, implement effective screening programmes, and develop evidence-based treatment strategies to mitigate delayed liver-related complications [8].

The use of steatometry in paediatric ultrasound diagnostics

Steatometry, a non-invasive ultrasound technique, has become a useful tool in paediatric hepatology for

assessing liver steatosis. This method determines the amount of fat in the liver and provides a reliable assessment of the severity of steatosis by measuring the attenuation of ultrasound waves as they pass through liver tissue [9-12].

Steatometry has a number of significant advantages in clinical practice with paediatric patients. It is a quick, bedside procedure that does not require sedation, making it particularly suitable for children [13]. It also avoids the risks associated with ionising radiation and invasive liver biopsy. Studies have shown its effectiveness in detecting and quantifying liver fat in children with metabolic dysfunction–associated steatotic liver disease (MASLD), a condition that is increasingly common in the paediatric population [14].

The standardisation and validation of ultrasound methods for measuring liver fat have been greatly facilitated by the work of G. Ferraioli, V. Cantisani, R. Bar, I. Sporea, S. Wilson and others. Their research demonstrates the importance of standardising procedures and training operators to ensure accurate and repeatable measurements in both adults and children [10, 14-19]. Liver disease in children is often assessed using ultrasound methods such as quantitative ultrasound (QUS) and controlled attenuation parameter (CAP). The degree of attenuation of the ultrasound wave caused by fat deposition in the liver is measured using CAP, which is integrated into transient elastography devices and has been validated against liver biopsy results [16]. Modern QUS methods, such as reverse scattering and ultrasound attenuation coefficients, have also shown promise in detecting liver steatosis in children [17].

In addition, children at risk for metabolic dysfunction–associated steatotic liver disease (MASLD) should undergo regular screening and monitoring for liver disease, including the use of non-invasive imaging methods such as steatometry, in accordance with European and American medical clinical guidelines [18].

Thus, steatometry is an important achievement in paediatric hepatology, as it provides a non-invasive, consistent and child-friendly way to assess liver fat levels. The introduction of steatometry into clinical practice can help control liver disease in children, and thus improve treatment outcomes.

AIM

The aim of this study was to assess the prevalence and staging of steatotic liver disease among overweight adolescents using non-invasive diagnostic methods, as well as to analyse metabolic correlations and the effectiveness of lifestyle interventions.

MATERIALS AND METHODS

Place and time of the study – the study was conducted at the Children's Clinical City Hospital (Uzhhorod, Ukraine) during the period 2023–2025.

Characteristics of the study and control group – the study included 42 adolescents aged 12–18 years with excess body weight. Inclusion criteria were: the presence of excess body weight or obesity (BMI >85th percentile for age and sex), as well as informed consent of patients and their parents. Exclusion criteria: viral hepatitis, autoimmune liver diseases, hereditary and metabolic liver diseases, use of hepatotoxic drugs. The study group included adolescents of both sexes (29 boys, 13 girls).

Description of the method – all patients underwent a comprehensive examination, including: laboratory methods: complete blood count (hemoglobin, erythrocytes, leukocytes, ESR, leukocyte formula), biochemical parameters: total protein, albumin, urea, creatinine, bilirubin and its fractions, ALT, AST, thymol test, fasting glucose, insulin, HOMA-IR index, lipid profile (total cholesterol, triglycerides, HDL, LDL), electrolytes and trace elements (potassium, sodium, calcium, magnesium, iron, zinc, selenium, etc.), blood pH level, urinalysis, stool examination for helminth eggs, rheumatic tests.

Instrumental methods: ultrasound of abdominal organs, liver, thyroid gland, spleen, heart, urinary system, ECG, EEG, steatometry and liver elastography by shear wave method using the Soneus P7 device (Ukraine).

The degree of steatosis was determined by the attenuation coefficient of the ultrasound wave.

After the initial examination, all patients underwent a 6-month lifestyle modification program, including: diet therapy according to the "Healthy Eating Plate" principle (recommendations of the Ukrainian Association of Dietitians), keeping a food diary, regular physical activity with cardiac monitoring, psychological counseling and motivational support, correction of micronutrient deficiencies.

Patients with stage S3 additionally received: therapy of depressive conditions, correction of functional disorders of the gastrointestinal tract and endocrine disorders, administration of ursodeoxycholic acid (UDCA) at a dose of ½ of the standard therapeutic dose for a long period, up to 3–4 months depending on the condition.

Assessment of parameters was carried out before and after 6 months of observation.

The study shows that metabolic disorders increase with the progression of the disease and, as a rule, are absent at the early stages. Steatometry is an informative non-invasive method for early diagnosis of liver steatosis in adolescents with excess body weight, allowing to quantitatively assess the degree of fatty infiltration of the liver.

Table 1. Characteristics of steatosis stages according to the number of patients, attenuation coefficient and presence of liver fibrosis

Stages of steatosis	Number of patients, %	Attenuation coefficient, dB/cm^3	Fibrosis, kPa
S1 (2.0 - 2.2 dB/cm^3)	52.4±7.7%	2.2±0.043	3.0± 0.04
S2 (2.3 - 2.9 dB/cm^3)	35.7±7.4%	2.6±0.052	3.4± 0.06
S3 (3.0 dB/cm^3 and above)	11.9±5.0%	3.0± 0.089	4.6± 0.09

Source: compiled by the authors of this study

ETHICS

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

RESULTS

STEATOMETRY AND STAGING

According to the results of steatometry at the beginning of the study, 88% (37) of participants were diagnosed with hepatic steatosis. The distribution by stages was as follows (Table 1):

- Stage S1 (mild steatosis): 52.4± 7.7%
- Stage S2 (moderate steatosis): 35.7± 7.4%
- Stage S3 (severe steatosis): 9± 5.0%.

According to the classification, steatometry indicators correlate well with the results of histological examination, where 5-33% fat corresponds to stage S1 steatosis, 34-66% to stage S2, and more than 66% to stage S3.

Shear wave elastography did not reveal any fibrotic changes in any of the patients (Table 1).

LABORATORY TEST RESULTS

In patients with MASLD, general clinical laboratory tests were uninformative. While metabolic disorders were observed in stages 2 and 3, no deviations from the norm were observed in stage S1. Biochemical indicators of liver function in patients with 0-2 steatosis remained within the reference ranges. Although bilirubin and its fractions mostly remained within normal limits, patients with stage S3 steatosis had minor deviations, such as a moderate increase in ALT and AST levels.

CORRELATION ANALYSIS

Multivariate correlation analysis revealed several key patterns:

- Stage S1 steatosis (Fig. 1) was asymptomatic, associated with an unbalanced diet (r=0.8), an increase in BMI after 10 years (r=0.8), and sometimes accompanied by carbohydrate metabolism disorders (HOMA index 3.0, r=1) and a slight decrease in vitamin D levels (r=0.7).
- Stage S2 steatosis (Fig. 2) correlated with increased BMI from 2-3 years of age (BMI > 30 at the time of inclusion in the study, r=0.7), hereditary factors (one parent with obesity, r=0.8), eating disorders (r=0.8), depression (r=0.6), dyspeptic symptoms (bloating, constipation, epigastric discomfort, r=0.8), HOMA index 8.0 (r=0.8), impaired glucose tolerance (IGT), elevated triglyceride levels (r=0.8) and significant micronutrient deficiencies (zinc, selenium, magnesium, iron, vitamin D).
- Stage S3 steatosis was associated with the development of early obesity (0-3 years, r=0.8), parental obesity (one or both parents, r=0.7), eating disorders with uncontrolled cravings for sweets (r=0.7), depression (r=0.8), sleep disorders (r=0.5), BMI > 30 <t0/ >at the time of inclusion (r=0.9), digestive and systemic symptoms (epigastric discomfort, bloating, constipation, headache, dizziness, weakness, arterial hypertension, r=0.8), HOMA index > 10.0 (r=0.9), impaired glucose tolerance (IGT), elevated triglyceride and cholesterol levels (r=0.8) and signs of steatohepatitis (elevated AST (r=0.8), ALT and inflammatory markers such as ASL-O, CRP > 2 mg/l).

LIFESTYLE INTERVENTION AND FOLLOW-UP

After the initial examination, patients with MASLD received therapeutic intervention and lifestyle modification. To encourage a healthy lifestyle, they received information sessions, participated in motivational consultations with a psychologist, and kept a food diary for a month. For six months, they followed the dietary recommendations of the Ukrainian Association of Dietitians (the 'Healthy Eating Plate'). Each of them was given a structured physical activity programme with cardiovascular monitoring, as well as psychological support to overcome emotional distress and prevent relapses. Micronutrient status was also corrected.

Patients with stage S3 MASLD were prescribed additional treatment for depressive symptoms, functional disorders of the gastrointestinal tract, endocrine disorders, and cardiovascular risk factors. To improve bile rheology, half the usual dose of ursodeoxycholic acid (UDCA) was prescribed for a prolonged period.

FURTHER ASSESSMENT

Testing of the control group showed that adherence to lifestyle modifications and dietary recommendations

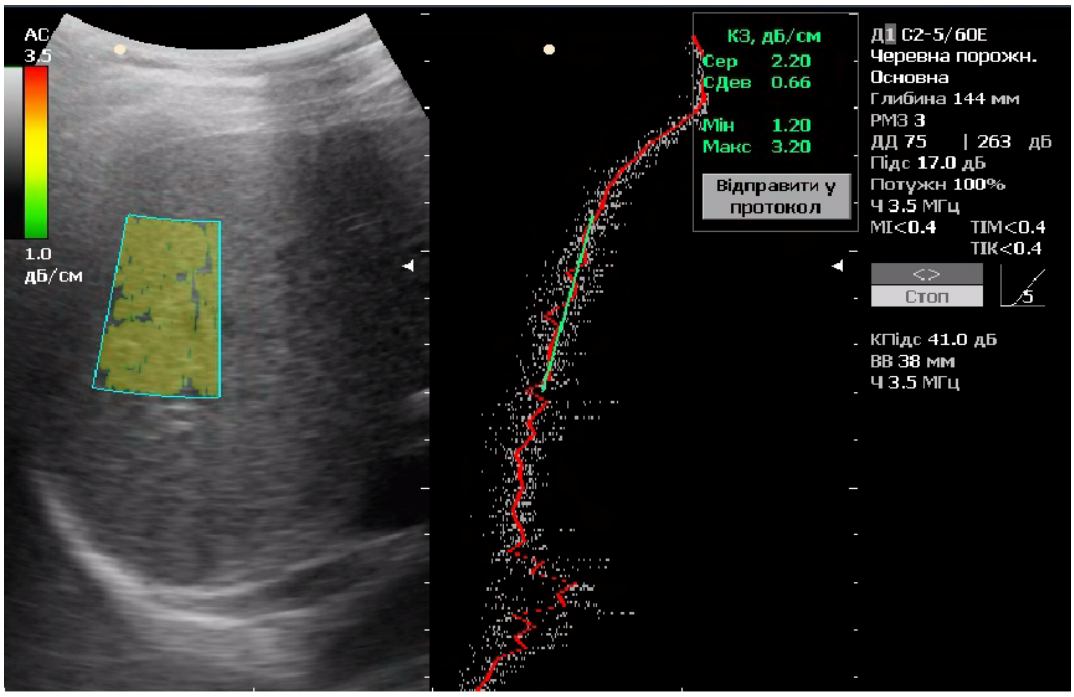


Fig. 1. Quantitative ultrasound liver steatometry, stage S1 steatosis
Picture taken by the authors

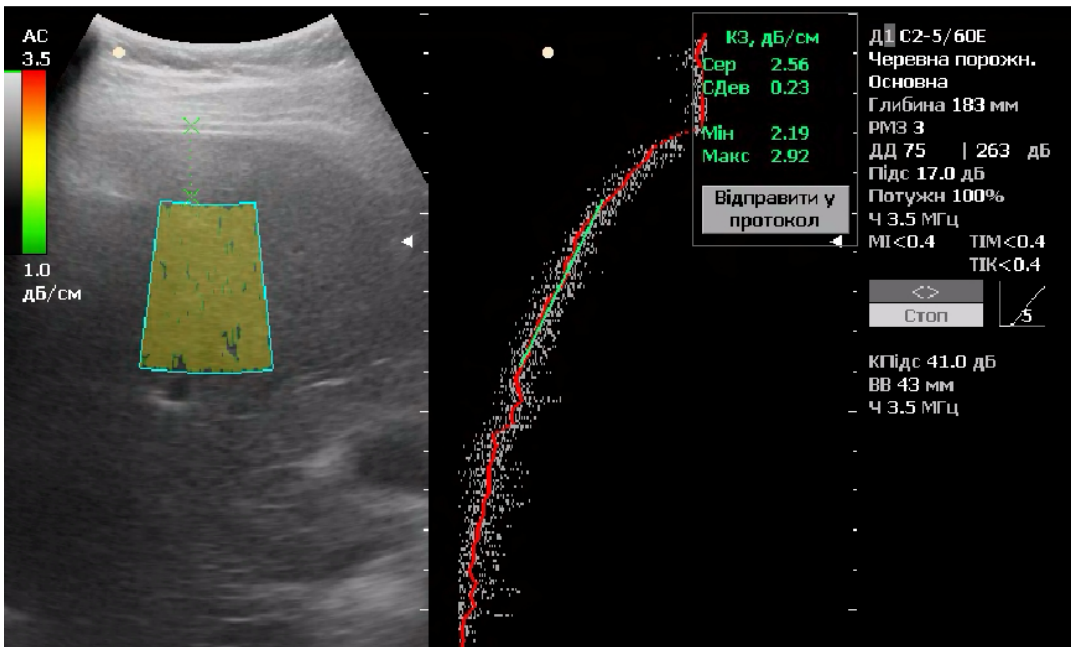


Fig. 2. Quantitative ultrasound liver steatometry, stage S2 steatosis
Picture taken by the authors

contributed to a significant improvement in liver condition, especially in patients with stage S1-2 steatosis. Steatometry data showed a probable 40% reduction (r=1.0) in the attenuation coefficient, as well as a reduction in triglyceride levels and the HOMA index. In patients with stage S3 steatosis, ALT and AST levels normalised, which also indicates an improvement in liver function.

The results obtained prove the value of early lifestyle modification in the treatment of mild and moderate steatotic liver disease in adolescents and the need for a multidisciplinary approach in more severe cases.

DISCUSSION

Formerly known as non-alcoholic fatty liver disease (NAFLD), metabolic dysfunction–associated steatotic liver disease (MASLD) has become the most common chronic liver disease in children and adolescents and is closely associated with the increase in obesity and metabolic syndrome worldwide [21]. 88% of participants in our study aged 12-18 who were overweight were diagnosed with steatotic liver disease; They were distributed as follows: 52.4% had mild steatosis (stage S1), 35.7% had moderate steatosis (stage S2), and 11.9% had severe steatosis (stage S3). These results are gener-

ally consistent with previous studies that have shown a strong association between adolescent obesity and the development of MASLD [22].

Among other things, no fibrotic changes were detected in any of the participants based on shear wave elastography results. These observations are consistent with reports that steatosis is common in obese adolescents, but fibrosis remains a less frequent but serious problem due to the potential for rapid progression of steatosis to fibrosis, and then to cirrhosis and complications related to liver function [23].

According to laboratory analyses, general clinical tests were proved ineffective for patients with MASLD. In patients with stages 0-2 steatosis, liver function markers remained within reference ranges, and only at stage 3 was there a moderate increase in ALT and AST. These results emphasise the need for imaging methods for early diagnosis and are consistent with previous studies, where researchers suggested that liver enzyme levels may remain within normal limits in the early stages of MASLD [24].

Our multiparametric correlation analysis revealed several key patterns:

• Stage S1 steatosis was asymptomatic, associated with an unbalanced diet, an increase in BMI after 10 years, and a slight decrease in vitamin D levels

• Stage S2 steatosis correlated with an increase in BMI at the age of 2-3 years, familial obesity, eating disorders, depression, dyspeptic symptoms, prediabetes (HOMA index 8.0), elevated triglyceride levels, and significant micronutrient deficiencies (zinc, selenium, magnesium, iron, vitamin D)

• Stage S3 steatosis was associated with early onset of obesity (at 0-3 years of age), parental obesity, intense craving for sweets, sleep disorders, arterial hypertension, severe insulin resistance (HOMA index >10.0), elevated triglyceride and cholesterol levels, and signs of steatohepatitis (elevated AST, ALT, C-reactive protein >2 mg/L).

The results obtained highlight the multifaceted aetiology of MASLD, which encompasses metabolic disorders, early-onset weight gain, dietary practices, psychological factors, and genetic predisposition [25]. Therefore, a multidisciplinary approach is necessary for the effective prevention and treatment of such patients.

Evidence has been obtained that diet and physical interventions aimed at lifestyle changes can improve steatosis and the risk of liver fibrosis in children. Our study used a 6-month programme that included structured physical activity, psychological support, and the 'Healthy Eating Plate' developed by the Ukrainian Association of Dietitians. Assessments conducted after the intervention showed that patients with stage S3 steatosis had normalised ALT and AST levels, reduced triglyceride levels, improved insulin sensitivity (reduced HOMA index) and a 40% reduction in the attenuation coefficient. These results are consistent with previous studies that have shown how effective lifestyle modification can be in influencing the course of MASLD in children and adolescents [24].

It is noteworthy that even a moderate reduction in excess weight significantly improved liver steatosis and metabolism, indicating that diet quality and increased physical activity play a key role in the treatment of MASLD. Similarly, previous studies have shown that lifestyle interventions significantly improve BMI, aminotransferase levels, and liver steatosis for children with MASLD [24].

The absence of fibrotic changes in the liver among the patients included in the study may be due to the relatively short duration of the disease and early intervention in this cohort. Longitudinal studies are needed to assess the long-term impact of lifestyle changes on the development of fibrosis in children with MASLD. In addition, more studies should be conducted in different populations to investigate genetic factors, such as PNPLA3 polymorphisms, which are associated with an increased risk of MASLD and fibrosis [22].

The study confirms the importance of early lifestyle intervention for the treatment of MASLD in adolescents. The implementation of comprehensive programmes targeting dietary habits, physical activity and psychological well-being can lead to significant improvements in liver health and metabolic function. Early detection and intervention are crucial to preventing disease progression and related complications. Future research should focus on identifying risk groups, elucidating the genetic and environmental factors that contribute to the development of MASLD, and developing individualised intervention strategies to effectively combat this growing problem in the healthcare system.

CONCLUSIONS

1. Steatometry is a modern, non-invasive and accurate ultrasound quantitative method for assessing liver fat content. It is practically the only method available for quantitative assessment of stage S1 steatosis in overweight children when biochemical markers remain unchanged and clinical symptoms are absent.
2. Assessment of the attenuation coefficient before and after treatment is useful for monitoring liver recovery and evaluating therapeutic efficacy.
3. Dietary adjustments, sleep hygiene, and appropriate physical activity during adolescence can significantly improve attenuation coefficient (AC) values

in stage S1 and S2 steatosis, even with minimal BMI reduction, without pharmacological intervention. Improvements in liver enzyme function, lipid profile, and micronutrient status in stage S2 and S3 steatosis contribute to the stabilisation or reduction of AC.

4. MASLD is an important but currently missing link in the clinical trajectory of overweight patients, although early diagnosis and treatment can prevent metabolic syndrome, steatohepatitis, and other complications in adulthood.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Decline zyxin and CALLY index as predictors of cardiovascular diseases in patients with T2DM

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ABSTRACT

Aim: To find out if a CALLY index and zyxin can be used as new, easy-to-find, and low-cost biomarkers for finding and classifying T2DM people who are more likely to have heart problems like an AMI or stroke early on.

Materials and Methods: A total of 60 T2DM patients (30 male and 30 female), along with 30 healthy controls matched by age and BMI between patients and controls. Various biochemical and inflammatory markers were assessed using ELISA and colorimetric methods. Lipid profiles and atherogenic indices such as CALLY index, metabolic inflammatory index (MII), AIP, CRI-I, and CRI-II index were also calculated.

Results: Results revealed that T2DM patients had significantly higher glucose levels, dyslipidemia, atherogenic indices while decreased zyxin. There was a significant increase in CRP and MII but lymphocyte counts and CALLY index were reduced. Receiver operation characterization (ROC) curve analysis showed that zyxin and CALLY index were the most effective markers for diagnosing cardiovascular risk in T2DM.

Conclusions: T2DM is associated with significant cardiometabolic disturbances characterized by dyslipidemia, chronic inflammation, immune imbalance, and structural protein alterations that increase cardiovascular risk. The significant elevation of MII in T2DM patients ($p < 0.001$), together with reduced zyxin and CALLY index levels, highlights enhanced metabolic and inflammatory stress contributing to endothelial dysfunction and cardiovascular complications. These findings support the potential use of MII, zyxin, and the CALLY index as simple and cost-effective biomarkers for early cardiovascular risk assessment in patients with T2DM.

KEY WORDS: inflammatory, type 2 diabetes, cardiovascular diseases, zyxin and CALLY index

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by high blood sugar levels, which can lead to major problems because it is long-lasting and hard to spot [1]. The major reasons for T2DM are that the body doesn't make enough insulin or can't use the insulin it does make properly .The onset of T2DM is often silent and many years may pass before diagnosis [2]. Many causes of T2DM are a combination of lifestyle factors, genetics, and age [3]. Key risk factors include obesity and physical inactivity, which contribute to the body becoming less responsive to insulin. Genetics and family history also play a significant role, as does increasing age, with the risk rising significantly for people aged 45 and older [4]. About 537 million people between the ages of 20 and 79 had T2DM in 2021. This condition caused 1.6 million deaths, and 47% of all diabetes deaths were before the age of 70 [5]. In Iraq, around 1.4 million of people have diabetes. Reported T2DM prevalence in Iraq ranges from 8.5% (IDF—age adjusted) to 13.9% [6]. Hyperglycemia, dyslipidemia, oxidative stress, and inflammation, common features of T2DM, are recognized cardiovascular risk factors [7]. Cardiovascular diseases (CVDs) are the main cause of death among patients with T2DM [8]. In T2DM, persistent low-grade inflammation is a vital connection between metabolic inefficiency and the onset of CVD. Elevated blood glucose levels induce inflammation, resulting in vascular damage, facilitating atherosclerosis, and heightening the risk of myocardial infarctions, cerebrovascular accidents, and cardiac insufficiency. This inflammatory condition is frequently induced by factors such as obesity and insulin

resistance, resulting in the secretion of cytokines and other pro-inflammatory substances. CVD frequently co-exists with DM, potentially due to the shared risk factors, including atypical inflammatory responses or aberrant lipid metabolism [9]. Recent findings indicate that atherosclerosis is not merely a consequence of T2DM within the context of metabolic syndrome, but also exhibits a comparable pattern of inflammation generated by metabolic stress [10]. Inflammation is regarded as a critical risk indicator for systemic atherosclerosis and is a notable characteristic of vascular problems resulting from diabetes [11]. High blood sugar levels may cause changes in the microvasculature and higher levels of inflammatory substances, such as CRP [12]. The CALLY index measures inflammation, nutrition, and immune system status by looking at serum CRP concentration, serum Albumin concentration, and peripheral lymphocyte count [13]. Zyxin, a part of focal adhesions, may be vital for keeping cardiomyocytes alive. Zyxin has a similar effect on cardiomyocytes, cardiac fibroblasts, and microvascular endothelial cells, which may protect the heart [14]. Zyxin functions as a mechanotransducer in vascular cells, perhaps enhancing cardiomyocyte survival [15].

AIM

Aim of this study is to find out if a CALLY index and zyxin can be used as new, easy-to-find, and low-cost biomarkers for finding and classifying T2DM people who are more likely to have heart problems like an AMI or stroke early on.

MATERIALS AND METHODS

SUBJECT

The present study comprised 60 Arab Iraqi patients with T2DM, consisting of 30 males and 30 females. The average age was 56.8 ± 3.73 years, and the body mass index (BMI) was 26.61 ± 2.51 (kg/m^2). The fasting blood glucose (FBG) was 169.93 mg/dl, and the HbA1c level was 8.4%. The "Al-Najaf Hospital" in Najaf, Iraq, recorded these patients from January to April 2025. Clinical manifestations, symptoms, and biochemical assays were employed for each patient to ascertain their diagnosis of diabetes mellitus. The present investigation excluded participants with any diseases, inflammation, or heart conditions.

CONTROLS

The selection process consisted of thirty people, 15 men and 15 women, all of whom appeared to be in good health. The average BMI of these individuals was 26.07 ± 3.35 and similar age to the patients 55.34±3.11. The workings of the FBG. FBG and HbA1c were measured to have mean values of 92.47±5.6 mg/dl and 5.55±0.45%, respectively. Individuals who were suffering from chronic, systemic disorders and anemia were not allowed to participate in the study.

BIOCHEMICAL RESEARCH

The diagnosis of diabetes was made in accordance with the criteria established by the American Diabetes Association (ADA). This was done based on the patient's medical history, the medication they were currently taking, or both. A HbA1c of 6.5% or a fasting blood glucose level of more than 126 mg/dl (or 7.1 mmol/L) was used to define type 2 diabetes. In accordance with the WHO guidelines, the measurements of height and weight were obtained, and the BMI was determined by applying the formula (weight/(height)^2) (kg/m^2). Consent was given by each patient to the control person. Using two gel tubes and two anticoagulant tubes, the blood was split into two separate tubes. In order to separate the serum from the blood in the gel tube, the blood was centrifuged for five minutes at a speed of four thousand revolutions per minute. After fifteen minutes at room temperature, the serum was transferred into brand-new tubes that were disposable.

INSTRUMENTS FOR EXPERIMENTATION

All analytical procedures were performed under strictly controlled laboratory conditions to minimize environmental interference. The instrumentation listed in Table 1 underwent daily calibration and standardized quality control checks to ensure linear measurement accuracy and high-resolution detection. To maintain the biochemical integrity of the specimens, automated processing was prioritized to reduce human-induced variability and manual handling errors. Furthermore, the integration of high-performance centrifugation and precision thermal incubation protocols ensured that all derived data points and calculated indices were based on stable, non-degraded samples. This rigorous methodological framework supports the analytical validity of the diagnostic thresholds and the statistical precision reported throughout this study.

CHEMICALS

As shown in Table 2, commercially available spectrophotometer and ELISA kits from certified manufacturers

Table 1. Instruments and apparatus for laboratories

Instruments	Company	Origin	Description
Glass gel tube	Q.L.lab	China	Used for sample collection and handling
Micropipette	Dragon	China	Used for accurate measurement and transfer of small liquid volumes
Water bath	Hettich	Germany	Maintains constant temperature for sample incubation
Centrifuge	Hettich	Germany	Separates components based on density by spinning
Refrigerator	Hitachi	Japan	Used for short-term storage of samples at 2–8°C
Deep freezer	Hitachi	Japan	Used for long-term storage at −20°C or −80°C
Spectrophotometer	Spctra721	Taiwan	Measures absorbance to determine concentration of analytes
ELIA Microplate reader	BioTek	USA	Measures optical density in ELISA assays
UniCel DxH 800 analyzer	Beckman	Japan	Automated hematology analyzer for blood cell analysis

Note: The table includes a brief description of each instrument and kit, including their function and principle of measurement
Source: Compiled by the authors of this study

Table 2. Chemical compounds and study kits

Type of Kits	Company/Country	Description
Blood Glucose	Biolabo/ France	Enzymatic colorimetric assay for glucose determination
Total cholesterol	Biolabo/ France	Enzymatic method for cholesterol measurement
Triglyceride	Biolabo/ France	Enzymatic colorimetric method
HDL-c	Biolabo/ France	Direct enzymatic assay for HDL cholesterol
Albumin	Biolabo/ France	Colorimetric assay for serum albumin
HbA1c	BT LAB/ China	ELISA-based assay for glycated hemoglobin
CRP	LTD/Britain	Immunoassay for C-reactive protein detection
Zyxin	BT LAB/ China	ELISA kit for protein quantification

Notes: The calculation for the CALLY index was: albumin concentration (g/dL) × lymphocyte count (10^9/L) ÷ [CRP (mg/dL) × 10] [16].
Lipids and atherogenic indices such as AIP, CRI-I, CRI-II and AC were also calculated.
LDL-C = TC- (TG/5+HDL-C)
VLDL-C = TG/5
CRI-I= TC/HDL-c
CRI-II= LDL-C / HDL-c
AIP= Log (TG/HDL-c)
AC= (TC - HDL-C/HDL-c)
Metabolic inflammatory index (MII)=Platelet*lymphocyte *HbA1c/ albumin
Source: Compiled by the authors of this study

were used for the quantitative determination of the studied biomarkers. All procedures were carried out according to the manufacturers' protocols to ensure the reliability and validity of the obtained results.

STATISTICAL ANALYSIS

The Kolmogorov-Smirnov test was used to examine the distribution types of the results group. The results were expressed for the variable normally distributed, like (mean ± standard deviation). The control and patient groups were compared by using a pooled t-test on the measured parameters. The distinction among groups is considered like different of statistically significant when $p < 0.05$. SPSS Statistics version 26 and IBM-USA performed all statistical analyses. While the numbers were structured using Excel, Microsoft Office 2016.

ETHICAL APPROVAL & INTERNATIONAL COMPLIANCE

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The research protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine of Kufa University prior to the commencement of recruitment. All participants provided written informed consent after being fully briefed on the study's objectives, methodology, and potential risks. To maintain participant privacy, all data were anonymized in strict compliance with local confidentiality regulations and international standards for human subject research.

Table 3. Lipid profile and atherogenic index between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
TC [mg/dl]	205.82±33.49	172.67±21.52	<0.001
TG [mg/dl]	230.87±92.58	127.3±44.52	<0.0001
HDL-c [mg/dl]	42.22±5.23	41.83±3.09	0.712
VLDL-c [mg/dl]	46.17±18.51	25.46±8.9	<0.0001
LDL-c [mg/dl]	117.42±32.49	103.37±15.69	0.058
TG/HDL-c	5.53±2.24	3.04±1.02	<0.0001
AIP	0.708±0.103	0.422±0.091	<0.001
CRI-I	5.01±1.03	4.13±0.51	<0.001
CRI-II	2.85±0.92	2.53±0.4	0.017
AC	3.97±1.03	3.13±0.51	0.001

Note: TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, VLDL: very low density lipoprotein, and LDL: low density lipoprotein. AIP: atherogenic plasma index AC: atherogenic coefficient, CRI-I: Castelli's Risk Index I, and CRI-II: Castelli's Risk Index II
Source: Compiled by the authors of this study

Table 4. Inflammatory markers between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
S.Albumin [g/dl]	4.51±0.36	4.61±0.33	0.225
CRP [mg/dl]	7.17±3.42	2.92±1.85	0.007
Lymph [* 10^9/L]	2.32±1.03	3.70±1.73	0.034
CALLY index	363.27±147.21	629.09± 290.31	<0.001
MII	132.18±62.79	61.85±25.61	<0.001

Note: MII; metabolic inflammatory index.
Source: Compiled by the authors of this study

Table 5. Zyxin parameter between patients and controls

Parameters	Patients Mean± SD	Control Mean± SD	p-value
Zyxin [ng/l]	1579.21±647.48	2081.65±444.99	0.0064

Source: Compiled by the authors of this study

Table 6. ROC curve to zyxin and CALLY index

Test result variable(s)	Cut-off concentration	Sensitivity [%]	Specificity [%]	Area	95% CI of AUC	P-value
Zyxin ng/l	1738.94	77	70	0.785	0.691-0.879	0.000
CALLY index	4273.58	76	69	0.755	0.653-0.858	0.000
MII	74.95	76	70	0.808	0.721-0.896	0.000

Source: Compiled by the authors of this study

RESULTS

All measurements were performed using the laboratory devices shown in Table 1. Some parameters were determined using a spectrophotometer, while other parameters were measured using ELISA kits listed in Table 2.

There is a significant increase in TC, TG, VLDL-c, AIP, CRI-I, CRI-II and AC in Patients than controls. While there is no significant difference in HDL-c, and LDL-c between patients and controls. Table 3 illustrated these lipids and atherogenic index.

In table 4, there is a significant increase in CRP, MII in patients than healthy group. While a significant decrease in lymphocytes and CALLY index in patients compared with controls.

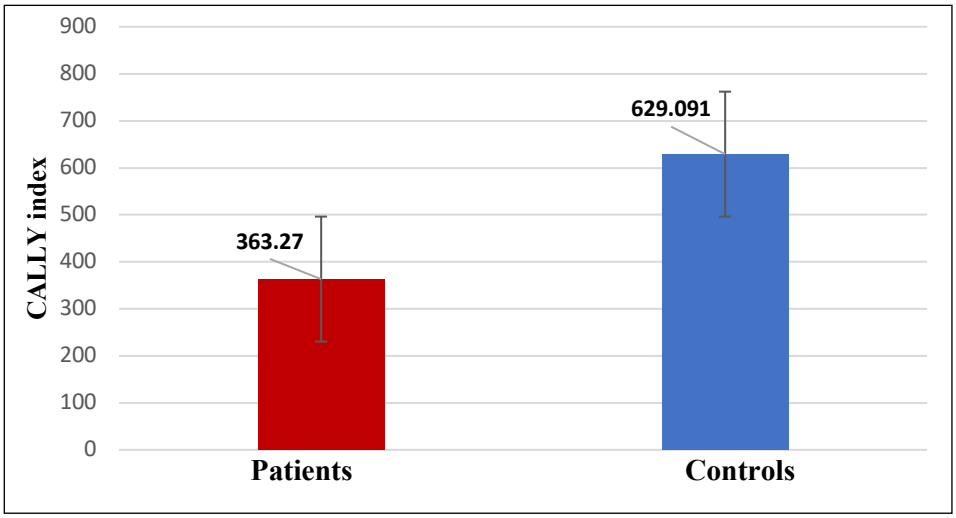


Fig. 1. Explain for comparison between patients and control groups in the CALLY index
Source: Own materials

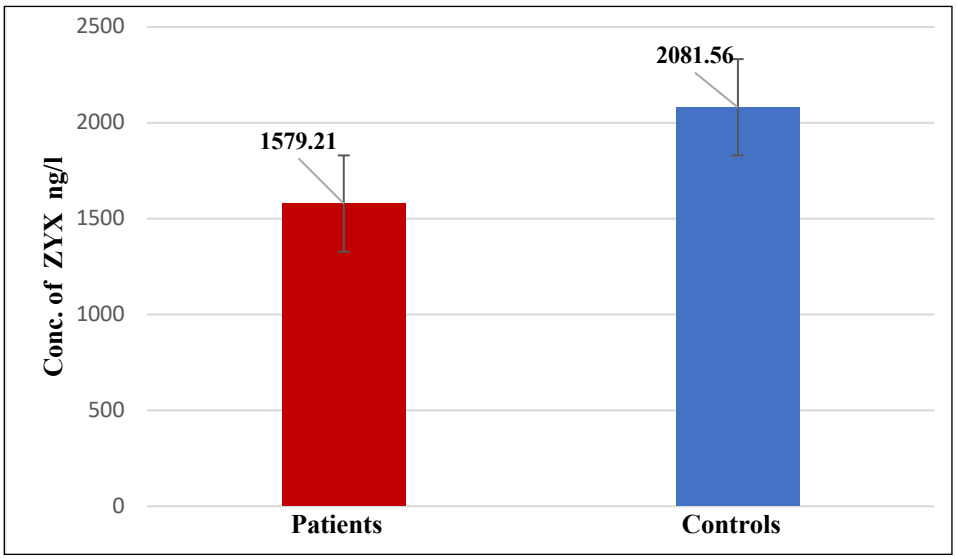


Fig. 2. Explain for comparison between patients and control groups in zyxin
Source: Own materials

The distribution of the CALLY index was compared between the patient group and the control group, as illustrated in Figure 1. The analysis revealed a significantly lower CALLY index in patients compared to healthy controls ($P < 0.001$), suggesting a distinct immunological status between the two cohorts.

There is a significant decrease in zyxin in patients as compared with controls showed table 5.

To investigate the potential of zyxin as a biological marker, its expression levels were compared between the patient group and the healthy control group. As illustrated in Figure 2, a significant decline in zyxin levels was observed in the patient cohort ($P < 0.05$), suggesting its involvement in the pathophysiological mechanism for patients with T2DM.

To determine the diagnostic efficacy and predictive power of both zyxin levels and the CALLY index in identifying patients with T2DM, a combined ROC curve analysis was performed. As illustrated in Figure 3, the AUC for each marker was calculated to compare their respective sensitivity and specificity. This analysis allows for a direct comparison of the clinical utility of these two parameters as potential biomarkers for the disease.

the diagnostic performance of the Metabolic Inflammatory Index (MII) was evaluated independently to determine its efficacy in identifying T2DM cases. As illustrated in Figure 4, a ROC curve was generated, showing the trade-off between sensitivity and specificity. The AUC was calculated to assess the overall accuracy of MII as a standalone metabolic-inflammatory marker

Table 6 showed the ROC curve analysis was performed to evaluated the diagnostic performed of the studies biomarkers. The area under the curve (AUC), sensitivity, and specificity were calculated to determine their ability to discriminate between patients and controls.

DISCUSSION

The number one cause of mortality worldwide is still CVDs. Individuals who suffer from T2DM are at a higher risk of developing cardiovascular diseases such as strokes, heart attacks, and heart failure, as well as peripheral artery dis-

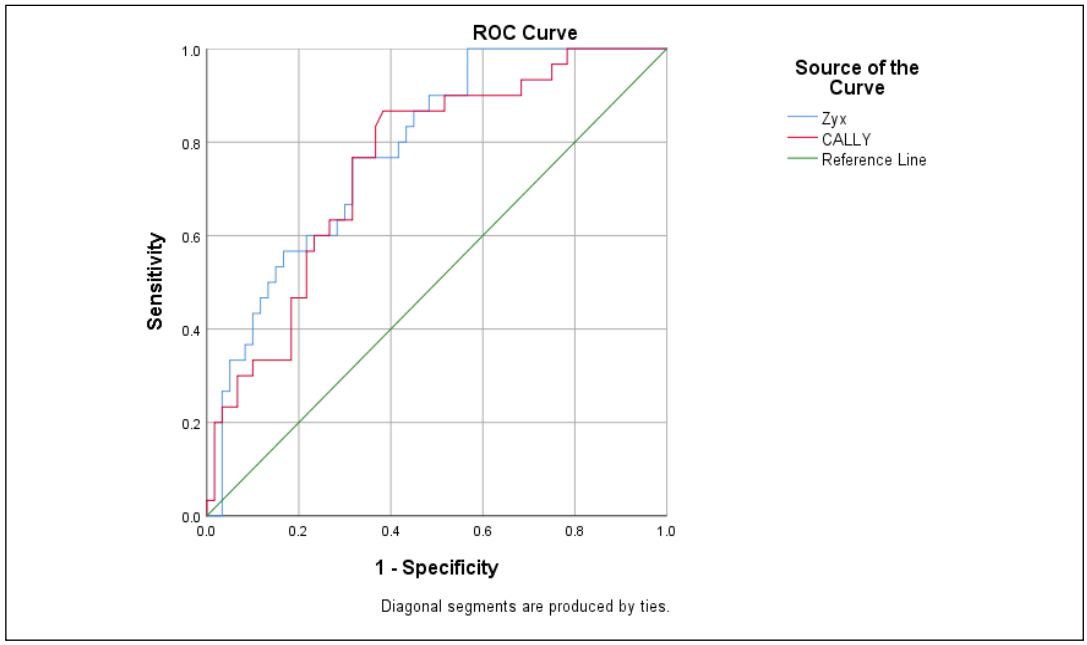


Fig. 3. Explain ROC curve to zyxin and CALLY index
Source: Own materials

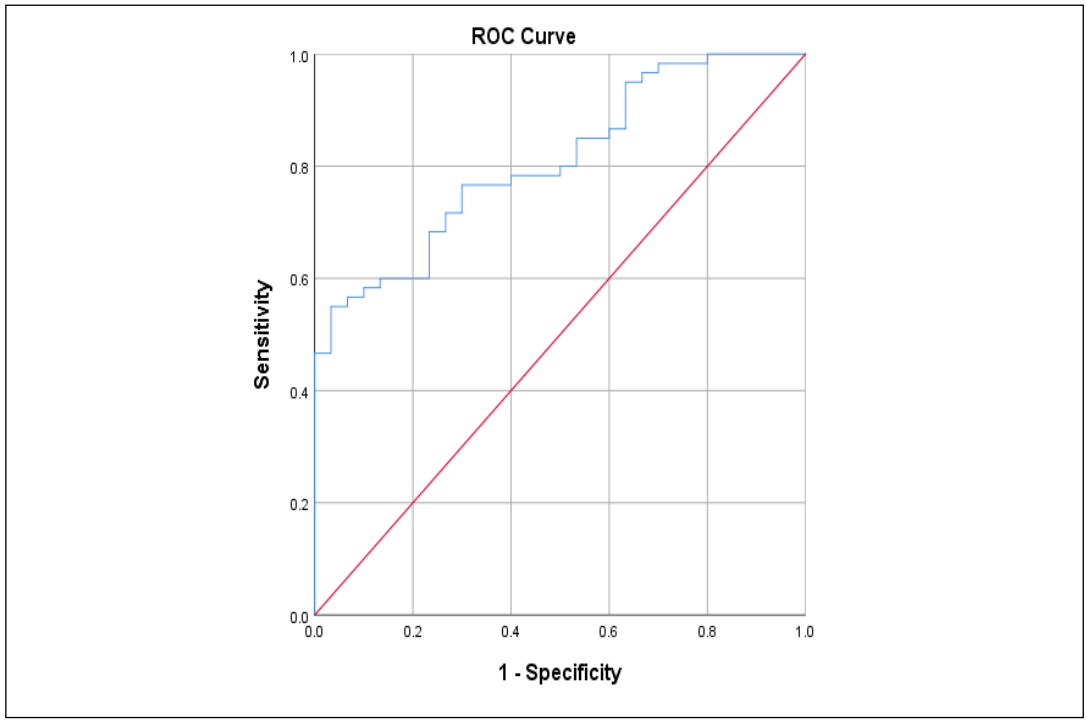


Fig. 4. Explain ROC curve to metabolic inflammatory index
Source: Own materials

ease, compared to individuals who do not have diabetes. This increased risk can be attributed to a number of factors, including insulin resistance, high blood sugar, and abnormal lipid metabolism. These variables are intricately intertwined and have a significant impact on one another. Low-grade chronic inflammation, in particular, is associated with a variety of pathways that contribute to the development and progression of CVD in individuals who have diabetes. In patients with T2DM, the CALLY index and zyxin were evaluated with the purpose of identifying CVD at an early stage. Diabetes and lipid profile are important predictors of metabolic abnormalities, such as dyslipidemia, hypertension, and cardiovascular diseases. The present study found that individuals with diabetes had significantly higher mean serum concentrations of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-c) compared to normative values showed in Table 3. This increase is either an insufficient supply of insulin or resistance to insulin, carbohydrates are diverted from muscle glycogen stores and instead used for de novo lipogenesis in the liver, which ultimately leads to an increase in the level of triglycerides in the plasma. When it comes to people who have diabetes, hypertriglyceridemia is the most common lipid condition that is noticed. In addition to this, the oxidation of LDL-c particles results in their increased incorporation into the wall of the artery by way of a receptor-independent mechanism.

This contributes to a rise in the number of people who have diabetes mellitus and also suffer from heart and brain illnesses. The measurement of cholesterol levels in plasma is a crucial factor in determining the probability of cardiovascular events occurring in individuals with diabetes [17]. People with T2DM frequently experience consequences from CVD, and this is particularly noticeable in those who also have other risk factors including obesity and dyslipidemia [18]. In individuals afflicted with T2DM, the occurrence of insulin resistance results in an increase in free fatty acids due to the lipolytic process, which in turn leads to an enhanced release of VLDL-c, including triglyceride [19]. In patients with T2DM, the TG/HDL-c ratio is a significant predictor of CVD risk and can also be used to identify patients at risk of developing T2DM. Elevated TG/HDL-C ratios are associated with increased CVD risk and poorer glycemic control in T2DM [20]. AIP outperforms standard lipid indicators in predicting CVD risk in T2DM patients, and elevated AIP is associated with an increased risk of major adverse cardiovascular events. AIP can be used as a risk stratification and prognosis tool, and its integration into clinical assessments could guide more targeted interventions [21]. Atherogenic Coefficient (AC), Castelli's Risk Index I (CRI-I), and Castelli's Risk Index II (CRI-II) are lipid ratios used to evaluate the risk of cardiovascular disease (CVD), especially in people with T2DM. These indices can be useful in identifying those who are at higher risk for CVD, and elevated levels of them are linked to increased risk. Since diabetes predicts a very high risk of coronary heart disease, it is not surprising that our T2DM patients have raised AIP and CRI I, CRI II, and AC cardiovascular markers. AIP and CRI I, CRI II, and AC have been found to significantly correlate in one study. These indicators show that the coronary arteries have a significant density of plaque [22]. Consequently, their increased prevalence among research participants suggests a significant risk of CAD that requires immediate attention. Dyslipidemia, recognized as a metabolic disorder, is often correlated with diabetes mellitus. Disturbances in lipid metabolism have been documented in individuals diagnosed with diabetes mellitus, which is concomitant with an elevated risk of cardiovascular atherosclerosis [23]. As established risk factors for CVD in this population.

CRP, lymphocyte, CALLY index and MII significantly differ between T2DM patients and controls Table 4. Chronic inflammation is a major factor in the onset and advancement of CVD in people with type 2 diabetes. In those with type 2 diabetes, elevated levels of inflammatory markers such as CRP are linked to an increased risk of CVD. This inflammation damages blood vessels and encourages the accumulation of plaque, which leads to atherosclerosis, a leading cause of CVD. The liver produces the protein known as CRP in reaction to internal inflammation. Active inflammation is indicated by a higher level of this protein in the circulation [22]. It is strongly linked with heart disorders since it serves as an indirect indicator of chronic inflammation in blood vessels, a contributing factor to atherosclerosis and coronary heart disease [24]. The correlation between CRP and cardiac health CVD, particularly atherosclerosis, involve not only the accumulation of lipids in the arteries but also represent chronic inflammatory disorders impacting the vascular walls [25]. According to this study, compared to non-diabetic individuals, diabetes patients had a higher maximal CRP, a lower minimal lymphocyte count, and a declining CALLY index. For the diagnosis and treatment of cardiovascular diseases, lymphocytes are essential. Their altered levels indicate inflammatory or immunological abnormalities that could promote the progression of the disease [26]. Lymphocytes are a category of leukocytes that facilitate adaptive immunity. They are categorized as T cells, which are responsible for cellular immunity. B cells generate antibodies, while Natural Killer (NK) cells directly target infected or tumor-like cells. Lymphocytes comprise around 20–40% of the overall leukocyte population [27]. Lymphocytes are a specialized category of white blood cells that are essential to the body's immunological response, particularly in recognizing and eliminating pathogenic organisms such as viruses and bacteria. Any change in their quantity may signify inflammation or immunological malfunction [28]. In T2DM, a decrease in lymphocyte count with increased risk of CVD. According to a study in the National Institutes of Health (NIH) on patients with T2DM both very low lymphocyte has been linked to higher cardiovascular mortality risk. The medical perspective on these disorders has progressed to recognize the immune system's function, especially chronic inflammation, as a crucial aspect in their etiology [29]. Lymphocytes are a prominent component of the immune system, actively participating in inflammatory and immunological responses inside the cardiovascular system [30]. Atherosclerosis is a persistent inflammatory condition in which lymphocytes are pivotal. T cells secrete cytokines that exacerbate the destabilization of atherosclerotic plaques, heightening the risk of problems.

CRP, albumin levels, and lymphocyte count are all combined to create the CALLY index, a composite biomarker. A greater CALLY index that shown in Figure 1 is strongly linked to a lower risk of cardiovascular and all-cause mortality in people with CVD, according to research. For cardiovascular risk stratification, the CALLY index could be a helpful tool. In this study decrease CALLY index in T2DM patients that indicator to more exposure patients to CVD than healthy people this result explained in figure 1. On the other hand, other study used CALLY index to deliver a thorough evaluation of a patient's with T2DM systemic inflammatory status,

nutritional state, and immunological competence [31]. A higher CALLY score indicates stronger overall health state of the patient [32]. Other study consider it may function as a protective factor or prognostic indicator in cardiovascular patients the CALLY index proves to be a straightforward and economical solution. A low CALLY index typically signifies elevated inflammation (high CRP), deficient nutritional status (low albumin), or immunological suppression (low lymphocytes), indicates an intensified inflammatory condition and compromised physiological resistance all of which are detrimental prognostic indicators in cardiovascular illnesses [33]. Chronic hyperglycemia in diabetes induces a persistent inflammatory and immune dysregulation state, characterized by elevated HbA1c, increased PLR, and reduced albumin levels. The integration of these parameters into MII suggesting enhanced metabolic and inflammatory imbalance associated with diabetic progression. This elevation may reflect increased cardiometabolic stress and supports the role of MII as a potential indicator of disease severity and cardiovascular risk in individuals with T2DM.

In Table 5 shown there is a significant decline in zyxin level ($p<0.05$) in T2DM patients than controls. This result explained Figure 2. Zyxin, a focal adhesion protein, plays a crucial role in various aspects of CVD and it is a mechanosensitive protein that facilitates cellular responses to mechanical stress, the regulation of the cytoskeleton and cellular motility playing an essential role in heart shape and communication pathways [34]. It appears to be involved in protecting against hypertension-induced cardiac dysfunction, potentially by promoting cardiomyocyte survival and inhibiting fibrosis. Additionally, zyxin is implicated in vascular repair and endothelial migration, suggesting a role in managing vascular injuries associated with CVD. Depending on these reasons, decrease zyxin in patients. Based on these scientific facts, patients in this study are more likely to develop cardiovascular diseases than healthy individuals, and these diseases can be predicted before symptoms appear, and their occurrence can be reduced. One possible reason for the decrease in Zyxin among patients with T2DM is the inefficiency of the heart muscle function due to diabetes. Consequently, the heart muscle cannot produce Zyxin protein, depending on the finding of the following research in circumstances such as heart failure or myocardial infarction, the heart may diminish its capacity to synthesis sufficient Zyxin, resulting in inadequate tissue regeneration and increased fibrosis [35]. As individuals age, Zyxin levels progressively diminish in small arteries, compromising their capacity to endure strain and heightening the likelihood of cardiovascular disease [15]. Therefore, the ages of patients and healthy individuals were taken to be identical or close to avoid such reasons, which is the effect of increasing age on the zyxin protein. Other study investigation has examined the function of zyxin in cardiac muscle activity. zyxin is essential for preserving optimal muscle architecture, cardiac growth, and functionality. Zyxin positively influences cardiomyocytes, and it lack may result in cardiomyocyte death and excessive fibrosis, thereby impairing cardiac function [36]. Recent research indicates that zyxin shortage in the heart results in detrimental effects, especially in hypertension situations. The deficiency of this protein enhances cardiomyocyte apoptosis, diminishing the heart's contractile capacity. Furthermore, research with genetically modified animals deficient in the zyxin gene has demonstrated that vascular smooth muscle cells maintain their contractile nature, hence inhibiting vascular remodeling [15, 37]. Zyxin modulates the expression of genes associated with fibrosis, and its deficiency may result in excessive collagen accumulation and cardiac fibrosis [34].

The diagnostic evaluation of Zyxin, the CALLY index, and MII demonstrates that all three parameters are highly significant biomarkers for the target condition ($p < 0.001$) illustrated in Table 6. Figure 3 shown that MII emerged as the most superior indicator, yielding an AUC of 0.808 (95% CI: 0.721–0.896), which signifies "excellent" discriminative ability according to standard diagnostic criteria. Figure 4 shown that zyxin and the CALLY index also provided robust results with AUC values of 0.785 and 0.755, respectively, indicating their reliability in clinical stratification. However, the relatively moderate specificity across all three markers (ranging from 69% to 70%) suggests that while these biomarkers effectively identify affected individuals, there is a consistent ~30% risk of false positives. Given that the 95% confidence intervals for all variables remain well above the 0.50 null-hypothesis threshold, these findings suggest that incorporating these biomarkers - particularly the MII - into a multi-parametric diagnostic model could significantly enhance overall clinical precision and predictive accuracy.

CONCLUSIONS

Type 2 diabetes mellitus is associated with a pronounced cardiometabolic derangement characterized by atherogenic dyslipidemia, systemic low-grade inflammation, immune dysregulation, and altered structural protein expression. Elevated TC, TG, LDL-c, and increased atherogenic indices (AIP, CRI-I, CRI-II, and AC) reflect enhanced hepatic *de novo* lipogenesis, increased VLDL secretion, impaired lipid clearance, and intensified LDL oxidation, collectively promoting endothelial dysfunction and atherogenesis. In addition, the significant increase in MII observed in T2DM patients further indicates aggravated metabolic and inflammatory imbalance contributing to cardiovascular risk progression.

Concomitantly, elevated CRP, reduced lymphocyte count, and a decreased CALLY index indicate persistent activation of inflammatory signaling pathways, impaired immunological homeostasis, and compromised protein synthetic ca-

pacity, all of which contribute to vascular injury and plaque instability. The significant reduction in zyxin levels further suggests disruption of focal adhesion signaling, cytoskeletal integrity, and mechanotransduction in cardiomyocytes and vascular smooth muscle cells, potentially facilitating adverse cardiac remodeling and fibrosis.

Together, these biochemical and molecular alterations underscore the multifactorial pathophysiology of cardiovascular risk in T2DM and support the combined use of lipid-derived indices, inflammatory biomarkers, MII, the CALLY index, and zyxin as integrated tools for early cardiovascular risk assessment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Alterations of thyroid and adipose tissue hormones in acute and convalescent COVID-19 patients

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ABSTRACT

Aim: To study and analyze studies data of thyroid function and adipose tissue hormones changes in patients with coronavirus infection.

Materials and Methods: The study was conducted on the basis of the Clinical Hospital «CMCH», Uzhhorod, outpatient department. The clinical presentation of the patients with Covid-19 verified diagnosis of was represented by damage to the upper respiratory tract, in particular nasopharyngitis. The age of the studied patients was 18±2.15 years.

Results: All thyroid status parameters varied within the reference values, but it is noteworthy that after treatment, a significant increase in TSH levels was observed both after treatment and in comparison with the control group ($p_1<0.01$; p3<0.01) and after 6 months шт 3.1 times ($p_2<0.01$; $p_4<0.01$). Representative dynamic values of ATPO were identified both after treatment and in comparison with the control group ($p_1<0.01$; $p_3<0.01$) and after 6 months. A significant decrease in ATPO was observed in 4.7 times ($p_2<0.01$; $p_4<0.01$). All levels of the studied adiponectin, leptin and C-peptide parameters varied within the reference range. Adiponectin values significantly decreased after treatment and after 6 months ($p_4<0.01$) in 4 times; Leptin levels significantly decreased after treatment in 2.8 times and after 6 months - in 3 times ($p_4<0.01$). C-peptide values ($p_4=0.12$) reached the level of the control group.

Conclusions: Investigation of thyroid function and adipose tissue hormones changes in patients with Covad-19 was conducted. All thyroid status parameters and adipose tissue hormones varied within the reference values with different variations.

KEY WORDS: Covid-19, upper respiratory tract infection, nasopharyngitis, thyroid hormones, adipose tissue hormones, patient

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INTRODUCTION

The response of each organism to a viral factor is unique and can be considered as a set of contributing factors for the development of certain clinical presentations. The persistence of the virus or viral fragments determines have triggered to the immune response, the duration of the disease and the possibility of developing complications. [1] From 20% to 60% of patients with COVID-19 have some endocrine disorders during the acute phase of the disease or during the convalescence period, according to numerous studies. Moreover, some hormonal disorders can persist for months after recovery, forming part of the symptom complex of "post-COVID syndrome" or "long COVID". Understanding the relationship between COVID-19 and the endocrine system is important for clinical practice, timely diagnosis, adequate treatment and prevention of long-term complications. [2]. If the immune response is ineffective in the first phase of SARS-CoV-2 infection, a second or late phase develops, which is based on large-scale virus replication and a "cytokine storm". This is accompanied by the generation of a large number of virions, which leads to massive damage of target tissues, with including lung tissue. Affected ACE2-expressing cells produce pro-inflammatory cytokines that recruit effector cells (macrophages, neutrophils) and release alarmins that induce the activity of inflammasomes (inflammasome from the English inflammation – inflammation) – a multi-protein oligomeric complex responsible for activating the inflammatory response. The functioning of the latter is accompanied by the release of a significant number of pro-inflammatory cytokines and, as result, the "cytokine storm"will developed, which enhance the participation of the above-mentioned macrophages and neutrophils, creating an extremely high level of inflammatory reaction [3].

The SARS-CoV-2 virus enters cells through the angiotensin-converting enzyme 2 (ACE2) receptor, which is widely expressed not only in the lungs, but also in many endocrine organs: the pancreas (islets of Langerhans), thyroid gland, adrenal glands, pituitary gland, testes, ovaries. The presence of these receptors makes the endocrine glands vulnerable to direct viral damage.

The virus can caused, after entering the cell[4]:
-direct cytopathic cell damage;
-local inflammatory reaction:
-glandular dysfunction;
-small vessel thrombosis with organ ischemia;
-autoimmune reactions against glandular tissues [5,6].

The coronavirus attacks fat cells and certain immune cells in fat tissue, creating an immune response that can lead to serious damage, according to the studies. This finding may explain why people, who are overweight or obese have an increasing risk in severe illness and death from COVID-19 [7].

AIM

The aim was to study and analyze studies data of thyroid function and adipose tissue hormones changes in patients with coronavirus infection

MATERIALS AND METHODS

The study was conducted on the basis of the Clinical Hospital «CMCH», Uzhhorod, outpatient department (2019-2021years). The clinical presentation of the patients with Covid-19 verified diagnosis of was represented by damage to the upper respiratory tract, in particular nasopharyngitis. The age of the studied patients (n=60) was 18±2.15 years(67,0±1,8% women, 33,0±2,6% men).

The developed treatment regimen was applied according to the treatment protocol of Ministry of Health of Ukraine [8] The Covid-19 strain named "Omicron" was detected in the studied contingent

Criteria for including of the examined contingent with diagnosed COVID-19: patients, aged 18,00±2.15 years, with a diagnosis of COVID-19, with clinical manifestations of confirmed results infection by the subjective and objective investigation, with laboratory data and the presence of informed consent to diagnostic and treatment measures conducting.

Exclusion/non-inclusion criteria: pztients before 18,00±2.15 years of age; oncological, autoimmune diseases; laboratory detection of TORCH infection, human immunodeficiency virus (HIV); viral diseases (rubella, syphilis, hepatitis, chickenpox), gonococcal infection; tuberculosis; malaria; severe defects.

A control group (n=28) was identical in age and sex, without clinical and laboratory manifestations of coronavirus infection (CI), was also formed.

Blood serum studies were performed on the ELISA analyzer VER-2000 (Siemens) (thyroid hormones by immunochemiluminescent method; leptin, adiponectin, C-peptide - by enzyme-linked immunosorbent assay). The study algorithm included observation for 6 months. All studies performed and treatment methods applied were carried out with the voluntary written consent of the patients, in compliance with all requirements for ensuring the anonymity and confidentiality of the results obtained. The methodology of scientific and practical monitoring was carried out in accordance with the criteria of the Helsinki Declaration of Human Rights of 1975 and its revision of 1983, the norms of the Council of Europe Convention on Human Rights and Biomedicine, as well as the current norms of Ukrainian legislation and the requirements of the local medical commission of the State Higher Educational Institution «Uzhhorod National University».

RESULTS

A very limited number of studies and reports describe the association between SARS-CoV-2 infection and thyroid diseases in children [7,8].

McCowan et al. [7] had studied 244 children with thyroid abnormalities, hypothyroidism or hyperthyroidism at a tertiary pediatric endocrine center in the United Kingdom before and after COVID-19 for identification any changes in their clinical presentation. They suggested that this finding may be related to the development of thyroiditis secondary to SARS-CoV-2 infection, which regressed before requiring treatment. This condition was associated with a significantly higher risk of death and severe inflammation (detected by high levels of IL-6), highlighting the importance of monitoring thyroid function test results in patients with severe COVID-19 infection [9]. The higher reported the thyroid dysfunction incidence where in the majority of analyzed patients. These patients had a mild course of COVID-19, which suggests a disease severity correlation with thyroid dysfunction [10,11].

A retrospective study of patients referred to a research center with a diagnosis of MIS-C showed that over 90% of patients had nonthyroidal illness syndrome (NTIS) [12]. Among the laboratory variants of NTIS, the most common was an isolated decrease in T3 free [12]. TG balance was restored in 100% of patients after twenty months[13].

Although data on thyroid function impairment during COVID-19 infection in the patients are still limited, the available evidence supports the theory that the virus

Table 1. Dynamic parameters of thyroid status in children with diagnosed coronavirus infection

Parameters	Control group N=28, M± m	1 group (n=60) , M± m		
		Before treatment	After treatment	After 6 month
TSH (0,4-4,0, мкIU/ml)	1.87 ± 0.46	0.41 ± 0.03	0.73 ± 0.19 (p_1<0.01; p_3<0.01)	1.25 ± 0.40 (p_2<0.01; p_4<0.01)
Free triiodothyronine (1,2-2,8, nmol/l)	1.33 ± 0.08	1.30 ± 0.28	1.40 ± 0.21 (p_1=0.11; p_3=0.18)	1.20 ± 0.31 (p_2=0.004; p_4=0.12)
Free thyroxine (12,5-21,0, nmol/l)	14.22 ± 0.49	15.00 ± 2.10	11.10 ± 1.42 (p_1<0.01; p_3<0.01)	13.84 ± 1.09 (p_2<0.01; p_4=0.08)
ATPO (< 35, IU/ml)	5.69 ± 0.11	6.10 ± 4.58	2.80 ± 1.86 (p_1<0.01; p_3<0.01)	1.32 ± 1.18 (p_2<0.01; p_4<0.01)

Notes: p_1 - significance of differences between the values of indicators before and after treatment; p_2 - significance of differences between the values of indicators after treatment and after 6 months; p_3 - significance of differences between the values of indicators after treatment and the parameters of the control group; p_4 - significance of differences between the values of indicators after 6 months and the parameters of the control group
Source: compiled by the authors of this study

Table 2. Dynamic characteristics of adipose tissue hormones in children with diagnosed coronavirus infection

Parameters	Control group N=28, M± m	1 group (n=60) , M± m		
		Before treatment	After treatment	After 6 month
Adiponectin (5-37, mkg/ml)	7.73 ± 0.86	31.20 ± 10.11	8.12 ± 1.65 (p_1<0.01; p_3<0.01)	8.34 ± 0.79 (p_2=0.22; p_4<0.01)
leptin (2,05-11,09, ng/ml)	6.97 ± 0.32	11.97 ± 2.28	3.81 ± 1.29 (p_1<0.01; p_3<0.01)	4.11 ± 0.55 (p_2=0.41; p_4<0.01)
C-Peptide (0,81-3,85, ng/ml)	1.43 ± 0.08	4.71 ± 1.52	2.28 ± 0.79 (p_1<0.01; p_3<0.01)	1.48 ± 0.49 (p_2<0.01; p_4=0.12)

Notes: p_1 - significance of differences between the values of indicators before and after treatment; p_2 - significance of differences between the values of indicators after treatment and after 6 months; p_3 - significance of differences between the values of indicators after treatment and the parameters of the control group; p_4 - significance of differences between the values of indicators after 6 months and the parameters of the control group
Source: compiled by the authors of this study

plays a role in thyroid dysfunction, which in most cases is transient[14]. Autoimmune thyroiditis and primary adrenal insufficiency have also been identified in the study. The role of COVID-19 in the etiopathogenesis of APS2 is unclear, but it may be a trigger for the rapid progression of both adrenal insufficiency and hypothyroidism [15]. A third potential mechanism is selective transient pituitary dysregulation, as secondary to either the direct cytotoxic effects of the virus on the pituitary or the indirect effects of the "cytokine storm" that can induce NTIS [16-19].

It should also be considered that the COVID-19 pandemic has limited access to healthcare services for the general population worldwide, which may have affected the severity of thyroid disease at diagnosis in adults [20]. It has been hypothesized that SARS-CoV-2 may enter thyroid cells via ACE2 and the transmembrane serine protease (2TMPRSS2), which is highly expressed in this gland [21,22]. The "cytokine storm" induced by the virus is characterized by a hyperactive Th1/Th17 immune response with overexpression of pro-inflammatory cytokines such as IL-6, which has been shown to be closely associated with thyroiditis [23]. Cases of undiagnosed subacute thyroiditis have been described in patients with COVID-19 with typical clinical presentation and onset within 5–30 days of illness onset, which may be explained by the use of glucocorticoids in COVID-19, which may mask cases of destructive thyroiditis. Subacute thyroiditis was most often associated with mild COVID-19.[24]. All of the above leads to pantropism – damage to all parenchymal organs, as well as mucous membranes.

We will consider the levels of thyroid status parameters (Table 1).

According to the table, all thyroid atatus indicators varied within the reference values, but it is noteworthy that after treatment, a significant increase in TSH levels was observed both after treatment and in comparison with the control group (p1<0.01; p3<0.01) and after 6 months in 3.1 times (p2<0.01; p4<0.01). Representative dynamic data of the ATPO indicator were identified, both after treatment and in comparison with the control group (p1<0.01; p3<0.01) and after 6 months a ATPO significant decrease in 4.7 times (p2<0.01; p4<0.01).

Adipose tissue has attracted considerable attention due to its role in immune response and inflammation through the secretion of adipokines. Adipokines, such as leptin and adiponectin, are secreted by adipose tissue and are involved in various physiological processes, with particular emphasis on their role in modulating immune responses and inflammation. Leptin and adiponectin are the most abundant adipokines in humans, playing crucial roles in regulating cardiac function, skeletal muscle, growth, and inflammation. Leptin, a pro-inflammatory adipokine, is involved in the control of food intake and energy expenditure, and also influences immune cell activation and cytokine production. In contrast, adiponectin, an anti-inflammatory adipokine, circulates at high levels in plasma and modulates immune cell function, counteracting the effects of leptin. Here, we provide an overview of the role of adipokines in immune response and inflammation. Furthermore, the leptin-adiponectin ratio (Adpn/Lep) has become an important indicator of various metabolic diseases and conditions. Further studies are needed to fully elucidate the mechanisms by which adipokines influence immune responses and to identify potential therapeutic targets for inflammatory and metabolic disorders[25].

We also have investigated adipose tissue hormones and the role of the metabolic component in the development of inflammation.(Table 2).

All levels of the studied indicators of adiponectin, leptin and C-peptide varied within the reference range. Adiponectin values significantly decreased after treatment and after 6 months (p4<0.01) in 4 times, when compared with the initial indicators. The value of the leptin level significantly decreased after treatment in 2.8 times and after 6 months in 3 times (p4<0.01). The value of C-peptide (p4=0.12) reached the level of the control group. Considering that C-peptide has a significant role in the secretion of inflammatory factors, including IL-6, IL-8 and correction of adhesion to endothelial cells of human vessels, the organism, after suffering from Covid-19, does not need the above-mentioned effects. Obesity has been identified as a risk factor for progression to severe COVID-19 [26].

The mechanisms underlying the association between obesity and disease severity following SARS-CoV-2 infection remain unclear. In principle, obesity may contribute to infection in several ways:

(a) down-modulation of antiviral responses [27];

(b) release of lipids that promote endothelial dysfunction and support intravascular coagulation [28];

(c) disruption of leptin and insulin signaling, thereby enhancing the inflammatory response [29];

(d) promotion of enhanced expression of SARS-CoV-2 receptors [30];

(e) representation of a large reservoir for viral replication with increased secretion of virus and inflammatory mediators [31]

Obesity is an independent risk factor for severe COVID-19, but there is still a lack of consensus on the mechanisms underlying this association. A hypothesis that has attracted considerable attention suggests that SARS-CoV-2 disrupts adipose tissue function either through direct infection or through indirect mechanisms. [32] Furthermore, the study suggested that healthcare providers should consider patient weight and body fat when administering COVID-19 vaccines and treatments. [33]

DISCUSSION

Despite less severe manifestations in the acute phase, two major long-term complications of COVID-19 have been reported in children: multisystem inflammatory syndrome in children (MIS-C) and prolonged COVID. Since symptoms usually begin four to six weeks after initial infection, it has been hypothesized that the virus remains in the intestine of children, causing irritation of its mucosa. For this reason, viral antigens have time to cross the intestinal barrier into the bloodstream, reaching other organs and causing a significant inflammatory response [34]. Prolonged COVID is a heterogeneous multisystem condition characterized by the persistence of signs and symptoms that occur three months after the onset of COVID-19 and persist for at least two months, and cannot be explained by an alternative diagnosis [35]. Lopez-Leon et al. [36] conducted a systematic review, showing a prevalence of prolonged COVID of 25.24%. The most frequently reported symptoms were mood changes (16.50%), fatigue (9.66%), sleep disturbances (8.42%), headache (7.84%), and respiratory symptoms (7.62%). The identified risk factors for developing prolonged COVID are older age, female gender, severe COVID-19, overweight/obesity, concomitant allergic diseases, and other long-term comorbidities [8]. There are no recommendations

for the diagnosis and treatment of prolonged COVID. The underlying pathogenetic mechanisms of these patients have not yet been identified [37].

SARS-CoV-2 is known to interact with host cells through its spike protein, binding to the membrane enzyme angiotensin-converting enzyme 2 (ACE2). After the virus enters the cells, the STAT3/NF-kB pathway is activated, which causes the production of pro-inflammatory cytokines and chemokines, leading to systemic hyperinflammation known as a "cytokine storm" [38]. Adipokines have been shown to influence inflammatory and immune responses in a variety of diseases. ACE2 is not only expressed in lung cells: it is ubiquitous, which explains the multiorgan involvement commonly seen in COVID-19. Further studies are needed to elucidate the mechanisms by which adipokines exert their immunomodulatory effects on all cells of the immune system[38].

CONCLUSIONS

1. All thyroid status parameters varied within the reference values, but it is noteworthy that after treatment, a significant increase in TSH levels was observed both after treatment and in comparison with the control group (p1<0.01; p3<0.01) and after 6 months by 3.1 times (p2<0.01; p4<0.01). Representative dynamic values of ATPO were identified both after treatment and in comparison with the control group (p1<0.01; p3<0.01) and after 6 months. A significant decrease in ATPO was observed in 4.7 times (p2<0.01; p4<0.01).
2. All levels of the studied adiponectin, leptin and C-peptide parameters varied within the reference range. Adiponectin values significantly decreased after treatment and after 6 months (p4<0.01) in 4 times when compared with the initial indicators. Leptin levels significantly decreased after treatment in 2.8 times and after 6 months - in 3 times (p4<0.01). C-peptide values (p4=0.12) reached the level of the control group. Considering that C-peptide has a significant role in the secretion of inflammatory factors, including IL-6, IL-8 and correction of adhesion to endothelial cells of human vessels, after suffering from Covid-19, does not need the above-mentioned effects.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

The correlation between body composition and the ability to perform physical activity in aerobic and anaerobic energy supply modes in females from lowland districts of the Transcarpathian region

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ABSTRACT

Aim: To establish correlations between indicators of aerobic and anaerobic productivity with the body composition of females of different somatotypes from the lowland districts of Transcarpathia.

Materials and Methods: A correlation analysis of physical health was conducted on 118 females (aged 16–20) from lowland Transcarpathia. Aerobic productivity was assessed $VO_{2\,max}$ using bicycle ergometry, while anaerobic capacity was measured using 10-second and 30-second Wingate tests ($WAnT_{10}$ and $WAnT_{30}$) and the 1-minute maximum quantity of external work (MQEW). Somatotypes were classified using the Heath-Carter method.

Results: The muscle component has a positive effect on the aerobic performance of females from lowland districts, but it has a moderate effect. Body mass index has a direct moderate correlation with the absolute $VO_{2\,max}$ and an inverse moderate correlation with the relative $VO_{2\,max}$. The correlation with the indicators of $MQEW_{rel}$, $WAnT_{30\,rel}$. and $WAnT_{10\,rel}$. is characterized as weak, which indicates a slight negative impact of the fat component on the anaerobic productivity of females living in the lowland districts of Transcarpathia

Conclusions: For females from lowland transcarpathia, fat and muscle components (including visceral fat) do not significantly impact aerobic or anaerobic performance across any somatotype. Instead, body mass index (bmi) is the primary determinant: higher bmi correlates with lower relative $VO_{2\,max}$ in endomorphs, but higher absolute anaerobic power $WAnT_{10}$ and $WAnT_{30}$ in ectomorphic and balanced types. Given conflicting literature, the specific influence of body composition on anaerobic capacity requires further clarification.

KEY WORDS: aerobic productivity, anaerobic productivity, physical health, fat, skeletal muscles

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INTRODUCTION

Physical health depends on the body's ability to adapt to environmental conditions, while maintaining normal functional parameters of all physiological systems [1,2]. Aerobic and anaerobic performance of the body are integral indicators of physical health. Aerobic and anaerobic performance indicators allow to assess physical health not only qualitatively, but also quantitatively [3]. The formation of physical health occurs under the influence of both endogenous and exogenous factors [4-6]. It should be noted that the set of various morphological factors which determine the somatotype (in particular, body composition) affects both functional capabilities of the body and predisposition to particular diseases [7-9]. The ability to demonstrate aerobic capabilities largely depends on the percentage of muscles which are the main consumer of oxygen [10]. Fat plays the role of a regulator of metabolic processes and is the main source of energy during prolonged low-intensity work, which is performed due to aerobic mechanisms of energy supply of muscle activity. Data from the scientific literature on the correlation of body composition with $VO_{2\,max}$ are contradictory. Therefore, to clarify the influence of somatotype components and body composition on the functional capabilities of females living in the mountainous districts of Transcarpathia, a correlation analysis should be conducted.

Establishing a correlation between human functional capabilities (aerobic and anaerobic productivity) that characterize the body's ability to adapt to external fac-

tors, and body composition of different somatotypes, which is largely genetically determined, will allow to individualize the prevention of certain diseases and choose effective treatment tactics, which is relevant and socially significant. [11-13].

AIM

The aim of the study is to establish correlations between indicators of aerobic and anaerobic productivity with the body composition of females of different somatotypes from the lowland districts of Transcarpathia.

MATERIALS AND METHODS

A correlation analysis of the level of physical health was conducted in 118 females in the postpubertal period of ontogenesis aged 16 to 20 years, residents of the lowland districts of the Transcarpathian region.

The level of physical health was assessed by indicators of the aerobic productivity of the body, namely, the maximum oxygen consumption ($VO2_{max\ rel}$.) was determined by the method of bicycle ergometry. To assess the level of aerobic productivity, the criteria of the University of Tartu Jürimäe J. [1], Nurmekivi A. [2] were used, which allow to assess the level of aerobic productivity of individuals from 10 years of age. This assessment method has become widely used, in particular, in the study of Ukrainian scientists Yu. Furman [8], V. Miroshnichenko [4].

The anaerobic productivity of the subjects was studied by the power of anaerobic alactic processes of energy supply of muscular activity, which was determined by the indicator of the maximum quantity of external work performed in 10 s. The power of anaerobic lactic processes of energy supply of muscular activity was also determined by the indicator of the maximum quantity of external work performed in 30 s. For this purpose, the 10-second and 30-second Wingate anaerobic tests $WAnT_{10}$ and $WAnT_{30}$, described by Yu.M. Furman and co-authors [15], were used. To assess the capacity of anaerobic lactic productivity, the value of the maximum quantity of external work for 1 minute (MQEW) was determined using the method of A. Shogy, G. Cherebetin [16].

All cycle ergometry tests were performed on a Christopeit Sport AX-1 cycle ergometer.

The somatotype was determined by the Heath-Carter method. This method allows for an anthropometric assessment by the relative fatness – endomorphy, the skeletal-muscular rubustness – mesomorphy, and the relative linearity of the body – ectomorphy [17].

Body composition was determined by the bioelectrical impedance method using the Body Composition Monitor "Omron BF511". This method allows to estimate the percentage of fat mass (subcutaneous and visceral fat) and the percentage of skeletal muscle [18].

Statistical processing of the obtained experimental data was carried out using Excel 7.0 and SPSS version 10.0. Correlation analysis was carried out using the Pearson correlation coefficient. The degree of correlation was assessed by the Chaddock criterion: very strong – $0,90 \le r_{xy} \ge 0,99$; strong – $0,7 \le r_{xy} < 0,9$; noticeable – $0,5 \le r_{xy} < 0,7$; moderate – $0,3 \le r_{xy} < 0,5$; weak – $0,1 \le r_{xy} < 0,3$. A relationship was considered significant at $p < 0.05$.

ETHICS

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

RESULTS

The study of the influence of the body composition, body mass index, on the aerobic performance of females from the lowland districts of Transcarpathia demonstrated that the fat component does not affect the absolute indicator of $VO_{2\,max}$. as there is no significant correlation. A slight positive influence of the fat component was found on the relative indicator of $VO_{2\,max}$, as indicated by a weak direct correlation (table 1). A similar trend was found when conducting a correlation analysis between the level of visceral fat and $VO_{2\,max}$. There is no correlation with the absolute indicator of $VO_{2\,max}$, while there is a weak direct correlation with the relative indicator.

The percentage of muscle in the body correlates only with the relative $VO_{2\,max}$. In this case, the correlation is characterized as direct moderate. Thus, the muscle component has a positive effect on the aerobic performance of females from lowland districts, but it has a moderate effect.

Body mass index has a direct moderate correlation with the absolute $VO_{2\,max}$ and an inverse moderate correlation with the relative $VO_{2\,max}$. Since the aerobic performance of the body is usually assessed by the relative $VO_{2\,max}$, it can be argued that higher BMI values determine to some extent the lower level of aerobic performance of females from lowland districts.

The results of the study of correlations of the body composition, BMI with indicators of aerobic productivity of females of different somatotypes of lowland districts are given in Table 2. The correlation of the fat percentage with the absolute indicator of $VO_{2\,max}$ in representatives of all somatotypes is insignificant ($p > 0.05$). A noticeable direct correlation was found with the relative indicator of $VO_{2\,max}$ in females of the endomorphic somatotype, and a

Table 1. The correlation between aerobic performance indicators and body composition in females from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
$V_{O2\ ma}x$	0.142	p > 0.05	0.042	p > 0.05	0.096	p > 0.05	0.339	p < .001
$VO_{2\ max.rel.}$	0.279	p < 0.01	0.361	p < 0.001	0.277	p < 0.01	-0.419	p < .001

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

Table 2. The correlation between aerobic performance indicators and body composition in females of different somatotypes from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
ectomorphic somatotype (n = 12)								
$VO_{2\ max}$	0.279	p > 0.05	0.235	p > 0.05	0.189	p > 0.05	0.357	p > 0.05
$VO_{2max\ rel.}$	-0.155	p >0.05	0.278	p > 0.05	-0.297	p > 0.05	-0.411	p > 0.05
endomorphic somatotype (n = 16)								
$VO_{2\ max}$	0.187	p > 0.05	0.019	p > 0.05	0.136	p > 0.05	0.225	p > 0.05
$VO_{2\ max\ rel.}$	0.538	p < 0.05	0.497	p < 0.05	0.343	p > 0.05	-0.737	p < 0.01
endomesomorphic somatotype (n = 38)								
$VO_{2\ max}$	0.198	p > 0.05	0.158	p > 0.05	-0.068	p > 0.05	0.337	p < 0.05
$VO_{2\ max\ rel.}$	0.486	p < 0.01	0.335	p < 0.05	0.319	p > 0.05	-0.672	p<0.001
balanced somatotype (n = 41)								
$VO_{2\ max}$	0.124	p > 0.05	-0.021	p > 0.05	0.092	p > 0.05	0.575	p< 0.001
$VO_{2\ max\ rel.}$	0.393	p< 0.05	-0.161	p > 0.05	0.236	p > 0.05	-0.498	p < 0.01
mesoectomorphic somatotype (n = 11)								
$VO_{2\ max}$	0.030	p > 0.05	-0.162	p > 0.05	0.378	p > 0.05	0.537	p > 0.05
$VO_{2\ max\ rel.}$	-0.296	p > 0.05	0.105	p > 0.05	-0.446	p > 0.05	-0.593	p > 0.05

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

moderate direct correlation in females of the endomesomorphic and balanced somatotypes. In representatives of other somatotypes, there is no correlation with the relative indicator of $VO_{2\ max}$. The level of visceral fat does not correlate with the absolute and relative indicators of $VO_{2\ max}$ in representatives of all somatotypes (Table 2). The muscle mass percentage does not correlate with the absolute indicator of $VO_{2\ max}$ in representatives of all somatotypes. A noticeable direct correlation between the muscle percentage and the relative $VO_{2\ max}$ index was found in representatives of the endomorphic somatotype, and a moderate direct correlation was found in representatives of the endomesomorphic somatotype. Thus, a positive effect of the muscle component on aerobic performance in females of the endomorphic and endomesomorphic somatotypes was established (Table 2).

Correlation analysis between BMI and absolute $VO_{2\ max}$ revealed a noticeable direct correlation in females of balanced somatotype and a moderate direct correlation in females of endomesomorphic somatotype. Research on the relationship between BMI and aerobic performance indicators revealed a strong inverse correlation with relative $VO_{2\ max}$ in females of endomorphic somatotype, a noticeable inverse correlation in females of endomesomorphic and mesoectomorphic somatotypes, and a moderate inverse correlation in females of balanced somatotype (Table 2).

The results of the study of the influence of the body composition, BMI on the anaerobic performance of females of lowland districts are shown in table 3.

The correlation of visceral fat content with absolute indicators of anaerobic productivity is insignificant (p > 0.05). The correlation of visceral fat content with relative indicators of anaerobic productivity of the body is characterized as a weak inverse correlation. Therefore, visceral fat does not have a significant effect on the anaerobic productivity of the subjects (Table 3).

The percentage of fat content has a significant inverse correlation only with relative indicators of anaerobic

Table 3. The correlation between anaerobic productivity indicators and body composition in females from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
MQEW	-0.028	p > 0.05	0.131	p > 0.05	0.157	p > 0.05	0.160	p > 0.05
$MQEW_{rel.}$	-0.280	p < 0.01	-0.124	p > 0.05	-0.203	p < 0.05	-0.144	p > 0.05
$WAnT_{30}$	0.140	p > 0.05	0.116	p > 0.05	0.046	p > 0.05	0.382	p < 0.001
$WAnT_{30\ rel.}$	-0.201	p < 0.05	-0.074	p > 0.05	-0.223	p < 0.05	0.272	p < 0.01
$WAnT_{10}$	0.161	p > 0.05	0.120	p > 0.05	0.151	p > 0.05	0.348	p < 0.001
$WAnT_{10\ rel.}$	-0.292	p < 0.01	-0.016	p > 0.05	-0.198	p < 0.05	0.193	p > 0.05

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

Table 4. The correlation between anaerobic alactic power indicators and body composition in females of different somatotypes from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
ectomorphic somatotype (n = 12)								
$WAn_{T1}0$	-0.203	p >0.05	0.163	p>0.05	-0.300	p >0.05	0.789	p < 0.01
$WAnT_{10\ rel}$	-0.310	p >0.05	0.202	p>0.05	-0.297	p >0.05	0.373	p > 0.05
endomorphic somatotype (n = 16)								
$WAnT_{10}$	-0.280	p >0.05	0.367	p>0.05	0.112	p >0.05	0.498	p < 0.05
$WAnT_{10\ rel}$	-0.399	p >0.05	-0.055	p>0.05	-0.407	p > 0.05	0.175	p > 0.05
endomesomorphic somatotype (n = 38)								
$WAnT_{10}$	-0.067	p> 0.05	0.209	p>0.05	-0.125	p > 0.05	0.226	p > 0.05
$WAnT_{10\ rel}$	-0.341	p< 0.05	0.008	p>0.05	-0.329	p < 0.05	0.046	p > 0.05
balanced somatotype (n = 41)								
$WAnT_{10}$	0.059	p >0.05	0.280	p>0.05	0.074	p > 0.05	0.787	p < 0.001
$WAnT_{10\ rel}$	-0.369	p <0.05	0.204	p>0.05	-0.333	p < 0.05	-0.481	p > 0.05
mesoectomorphic somatotype (n = 11)								
$WAnT_{10}$	-0.255	p >0.05	0.204	p>0.05	-0.300	p >0.05	0.460	p>0.05
$WAnT_{10\ rel}$	-0.336	p >0.05	0.157	p>0.05	-0.287	p>0.05	0.220	p>0.05

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

productivity. At the same time, the correlation with the indicators of $MQEW_{rel.}$, $WAnT_{30\ rel.}$ and $WAnT_{10\ rel.}$ is characterized as weak, which indicates a slight negative impact of the fat component on the anaerobic productivity of females living in the lowland districts of Transcarpathia (Table 3).

The percentage of muscle content does not have any significant correlation with any of the indicators of anaerobic productivity (Table 3).

Body mass index has a moderate direct correlation with absolute indicators of anaerobic alactic and lactic productivity ($WAnT_{10}$ and $WAnT_{30}$) and a weak direct correlation with the relative $WAnT_{30}$ indicator (Table 3).

Correlation analysis of the body composition, BMI with indicators of anaerobic alactic productivity of females from lowland districts of different somatotypes revealed the absence of correlation between the percentage of body fat and the absolute indicator of $WAnT_{10}$ in representatives of all somatotypes (p > 0.05) (Table 4). The correlation of fat percentage with the relative indicator of $WAnT_{10}$ in females of endomesomorphic and balanced somatotypes is characterized as a moderate inverse correlation. There is no correlation in representatives of other somatotypes.

The correlation of visceral fat content with anaerobic alactic power has similar tendency. Thus, the correlation of visceral fat content with the absolute indicator of $WAnT_{10}$ is insignificant; the correlation with the relative indicator of $WAnT_{10}$ in representatives of endomesomorphic and balanced somatotypes is characterized as moderate inverse, and in representatives of other somatotypes as insignificant (Table 4).

Table 5. The correlation between anaerobic lactic power indicators and body composition in females of different somatotypes from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
ectomorphic somatotype (n = 12)								
$WAn_{T3}0$	0.107	p > 0.05	0.201	p > 0.05	0.135	p > 0.05	0.792	p < 0.01
$WAnT_{30\,rel}$	-0.239	p > 0.05	0.384	p > 0.05	-0.207	p > 0.05	0.118	p > 0.05
endomorphic somatotype (n = 16)								
$WAnT_{30}$	0.142	p > 0.05	0.036	p > 0.05	-0.179	p > 0.05	0.220	p > 0.05
$WAnT_{30\,rel}$	-0.322	p > 0.05	0.456	p > 0.05	-0.290	p > 0.05	0.025	p > 0.05
endomesomorphic somatotype (n = 38)								
$WAnT_{30}$	0.125	p > 0.05	0.075	p > 0.05	0.115	p > 0.05	0.026	p > 0.05
$WAnT_{30\,rel}$	-0.338	p < 0.05	0.228	p > 0.05	-0.241	p > 0.05	0.277	p >0.05
balanced somatotype (n = 41)								
$WAnT_{30}$	0.267	p > 0.05	0.285	p > 0.05	0.062	p > 0.05	0.722	p < 0.001
$WAnT_{30\,rel}$	-0.258	p > 0.05	0.276	p > 0.05	-0.177	p > 0.05	-0.015	p > 0.05
mesoectomorphic somatotype (n = 11)								
$WAnT_{30}$	0.078	p > 0.05	0.233	p > 0.05	-0.111	p > 0.05	0.624	p < 0.05
$WAnT_{30\,rel}$	-0.248	p > 0.05	0.115	p > 0.05	-0.164	p > 0.05	-0.100	p > 0.05

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

Analysis of the correlation between the percentage of muscle component with anaerobic alactic productivity of females of different somatotypes did not reveal a significant correlation with either absolute or relative indicators of $WAnT_{10}$ (Table 4).

A strong direct correlation was established between BMI and the absolute indicator of $WAnT_{10}$ in representatives of ectomorphic and balanced somatotypes; a moderate direct correlation was established in representatives of the endomorphic somatotype; however, no correlation was established in representatives of other somatotypes. There is no correlation between BMI and the relative index of $WAnT_{10}$ in representatives of all somatotypes (p > 0.05) (Table 4).

The results of the correlation analysis between body composition, body mass index and absolute and relative indicators of anaerobic lactic power of females of different somatotypes living in the lowland districts of Transcarpathia are shown in Table 5.

The obtained data indicate that the percentage of body fat does not correlate with the absolute indicator of $WAnT_{30}$ in representatives of all somatotypes (p > 0.05). Analysis of the correlation of fat percentage with the relative indicator of $WAnT_{30}$ revealed a moderate inverse correlation in representatives of the endomesomorphic somatotype, and an insignificant correlation in representatives of other somatotypes (Table 5).

The level of visceral fat does not correlate with either absolute or relative indicators of $WAnT_{30}$ in females of all somatotypes (Table 5).

The muscle percentage in the body does not affect the anaerobic lactic power of females of different somatotypes from lowland districts, as indicated by the absence of a significant correlation with absolute and relative indicators of $WAnT_{30}$ in representatives of all somatotypes (Table 5).

Body mass index has a strong direct correlation with the absolute $WAnT_{30}$ index in females of ectomorphic and balanced somatotypes, and a noticeable direct correlation in females of mesoectomorphic somatotype. The correlation of BMI with the relative $WAnT_{30}$ index is insignificant in representatives of all somatotypes (Table 5).

The results of the study of the relationship between body composition, BMI and the capacity of anaerobic lactic productivity of females of different somatotypes from lowland districts are presented in Table 6.

The percentage of body fat does not have a significant effect on the level of the absolute index of MQEW in representatives of all somatotypes. A moderate inverse correlation was found between the fat component and the relative index of MQEW in females of the endomesomorphic and balanced somatotypes. No correlation between the percentage of body fat and the relative index of MQEW was found in representatives of other somatotypes (p > 0.05) (Table 6).

The percntage of visceral fat does not affect anaerobic lactic capacity, as indicated by the absence of a

Table 6. The correlation between anaerobic lactic capacity indicators and body composition in females of different somatotypes from lowland districts of Transcarpathia (n = 118)

Indicators	Fat component		Muscle component		Visceral fat		Body mass index	
	r	p	r	p	r	p	r	P
ectomorphic somatotype (n = 12)								
MQEW	-0.237	p > 0.05	0.324	p > 0.05	0.173	p > 0.05	-0.353	p > 0.05
$MQEW_{rel.}$	-0.206	p > 0.05	0.291	p > 0.05	-0.138	p > 0.05	0.319	p > 0.05
endomorphic somatotype (n = 16)								
MQEW	0.154	p > 0.05	0.084	p > 0.05	-0.167	p > 0.05	0.546	p < 0.05
$MQEW_{rel.}$	-0.239	p > 0.05	0.276	p > 0.05	-0.063	p > 0.05	0.113	p > 0.05
endomesomorphic somatotype (n = 38)								
MQEW	-0.283	p > 0.05	0.139	p > 0.05	-0.156	p > 0.05	0.449	p < 0.01
$MQEW_{rel.}$	-0.331	p < 0.05	0.007	p > 0.05	-0.141	p > 0.05	-0.111	p > 0.05
balanced somatotype (n = 41)								
MQEW	0.136	p > 0.05	0.300	p > 005	0.035	p > 0.05	0.523	p < 0.001
$MQEW_{rel.}$	-0.349	p < 0.05	0.017	p > 0.05	-0.261	p > 0.05	0.108	p > 0.05
mesoectomorphic somatotype (n = 11)								
MQEW	-0.117	p > 0.05	0.371	p > 0.05	-0.394	p > 0.05	0.277	p > 0.05
$MQEW_{rel.}$	-0.301	p > 0.05	0.223	p > 0.05	-0.115	p > 0.05	0.161	p > 0.05

Note: r is the correlation coefficient; p is the significance level
Source: compiled by the authors of this study

significant correlation with both absolute and relative indicators of MQEW in representatives of all somatotypes (Table 6).

The percentage of muscle in the body also has no significant correlation with the absolute and relative indicators of MQEW in representatives of all somatotypes (p > 0.05) (Table 6).

BMI has a noticeable direct correlation with the absolute indicator of MQEW in females of endomorphic and balanced somatotypes and a moderate direct correlation in females of endomesomorphic somatotype. The correlation of BMI with the relative indicator of MQEW is insignificant (p > 0.05) in representatives of all somatotypes.

DISCUSSION

Thus, the study has found that in females of different somatotypes living in the lowland districts of Transcarpathia, body composition affects the aerobic and anaerobic capabilities of the body in different ways. Such data are consistent with studies conducted among females in the first period of adulthood, which also established significant differences in representatives of different somatotypes, both in terms of aerobic [3] and anaerobic [10] productivity, as well as with studies conducted among females aged 17-19, residents of the Podillia region of Ukraine [4, 7].

Analysis of the correlation of body composition with aerobic performance in females of different somatotypes from the lowland districts of Transcarpathia revealed the strongest correlation with body mass index. At the same time, inverse correlation was established with the relative $VO_{2\ max}$ indicator, and direct correlation with the absolute indicator. The degree of such correlation in representatives of different somatotypes is different (strong in representatives of the endomorphic somatotype, noticeable in representatives of the endomesomorphic somatotype, and moderate or the correlation is insignificant in representatives of other somatotypes). Such data are consistent with the findings of V. Miroshnichenko et al. [3], who revealed similar tendencies in females in the first period of adulthood. It should be noted that in the scientific literature, information on the correlation of the body composition with aerobic performance in individuals of different somatotypes is available only concerning certain age and gender categories. Thus, S. Hasmyati at al. [19] claim that BMI is a reliable predictor of aerobic capacity in females aged 20-32 years, provided that it is within the values that correspond to the norm. However, the opinion of J.R. Alkandari, B. Nieto [20] about the negative impact of the fat component on the ability to demonstrate aerobic capacity was not confirmed by our study. It should be noted that the above authors studied Kuwaiti women whose region differs significantly from Ukraine

in geographical, climatic and social characteristics. Therefore, these factors may account for the differences.

The relationship between body composition and anaerobic performance indicators in females of different somatotypes from the lowland districts of Transcarpathia is characterized by a strong direct correlation of BMI with absolute indicators of $WAnT_{10}$ and $WAnT_{30}$ in representatives of ectomorphic and balanced somatotypes. The correlation of fat and muscle components with indicators of $WAnT_{10}$ and $WAnT_{30}$ indicates the absence of their significant influence on the anaerobic capabilities of the females. Such data are consistent with the findings of V. Miroshnichenko et al. [21], which confirm a strong correlation between BMI and indicators of $WAnT_{10}$ and $WAnT_{30}$ in females aged 25-35 of certain somatotypes, and the absence of a significant influence of fat and muscle components on anaerobic performance in representatives of all somatotypes. N. Kucukkubas et al. [22] found that there was no correlation between body fat percentage and anaerobic performance test results in female athletes specializing in Zumba, cross-country running, basketball, football, tennis and volleyball. However, some studies do not align with our data. For example, M. Kale, E. Akdoğan [23] believe that endomorphism (relative fatness) has a negative effect on anaerobic performance of handball players. Pei Yang et al. [24] obtained somewhat contradictory results, indicating a strong direct correlation between visceral fat content and hand strength and an inverse correlation with body fat content. Such correlation can be conditionally extrapolated to the anaerobic alactic performance, since strength is determined precisely by the development of the anaerobic alactic energy supply system for muscle activity. Therefore, the data on the influence of fat component on the power of anaerobic energy supply processes are contradictory.

There is also no consensus on the influence of the muscle component on the anaerobic capabilities of females. Our studies prove the absence of a significant correlation between the percentage of muscle and anaerobic performance according to the $WAnT_{10}$, $WAnT_{30}$ tests, and the MQEW. This is indicated by the correlation that does not exceed a moderate level, or its insignificance. To some extent, these results are consistent with the data obtained by M. Kale, E. Akdoğan [23], who established the absence of a significant correlation between fat-free body mass and performance in anaerobic tests. No correlation was found between the percentage of muscle in the body and performance in anaerobic tests $WAnT_{10}$, $WAnT_{30}$, and MQEW in females aged 25-35 who did not do sports [21]. On the other hand, J. Zera et al. [25] proves that muscle percentage in males and females can be a predictor of higher levels of anaerobic performance, determined by the $WAnT_{30}$ test.

CONCLUSIONS

Fat and muscle components, as well as visceral fat, do not have a significant impact on the ability to demonstrate aerobic and anaerobic capabilities of the body in females from the lowland districts of Transcarpathia, regardless of somatotype, and in representatives of all studied somatotypes. The greatest impact on the aerobic and anaerobic capabilities is exerted by body mass index. In representatives of the endomorphic somatotype, higher values of body mass index determine lower values of the relative $VO_{2\,max}$ indicator. In representatives of the ectomorphic and balanced somatotypes, higher values of body mass index determine higher values of the absolute indicators $WAnT_{10}$ and $WAnT_{30}$. Data from the scientific literature on the impact of fat and muscle components on the anaerobic capabilities of the body are contradictory and require clarification.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Efficacy and safety of apixaban versus warfarin in LVAD patients: A propensity-matched analysis

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ABSTRACT

Aim: The primary endpoint was the incidence of major HRAEs. Secondary endpoints included LVAD thrombosis, stroke, major bleeding, non–central nervous system thromboembolism, and all-cause mortality.

Materials and Methods: We conducted a multicenter retrospective cohort study using the TriNetX database, identifying LVAD patients receiving either warfarin or apixaban. Two cohorts were established based on anticoagulation regimen, and propensity score matching was employed to minimize baseline differences.

Results: After matching, 3,129 patients were included in each group. HRAEs occurred less frequently in the apixaban cohort (19.7%) than in the warfarin cohort (28.4%) (OR: 0.618; 95% CI: 0.513–0.744; $P < 0.001$). Apixaban was also associated with significantly fewer LVAD thrombosis events (OR: 0.088), strokes (OR: 0.721), and major bleeding events (OR: 0.528). Rates of non-CNS thromboembolism and all-cause mortality were similar between groups.

Conclusions: Although warfarin remains the standard anticoagulant for LVAD recipients, apixaban demonstrated lower rates of major adverse events in this large retrospective analysis. Further prospective and randomized studies are warranted to confirm these findings and inform future clinical practice.

KEY WORDS: left ventricular assist device (LVAD), heart failure with reduced ejection fraction (HFrEF), anticoagulation, apixaban, warfarin

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INTRODUCTION

Heart failure with reduced ejection fraction (HFrEF) is a prevalent disease, affecting approximately 1–1.5% of the general population and up to 5% of individuals over 65 years of age [1, 2]. The main focus of heart failure treatment is to support the native heart function through medications and devices. However, some end-stage cases require a heart transplant as the native heart function with all available options can not support the survival of the patient anymore [1, 2].

However, donor hearts are limited, and the waiting time can be too long for patients who have reached the terminal stage of heart failure. This challenge led to the development of left ventricular assist devices (LVADs), which were used as a bridge to transplantation at first. [3, 4] LVAD technology has come a long way since its first introduction in the 1960s [4] with the current use of third-generation devices [5]. LVADs today are not only used as a bridge to transplant but also as destination therapy and, in certain patients, as a bridge to myocardial recovery [3, 4].

At the time of introduction of the initial LVADs, there was only one class of oral anticoagulant, which was vitamin K antagonists (VKAs) with warfarin being the most frequently used agent in the class [6]. Warfarin became the standard of treatment for patients with LVADs, due to the absence of other choices and its well-established efficacy [7]. With the introduction of direct oral anticoagulants (DOACs) in 2010, these medications increasingly replaced warfarin in other indications, such as atrial fibrillation and venous thromboembolism [8, 9].

Encouraged by the success of DOACs for other indications, researchers began investigating DOACs as a potential effective anticoagulant in LVAD patients [10, 11]. Studying the effectiveness of anticoagulant therapies is challenging in this group because of the relatively low number of LVAD patients and the complexity of their medical care. Warfarin is the current standard of care in long-

term LVAD patients, and DOACs in this setting are used off-label or experimental [5]. The objective of the current research is to evaluate the efficacy and safety of apixaban as an alternative to warfarin in patients with LVADs.

AIM

This study compared the outcomes of warfarin versus apixaban in patients with LVADs, aiming to address a gap in the existing literature. The primary endpoint was the occurrence of major hemocompatibility-related adverse events (HRAEs). Secondary endpoints included individual elements of the primary endpoint: LVAD thrombosis, stroke, major bleeding, non-central nervous system thromboembolism, and all-cause mortality.

MATERIALS AND METHODS

DATA SOURCE

This study was conducted using the TriNetX research network, a federated database providing access to electronic health records (EHRs) from 154 healthcare organizations (HCOs). 153 healthcare organizations responded with patients. The TriNetX platform aggregates deidentified patient data, ensuring compliance with HIPAA deidentification standards.

PATIENT POPULATION

We conducted a retrospective observational cohort study of adult patients (≥18 years old) with LVADs, stratified by the anticoagulation agent they were given, either apixaban or warfarin. Cohort 1 (apixaban group) included patients with LVADs who were started on apixaban, and Cohort 2 (warfarin group) included patients with LVADs who were started on warfarin. Patients with LVADs were identified using ICD-10 codes, and anticoagulation drugs were confirmed using RxNorm codes for apixaban and warfarin prescriptions. Additional details regarding cohort definition and study window definitions, including the relevant ICD-10, RxNorm, and Current Procedural Terminology codes, are available in the Supplemental Appendix.

STUDY ENDPOINTS

The index event was defined as the first recorded administration of either apixaban or warfarin for patients with LVAD, identified using medication codes RxNorm 1364430 (apixaban) and RxNorm 11289 (warfarin). LVAD was identified based on ICD-10 codes Z95.811. Patients were assigned to cohorts based on the initial anticoagulation agent; those who received apixaban without warfarin were placed in the apixaban cohort, while those who received warfarin without apixaban were placed in the warfarin cohort. The index date, defined as the time of initial anticoagulation agent administration, marked the beginning of the observation window for evaluating outcomes.

The primary outcome of interest was the incidence of major hemocompatibility-related adverse events (HRAEs) after anticoagulation agent initiation. Secondary outcomes included individual elements of the primary outcome: LVAD thrombosis, stroke, major bleeding, non central nervous system thromboembolism, and all cause mortality. The Supplemental Appendix elaborates on outcome definitions and ICD-10 codes.

STATISTICAL ANALYSIS

Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are presented as number (percentage), as appropriate. Baseline characteristics were compared between the apixaban and warfarin groups using independent samples Student's t-tests for continuous variables and chi-square tests for categorical variables. To mitigate baseline differences between cohorts, 1:1 propensity score matching was performed using greedy nearest neighbor matching with a caliper of 0.1 times the pooled SD of the linear propensity scores. Variables included in the matching process were age, sex, race, , comorbidities (hypertension, hyperlipidemia, ischemic heart diseases, cerebrovascular diseases, atrial fibrillation and flutter, cardiomyopathy, diabetes mellitus, overweight and obesity, disorders of the thyroid gland, chronic obstructive pulmonary disease (COPD), asthma, chronic kidney disease (CKD), liver diseases, and nicotine dependence), medication use (aspirin, beta blockers and related agents, diuretics, antilipemic agents, and antiarrhythmics) and laboratory result(cholesterol, hemoglobin A1c, iron, ferritin, blood urea nitrogen, and creatinine). The standardized mean difference represents the difference between the means of two groups in terms of SD units, and is used to assess balance in measured variables in the sample weighted by the inverse probability of treatment. Variables were selected based on their potential effect on overall and HRAEs outcomes.

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

Table 1. Baseline characteristics of patients in apixaban and warfarin groups before and after propensity score matching (PSM)

	Before PSM			After PSM		
	Before Matching (Apixiban group, n=7,219)	Before Matching (Warfarin group, n=4,730)	Standardized Difference	After Matching (Apixiban group, n=3,129)	After Matching (Warfarin group, n=3,129)	Standardized Difference
Demographics						
Current Age (Mean ± SD)	70.5± 13.9	62.5± 15.1	0.551	65.4± 15.3	65.5 ± 13.9	0.003
Age at Index (Mean ± SD)	68.5 ± 14.0	59.4± 15.2	0.622	62.8 ±15.3	62.9 ±13.7	0.004
Female (%)	2,539 (35.2%)	1,279 (27.0%)	0.176	949 (30.3%)	921 (29.4%)	0.020
Male (%)	4,310 (59.7%)	3,277 (69.3%)	0.201	2,040 (65.2%)	2,059 (65.8%)	0.013
White (%)	5,012 (69.4%)	2,759 (58.3%)	0.233	1,953 (62.4%)	1,941 (62.0%)	0.008
Black or African American(%)	1,244 (17.2%	1,256 (26.6%)	0.227	731 (23.4%)	729 (23.3%)	0.002
Comorbid conditions						
Hypertension	6,381 (88.4%)	3,756 (79.4%)	0.246	2,589 (82.7%)	2,579 (82.4%)	0.008
Dyslipidemia	5,700 (79.0%)	3,079 (65.1%)	0.313	2,212 (70.7%)	2,214 (70.8%)	0.001
Cardiomyopathy	3,290 (45.6%)	3,234 (68.4%)	0.473	1,871 (59.8%)	1,880 (60.1%)	0.006
Ischemic heart diseases	5,553 (76.9%)	3,311 (70%)	0.157	2,317 (74.0%)	2,317 (74.0%)	<0.001
Atrial fibrillation and flutter	5,627 (77.9%)	2,790 (59.0%)	0.417	2,062 (65.9%)	2,052 (65.6%)	0.007
Diabetes mellitus	3,421 (47.4%)	2,240 (47.4%)	0.001	1,510 (48.3%)	1,513 (48.4%)	0.002
chronic obstructive pulmonary disease	1,774 (24.6%)	977 (20.7%)	0.094	672 (21.5%)	702 (22.4%)	0.023
Asthma	1,167 (16.2%)	614 (13.0%)	0.090	437 (14.0%)	430 (13.7%)	0.006
Cerebrovascular diseases	2,767 (38.3%)	1,823 (38.5%)	0.004	1,199 (38.3%)	1,170 (37.4%)	0.019
Chronic kidney disease	3,415 (47.3%)	2,413 (51.0%)	0.074	1,533 (49.0%)	1,547 (49.4%)	0.009
Diseases of liver	2,145 (29.7%)	1,554 (32.9%)	0.068	1,053 (33.7%)	1,027 (32.8%)	0.018
Nicotine dependence	1,633 (22.6%)	1,080 (22.8%)	0.005	752 (24.0%)	772 (24.7%)	0.015
Overweight and obesity	3,276 (45.4%)	1,980 (41.9%)	0.071	1,349 (43.1%)	1,342 (42.9%)	0.005
Disorders of thyroid gland	2,131 (29.5%)	1,260 (26.6%)	0.064	857 (27.4%)	813 (26.0%)	0.032
Medication use						
Aspirin	5,641 (78.1%)	3,684 (77.9%)	0.006	2,392 (76.4%)	2,399 (76.7%)	0.005
Beta-blockers	6,219 (86.1%)	3,759 (79.5%)	0.178	2,554 (81.6%)	2,555 (81.7%)	0.001
Diuretics	5,980 (82.8%)	4,130 (87.3%)	0.126	2,712 (86.7%)	2,704 (86.4%)	0.007
Antilipemic agents	5,717 (79.2%)	3,254 (68.8%)	0.239	2,273 (72.6%)	2,315 (74.0%)	0.030
Antiarrhythmics	6,463 (89.5%)	4,013 (84.8%)	0.141	2,708 (86.5%)	2,699 (86.3%)	0.008
Laboratory						
Total cholesterol (md/dL)	145.4 ± 44.7	140.7 ± 45.8	0.104	142.7 ± 45.5	140.9 ± 46.4	0.039
Hemoglobin A1c (%)	6.3 ± 1.5	6.3 ± 1.5	0.005	6.4 ± 1.6	6.3 ± 1.5	0.019
Iron (mcg/dL)	58.6 ±43.9	58.2 ± 43.4	0.010	58.2 ± 44.1	58.7 ± 41.6	0.011
Ferritin (ng/mL)	476.9 ± 2084.2	345.1 ± 713.8	0.085	552.0 ± 2535.0	372.1 ±747.2	0.096
Blood urea nitrogen (mg/dL)	25.8 ± 16.1	25.3 ±15.2	0.032	26.8 ± 17.1	25.8 ±15.5	0.063
Creatinine (mg/dL)	1.5 ±3.0	1.4 ± 2.9	0.017	1.6 ±2.7	1.4 ±1.2	0.079

Source: Compiled by the authors of this study

analyses were conducted using integrated R (The R Foundation) within the TriNetX platform.

ETHICS APPROVAL

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

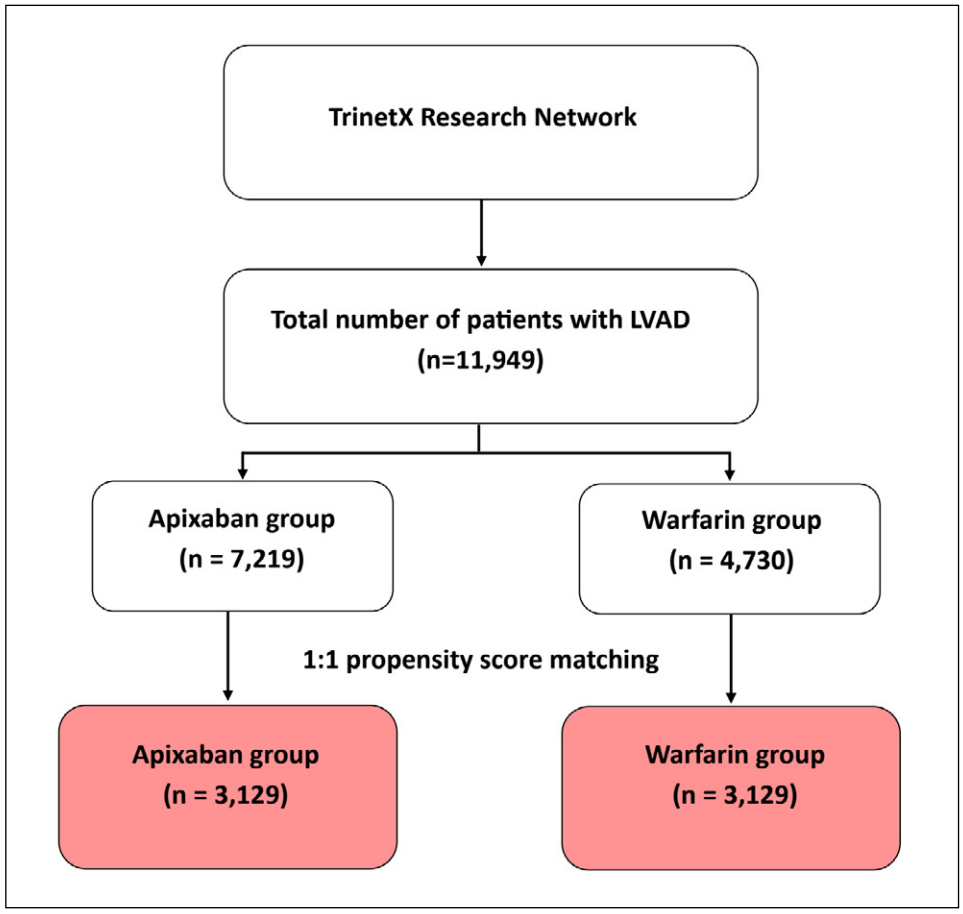


Fig. 1. Flow diagram of patient selection and cohort derivation following propensity score matching.
Source: Own materials

determination as defined in Section §164.514(b)(1) of the HIPAA Privacy Rule.

RESULTS

STUDY POPULATION

This retrospective cohort study identified a total of 11,949 patients with LVAD who were receiving apixaban or warfarin. Among them, 7,219 patients received apixaban , and 4,730 patients received warfarin. After applying 1:1 propensity score matching (PSM) to balance baseline characteristics, 3,129 patients were included in each cohort (apixaban and warfarin groups) for the final analysis (Fig. 1).

PATIENT CHARACTERISTICS

The baseline characteristics of the study cohorts, before and after propensity score matching (PSM), are shown in Table 1. In the unmatched cohort, patients receiving apixaban were slightly older at index (mean age:68.5 ± 14.0 years *vs.* 59.4 ± 15.2 years, $P < 0.001$) compared to those receiving warfarin. The proportion of male patients was slightly lower in the apixaban group (59.7%% vs. 69.3%, $P < 0.001$), while the apixaban group had a higher proportion of female patients (35.2% *vs.* 27.0%, $P < 0.001$). Regarding racial distribution, the apixaban group was more likely to be white (69.4% *vs.* 58.3%) compared to the warfarin group, while black or affrican american more in warfarin group comparing to apixaban group (26.6% *vs.* 17.2%, $P < 0.001$).

Before matching, the apixaban group exhibited a higher prevalence of hypertension (88.4% *vs.* 79.4%, $P < 0.001$), ischemic heart disease (76.9% *vs.* 70.0%, $P < 0.001$), atrial fibrillation and flutter (77.9% *vs.*59.0%, $P < 0.001$), overweight and obesity (45.4% *vs.* 41.9%, $P < 0.001$), thyroid gland disease (29.5% *vs.* 26.6%, $P < 0.001$), chronic obstructive pulmonary disease (24.6% *vs.* 20.7%, $P < 0.001$), asthma (16.2% *vs.* 13.0%, $P < 0.001$), hyperlipidemia (79.0% *vs.* 65.1%, $P < 0.001$), and conversely, cardiomyopathy (68.4% *vs.* 45.6%, $P < 0.001$), chronic kidney disease (51.0% *vs.* 47.3%, $P < 0.001$), diseases of liver (32.9% *vs.* 29.7%, $P < 0.001$) were more frequently observed in the warfarin group while cerebrovascular diseases, diabetes mellitus and nicotine dependence were almost the same in the two groups.

After propensity score matching (PSM), the two cohorts were well balanced across key baseline characteristics, including age, sex, race, medications, laboratory results, and comorbidities, with standardized mean differences (SMDs) < 0.1 for most variables, indicating

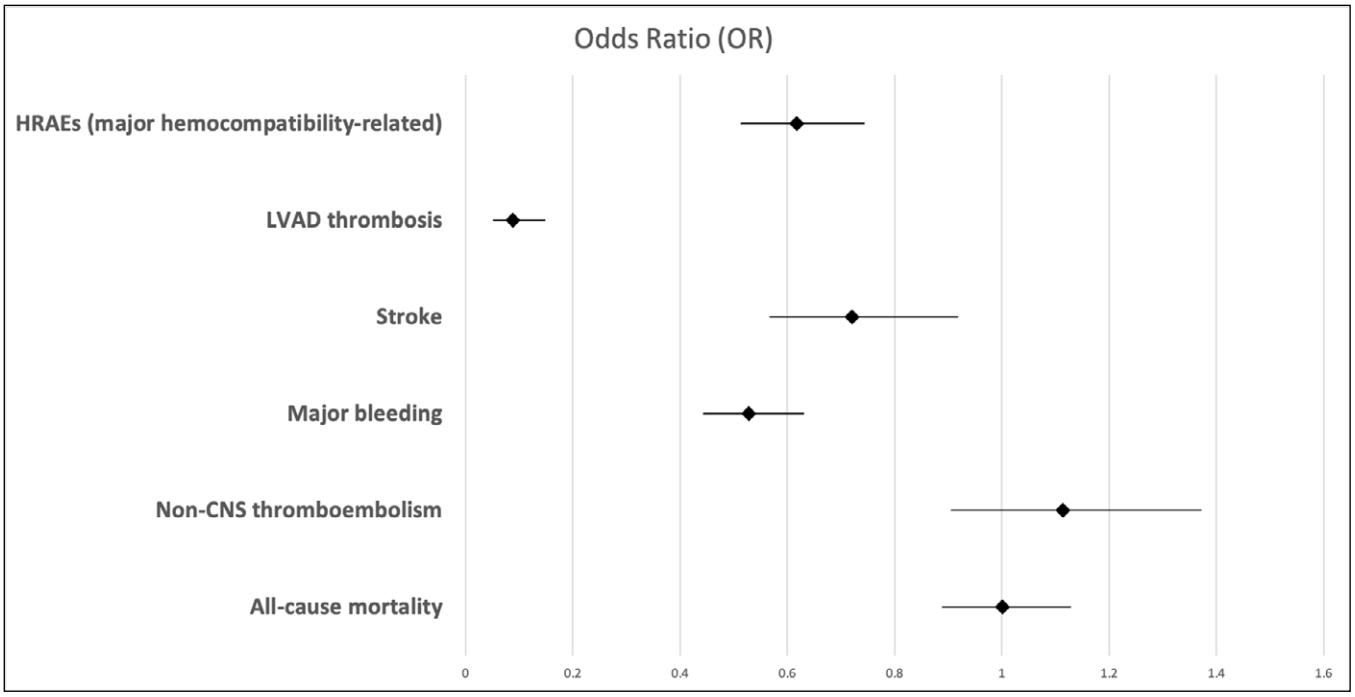


Fig. 2. Forest plot of primary and secondary clinical outcomes: apixaban *vs.* warfarin in lvad patients
Source: Own materials

a well-matched cohort. The final matched cohort consisted of 3,129 patients in the apixaban group and 3,129 patients in the warfarin group.

PRIMARY ENDPOINT: MAJOR HEMOCOMPATIBILITY-RELATED ADVERSE EVENTS (HRAES)

After matching, during the follow-up period, a total of 374 patients (28.4%) in the warfarin cohort experienced major hemocompatibility-related adverse events, compared to 234 patients (19.7%) in the apixaban cohort. Receiving apixaban was associated with a significantly lower risk of HRAEs (OR: 0.618; 95% CI: 0.513 - 0.744; P < 0.001). Kaplan–Meier survival analysis revealed a significantly lower incidence of new-onset atrial fibrillation in the apixaban group compared to the warfarin group (log-rank P < 0.001). The hazard ratio for having HRAEs was 0.649 (95% CI: 0.551-0.765), indicating that patients receiving warfarin had more risk of developing HRAEs relative to those receiving apixaban. The proportional hazards assumption was not violated ($P = 0.001$) (Table 2, Fig. 2).

Secondary endpoints: LVAD thrombosis, stroke, major bleeding events, non-central nervous system thromboembolism, and all-cause mortality

After matching, the secondary outcomes demonstrated significant differences between the apixaban group and the warfarin group. LVAD thrombosis events were less frequently reported in the apixaban group, with an odds ratio (OR) of 0.088 (95% CI: 0.051–0.149, $P < 0.001$), and a hazard ratio of 0.094 (95% CI: 0.055–0.159). Stroke was also less frequently reported in the apixaban group, with an OR of 0.721 (95% CI: 0.567–0.918, $P = 0.008$), and a hazard ratio of 0.775 (95% CI: 0.613–0.980). Major bleeding events were less commonly reported in the apixaban group, with an OR of 0.528 (95% CI: 0.443–0.631, $P < 0.001$), and a hazard ratio of 0.557 (95% CI: 0.473–0.657). non-central nervous system thromboembolism events showed no difference between the two groups, with an OR of 1.114 (95% CI: 0.905–1.372, $P = 0.308$), and a hazard ratio of 1.153 (95% CI: 0.946–1.406). All cause mortality showed no difference between the two group OR of 1.001 (95% CI: 0.888–1.129, $P = 0.987$), and the hazard ratio was 1.072 (95% CI:0.964–1.192) (Table 2, Fig. 2).

DISCUSSION

Warfarin has been the standard anticoagulant since the introduction of LVADs. At the time of LVAD development in the 1960s, warfarin was the only oral anticoagulant available. [12] With the emergence of DOACs in 2010s as an alternative to warfarin in nonvalvular atrial fibrillation, their use for anticoagulation in other indications has been extensively investigated. [13] This interest was driven by the simpler clinical use of these medications, without the need for INR monitoring, and the possibility of a lower incidence of bleeding and/or a better protective effect against thrombosis.

Although apixaban lacks the extensive evidence supporting its use in LVAD patients, it has been used on a case-by-case basis. In this large multicenter retrospec-

Table 2. Primary and secondary clinical outcomes: Apixaban *vs.* warfarin in LVADs patients

Outcome	Risk of Event, % (Apixaban group)	Risk of Event, % (Warfarin group)	Odds Ratio (95% CI)	P Value
Primary Outcome				
Major Hemocompatibility-Related Adverse Events (HRAEs)	234 (19.7%)	374 (28.4%)	0.618 (0.513 – 0.744)	P < 0.001
Secondary Outcome				
LVAD thrombosis	15 (0.5%)	152 (5.3%)	0.088 (0.051 – 0.149)	P < 0.001
Stroke	120 (4.8%)	168 (6.5%)	0.721 (0.567 – 0.918)	P = 0.008
Major bleeding	231 (10.5%)	368 (18.2%)	0.528 (0.443 – 0.631)	P < 0.001
Non-central nervous system thromboembolism	192 (10.0%)	200 (9.1%)	1.114 (0.905 – 1.372)	P = 0.308
All cause mortality	679 (21.9%)	680 (21.9%)	1.001 (0.888 – 1.129)	P = 0.987
Values are n (%)				

Source: Compiled by the authors of this study

tive cohort study, we aimed to investigate apixaban as a potential oral anticoagulant for patients with LVADs.

In the DOT-HM3 study, Netuka et al. (2024) demonstrated the feasibility of using a direct oral anticoagulant, with or without aspirin, in patients chronically supported with an HM3 LVAD for six months [10]. In the DOAC LVAD study, Mehta et al. (2025) found no statistically significant differences between warfarin and apixaban in the incidence of death, stroke, device thrombosis, major gastrointestinal bleeding, aortic root thrombus, or arterial non-CNS thromboembolism [11].

The results of our study suggest that apixaban is more effective in preventing major hemocompatibility-related adverse events, defined as a composite of LVAD thrombosis, stroke, major bleeding, non-central nervous system thromboembolism, and all-cause mortality. These findings do not suggest that apixaban is an equivalent alternative to warfarin, but they suggest that it may be a superior anticoagulation option for LVAD patients.

A major limitation across all previous studies investigating anticoagulation in LVAD patients is the small sample size. In our large multicenter retrospective cohort, we included 6,258 patients after propensity score matching. The statistical power of the analysis was substantially increased, enabling the detection of differences that smaller previous studies were unable to identify.

The results were further analyzed according to secondary endpoints. LVAD thrombosis was notably less frequent in the Apixaban cohort compared to the Warfarin cohort. Previous randomized controlled trials (DOT-HM3 study and DOAC LVAD study) [10, 11] were underpowered to detect this rare complication; both arms in these trials reported zero cases of pump thrombosis. While these results suggest that both anticoagulants are generally safe regarding pump thrombosis, they do not provide definitive evidence as to which agent is superior in this regard. In our study, there was a large difference between two groups with an odds ratio (OR) of 0.088, indicating a potentially better safety profile for apixaban compared to warfarin.

The incidence of stroke was lower in the apixaban group compared to the warfarin group, with an odds ratio of 0.721. Similarly, major bleeding events were less frequently reported in the apixaban group, with an OR of 0.528. These findings are not only statistically significant, but also clinically significant as they could represent a breakthrough in the management of LVAD patients. The previous RCTs and retrospective studies did not show a similar effect on LVAD patients, which can be attributed to the small size of the sample.

Although prior RCTs were unable to establish this relationship in LVAD patients, the use of warfarin was compared against apixaban for the prevention of thromboembolic events in atrial fibrillation In the ARISTOTLE study, Granger et al. (2011) demonstrated that apixaban was superior to warfarin in reducing the risk of stroke or systemic embolism in patients with atrial fibrillation, while also showing a lower risk of major bleeding [9]. These results align with our findings, supporting the notion that apixaban may offer a more favorable safety profile without compromising efficacy. Taken together, both the reduced incidence of stroke and major bleeding suggest that apixaban could be a preferable anticoagulant option, particularly in LVAD patients who are at higher risk for bleeding complications and thromboembolic events.

Our study found no significant differences between the two groups in non–central nervous system thromboembolic events or all-cause mortality, consistent with previous RCTs [11, 10] and the most recently published systematic review [14].

STUDY LIMITATION

Our study involved a large number of patients and used propensity score matching to balance baseline characteristics between the two cohorts and reduce confounding. But, Our study was conducted using the TriNetX database, which identifies diagnoses according to ICD codes. This system is susceptible to coding errors by its nature and unable to determine medication dose and adherence. Although this study was unable to quantify the potential effect of previously mentioned limitations on the study findings, the two cohorts would likely be affected similarly, minimizing the effects on outcomes.

Furthermore, since it is a retrospective cohort study, our analysis is constrained by the nature of observational data, as they are unable to establish causal relationships due to potential residual confounding and selection bias in addition to unmeasured variables. While they can identify associations and generate hypotheses, retrospective designs do not provide the same level of evidence provided by prospective, randomized trials and are therefore limited in their ability to generate fully informative or definitive conclusions.

CONCLUSIONS

In conclusion, this large retrospective study found that using apixaban in LVAD patients was associated with a lower incidence of major hemocompatibility-related adverse events, including LVAD thrombosis, stroke, and major bleeding. These findings suggest that apixaban could potentially be a safer option than warfarin. A safer anticoagulant can improve overall LVAD safety and could shift clinical practice toward using apixaban as a preferred anticoagulant in LVAD patients. However, due to the limitations of retrospective studies, it is important to interpret these results carefully. Large randomized clinical trials are necessary to confirm these findings.

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AVAILABILITY OF DATA AND MATERIALS

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

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

APPENDIX A – TEXT REPRESENTATION OF THE COHORTS DEFINITION

This section lists all terms used in the definitions of the two cohorts.

Query Criteria for Cohort 1 (Apixaban)

Patients must have:
Age (Age) (at least 18 years (most recent occurrence).
Patients cannot have:
- Warfarin (NLM:RXNORM:11289).
All the following must be satisfied:
- Apixaban: The terms in this group occurred between Sep 10, 2020 and Sep 10, 2025
- Patients must have all of the following:
- Presence of heart assist device (UMLS:ICD10CM:Z95.811); and
- Apixaban (NLM:RXNORM:1364430).

Query Criteria for Cohort 2 (warfarin)

Patients must have:
- Age (Age) (at least 18 years (most recent occurrence).
Patients cannot have:
- Apixaban (NLM:RXNORM:1364430).
All the following must be satisfied:
- Warfarin: The terms in this group occurred between Sep 10, 2020 and Sep 10, 2025
Patients must have all of the following:
- Presence of heart assist device (UMLS:ICD10CM:Z95.811); and
- Warfarin (NLM:RXNORM:11289).

APPENDIX B – TEXT REPRESENTATION OF THE ANALYSIS SETUP

This section contains the Index Event definition for each cohort.
The index event for Cohort 1 (Apixaban) is defined as the following:
All the following must be satisfied:
- Apixaban: The terms in this group occurred between Sep 10, 2020 and Sep 10, 2025
Patients must have all of the following:
- Presence of heart assist device (UMLS:ICD10CM:Z95.811); and
- Apixaban (NLM:RXNORM:1364430).
The index event for Cohort 2 (warfarin) is defined as the following:
All the following must be satisfied:
- Warfarin: The terms in this group occurred between Sep 10, 2020 and Sep 10, 2025
Patients must have all of the following:

- Presence of heart assist device (UMLS:ICD10CM:Z95.811); and
- Warfarin (NLM:RXNORM:11289).

APPENDIX C – TEXT REPRESENTATION OF THE OUTCOMES DEFINITION

This analysis includes the following outcomes: major hemocompatibility-related adverse events (HRAEs)

Patients must have any of the following:

- Thrombosis due to cardiac prosthetic devices, implants and grafts (UMLS:ICD10CM:T82.867); or
- Thrombosis due to cardiac prosthetic devices, implants and grafts, initial encounter (UMLS:ICD10CM:T82.867A); or
- Thrombosis due to cardiac prosthetic devices, implants and grafts, subsequent encounter (UMLS:ICD10CM:T82.867D); or
- Thrombosis due to cardiac prosthetic devices, implants and grafts, sequela (UMLS:ICD10CM:T82.867S); or
- Cerebral infarction (UMLS:ICD10CM:I63); or
- Hemorrhage, not elsewhere classified (UMLS:ICD10CM:R58); or
- Gastrointestinal hemorrhage, unspecified (UMLS:ICD10CM:K92.2); or
- Hemorrhage from respiratory passages (UMLS:ICD10CM:R04); or
- Nontraumatic intracranial hemorrhage, unspecified (UMLS:ICD10CM:I62.9); or
- Gross hematuria (UMLS:ICD10CM:R31.0); or
- Other abnormal uterine and vaginal bleeding (UMLS:ICD10CM:N93); or
- Embolism and thrombosis of thoracic aorta (UMLS:ICD10CM:I74.11); or
- Pulmonary embolism with acute cor pulmonale (UMLS:ICD10CM:I26.0); or
- Pulmonary embolism without acute cor pulmonale (UMLS:ICD10CM:I26.9); or
- Embolism and thrombosis of abdominal aorta (UMLS:ICD10CM:I74.0); or
- Embolism and thrombosis of other and unspecified parts of aorta (UMLS:ICD10CM:I74.1); or
- Embolism and thrombosis of arteries of the upper extremities (UMLS:ICD10CM:I74.2); or
- Embolism and thrombosis of arteries of the lower extremities (UMLS:ICD10CM:I74.3); or
- Embolism and thrombosis of arteries of extremities, unspecified (UMLS:ICD10CM:I74.4); or
- Embolism and thrombosis of iliac artery (UMLS:ICD10CM:I74.5); or
- Embolism and thrombosis of other arteries (UMLS:ICD10CM:I74.8); or
- Embolism and thrombosis of unspecified artery (UMLS:ICD10CM:I74.9); or
- Other venous embolism and thrombosis (UMLS:ICD10CM:I82).

LVAD thrombosis

Patients must have any of the following:

- Thrombosis due to cardiac prosthetic devices, implants and grafts (UMLS:ICD10CM:T82.867); or

- Thrombosis due to cardiac prosthetic devices, implants and grafts, initial encounter (UMLS:ICD10CM:T82.867A); or
- Thrombosis due to cardiac prosthetic devices, implants and grafts, subsequent encounter (UMLS:ICD10CM:T82.867D); or
- Thrombosis due to cardiac prosthetic devices, implants and grafts, sequela (UMLS:ICD10CM:T82.867S).

Stroke

Patients must have:

- Cerebral infarction (UMLS:ICD10CM:I63).

Major bleeding

Patients must have any of the following:

- Gastrointestinal hemorrhage, unspecified (UMLS:ICD10CM:K92.2); or
- Hemorrhage, not elsewhere classified (UMLS:ICD10CM:R58); or
- Hemorrhage from respiratory passages (UMLS:ICD10CM:R04); or
- Nontraumatic intracranial hemorrhage, unspecified (UMLS:ICD10CM:I62.9); or
- Gross hematuria (UMLS:ICD10CM:R31.0); or
- Other abnormal uterine and vaginal bleeding (UMLS:ICD10CM:N93).

Non-central nervous system thromboembolism

Patients must have any of the following:

- Pulmonary embolism with acute cor pulmonale (UMLS:ICD10CM:I26.0); or
- Pulmonary embolism without acute cor pulmonale (UMLS:ICD10CM:I26.9); or
- Embolism and thrombosis of abdominal aorta (UMLS:ICD10CM:I74.0); or
- Embolism and thrombosis of other and unspecified parts of aorta (UMLS:ICD10CM:I74.1); or
- Embolism and thrombosis of arteries of the upper extremities (UMLS:ICD10CM:I74.2); or
- Embolism and thrombosis of arteries of the lower extremities (UMLS:ICD10CM:I74.3); or
- Embolism and thrombosis of arteries of extremities, unspecified (UMLS:ICD10CM:I74.4); or
- Embolism and thrombosis of iliac artery (UMLS:ICD10CM:I74.5); or
- Embolism and thrombosis of other arteries (UMLS:ICD10CM:I74.8); or
- Embolism and thrombosis of unspecified artery (UMLS:ICD10CM:I74.9); or
- Other venous embolism and thrombosis (UMLS:ICD10CM:I82); or
- Embolism and thrombosis of thoracic aorta (UMLS:ICD10CM:I74.11).

All cause mortality

Patients must have:

- Deceased (Deceased).

ORIGINAL ARTICLE

CONTENTS

Predictors of complicated course of acute myocardial infarction with ST-segment elevation in patients with obesity, overweight and normal body weight

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ABSTRACT

Aim: The aim of the study was to clarify the influence of smoking and other risk factors (RFs) on the occurrence of complications in patients with normal body weight, overweight (OW) and obesity.

Materials and Methods: Patients (n=158) with acute ST-segment elevation myocardial infarction (STEMI) were divided into 3 groups depending on body mass index (BMI): Group I included 52 patients with normal body weight (mean age - 60.83±11.94 years); Group II - 51 patients with OW (mean age - 62.04±8.55 years); Group III - 55 patients with obesity of I-III degree (mean age - (0.96±11.31 years) (p>0.05).

Results: The most common RFs were hypertension (88.46-94.55%), dyslipidemia (DLP) (50.00-76.36%), smoking (69.23-69.09%). The relative risk of acute left ventricular aneurysm in Group I with DLP is 2.08 times higher (RR=2.08 [0.37; 11.62], p=0.029), and among smokers, this risk increases by 25% (RR=1.25 [0.22; 7.18], p=0.049).

The relative risk of major complications in Group II in the presence of 5-6 RFs is 1,5 times higher (RR=1.58 [0.61; 10.94], p=0.016). The relative risk of all complications in Group III in the presence of DLP is 1.23 times higher (RR=1.23,

with 95% CI from 0.91 to 1.65, p=0.017), and in the presence of smoking is 8.37 times higher RR=8.37 [2.11; 33.16], p<0.001).

Conclusions: Complications are observed three times more often in obese patients and about twice as often in overweight patients. Significant predictors of complications are smoking, dyslipidemia, simultaneous exposure to 5-6 risk factors.

Smoking increases the risk of complications by 8 times in obese patients.

KEY WORDS: acute myocardial infarction, overweight, obesity, risk factors, smoking

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INTRODUCTION

Obesity is one of the major risk factors and predictors of coronary heart disease (CHD) and premature death. Individuals with mild obesity have a life expectancy 3–5 years shorter than those with normal body weight, while severe obesity can reduce life expectancy by up to 15 years [1–4]. The 26-year Framingham study involving 5209 participants established a clear relationship between obesity and the development of cardiovascular diseases (CVD). Researchers note that obesity is an independent risk factor (RF) for cardiovascular events, especially in women [5]. The distribution of adipose tissue is important in the development of cardiometabolic diseases. For example, according to the results of an American study that included 2683 postmenopausal women with a normal BMI (from 18.5 to 24.9 kg/m2) without CVD at the beginning of the study, it was found that women with a higher amount of adipose tissue in the abdominal area and a lower level in the thigh area have an increased risk of developing CVD [6]. These findings highlight the importance of fat distribution in specific body areas in the development of CVD.

One of the most aggressive risk factors for acute myocardial infarction (AMI) is smoking [7]. The great number of studies demonstrate a causal relationship between smoking and CVD occurrence'. It has been proven that each cigarette smoked increases the risk of non-fatal AMI by 5.6% [8]. Smoking one pack of cigarettes per day for 20 years leads to the intake of approximately 2 kg of various chemicals (sulfur, chlorine, calcium, potassium) and heavy metals (cadmium, lead, chromium, iron, manganese, strontium, nickel, zinc) enter the body [9]. The effect of passive smoking on the human body is no less dangerous [10]. The combination of smoking with

obesity and arterial hypertension (AH) is particularly dangerous. When smoking is combined with abdominal obesity, cardiovascular risk increases by 5.5 times [11]. In particular, one study examining the long-term consequences of ST-segment elevation AMI (STEMI) found that smoking was the strongest predictor of secondary cardiovascular events in individuals under 35 years of age. The association with obesity significantly increases this risk [12].

Since AMI in the context of obesity remains an important issue in modern medicine, a detailed study of the influence of various risk factors—primarily smoking—on the course of this combined pathology may form the basis for identifying key pathogenetic mechanisms and contribute to the development of pathogenetically based treatments [13,14].

AIM

The aim of our study was to determine the influence of smoking and other risk factors on the occurrence of complications in the early post-infarction period in patients with overweight (OW) and obesity.

MATERIALS AND METHODS

158 patients with STEMI aged 32 to 86 years who were hospitalized at the Department of Cardiology and Reperfusion Therapy of the Lviv Territorial Medical Association «Multidisciplinary Clinical Hospital of Intensive Care and Emergency Medical Care» were examined. The inclusion criteria were: men and women over the age of 18 years diagnosed with STEMI. The clinical diagnosis of STEMI was established on the basis of clinical, laboratory and instrumental studies, in accordance with the recommendations of the Ukrainian Association of Cardiologists (2021) and the European Society of Cardiology (2023) [15]. Also, the patient's informed consent to participate in the study was mandatory. Exclusion criteria included hereditary syndromes associated with obesity, as well as secondary, diencephalic, and mixed forms of obesity.

All patients with AMI were divided into three groups: the first Group consisted of 52 patients with AMI with normal body weight (BMI – 18.5-24.9 kg/m2), the second Group – 51 patients with AMI with OW (BMI – 25.0-29.9 kg/m2), the third Group – 55 patients with AMI and obesity of I-III degrees (BMI ≥30.0 kg/m2). In all cases, the patients were comparable between the groups in terms of age and sex.

According to the criteria of the American BRFSS system (The Behavioral Risk Factor Surveillance System), smokers were considered to be people who smoke daily, sometimes or are exposed to passive tobacco smoke. When classifying the status of a smoker, the following criteria were used:

1) smokers – persons who smoke daily or occasionally and have smoked 100 or more cigarettes in their lifetime;

2) former smokers – persons who have quit smoking and now do not smoke at all, but have smoked more than 100 cigarettes in their lifetime;

3) non-smokers – persons who have not smoked 100 cigarettes in their lifetime.

The survey recorded the number of cigarettes smoked per day and the duration of smoking (in years).

Statistical analysis of the obtained results was performed using both descriptive and analytical methods. Medico-statistical analysis of the received data was produced by descriptive and analytical methods using Microsoft Excel 2016, Statistica 10 and IBMSPSS Statistics 20 programs. A Gaussian distribution was established, which allowed us to determine the standard deviation (SD) and error (M±m) for each of the studied mean values (Mean). Comparison of two sets of mean values was carried out using the unpaired Student's t-test (t). Pearson's $\chi 2$ test was used to compare two sets of categorical variables and frequencies. The calculation of the correlational dependence between the studied parameters was carried out using the Pearson linear correlation method (r). Averages (Mean) were presented as the arithmetic mean and its standard error (M±m). Comparison of 2 groups of mean values was performed using the unpaired Student's t-test. To describe relative values, percentages and their errors ($P \pm m_p$, %) were calculated. The calculation of the correlational interdependence between the studied indicators was carried out using the Spearman method. The results were considered reliable at a minimum significance level of $p<0.05$. To compare the magnitude of the influence of risk factors in the studied groups, the relative risk indicator (RR) was calculated.

During the study, all ethical standards were upheld in accordance with the principles of the World Medical Association's Declaration of Helsinki, the Council of Europe's Convention on Human Rights and Biomedicine, ICHGCP, and current regulatory legal acts of Ukraine. The set of diagnostic procedures was approved by the Bioethical Commission of the State non-profit enterprise «Danylo Halytsky National Medical University in Lviv». The main source of information was the inpatient medical record (form 003/0). No violations were identified during the study (protocol No. 2, dated 09/26/2022).

RESULTS

The prevalence of STEMI risk factors was analyzed across the three patient groups (Table 1). Along with hyper-

Table 1. Prevalence of the main risk factors in patients with STEMI depending on BMI ($P\pm m_p$, %)

Indicators	Group I (n=52)	Group II (n=51)	Group III (n=55)
	$P\pm m_p$, %	$P\pm m_p$, %	$P\pm m_p$, %
Hypertension	88.46±4.43	92.16±3.76	94.55±3.06
DLP	50.00±6.93	62.75±6.77	76.36±5.73##
T2DM	17.31±5.25	31.37±6.5	47.27±6.73##
Smoking	69.23±6.40	56.86±6.94	69.09±6.23
Excessive alcohol consumption	40.38±6.80	43.14±6.94	32.73±6.33
Hereditary factors	19.23±5.47	25.49±6.10	36.36±6.49#
Occupational hazards	32.69±6.51#	25.49±6.10	16.36±4.99
PIC*	25.00±6.00	15.69±5.09	16.36±4.99

Note: #– p<0.05, ##– p<0.01, significance of the difference between the indicators of Groups I and III; *–Type 2 diabetes mellitus;
Source: compiled by the authors of this study

Table 2. Relative risk (RR) of complications depending on the presence of risk indicators

Indicators	RR value [95% CI]		
	Group I	Group II	Group III
60-69 years	1.02 [0.65-1.60]	1.09 [0.74-1.62]	1.45[0.77-2.73] *
≥70 years	0.87 [0.67-1.13]	1.08 [0.79-1.46]	1.28[0.07-1.2] *
VAI>1.1**	0.91[0.82-1.0]	0.86 [0.22-3.45]	1.12[0.99-1.26] *
Hypertension	1.19 [0.16-9.09]	1.14 [0.94-1.38]	0.56 [0.05-5.8]
T2DM	1.04 [0.74-1.46]	1.09 [0.74-1.62]	1.37[0.83-2.28] *
Dyslipidemia	1.00 [0.50-1.99]	1.42 [0.87-2.32] *	1.23 [0.91-1.65]
Smoking	1.48 [0.77-2.82] *	1.23 [0.73-2.06]	8.37[2.11-3.16] *
LDL-C >1.4 mmol/l	1.22 [0.80-1.87] *	1.42 [0.87-2.32] *	1.43[0.91-2.26] *
HDL-C <1.0 mmol/l	1.61 [0.73-3.55] *	1.51 [0.69-3.26] *	1.54[0.96-2.49] *
GFR<60 ml/min/1.73 m^2	1.43 [0.38-5.39] *	1.42 [0.87-2.32] *	1.56 [0.84-2.89] *
hospitalization after 12 hours	1.16 [0.75-1.78]	1.61 [0.89-2.93] *	1.51[0.97-2.36]
5-6 FR simultaneously	1.43[0.19-10.57] *	1.58 [0.61-10.94] *	1.39 [1.08-1.8] *

Note: *– p<0,05; **–visceral adiposity index
Source: compiled by the authors of this study

tension and dyslipidemia (DLP), smoking was found to be one of the most common risk factors among the surveyed individuals. Daily active or passive smoking was observed in 69.23±6.40% of patients in Group I, 56.86±6.94% of patients in Group II, and 69.09±6.23% of patients in Group III (p>0.05). The duration of smoking was analyzed, no significant difference was found between the studied groups (22.42±0.9 years (I), 23.86±1.29 years (II), 23.26±1.34 years (III), p>0.05). It was found that patients of Group I smoked a slightly larger number of cigarettes per day (21.33±0.96 pcs.), compared to individuals of Groups II and III (18.45±0.94 pcs. (II), 20.00±0.63 pcs. (III), p_{1-2}=>0.05, $p_{1-3>}$0.05, p_{2-3}>0.05), although there was no significant difference either. It was also found that only 2 individuals in Group II were exposed to the harmful effects of passive smoking, while the remaining patients were active smokers.

The frequency of complications during the hospital period of AMI was analyzed. The highest proportion of patients with complicated AMI was recorded in the obese group (52.73±6.73%), compared with OW (39.22±6.84%) and patients with normal (19.23±5.47%) body weight (p_{1-2}=0.026, p_{1-3}<0.001) (Fig. 1). In particular, the course of AMI was complicated by the formation of acute LV aneurysm (p_{1-3}=0.005, p_{2-3}=0.028) three times more frequently in obese patients and approximately twice as often in overweight patients, compared with patients with normal body weight. A similar trend was observed in the groups regarding the occurrence of acute heart failure (Killip Class III-IV) (p_{1-3}=0.049).

Rhythm and conduction disturbances were observed across all groups, but occurred three times more fre-

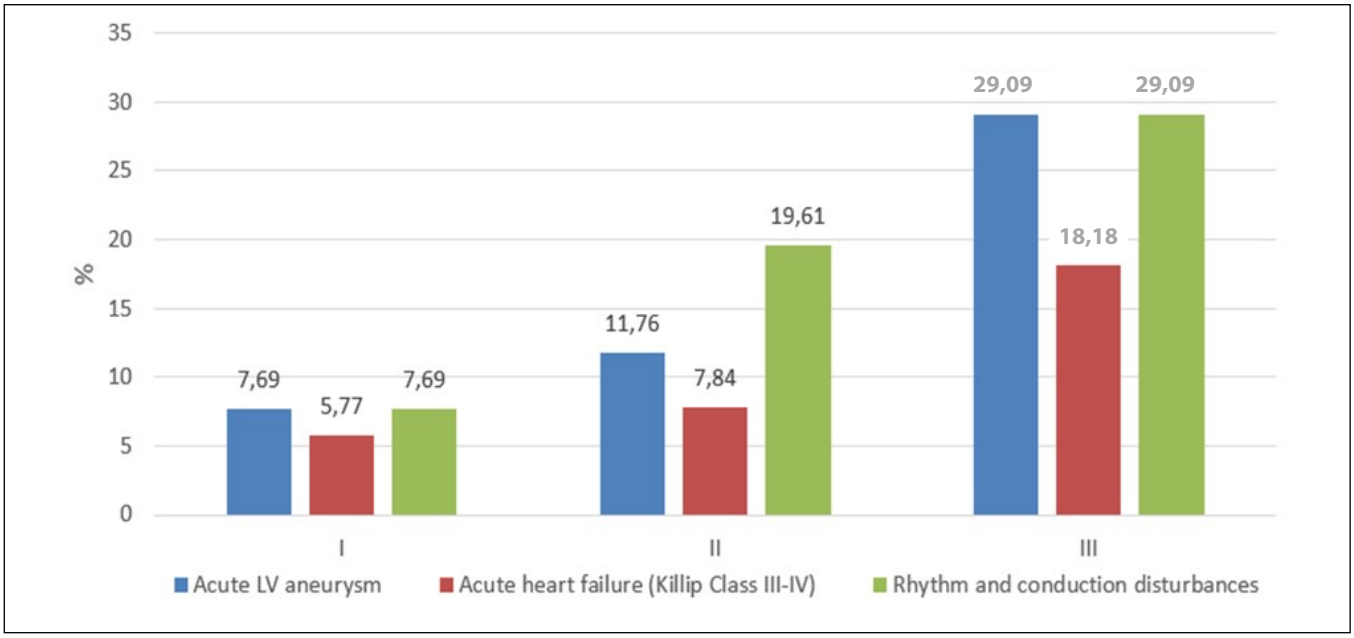


Fig. 1. Complications during the hospital period STEMI (P±mp, %)
Picture taken by the authors

quently in obese patients (Group III) ($p_{1-3=}$0.005) and approximately twice as often in overweight patients compared to patients with normal body weight. In particular, ventricular fibrillation (VF) with successful defibrillation was diagnosed in 1.92±0.9% of persons in Group I, 1.96±0.9% of persons in Group II and 5.45±2.06% of persons in Group III. Other rhythm disturbances (such as atrial fibrillation and ventricular tachycardia) were observed in 6 patients (10.91±4.2%) in Group III, 4 patients (7.84±3.76%) in Group II and 1 patient (1.92±0.9%) in Group I (p>0.05). Conduction disorders were noted in 3.85±1.67% of patients in Group I, 9.8±4.16% of patients in Group II, and among patients of III Group – in 12.73±4.49%.

We analyzed the impact of individual risk factors on the likelihood of adverse events during the early post-infarction period in patients with different body weights (Table 2). It was found (p=0.014) that the relative risk of major STEMI complications in smokers with normal body weight (Group I) is 1.48 times higher than among non-smokers (RR=1.48, with a 95% confidence interval (CI) of 0.77 to 2.82). In Group I, the presence of 5–6 risk factors (RFs) increased the complication risk by 43% (RR=1.43[0.19; 10.57], p=0.021), with LDL-C level>1.4 mmol/l – by 22% (RR=1.22 [0.80; 1.87], p=0.048), with HDL-C level<1.0 mmol/l – by 61% (RR=1.61 [0.73; 3.55], p=0.016), and with GFR<60 ml/min – by 43% (RR=1.43 [0.38; 5.39], p=0.035) (Table 2).

We analyzed the impact of individual RFs on the risk of developing major complications of AMI in each of the patient groups. In particular, it was reliably established (p=0.038) that the relative risk of acute LV aneurysm in patients with STEMI and normal body weight (Group I) who have DLP is 2.08 times higher (RR=2.08 [0.37; 11.62], p=0.029), furthermore, among smokers, this risk increases by 25% (RR=1.25 [0.22; 7.18], p=0.049). Risk of acute LV aneurysm increases by 3.33 times (RR=3.33 [0.61; 18.29], p=0.006) with late hospitalization in these patients. The relative risk of rhythm and conduction disturbances occurrence in smokers with STEMI and normal body weight (Group I) is 1.42 times higher than in patients who do not smoke (RR=1.42 [0.52; 3.84], p=0.038). Thus, in patients with normal body weight (Group I) the dominant RFs are smoking, DLP and late hospitalization.

The relative risk of major complications in patients with STEMI and OW (Group II) in the presence of 5-6 FRs is one and a half times higher (RR=1.58 [0.61; 10.94], p=0.016). A similar situation is observed in patients hospitalized after 12 hours (RR=1.61 [0.89; 2.93], p=0.046). In particular, the relative risk of acute LV aneurysm in patients of Group II doubles in the presence of T2DM (RR=2.00 [0.32; 12.55], p=0.040), a similar trend is observed in DLP (RR=2.00 [0.63; 6.32], p=0.011). This risk is also two and a half times higher with HDL-C levels <1.0 mmol/l (RR=2.53 [0.41; 15.66], p=0.022) and with GFR <60 ml/min/1.73 m^2 (RR=2.40 [0.39; 14.88], p=0.026).

Analysis of the prerequisites for the acute left ventricular failure (Killip class III-IV) occurrence in patients with STEMI and OW (Group II) showed that the risk is 2.64 times higher in the presence of DLP (RR=2.64 [0.48; 14.58], p=0.010). A similar relative risk of acute left ventricular failure (Killip class III-IV) in Group II is associated with smoking, and is 2.38 times higher in

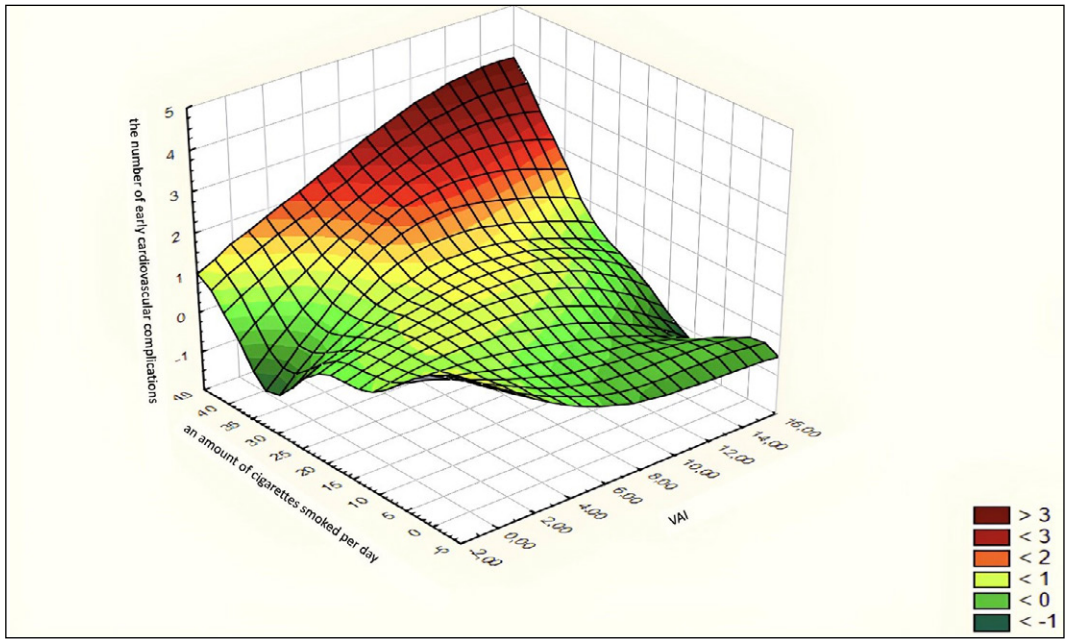


Fig. 2. Dependence of the number of early cardiovascular complications in patients with STEMI, an amount of cigarettes smoked per day and VAI
Picture taken by the authors

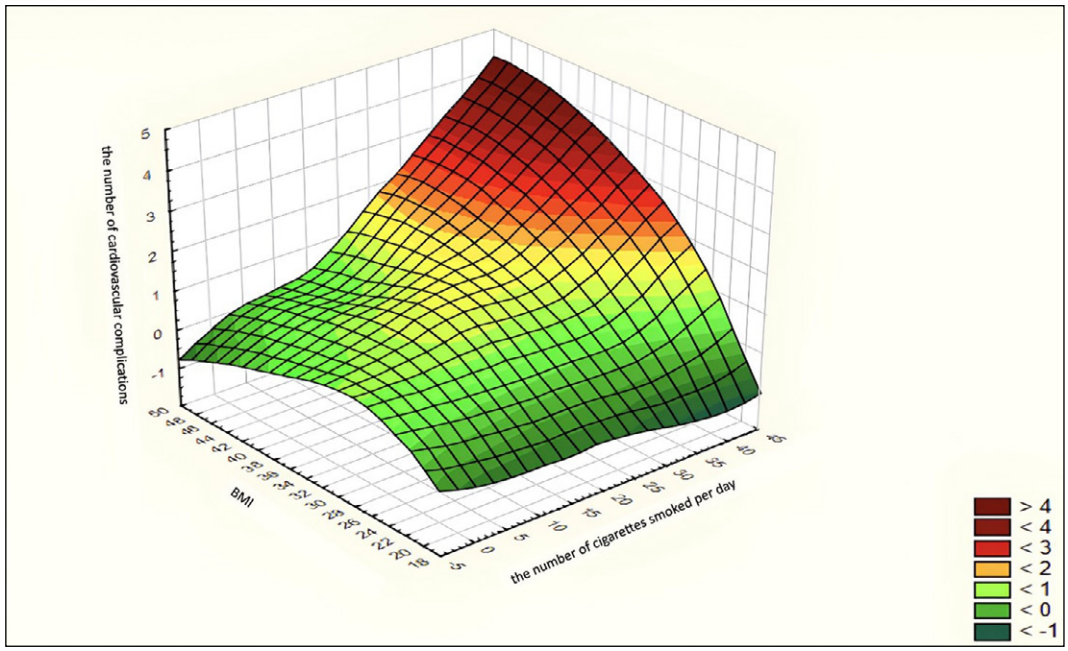


Fig. 3. Relationship between the number of cigarettes smoked per day, BMI and the number of cardiovascular complications in patients with STEMI
Picture taken by the authors

smokers compared to non-smokers (RR=2.38 [0.43; 13.22], p=0.018).

The risk of rhythm and conduction disturbances in smokers increases by one and a half times (RR=1.52 [0.69; 3.38], p=0.023), in the presence of DLP – by two times (RR=2.07, [0.57; 7.55], p=0.020), in the presence of more than five RFs – by 2.20 times (RR=2.20 [0.31; 15.38], p=0.039), with GFR <60 ml/min – by 2.36 times (RR=2.36 [0.90; 6.20], p=0.017). Therefore, the most significant RFs in Group II patients are smoking, T2DM and DLP.

In patients in Group III, the relative risk of major complications increases 1.45-fold in those aged over 60 years compared to younger individuals (RR=1.45 [0.77; 2.73], p=0.024) (Table 2). Additionally, the relative risk of all complications in the presence of DLP is 1.23 times higher (RR=1.23, [0.91; 1.65], p=0.017), and T2DM is one and a half times higher (RR=1.37 [0.83; 2.28], p=0.021). In obese patients with more than five RFs, the relative risk of complications increases by 39% (RR=1.39 [1.08; 1.80], p=0.021). With late hospitalization (i.e., more than 12

hours after AMI onset), the risk increases by 51% (RR=1.51 [0.97; 2.36], (p=0.024), with LDL-C level>1.4 mmol/l – by 43% (RR=1.43 [0.91; 2.26], p=0.012), with HDL-C level<1.0 mmol/l – by 54% (RR=1.54 [0.96; 2.49], p=0.012), and with GFR<60 ml/min – by 56% (RR=1.56 [0.84; 2.89], p=0.015). Special attention should be paid to the smoking factor, which increases the risk of major complications by as much as 8.37 times RR=8.37 [2.11; 33.16], p<0.001). Particularly, the relative risk of acute LV aneurysm in obese smokers increases by 6.56-fold (RR=6.56 [0.95; 45.44], p=0.011).

To better illustrate the combined effect of multiple factors on the occurrence of cardiovascular complications in patients with STEMI, we present 3D diagrams. Fig. 2 demonstrates a direct relationship between the number of complications, the number of cigarettes smoked per day, and the visceral adiposity index (VAI).

The increase in the number of cardiovascular complications in patients with STEMI, BMI level, and cigarettes smoked per day Fig. 3 demonstrates.

DISCUSSION

The results of our study confirm the fact that the presence of multiple RFs significantly determines the development of a complicated course of AMI, regardless of the patient's body weight. However, it is among individuals with OW and obesity (BMI >25 kg/m^2) that these factors have a more pronounced negative impact. The presence of ≥5 RF credibly increases the risk of major complications by one and a half times in patients with normal body weight. This is statistically proven (RR=1.58; p=0.016). According to the data of the large international INTERHEART study, more than 90% of the first myocardial infarction' risk in different populations is explained by nine factors that are potentially modifiable [16].

In the group of OW patients (group II), the presence of metabolic disorders plays an important role. The risk of acute LV aneurysm doubles in the presence of T2DM and DLP (RR=2.00; p=0.040 and p=0.011, respectively). Diabetes is equivalent to previous MI in predicting recurrent cardiovascular events. Similar results were demonstrated in the UKPDS study [17].

Low HDL-C (<1.0 mmol/L) is associated with a 2.5-fold increased risk of LV aneurysm (RR=2.53; p=0.022). This correlates with the findings of the Framingham Heart Study, where low HDL-C was identified as an independent predictor of CHD [18]. A negative impact of late hospitalization (>12 h after symptom onset) was also noted, increasing the risk of complications in both patients groups with OW (RR=1.61; p=0.046) and obesity (RR=1.51; p=0.024). This is confirmed by current recommendations of the European Society of Cardiology (ESC), which emphasize the crucial role of "time to reperfusion" in STEMI [19].

Smoking deserves special attention, as in obese patients it is associated with a sharp increase in the risk of major complications by 8.37 times (p<0.001), and the probability of developing LV aneurysm increases by 6.56 times (p=0.011). These data noticeably exceed those obtained in the large Global Burden of Disease analysis (GBD), which estimated smoking as a risk factor for cardiovascular death (RR ≈ 2.5) [20], and suggest the joint effect of smoking and obesity.

The risk of acute left ventricular failure (Killip III–IV) in OW individuals is increased in the presence of DLP (RR=2.64; p=0.010) and smoking (RR=2.38; p=0.018). The combined effect of several risk factors (≥5) leads to an increase in the possibility of rhythm and conduction disturbances by 2.2 times (p=0.039), and with a decrease in GFR <60 ml/min – by 2.36 times (p=0.017). This is coincides with the REACH study, which identified chronic kidney disease as an important predictor of poor prognosis in patients with atherothrombosis [21].

In the obese group (group III), individuals older than 60 years had a 1.45-fold higher risk of complications (p=0.024), and the presence of T2DM, DLP, high LDL-C, low HDL-C, and reduced GFR also significantly worsened the prognosis. These results are consistent with the IDF criteria for metabolic syndrome, which indicate a cardiometabolic "explosive" combination of RF [22].

The 3D diagrams we used also confirmed an evidence of a dose-dependent association between the number of cigarettes smoked, body mass index, and the number of complications, indicating a risk accumulation effect. This is consistent with the concept of "total risk burden" that underlies modern risk assessment scales, in particular SCORE2 [23].

Our results confirm that the presence of multiple risk factors significantly worsens the prognosis of STEMI, regardless of the patient's body weight. However, smoking, type 2 diabetes, DLP, reduced renal function, and late hospitalization in obese patients are particularly dangerous. A study of Chinese patients aged ≥60 years showed that smokers had higher levels of C-reactive protein and a higher incidence of acute MI (16.7% in smokers vs. 7.0% in nonsmokers; p=0.008) and worse 10-year survival [24]. Among persons younger than 60 years of age who had a STEMI, continued smoking at one year was associated with a 2.51-fold increased risk of MACE and a 2.52-fold increased risk of mortality compared with nonsmokers [25]. However, those who quit smoking had similar outcomes to nonsmokers. In a multinational cohort study, smokers had a higher risk of MACE and mortality over 5 years, as well as an increased risk of lung cancer.[26] A recent review (Global Heart, 2024–2025) confirms that smoking accelerates atherosclerosis, causes endothelial dysfunction, hypercoagulability, oxidative stress, and that quitting smoking even for a few months significantly reduces cardiovascular risk [27].

In our study, smoking increased the risk of complications

by 8.37 times (p<0.001), the risk of developing LV aneurysm by 6.56 times (p=0.011) in the obese group. This is strong evidence of the synergistic effect of smoking and obesity, consistent with current evidence of increased endothelial dysfunction and thrombosis with the simultaneous action of multiple RFs [27].

In the aforementioned study of young STEMI patients, smoking cessation within a year reduced the risk of MACE to that of nonsmokers [25]. This is supported by the results of the Global Heart Review, which shows that the benefits of smoking cessation begin to be apparent after 1-2 years [27].

Several large registries, including ISACS-STEMI (16,000 patients, COVID pandemic), show the "smoker's paradox" [28], i.e. reduced in-hospital and 30-day mortality in smokers after PCI. However, subsequent analyses suggest that age plays a role, as smokers are generally younger. Furthermore, the short-term benefits of rapid reperfusion outweigh the systemic long-term effects of smoking. It should be emphasized that this paradox does not imply a positive effect of smoking; it reminds us of the need to adjust for age and clinical factors in statistical analyses. Thus, smoking, type 2 diabetes, DLP, reduced GFR, and late hospital admission are the main predictors of a complicated AMI in OW and obese patients. This requires a more intensive multidisciplinary approach to the management of such patients, starting from the outpatient observation stage.

CONCLUSIONS

The total number of complications is observed three times more frequently in obese patients and approximately twice as frequently in overweight patients compared to those with normal body weight. Regardless of body mass index, significant predictors of complications include smoking, dyslipidemia, late hospitalization, and the simultaneous presence of five to six risk factors. At the same level of smoking intensity, the risk of complications in the early post-infarction period increases with a higher body mass index. Smoking increases the likelihood of complications by 1.5 times in patients with normal body weight and by eight times in obese patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Clinical effectiveness of various methods of inguinal hernia repair

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ABSTRACT

Aim: To improve the results of surgical treatment of patients with inguinal hernias.

Materials and Methods: The paper analyzes the results of surgical treatment of 198 patients who underwent inguinal hernia repair in the period from 2018 to 2024. The study was conducted at the clinical base of the Department of General Surgery of Uzhhorod National University - KNP «Uzhgorod City Multidisciplinary Clinical Hospital» of the Uzhgorod City Council of the SE «Clinical Hospital of Planned Treatment» The study was a bidirectional (ambidirectional study), namely, it included: 1) retrospective analysis ("case-control", randomized multicenter analysis); 2) prospective observation and treatment. The inclusion criteria in the study were: the presence of acquired inguinal hernia, hernioplasty according to the Lichtenstein, Shouldis method, TAPP hernioplasty (transabdominal preperitoneal hernioplasty) and TAPP with suturing of the internal inguinal ring (TAPP with SIIR) and TEP - total extraperitoneal allohernioplasty, which was performed in the preperitoneal space without entering the abdominal cavity.

Results: The smallest number of complications was recorded after laparoscopic TAPP and TAPP-SIIR hernioplasty, including in people with overweight and obesity. The results obtained were statistically processed with the inclusion of all possible risk factors that could affect the postoperative outcome of each specific patient. Based on the obtained values, χ2 in all cases was equal to or greater than the value of 3.99, which exceeds the critical value of 3.84; p=0.05..

Conclusions: A comprehensive differentiated approach to preoperative preparation of patients with inguinal hernias with correction of comorbid pathology, body mass index and factors that increase intra-abdominal pressure significantly reduces the frequency of early postoperative complications compared with standard treatment tactics (χ^2>3.84; p≤0.05).

KEY WORDS: inguinal hernias, «Trans Abdominal Pre-Peritoneal» – hernioplasty, «Total Extra Peritoneal» – allohernioplasty, Lichtenstein method, Shouldis method

Wiad Lek. 2026;79(5):997-1002. doi: 10.36740/WLek/220831 DOI

INTRODUCTION

About 20 million hernia operations are performed annually in the world. Inguinal hernias (IG) account for about 75% of all abdominal wall hernias. The incidence of IG in adults increases with age, especially after 70 years. Prognostic calculation of the risks of developing IG indicates that this pathology can affect almost 25% of men and 2% of women in one way or another during their lives. At the same time, men account for almost 90% of all cases of the disease. The share of emergency surgical treatment of IG in developed countries is 2.5-7.7%, and in developing countries it can reach 76.9%. Mortality in emergency repair of inguinal hernia is from 1.7% to 7.0%, but in cases of necrosis and intestinal resection reaches 25%. The high frequency of recurrence of inguinal hernias only increases the urgency of the problem. For example, after autoplastic methods of plastic surgery, recurrences range from 10% to 30%, and after plastic surgery according to Shouldice, recurrences reach 6% (on average 3.6%); after prosthetic plastic surgery according to Liechtenstein - 2.4% (on average 0.8%) [1-4].

In some articles, the basis of research of which were hospitals specializing only in herniotomy, the frequency of recurrences for decades does not exceed 1%. Undoubtedly, the use of endoscopic methods of hernioplasty has allowed to reduce the number of recurrences. For example, in countries, the cumulative percentage of such complications, calculated for a 5-year period, is generally slightly more than 3.7%. The success of operations also depends on the presence or absence of various concomitant aggravating diseases: overweight (OB), hypertension, diabetes mellitus, obesity. At the same time, comparative studies on the influence of concomitant comorbid factors on the outcome after inguinal hernia repair are reported extremely rarely in the modern literature, which does not satisfy specialists and requires a comprehensive study of this issue, which necessitates a deeper study of this issue [5-8].

The modern stage of herniology development is characterized by the transition from tension techniques to alloplasty according to the "tension-free" principle, which has significantly reduced the frequency of recurrence of inguinal hernias. Open hernioplasty according to Lichtenstein has long remained the standard of treatment due to its technical simplicity and reliability of results. At the same time, laparoscopic techniques TAPP and TEP have become widely used, which provide preperitoneal placement of a mesh implant with overlapping of all potential hernial defects. Their use is associated with less intensity of postoperative pain, faster rehabilitation and the possibility of simultaneous correction of bilateral and recurrent hernias. The TEP technique has the additional advantage of being performed without entering the abdominal cavity, which reduces the risk of intra-abdominal complications [6,9-11].

The choice of the optimal method of hernioplasty should be based on an individualized approach, taking into account the patient's clinical characteristics and risk factors [12-15].

AIM

To improve the results of surgical treatment of patients with inguinal hernias.

MATERIALS AND METHODS

The paper analyzes the results of surgical treatment of 198 patients who underwent inguinal hernia repair in the period from 2018 to 2024. The study was conducted at the clinical base of the Department of General Surgery of Uzhhorod National University - KNP «Uzhgorod City Multidisciplinary Clinical Hospital» of the Uzhgorod City Council of the SE «Clinical Hospital of Planned Treatment» The study was a bidirectional (ambidirectional study), namely, it included: 1) retrospective analysis ("case-control", randomized multicenter analysis); 2) prospective observation and treatment. The inclusion criteria in the study were: the presence of acquired inguinal hernia, hernioplasty according to the Lichtenstein, Shouldis method, TAPP hernioplasty (transabdominal preperitoneal hernioplasty) and TAPP with suturing of the internal inguinal ring (TAPP with SIIR) and TEP - total extraperitoneal allohernioplasty, which was performed in the preperitoneal space without entering the abdominal cavity.

The following were considered indications for TEP:

- primary unilateral and bilateral inguinal hernias
- absence of entrapment
- absence of pronounced adhesions after previous operations on the abdominal organs.

When performing TEP, a mesh polypropylene implant of at least 10×15 cm in size was used with the overlap of all potential hernial defects of the myopectineal foramen. Ranking of patients by body weight was performed based on the WHO classification, while calculating the body mass index (BMI) in kg/m2. According to the Asian overweight classification, patients were considered underweight if their BMI was <18.5; normal weight (18.5 BMI<23); overweight (23≤BMI<25); obese (if BMI≥25) and severely obese (if BMI≥30 kg/m2). Statistical analysis was performed using Statistica 10.0 and SPSS ver. 10.0.

FRAMEWORK

The work was carried out in the framework of research work 0124U002167 «Monitoring of traumatic disease against the background of chronic stress».

RESULTS

The study involved 33 (16%) women, 165 (84%) men. Patients were divided into two groups: group B (study) 97 patients (49%), for whom the proposed treatment complex was used, and group A (control) 101 patients (51%) - for whom the proposed algorithm was not used. There were 139 (70.0%) patients with normal BMI, 41 (21%) with overweight, 18 (9%) with obesity. The age composition of the operated patients ranged from 18 to 78 years. The average age of all patients was 59.2±10.6 years. There were 34 (17%) young people, according to the WHO classification «from 18 to 44 years». Almost all of them were overweight (BMI≥23) or engaged in heavy physical labor.

Among the older age group, patients with normal weight and the presence of one or more concomitant aggravating diseases prevailed. 156 patients were operated on in a planned manner, which is 79%. Emergency surgery was performed in 42 people, or 21% of patients. Hernias were more often located on the right - in 57.0% of patients, on the left - in 43.0%, including bilateral hernias observed in 8 (4%) people. Among the patients of the study group with concomitant aggravating factors, who underwent surgical intervention in a planned manner, preoperative preparation was carried out in cooperation with related specialists in order to correct indicators of glycemia, hypertension, spirometry, heart function, body weight. At the same time, necessary measures were taken to minimize factors contributing to increased intra-abdominal pressure: treatment of benign prostatic hyperplasia, combating constipation, etc. The choice of anesthetic care depended on the surgical technique. In laparoscopic hernioplasty, general anesthesia was used, in open surgical

Table 1. General characteristics of surgical interventions in both groups

Hernioplasty method	Number of patients, n = 198	
	Group A	Group B
TAPP	25 (25%)	15 (15.5%)
TAPP with SIIR	3 (2%)	30 (30.9%)
TEP	22 (22%)	24 (24.7%)
Lichtenstein method	30 (30%)	22 (22.7%)
Shouldis method	8 (8%)	6 (6.2%)
Autohernioplasty	13 (13%)	0
Total	101	97

Source: compiled by the authors of this study

methods, spinal anesthesia. In rare cases, with a high risk of infarction, thromboembolism, etc. in elderly patients with severe concomitant pathology of the cardiovascular and respiratory systems, local infiltration anesthesia was used. In the study group, surgical treatment was primarily based on the latest guidelines of the international «Hernia Surgery Group». In all cases, a mesh graft was used, and the posterior wall of the inguinal canal was plasticized. The operations were performed by open methods (according to Liechtenstein, Shouldice) and laparoscopically (according to the TAPP method). TAPP surgery was performed in two versions: a) TAPP without suturing the internal inguinal ring; b) TAPP with suturing the internal inguinal ring. Videolaparoscopic hernioplasty was not used for incarcerated inguinal hernias. In young individuals, in order to preserve the spermatic cord membranes and prevent adverse effects on testicular function and spermatogenesis, surgical interventions were performed using the TAPP or Shouldice method. The distribution of surgical intervention methods in the control and study groups is presented in Table 1.

In terms of the frequency of early postoperative complications, TEP was comparable to TAPP and was characterized by less trauma compared to open methods of hernioplasty. In the algorithm of management of patients with inguinal hernias proposed by us, one of the leading links is the correction of concomitant complicating factors in cooperation with therapists, cardiologists, endocrinologists and dietitians.

Thus, all the studied patients of group B with diabetes mellitus achieved a preoperative glycemia level of no higher than 8.5 mmol/l, except for one patient who was operated on urgently due to pinching (glycemia level 11.4 mmol/l).

Patients with concomitant arterial hypertension were selected for conservative treatment in order to reduce blood pressure to no higher than 150/90 mmHg.

Patients with concomitant heart failure were treated conservatively to maintain an ejection fraction of no lower than 55%, diuretics were used to eliminate edema.

A few months before the surgical treatment of patients with obesity of the I-II degree, in cooperation with a dietician, it was possible to include patients with excess body weight in the group. And patients who did not fall into this category according to the BMI index within the allotted time, had their surgical intervention postponed until the target weight indicators were achieved. body.

To prevent and reduce the severity of inguinal pain in all patients of group B, a TAP block and the administration of analgesics were performed before surgery according to the pain control scheme proposed by the European Herniology Association. The severity of pain was assessed 6 hours after surgical treatment using the VAS scale.

In patients of the study group, endoprostheses made of bioinert monofilament polypropylene were used for allohernioplasty, with a surface density of 34 g/m2, not less than 6x11 cm in size. Based on the statement of modern authors that any inguinal hernia is a consequence of stretching or the occurrence of a defect in the transverse fascia, in all cases of Lichtenstein plastic surgery, the internal inguinal ring was formed by applying a continuous suture (or knotted sutures) to the transverse fascia according to Joffe.

Considering that with the laparoscopic TAPP technique, the internal inguinal ring is the site of formation of recurrent hernias, in order to To reduce the risk of recurrence, the study group underwent intracorporeal suturing of this ring, after which a mesh implant was applied and fixed.

Laparoscopic methods of surgical treatment were not used for incarcerated hernias. In emergency cases, Shouldis inguinal canal repair was preferred. Large, inguinal-scrotal incarcerated hernias without signs of hernial sac inflammation were operated on using the Lichtenstein method under the cover of antibacterial therapy.

Analysis of the frequency and nature of postoperative complications shows that complications are most often

Table 2. Complications of different hernioplasty methods

Complications	Shouldis method		Lichtenstein method		TAPP		TEP		Autohernioplasty	
Group	A	B	A	B	A	B	A	B	A	B
Scrotal swelling	1	0	6	1	3	1	1	0	8	-
Infiltrate	2	1	5	2	2	0	1	0	10	-
Seroma	0	0	3	4	2	1	1	0	6	-
Purulent complications	0	0	1	0	1	0	0	0	3	-
Acute urinary retention	1	1	2	1	0	0	1	1	4	-
Total	4	2	17	8	8	2	4	1	31	

Source: compiled by the authors of this study

Table 3. Results of different hernioplasty methods

Complications	Shouldis method		Lichtenstein method		TAPP		TEP		Autohernioplasty	
Group	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
Pain severity (Visual Analogue Scale)	5.1	4.2	5.4	4.4	4.6	3.5	3.9	3.1	6.7	-
Average hospital stay (days)	6.1	4.4	5.4	3.9	3.1	2.9	2.5	2.3	9.4	-
Recurrence of the disease (after a year)	0	0	1	0	2	0	0	0	3	-

Source: compiled by the authors of this study

recorded after open methods of hernioplasty (Table 2).

Moreover, among people with overweight and obesity, these complications were more common.

It is characteristic that seroma was detected when using a mesh implant (according to the Lichtenstein method) in both groups. This is explained by the rather high traumatic nature of this surgical intervention. However, this figure is much lower in patients who underwent outpatient preparation for surgical treatment in group B.

The smallest number of complications was recorded after laparoscopic TAPP and TAPP-SIIR hernioplasty, including in people with overweight and obesity (Table 3)

The use of laparoscopic hernioplasty techniques is accompanied by less intensity of postoperative pain syndrome, a shorter hospitalization period, and a lower incidence of local complications compared to open plastic surgery methods.

The results obtained were statistically processed with the inclusion of all possible risk factors that could affect the postoperative outcome of each specific patient. Based on the obtained $\chi 2$ values, the presence of a statistical relationship between the risk factors under study and the outcome was confirmed at the appropriate level of significance (the chi-square criterion in all cases was equal to or exceeded the value of 3.99, which is greater than the critical value of 3.84; $p=0.05$).

DISCUSSION

Throughout the history of herniology, a large number of surgical methods for the treatment of hernias have been proposed. Today, there are 80–350 methods for plastic surgery of the inguinal canal with local tissues. Most surgeons for all forms of inguinal hernias prefer operations aimed at strengthening the posterior wall of the inguinal canal. They consider it a significant prospect in improving treatment results to give preference to "non-tension" methods of hernioplasty compared to "tension" ones.

The most popular in the world has become the multilayer deep plastic surgery of the posterior wall of the inguinal canal, proposed by E. Shouldice. The method is recommended as the best when it is impossible to use a mesh in hernioplasty [1-4].

Over the past 30 years, the main method for the treatment of inguinal hernias has been and is the Lichtenstein operation [6]. With the development of laparoscopic techniques, hernioplasty methods using laparoscopic techniques (TAPP and TEP) are gaining increasing popularity among both patients and surgeons. The technique of laparoscopic hernioplasty without mesh endoprosthesis fixation helps to reduce the severity of postoperative pain. The laparoscopic method of inguinal hernia repair has a number of advantages, such as a lower incidence of postoperative complications, less severe postoperative pain, reduced hospitalization time, reduced costs for patient treatment and the duration of his disability. The most popular technique for surgical treatment of inguinal hernias today has become laparoscopic extraperitoneal hernioplasty [8].

The inclusion of the TEP technique in the range of laparoscopic interventions allowed:

- to avoid contact with the abdominal organs;
- to reduce the risk of intra-abdominal complications;
- to reduce the intensity of postoperative pain;
- to reduce the duration of hospitalization.

CONCLUSIONS

1. A comprehensive differentiated approach to preoperative preparation of patients with inguinal hernias with correction of comorbid pathology, body mass index and factors that increase intra-abdominal pressure significantly reduces the frequency of early postoperative complications compared to standard treatment tactics (χ^2>3.84; p≤0.05).
2. The use of laparoscopic hernioplasty techniques (TAPP and TEP) is accompanied by a lower intensity of postoperative pain syndrome, a shorter hospitalization period and a lower frequency of local complications compared to open methods of plastic surgery.
3. Intracorporeal suturing of the internal inguinal ring when performing TAPP reduces the risk of pseudorecurrence and recurrent inguinal hernias.
4. The highest frequency of postoperative complications and the longest period of inpatient treatment are noted after autohernioplasty, which limits the feasibility of its use in modern surgical practice.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Effectiveness of intravenous infusion protocol in improving quality of care and reducing complications among hospitalized patients

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ABSTRACT

Aim: This study aimed to evaluate the effectiveness of a structured intravenous infusion protocol in improving the quality of care and reducing IV-related complications among hospitalized patients.

Materials and Methods: A purposive sample of 72 nurses was divided equally into a study group (n = 36), who received the structured IV infusion training, and a control group (n = 36), who followed routine practice. Data were collected using a validated Quality of Care Scale, which included five domains: effectiveness, patient-centered, timeliness, efficiency and equity.

Results: Results showed a marked improvement in the study group's performance after the intervention. The mean of scores for all domains and overall quality of care was low in the pretest (i.e. Effectiveness 0.63, Timeliness 0.64, and Efficiency 0.61, overall, 0.63) while post-test scores increased markedly to 1.42, 1.42, 1.38 and 1.41 respectively. The IV-related complications have decreased in the study group following the intervention (posttest) including phlebitis, infiltration and infection. Although these reductions were not significant, a high significant improvement was observed in pain incidence, which dropped from 25% to 2.8% ($p<0.01$).

Conclusions: the structured intravenous infusion protocol effectively improved nurses' performance resulting in enhancing patients' quality of life. The significant decline in pain reflects an important enhancement in patient comfort and nursing performance.

KEY WORDS: intravenous infusion, IV protocol, quality of care, complications

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INTRODUCTION

Intravenous (IV) infusion is one of the oldest and fundamental treatment modalities in healthcare interventions. Whether being used as treatment, intervention (crystalloid transfusion or fluid resuscitation) or essential sustenance (in total parenteral nutrition), only a few therapies are delivered directly into the veins and to the target organ via the IV route [1]. The widespread prevalence of infusion therapy supports its clinical significance, but also serves as the potential root cause of inaccuracy and adverse events because of the complexity of infusion therapy, including prescribing, preparation, administration, monitoring, and discontinuation. For instance, inappropriate use of IV fluids and medications is linked to prolonged length of stay, higher complication rates, and higher costs [2]. Considering the severity of the stakes, the use of standardized intravenous-infusion protocols (VIPs) is a rational approach to improving quality of care and preventing complications in inpatient settings [3]. Thus, the quality of care rendered through IV infusions is dependent on many factors: consistency of fluid or medication selection, dosage and rate-infusion knowledge, understanding of compatibility and stability problems, operation of infusion pumps, documentation of therapy, and monitoring of the patient response and complications [4]. It has been noted in the literature that while infusion therapy is an essential aspect of patient care, there is still considerable variability in practice with little monitoring of best practices within hospitals. Therefore, the implementation of a standardized infusion protocol may help unite and streamline these processes [5]. In addition to harmonization of procedures, the implementation of infusion protocols is also associated with patient safety and care outcomes. Standardization of intravenous (IV) medications is known to decrease medication errors and medication-related adverse events in inpatient settings. Protocolized approaches to fluid and electrolyte therapy lead to decrease in inappropriate fluid usage that in some studies can

affect up to one in five patients receiving intravenous therapy. Consequently, infusion protocols are not merely operational guidelines; they are quality-advancing instruments with a direct impact on patient-oriented outcomes [6]. Recent studies have reaffirmed hospital complications related to IV therapy. For instance, a hospital audit identified opportunities for documented standardized protocols for both intermittent and continuous infusions, primary motivators for reuse of sets, and bloodstream infection risk; and as a result, achieved reduced rates of inappropriate reuse of sets, decreased risk of bloodstream infection, improved documentation and improved pump-use safety [7]. Likewise, a cross-sectional survey of IV fluid administration by nurses and midwives determined IV practice deficiencies (e.g. incorrect infusion rates or inadequate monitoring) that were linked to greater complication rates. These results confirm that the infusion process is prone to human, technical and organizational errors and that protocols can address these failings [4]. Quality of care is a multidimensional concept which goes beyond access as it includes effectiveness, efficiency, safety, timeliness, patient-centeredness and equity. Accordingly, IV infusion protocols have been shown to be directly related to organizational priority and clinical priority. Infusion protocols underpin evidence-based practice by standardizing practitioner behavior and minimizing unwarranted variation [8]. Implementation of standardized IV medication protocols incorporated in a systematic review was associated with improved guideline adherence and a reduction in the incidence of medication errors and improvement in workflow efficiency that collectively enhance the quality of healthcare delivery [5]. For example, within inpatient care, where IV infusion is one of the most common procedures, such standardized excellence could translate to significant complication reductions, shorter lengths of stay, and improved patient experience [9]. The existing literature, however, demonstrated that most of the studies of infusion protocols were addressed only at medication infusion and often forgot to introduce the term of infusion therapy that includes fluids, electrolytes, nutrition and several-component therapies [10]. Additionally, there have been limited studies in non-homogeneous inpatient wards (surgical, medical, critical care) or hospitalized populations from non-high-income settings. Similarly, few studies addressed the long-term outcomes of complication rates, re-admission rates, or cost outcomes by different infusion protocols. This diminished generalizability suggests that more research is necessary to assess more global infusion initiatives and quality of care outcomes in large hospital populations.

AIM

The aim of the current study assessment was to determine if VIP is associated with better patient care indicators, that is fewer actual complication rates in Iraqi hospitals and to evaluate the effectiveness of a structured intravenous infusion protocol in improving the quality of care and reducing IV-related complications among hospitalized patients

MATERIALS AND METHODS

This quasi-experimental study was conducted to assess the impact of an intravenous (IV) treatment protocol on quality nursing care and complications related to IV among hospitalized patients. The current study was carried out in Al-Hussein Teaching Hospital, Diwaniyah, Iraq, from November 1st 2024 to May 10th 2025. Seventy-two nurses meeting the inclusion criteria were selected through purposive sampling. They were randomly assigned into two groups: the study group included 36 nurses who received IV infusion protocol training program, and a control group included 36 nurses without special education on the hospital that continued with the usual activities of the hospital. Nurses who were working in inpatient settings, had at least 1 year of clinical experience performing IV therapy, and agreed to participate voluntarily were eligible. Nurses on long leave and not directly practicing intravenous therapy were excluded. The reason we used purposive sampling was that the participants with IV care experiences could be included in our study as much as possible to maximize practical and accurate results. Patients who received the intravenous (IV) infusion intervention from both the study and control nurse groups were comparable with respect to potential confounding variables that could influence IV-related complications, including age, sex, type of infused solution, catheter gauge, and insertion site. The study instrument was constructed by the researchers to gather information. The instrument was divided into three main sections. Part I: Socio-demographic data such as age, sex, educational level, prior training on IV infusion protocol awareness, number of training sessions attended, type of previous trainings, and hours spent being trained were collected in the first part. The second part is the Quality of Care Scale to assess key dimensions of nursing care during IV infusion, including: effectiveness, patient-centered, timeliness, efficiency and equity. Content validity of the instrument was confirmed by a panel of ten experts who are well-versed in nursing education and clinical practice; a S-CVI/Ave (scale-level content validity index) score of 0.97 was obtained, representing excellent content validity. The Cronbach's alpha to measure reliability

Table 1. Descriptive statistics (frequency and percentage) for the demographic data of both study and control groups

Demographic data		Control Group		Study Group		χ^2 P value
		Freq. (N=36)	[%]	Freq. (N=36)	[%]	
Age / Years	22-28	7	19.4	8	22.2	0.21 0.99 (NS)
	29-35	9	25	9	25	
	36-42	8	22.2	7	19.4	
	43-49	6	16.7	6	16.7	
	≥ 50	5	13.9	6	16.7	
Gender	Male	16	44.4	15	41.7	0.06 0.81 (NS)
	Female	20	55.6	21	58.3	
Educational Status	School of Nursing	9	25	8	22.2	0.13 0.98 (NS)
	Institute of Nursing	11	30.6	12	33.3	
	College of Nursing	12	33.3	13	36.1	
	Postgraduate	4	11.1	4	11.1	
Training on IV protocol	Yes	22	61.1	23	63.9	0.06 0.80 (NS)
	No	14	38.9	13	36.1	
No. of Training Sessions	0	14	38.9	13	36.1	0.07 0.96 (NS)
	1-2	13	36.1	14	38.9	
	≥ 3	9	25	9	25	
Duration of Training/ Hours	0	14	38.9	13	36.1	0.25 0.96 (NS)
	1-5	10	27.8	12	33.3	
	6-10	6	16.7	6	16.7	
	≥ 11	5	13.9	6	16.7	

NS: Non-Significant at P>0.05
Source: Compiled by the authors of this study

Table 2. Differences in mean of scores of overall and domains of Quality-of-Care between (pre-test and post-test) measurements for the study group

Domains	Study Group Tests	Mean	SD	Paired T-Test	df	P-value
Effectiveness	Pre-test	0.63	0.18	8.92	35	0.001 HS
	Post-test	1.42	0.18			
Patient-centered	Pre-test	0.65	0.21	9.05	35	0.001 HS
	Post-test	1.42	0.12			
Timeliness	Pre-test	0.64	0.24	9.18	35	0.001 HS
	Post-test	1.42	0.11			
Efficiency	Pre-test	0.61	0.21	8.75	35	0.001 HS
	Post-test	1.38	0.31			
Equity	Pre-test	0.61	0.21	7.85	35	0.001 HS
	Post-test	1.42	0.11			
Overall Quality-of-Care	Pre-test	0.63	0.22	10.02	35	0.001 HS
	Post-test	1.41	0.18			

SD: standard deviation, df: degree of freedom, HS: high significance at P<0.01
Source: Compiled by the authors of this study

for all scale items was investigated and showed a high internal consistency, with values greater than 0.85. Expert validation, pilot testing and repeated measures were used to guarantee the validity and reliability of the instruments. Before conducting the main study, a pilot-study was carried out with eight nurses to test the clarity, applicability and feasibility of data collection tools. Revisions to enhance clarity and the organization of the observation checklist were incorporated according to participant feedback. The results of the pilot study are not included in the main analysis to avoid bias. The educational intervention for study group was

Table 3. Differences in means of scores for overall and domains of quality of care between (pre-test and post-test) measurements for the control group

Domains	Control Group Tests	Mean	SD	Paired T-Test	df	P-value
Effectiveness	Pre-test	0.61	0.19	1.42	35	0.16 NS
	Post-test	0.71	0.2			
Patient-centered	Pre-test	0.64	0.2	1.28	35	0.21 NS
	Post-test	0.73	0.21			
Timeliness	Pre-test	0.65	0.21	1.11	35	0.27 NS
	Post-test	0.72	0.22			
Efficiency	Pre-test	0.59	0.2	1.34	35	0.19 NS
	Post-test	0.68	0.21			
Equity	Pre-test	0.6	0.2	1.47	35	0.15 NS
	Post-test	0.72	0.22			
Overall Quality-of-Care	Pre-test	0.62	0.2	1.38	35	0.17 NS
	Post-test	0.71	0.21			

SD: standard deviation, df: degree of freedom, NS: Non-Significance at P>0.05
Source: Compiled by the authors of this study

Table 4. Comparison of complications of IV Therapy at the pre-test measurement between study and control groups

Demographic data		Control Group		Study Group		P value
		Freq. (N=36)	%	Freq. (N=36)	%	
Phlebitis	Yes	2	5.6	3	8.3	0.64 NS
	No	34	94.4	33	91.7	
Infection at the insertion site	Yes	3	8.3	2	5.6	0.64 NS
	No	33	91.7	34	94.4	
Occlusions	Yes	1	2.8	1	2.8	1.00 NS
	No	35	97.2	35	97.2	
Extravasation	Yes	1	2.8	2	5.6	0.55 NS
	No	35	97.2	34	97.2	
Bleeding at the insertion site	Yes	2	5.6	1	2.8	0.55 NS
	No	34	94.4	35	97.2	
Pain	Yes	8	22.2	9	25	0.78 NS
	No	28	77.8	27	75	

NS: Non-Significant at P>0.05
Source: Compiled by the authors of this study

a predetermined training program developed based on standard IV infusion guidelines. It was a two-week program with four intensive sessions that combined theoretical lectures and practical demonstrations. The theoretical aspects drew from principles of IV therapy, infection control, safe administration of IV fluids and medication, as well as documentation. Workshops were skill based, including correct IV insertion and trouble-shooting (IV complications – phlebitis or infiltration) as well as adherence to aseptic technique by applying set criteria. Each session varied in length (1-1.5 hours) and was accompanied by educational materials including a pictorial manual, checklists and visual prompts. The control group was not given any form of training or intervention during the whole trial period and maintained their routine nursing mode. Two measurements were scheduled for the two groups: one before training program (pre-test) and the other, two weeks after completion (post-test). Each nurse was observed by the researcher three times during each phase of the study, all with the same observation list. The average of the 3 observation scores was taken for analysis to attenuate individual variability and observer effects. Based on these observations, scores were recorded as follows: (0 = Not done; 1 = partially done; 2 = completely done). For each domain of nursing practice and quality of care, scores were summed and converted to mean scores. Higher mean scores indicated improved practice

Table 5. Comparison of complications of IV Therapy at the post-test measurement between study and control groups

Demographic data		Control Group		Study Group		P value
		Freq. (N=36)	[%]	Freq. (N=36)	[%]	
Phlebitis	Yes	3	8.3	0	0.0	0.07 NS
	No	33	94.4	36	100	
Infection at the insertion site	Yes	2	5.6	0	0.0	0.15 NS
	No	34	91.7	36	100	
Occlusions	Yes	1	2.8	0	0.0	0.07 NS
	No	35	97.2	36	100	
Extravasation	Yes	1	2.8	0	0.0	0.07 NS
	No	35	97.2	36	100	
Bleeding at the insertion site	Yes	2	5.6	0	0.0	0.15 NS
	No	34	94.4	36	100	
Pain	Yes	9	25	1	2.8	0.01 HS
	No	27	77.8	35	97.2	

NS: Non-Significant at P>0.05

Source: Compiled by the authors of this study

performance and quality of care. A larger mean score demonstrated better nursing performance and quality of care after the intervention.

Statistical analysis was conducted using IBM SPSS Statistics version 27. Descriptive statistics such as frequency, percentage, mean, and standard deviation were employed in the description of demographic information and performance scores. Comparisons between the study and control groups, as well as within each group (pre to post) were made using inferential statistics (paired and independent t-tests). A p-value equal to or lower than 0.05 was considered statistically significant. All ethical issues were approved by the Scientific Research Ethics Committee at the Faculty of Nursing, University of Kufa. Approval for the study was obtained from Al-Hussein Hospital administration. Informed consent was obtained from all participants, with participation being entirely voluntary. All-round confidentiality and anonymity of participants were ensured throughout the research. Nurses were told that they could quit the study at any time without being penalized.

RESULTS

The demographic characteristics of participants in the study and control groups are shown in table 1. The results reveal an even distribution across all demographic factors with no statistically significant differences between groups (Chi-square test, $p > 0.05$). This implies that both groups were similarly homogeneous prior to the intervention.

Table 2 showed a statistically significant difference for all quality of care domains between study group before and after implementation of IV fluid protocol. Pre-test scores were poor-to-moderate in for most domains (Effectiveness mean = 0.63, Timeliness mean = 0.64, and Efficiency mean = 0.61), whilst post-test scores had shifted significantly towards the moderate-to-good (Effectiveness mean = 1.42, Timeliness mean = 1.42, Efficiency mean = 1.38). The paired t-test values were between 7.85 and 10.02, all statistically significant ($p < 0.001$), which indicate strong improvements in all domains. The Overall Quality-of-Care domain showed the largest gain ($t = 10.02$; $p < 0.001$), suggesting that the overall quality and safety of nursing performance significantly improved with the introduction of the structured IV fluid protocol.

The results of the control group revealed only a slight improvement in quality-of-care scores from the pre-test to the post-test across all domains, but the changes did not reach statistical significance. For example, the mean score for effectiveness increased from 0.61 ± 0.19 at baseline to 0.71 ± 0.20 post-test ($t=1.42$, $p=0.16$), while patient-centered care rose from 0.64 ± 0.20 to 0.73 ± 0.21 ($t=1.28$, $p=0.21$). Similarly, timeliness improved from 0.65 ± 0.21 to 0.72 ± 0.22 ($t=1.11$, $p=0.27$), and efficiency from 0.59 ± 0.20 to 0.68 ± 0.21 ($t=1.34$, $p=0.19$). The equity domain showed a comparable trend (0.60 ± 0.20 to 0.72 ± 0.22; $t=1.47$, $p=0.15$). The overall quality-of-care score also increased marginally from 0.62 ± 0.20 to 0.71 ± 0.21 ($t=1.38$, $p=0.17$) as shown in (Table 3).

The results of complications revealed that there were no statistically significant differences between the study and control groups regarding IV therapy-related complications at the pre-test stage ($p>0.05$ for all items). The frequencies of phlebitis (5.6% for control in comparison to 8.3% for study), infection at the insertion site (8.3% *vs.* 5.6%), occlusions (2.8% *vs.* 2.8%), extravasation (2.8%

vs. 5.6%), bleeding (5.6% *vs.* 2.8%), and pain (22.2% *vs*. 25%) were relatively comparable between both groups (Table 4).

On the other hand, table 5 explained that the IV-related complications have decreased in the study group following the intervention (post-test measurement) including phlebitis, occlusion, infiltration extravasation, bleeding and infection at the insertion site. Although these reductions were not significant, a high significant improvement was observed in pain incidence, which declined from 25% to 2.8% ($p<0.01$).

DISCUSSION

This study results revealed that, there are a highly significant overall improvements in all domains of the Quality-of-Care Scale post educational intervention among the interventional compared to control. All the domains tested (effectiveness, patient-centeredness, timeliness, efficiency and equity) reflected a uniform increase in the average scores of quality of care with p values<0.001 in all instances—indicating that there is high degree statistical significant difference between these domains. This progress supports the importance of structured training curricula in improving nurses' practice and performance and the quality of patient care. Regarding effectiveness, the mean score grew from 0.63 ± 0.18 at pre-test to 1.42 ± 0.24 at post-test in compliance with clinical orders and documentation criteria. The mean in control group was still lower (0.71 ± 0.21), indicating no spontaneous improvement without therapy. This is consistent with previous studies that have shown nurse education programs are beneficial in increasing adherence to medication regimes, administering medications and recording the correct amounts of medication. Increased effectiveness may also derive from increased knowledge of evidence-based practice and SOPs, resulting in safety patient care [11-12]. In patient-centered nursing care, the pre- and post-test results indicated that the mean score increased significantly (pre = 0.65 ± 0.20; post = 1.42 ± 0.25), suggesting that there was a change on a better side for behaviors of communication and empathy among nurses after training to participants in this dimension of patient-centered nursing care ($p <0.05$). Control group remained 0.73 ± 0.22, which is little changes in its level of improvement. This improvement corresponds to the findings of Fawzy and Hamed [12], who found that patient-centered interventions enhanced the ability of nurses to engage patients in care decision making as well as help in increasing comfort and decreasing anxiety levels. At the same time WHO (2020) highlighted that an ideal aspect of care quality is patient-centeredness, which creates trust and leads to adherence. The educational intervention in this study seemed to enable the gap of communication between a patient and caregiver efficiently [13]. In the timeliness domain as well, progress was also marked. 0.64 ± 0.20 before/1.42 ± 0.24 after pre and post-tests respectively: care—acuity merited med/IV as specified by orders Meets patient outcomes and satisfaction, is subject to the actual time of medicine or IV. The higher scores on second parts show the subjects have been led astray, from paying more attention to quickly responding time and arrangement of order than to accuracy in their exiting or migrating movement of work location (08). This reflects the work of Prosser et al. [14]. They discovered that teaching nurses how to use clinical workflows can effectively reduce infusion delays by helping nurses reach the maximum safe infusion rate faster. The control group showed no significant change between pre and post assessment of patients' quality of care. The domain of efficiency also improved (0.61 ± 0.20 → 1.38 ± 0.24), indicating that more resources are being used, there is greater technical skill, and there is greater procedural capability on the post-test instruments. In post-test evaluation, the majority of the items were improved especially in regards to preparation of materials, selection of appropriate type of cannula and prevention of complications such as vein shredding or bleeding. This corroborates the findings by Kim et al. [15] who stated that increasing nurses' knowledge and self-efficacy in intravenous injection management are important predictors of intravenous care practice among adult care nurses. Therefore, targeted education and intervention programs should be implemented to improve adult nurses' knowledge and confidence in managing intravenous injections. The equity dimension also significantly improved, with an average of the scores' rise from 0.61 ± 0.21 to 1.42 ± 0.25. This suggests that, as the years progressed, nurses integrated fair and equal access to care among all populations of patients. The better results of this last period prove that the teaching has not only improved technical indicating competences but ethical and equitable values in care. Previous research, have likewise highlighted the role of continuous professional development programs in transforming a culture of equality, inclusivity and patients' dignity as part for delivering healthcare [16]. Comparison between assessment and control groups at post-test The study group had significantly higher scores than the control group in each of the five domains at baseline (p 120 % rise in mean scores-indicates that a structured competence based education equips for improved care

and subsequent patient status. In general terms, the results confirm the Donabedian model of healthcare quality that stresses structure, process and outcome. The intervention directly addressed the "process" domain, such as practice improvement, knowledge, and behavior change that led to better quality of outcomes [17]. Chen et al., also reported the same results, they performed a meta-analysis study and showed that nurses in tertiary hospitals significantly advanced nurse safety and quality after receiving competence education [18]. The present results further ease the evaluation of IV therapy related complication in both intervention and control group before and after the educational intervention. Such complications comprise phlebitis, site infection, occlusion, extravasation and bleeding or pain. No significant differences between groups were detected at the baseline pre-test ($p>0.05$), indicating that there were no group performance differences in IV management and complications prevention. By the post-test period, a significant decrease in complications related to IV therapy, including pain ($p=0.01$), was reported in the study group with at least other complications showing smaller decreases that were not statistically significant though very clinically meaningful. At baseline, participants in the study group compared with those in the control group were less likely to experience a complication (phlebitis 5.6% *vs* 8.3%, site infection 8.3% *vs* 5.6% for control and study group respectively). Other complications such as occlusions, extravasation and bleeding were uncommon with less than 6% of patients having these. These findings are typical complication rates presented in the clinical literature before targeted nursing initiatives [19]. The lack of difference between groups would suggest that both nurse groups were engaging in similar (potentially suboptimal) infection control practice and IV line management at baseline, further to this the lower rate of complications found across this study could also account for non-significant results. However, the outcomes of the study group improved significantly as after the educational intervention, the post-test results. The rate of phlebitis, infection, occlusion, and extravasation declined to zero, compared with the persistent rates of 8.3%, 5.6% and 2.8% in the control group respectively. While these differences were not statistically significant ($p > 0.05$), the direction represents clinically relevant gains suggesting translation of the intervention to clinical benefit by reducing IV-associated complications. Privitera et al. also recorded similar findings in regards to structured training and compliance with aseptic techniques reducing infections dislocations and occlusions in hospital wards [20]. As for side effects, the only side effect that achieved statistical significance was pain, which significantly decreased from 25% in the control group to 2.8% in the experimental group ($p = 0.01$). This finding suggests that education about proper cannulation and vein selection, and securement after cannulation may improve the comfort of the patients [19]. Despite the controls failing to make any impact to reduce the pain incidence (25%), 91% of the treatment group were able to alleviate their pain, underscoring the significance underlying clinical training skills. These results are in line with Gorski et al, who highlighted less patient suffering and discomfort during IV insertion as an important aspect of quality care and correlates highly with vein assessment and catheter stabilization [21]. In this study group, a decrease in complications in all categories was observed, possibly due to adherence to hand hygiene, aseptic technique, and close monitoring as taught in the intervention. Adequate cleansing over the insertion site, use of aseptic apparatus and specific protocols based on evidence would certainly play a role in their mitigation in terms of contamination via microbes as well as mechanical impact [22]. In addition, the larger bed numbers for low occlusion and extravasation were also due to the improved awareness of the timely replacement of IV lines and minimal handling. The educational strategy is likely to boost the confidence and accountability of nurses, which may encourage the application of practice more frequently [23]. In contrast, as per non-control group data, the control group revealed either equal or worse post-test complication rates; which is a reflection of the spontaneous ups and downs that occur in routine day-to-day practice, in the absence of intervention. Even more importantly, this means that the IV therapy safety will be sustainably improved by organized continuing practice and reinforcement, rather than by experience. Kaur et al. [24] also published similar findings in their study demonstrating that a widely implemented competence-based training was linked with improved adherence to safety practices in hospitals with a significant reduction of IV placement-associated complications. Avoid the importance of these results is only but convenience – the large international clinical trial. For example, complications including phlebitis, infection and pain may lead to prolonged hospitalization, increased healthcare cost and poor patient experience [25]. As a result, it is clinically and economically advantageous to limit such complications via focused nursing education. This is also consistent with WHO (2020) recommendations for global safe injection practices, where educating and monitoring are essential for ensuring lasting reductions in IV-related preventable injuries [13].

CONCLUSIONS

The implementation of IV infusion protocol has effectively enhanced nurses' practice regarding all domains of patients' quality of life after applying IV infusion interventions. All domains of patients' quality of life have been improved after receiving the protocol, including: effectiveness, patient-centeredness, timeliness, efficiency, and equity. The protocol intervention has clearly improved IV-related complications—particularly in pain reduction. Continuous in-service education, skill reinforcement, and regular performance monitoring are recommended to sustain these improvements and ensure safe and high-quality nursing care in IV therapy.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

CONTENTS

Neurodynamic correlates of chronic war-related stress: EEG evidence of cognitive and emotional vulnerability in women and men

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ABSTRACT

Aim: This study aimed to investigate whether chronic war-related stress producessex-differentiated reorganization of large-scale cortical functional connectivity, as measur ed by EEG coherence, and to identify neurodynamic markers of cognitive and emotional vulnerability in civilian populations exposed to ongoing armed conflict.

Materials and Methods: Fifty-three students of Taras Shevchenko National University of Kyiv (aged 18–24) voluntarily participated in the study. Pre-war groups (n = 24, nfem = 15) were examined between 2010 and 2012, before the onset of Russia's hybrid and full-scale invasion and the COVID-19 pandemic. War groups (n = 29, nfem = 18) were assessed during the ongoing full-scale invasion (2022–2024). EEG recordings were obtained at rest and during cognitive load induced by a mental arithmetic task. Magnitude-squared coherence was analyzed in θ1 (3.9–4.9 Hz), θ2 (5–6 Hz), β1 (13–20 Hz), and β2 (20–30 Hz) bands.

Results: Pre-war maps showed posterior-dominant coupling with robust P3–P4 and O1–O2 homotopy and a preserved Fz–Cz–Pz axis. Men additionally exhibited stronger C3–C4 and midline integration, whereas women showed a right-posterior bias and weaker fronto-posterior coupling. During the war, men demonstrated strengthened fronto-temporal coupling and accentuated dorsal midline links, while women displayed diffuse θ reinforcement over right temporoparietal and posterior midline regions, emergent cross-hemispheric fronto-temporal links, and persistently attenuated long-range coupling. In β bands, posterior homotopy weakened in both sexes, with frontal strengthening more pronounced in women.

Conclusions: Chronic war stress drives posterior-to-anterior reweighting of coherence, supporting reactivity through salience/control hubs at the expense of efficiency. Women's weaker long-range fronto-posterior coordination suggests heightened vulnerability and points toward sex-specific intervention strategies.

KEY WORDS: Electroencephalography, Neuropsychological Tests, Sex Characteristics, Armed Conflicts

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INTRODUCTION

Chronic stress is one of the key factors affecting human health, particularly in terms of mental state and cognitive abilities. Recent studies have demonstrated that chronic psychological stress can lead to substantial alterations in brain function, particularly in areas responsible for cognitive functions, emotional stability, and adaptive processes [1]. Changes in the electrical activity of the cerebral cortex can serve as important indicators of the impact of stress on the central nervous system, with long-term consequences for mental health and cognitive functioning [2].

In the active combat areas, civilians are often exposed to a unique stressor, which includes both external (physical threats, perceived danger) and internal (emotional and psychological) factors. The peculiarity of their situation lies in the specific sensitivity to prolonged psycho-emotional stress, which places an additional burden on their body and, in particular, on the nervous system. It is known that chronic stress significantly increases the risk of developing anxiety and depressive disorders, cognitive dysfunction, and even neurodegenerative diseases. In addition, prolonged exposure to stressors, such as constant air anxiety and night attacks, can cause sleep and circadian rhythm disturbances, which further worsen the state of mental health [3].

The relevance of the study is also emphasized by the need to develop and improve methods for assessing and correcting the cognitive and emotional state in women experiencing chronic stress. Understanding the mechanisms underlying changes in neurodynamics can help create effective programs of psychological support, rehabilitation, and adaptation [4,5].

In the context of modern research, chronic stress is a factor that can significantly change the functioning of the central nervous system [6]. Prolonged stress is associated with negative changes in the brain, particularly in its cortical structures, which play a crucial role in regulating cognitive processes. Civilian population in active military conflict are particularly vulnerable to the effects of prolonged stress. The constant feeling of danger, emotional tension associated with threats to life and health, can significantly affect their ability to respond adequately to stressors. Sleep disturbances, increased anxiety, and changes in the functionality of the cerebral cortex often accompany chronic stress in these conditions. Understanding the impact of these factors on cognitive functions and the general psycho-emotional state of women will help in developing strategies to improve their health.

The primary objective of the study was to investigate changes in the electrical activity of the cerebral cortex in the civilian population residing in an active military conflict zone. Using electroencephalography (EEG), it was planned to assess the impact of chronic stress on neurodynamic processes during cognitive tasks.

The study aims to identify possible cognitive impairments and changes in neurodynamics resulting from prolonged stress. Assessment of such changes allows us to form a general idea of the impact of chronic stress on brain activity. It indicates the need to develop approaches to their correction and prevention.

AIM

This study aimed to investigate whether chronic war-related stress producessex-differentiated reorganization of large-scale cortical functional connectivity, as measur ed by EEG coherence, and to identify neurodynamic markers of cognitive and emotional vulnerability in civilian populations exposed to ongoing armed conflict.

MATERIALS AND METHODS

Fifty-three volunteer students of Taras Shevchenko National University of Kyiv, aged 18-24, participated in the study on a volunteer basis. The data of the first group of subjects were obtained between 2010 and 2012, that is, before the start of Russia's hybrid and full-scale invasion of Ukraine, as well as the COVID-19 pandemic (n = 24, n_{fem}=15). EEG data of the second group of volunteers were recorded during the full-scale invasion (n = 29, n_{fem}=18).

Participants were informed about the content of the stimulation program; written informed consent was obtained from each subject following the World Medical Association (WMA) Declaration of Helsinki – Ethical Principles of Medical Research Involving Humans (Helsinki, Finland, June 1964), Declaration of Principles of Tolerance (28th Session of the General Conference of UNESCO, Paris, November 16, 1995), Conventions for the Protection of Human Rights and Human Dignity in the Use of Biology and Medicine: Convention on Human Rights and Biomedicine (Oviedo, April 4, 1997). For the sake of the study, ethical committee approval was obtained (Protocol #4, 26.06.2025).

A routine EEG was recorded using the Neurocom hardware and software complex (KhAI "Medica", Kharkiv, Ukraine). The electrodes were placed according to the international electrode placement system "10-20%. According to the study design, participants were asked to perform sequential subtraction of a 2-digit number (the subtractor) from a 4-digit number (the reduced number) (e.g., 4753 and 17, 3141 and 42, etc.) with their eyes closed. Mental arithmetic performance is a standardized stress-inducing experimental protocol [7,8]. Serial subtraction for 15 minutes is considered psychosocial stress [9]. In this way, our study design required subjects to engage in intensive cognitive activity. The intensive mental load is accompanied by a change in the emotional background when the subject makes an additional effort to resolve tasks, allowing for the discussion of evoked emotions in this case. Additionally, an EEG was recorded in a resting state with eyes closed before and after the task for 1 minute.

Further processing of the obtained data was carried out using the EEGLab software package [10] based on the MATLAB software environment. The main algorithm used for data analysis was magnitude-squared coherence. Permutation statistics were applied to detect strong coherent connections.

Magnitude-squared coherence (MSC) is an objective frequency-domain response detection method introduced by Dobie and Wilson [11] for analyzing evoked potentials. These researchers found that the technique can yield essential results in the field of research on the cortical response of the human brain. Magnitude-squared coherence (MSC) has been used in many areas of signal processing, for example, to measure the coherence between two realized complex signals [12, 13]. The MSC between the two one-dimensional wide-sense stationary signals $x(t)$ and $y(t)$ is defined as

$Coh^2 =$ (1),

where is the cross-spectral density estimate between the two signals ($x(t)$ and $y(t)$), and are the auto-spectral density estimates for the $x(t)$ and $y(t)$ signals, respectively.

The MSC measure in human EEG is a real number between 0 and 1 in each band and subband. If MSC is 0 for all frequencies, the two signals are not linearly de-

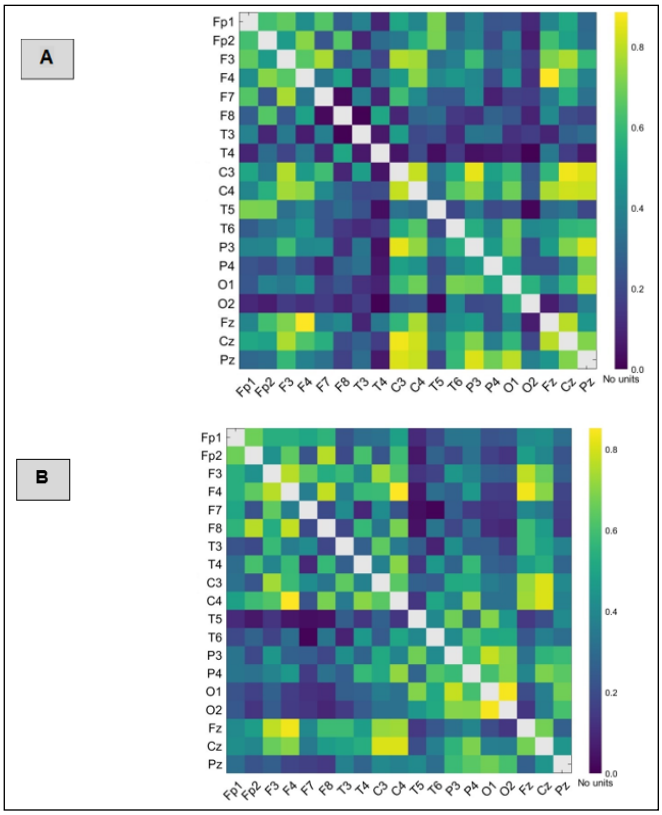


Fig. 1. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in θ1 EEG subband in (A) male group and (B) female group before the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
Picture taken by the authors

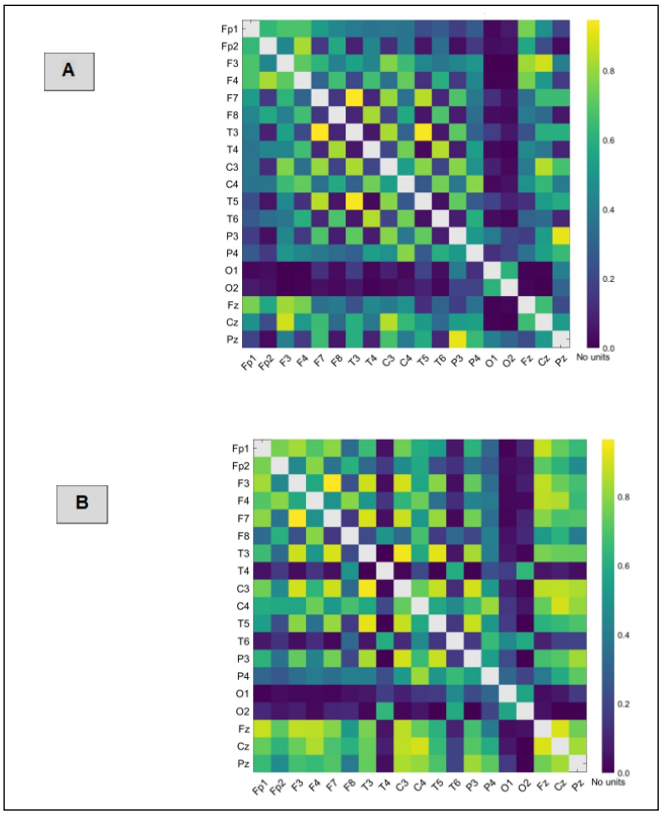


Fig. 2. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in θ1 EEG subband in (A) male group and (B) female group after the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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pendent; if MSC is 1 for all frequencies, the two signals are connected via a linear time-invariant (LTI) system. In other words, MSC is a measure that evaluates the degree to which one signal is predicted by another signal using a linear model and is implemented to analyze linear systems [14]. MSC can also be considered as a measure of the similarity of the frequency content of two signals [14]. The MSC is characterized by symmetry, which means that the MSC between two signals, *x(t)* and *y(t)*, is the same as the MSC between *y(t)* and *x(t)*.

RESULTS

Prior to war exposure, θ1 coherent connections were concentrated over the posterior cortices (Fig.1). In men (Fig.1, A), the distribution peaked along the posterior midline (Pz/Oz) with robust homotopic interhemispheric links (O1-O2, P3-P4) and a continuous dorsal axis (Fz-Cz-Pz), indicating preserved long-range integration. Women (Fig.1, B) exhibited a comparable posterior emphasis, but with a more pronounced right temporo-parietal bias (T4-P4/O2) and relatively attenuated fronto-posterior midline coupling, suggesting a more locally clustered posterior network.

Under ongoing war conditions (Fig.2), θ1 coherence shifted anteriorly. In men (Fig.2,A), fronto-temporal coupling (F3/F4-T3/T4) strengthened, while posterior interhemispheric links remained present but less dominant than before the war; dorsal midline connectivity (Fz-Cz) was accentuated. Women (Fig.2, B) exhibited diffuse θ1 reinforcement with maxima over the right temporoparietal and posterior midline regions (T4-P4 to Pz/Oz). Interhemispheric occipital and parietal links remained strong, yet fronto-posterior midline integration continued to be comparatively weaker than in men.

Before war exposure (Fig.3), θ2 coherence again manifested within the posterior association cortices. Men (Fig.3, A) displayed pronounced homotopic coupling

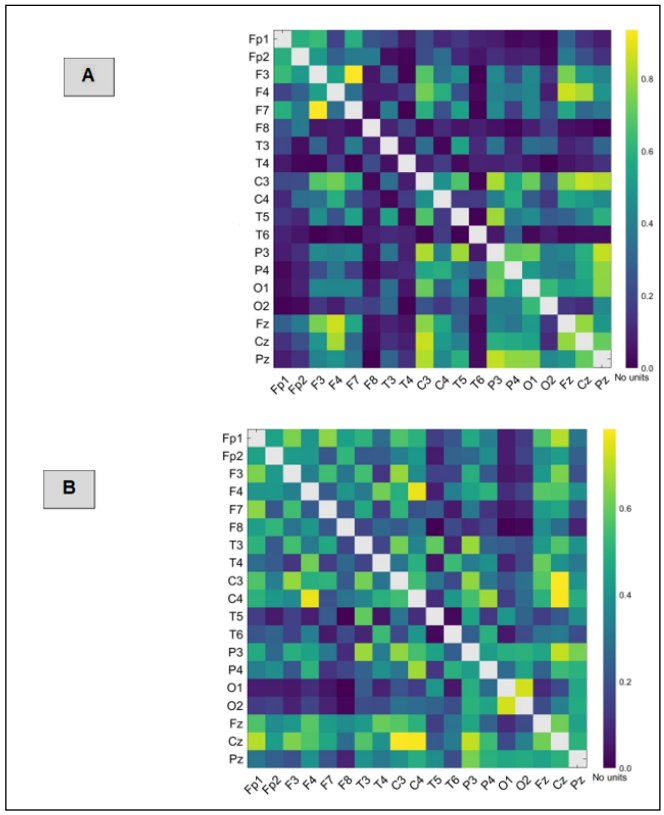


Fig. 3. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in θ2 EEG subband in (A) male group and (B) female group before the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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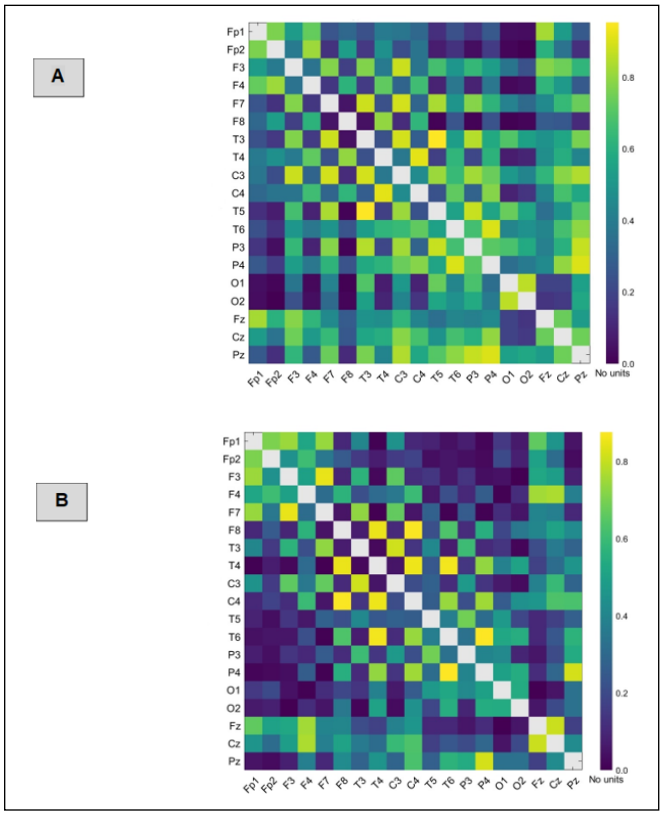


Fig. 4. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in θ2 EEG subband in (A) male group and (B) female group after the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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(P3–P4, O1–O2) with extensions into temporo-parietal junctions (T5/T6–P3/P4) and moderate Fz–Pz integration. Women (Fig.3, B) presented a similar pattern but with more focal right-lateral peaks (T6–P4/O2) and comparatively weaker long-range fronto-posterior connectivity, consistent with stronger local posterior clustering.

During war (Fig.4), θ2 coherence became more fronto-temporal. In men (Fig.4, A), bilateral frontal and temporal strengthening (F3/F4-T3/T4) occurred alongside preserved, though relatively de-emphasized, posterior homotopic links; the dorsal midline connectivity (Fz–Cz–Pz) also intensified. Women (Fig.4, B) showed marked right-lateral augmentation spanning T6-P4-O2 with robust O1-O2 and P3-P4 coupling; cross-hemispheric fronto-temporal connections emerged, while Fz-Pz remained less prominent than in men.

Posterior hubs dominated pre-war β1 coherence (Fig.5). Men showed strong parietal–occipital interhemispheric links (P3-P4, O1-O2), stable posterior-midline coupling (Pz–Oz), and moderate sensorimotor homotopy (C3-C4) (Fig.5, A). Women exhibited a similar posterior concentration with slightly reduced parietal interhemispheric strength and a subtle right-posterior tilt; long-range fronto-posterior coupling was less pronounced (Fig.5, B).

With war exposure, β1 coherence showed a redistribution from posterior toward anterior networks (Fig.6). In men (Fig.6, A), posterior homotopic coupling decreased relative to pre-war levels, while frontal and fronto-temporal links (including Fz-Cz and F3/F4-T3/T4) strengthened. The strongest residual posterior connections included Pz-Oz and C3-C4. Women (Fig.6, B) displayed a clearer attenuation of posterior interhemispheric links (notably P3-P4 and O1-O2), accompanied by compensatory increases over anterior salience/control territory (Fz/Cz and F4-T4). Long-range fronto-posterior coupling remained comparatively weak, consistent with a shift toward more local anterior coherence.

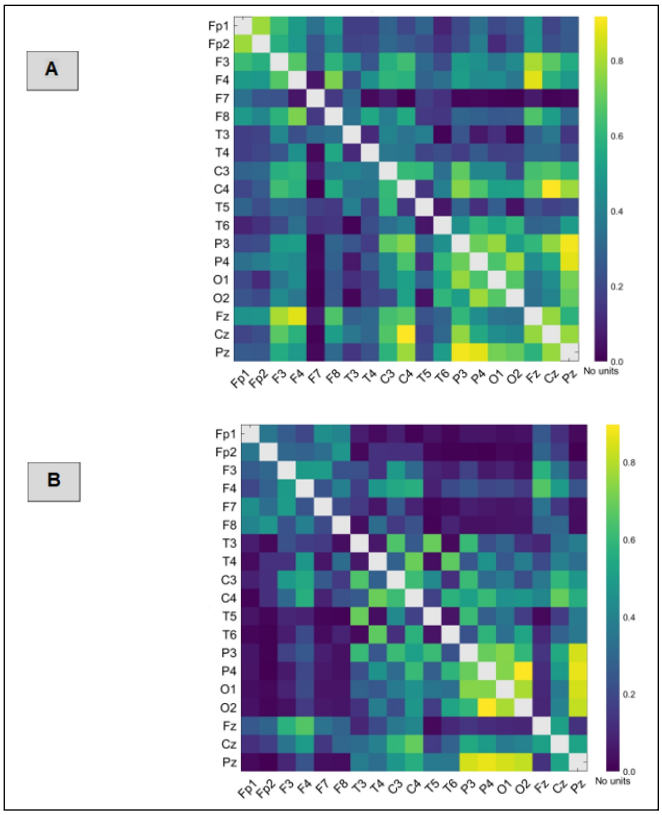


Fig. 5. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in β1 EEG subband in (A) male group and (B) female group before the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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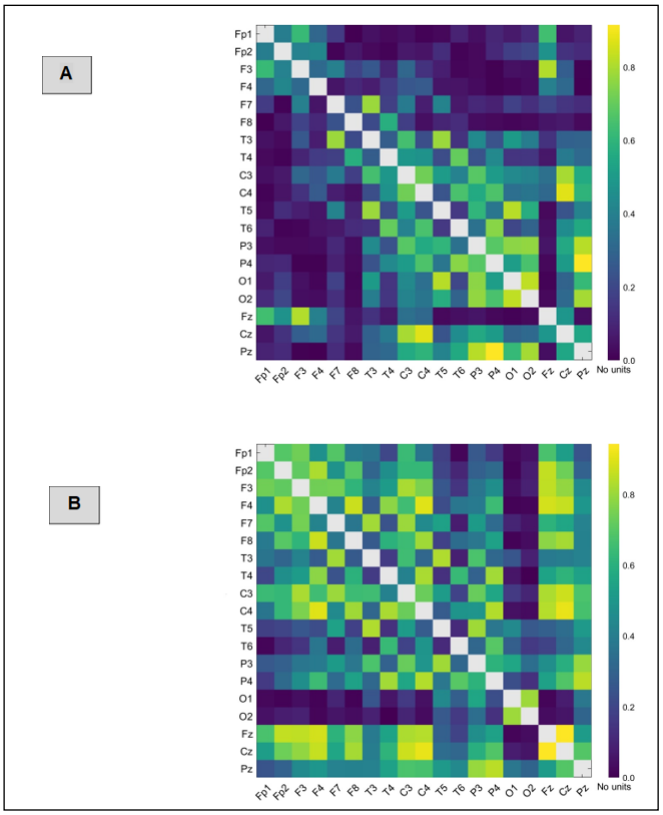


Fig. 6. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in β1 EEG subband in (A) male group and (B) female group after the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
Picture taken by the authors

Before the war (Fig. 7), β2 coherence analysis highlighted sensorimotor and posterior hubs. Men (Fig.7, A) demonstrated strong C3-C4 and robust homotopic posterior links (P3-P4, O1-O2) with moderate fronto-central coupling (Fz-Cz). Women (Fig.7, B) retained prominent posterior homotopy, but with a right-lateral tendency (T6-P4/O2). C3-C4 connection was evident, yet less dominant than in men, and fronto-posterior midline integration remained subdued.

During war, β2 coherence shifted toward anterior control networks (Fig. 8). Men exhibited (Fig. 8, A) enhanced fronto-central and fronto-temporal coupling (Fz/Cz-F3/F4-T3/T4) with reduced, but still detectable, posterior homotopy; the C3-C4 link persisted among the strongest. Women (Fig.8, B) likewise exhibited diminished posterior interhemispheric coupling and emergent right-weighted fronto-temporal coherence (F4-T4/T6), with modest increases along the frontal midline and persistently weak long-range fronto-posterior integration.

DISCUSSION

Across cohorts, the coherence maps converge on two network-level themes: a posterior-dominant baseline architecture and a stress-linked redistribution toward anterior control/salience networks. Pre-war, both θ and β ranges show robust homotopic parietal and occipital coupling (P3–P4, O1–O2) with a preserved dorsal midline axis (Fz–Cz–Pz), a configuration consistent with efficient posterior association-cortex integration for visuospatial/attentional processing and context maintenance [15-17]. Reduced parietal–occipital coupling under load is well-known to compromise sensory integration and spatial updating, particularly when rapid, coordinated responses are required [17]. Stress-induced alterations in occipital/posterior dynamics are likewise expected to degrade visual information flow and stability [18].

Mechanistically, chronic threat engages neurochemical and neuroendocrine cascades that perturb

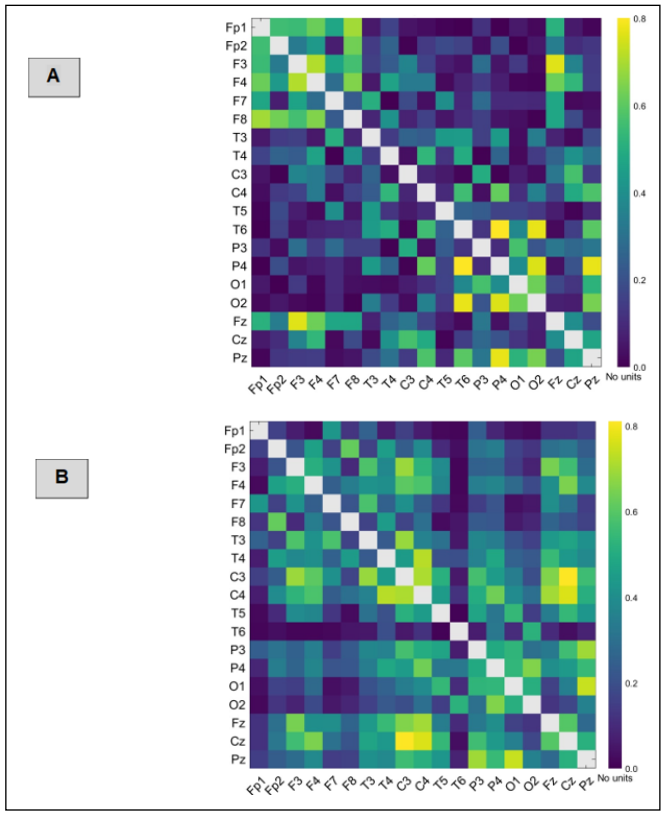


Fig. 7. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in the β2 EEG subband in (A) male group and (B) female group before the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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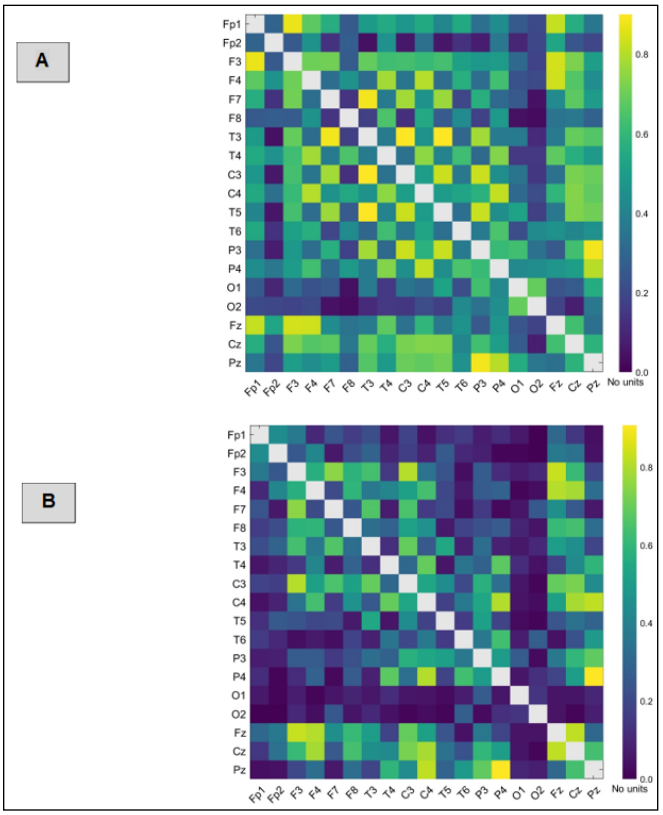


Fig. 8. Topographical distribution of connections (magnitude squared coherence) upon mental arithmetic task performance in β2 EEG subband in (A) male group and (B) female group after the full-scale russian invasion. Both axes represent the complete list of EEG electrode sites used for recording, and each square at the intersection of two channels denotes the strength of coherence between them. The colorbar indicates the magnitude-squared coherence coefficient (range 0 to 1), with warmer colors reflecting stronger coupling. The strong coherent connections (≥0.8) were validated using permutation analysis ($p<0.05$)
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excitation–inhibition balance and erode large-scale synchrony. Excess glutamatergic drive and altered inhibitory tone under sustained stress impair coherent communication between sensory–associative hubs [19, 20], while prolonged HPA-axis activation and cortisol exposure further destabilize network efficiency and cognitive control [21, 22]. In this context, the observed decrements in posterior β1/β2 homotopic links (notably P3-P4, O1-O2) in the during-war cohort provide a plausible readout of weakened large-scale integration required for visuospatial processing and stimulus-driven stability.

Concurrently, the maps show compensatory anterior upshifts. During war, men exhibit strengthened frontal and frontotemporal coupling, with reinforcement along the dorsal midline. Women display diffuse θ strengthening, with maxima over the right temporoparietal and posterior midline regions, and emergent cross-hemispheric frontotemporal links, yet persistently weaker long-range frontoposterior coupling. These patterns suggest increased reliance on salience appraisal, interoceptive monitoring, and rapid response selection to sustain performance as posterior integration wanes [23]. In particular, engagement of the anterior insular cortex and putaminal circuits is consistent with mobilizing affective salience and gating motor-cognitive responses under load [23, 24]. Right-lateral θ increases across temporo-parietal connections further indicate a bias toward emotion-laden, intuitive appraisal streams, which can enhance vigilance but compete with sustained rule-based control [24-26].

Hippocampal interactions help explain the coexistence of heightened vigilance with fatigue and distractibility. θ-linked hippocampal dynamics support the consolidation of emotionally salient content and context updating; in women, stress-hormone modulation can amplify verbal/episodic contributions and shape coping strategies [27, 28]. As a result, right-posterior

θ reinforcement may promote rapid appraisal at the expense of sustained executive stability [29]. Against this backdrop, vmPFC recruitment emerges as a key determinant: effective vmPFC coupling with posterior cingulate and limbic nodes buffers affective reactivity and supports reappraisal, thereby preserving control despite network inefficiency [30, 31]. Variability in vmPFC engagement under chronic threat likely differentiates individuals who maintain cognitive efficiency from those who exhibit slower decision-making, increased fatigue, and behavioral inhibition.

Altogether, the topographies delineate a stress-adapted yet efficiency-limited regime: (i) posterior β coherence decreases that index weakened large-scale integration, (ii) anterior θ and β band coherence increases that reflect compensatory salience and control, and (iii) sex-specific configurations – men retaining relatively stronger dorsal midline/sensorimotor homotopy, women showing clearer right-posterior emphasis at baseline and a more substantial shift toward local anterior coupling during war [15, 16, 30]. From a systems perspective, oscillatory network state exerts a first-order influence on attention and cognitive throughput; as coupling reweights toward anterior hubs, reactivity is preserved but efficiency declines [32, 33].

Due to the principal deficit manifestation in reduced posterior β homotopy and weakened long-range fronto-posterior coupling – especially in women – interventions that (a) train fronto-parietal coordination under load, (b) stabilize visuospatial/posterior integrative hubs, and (c) modulate vigilance (e.g., graded executive tasks with paced breathing/attention regulation) may help rebalance the system [28-31]. Program design can leverage preserved midline/sensorimotor strengths in men and prioritize long-range fronto-posterior integration in women.

Despite providing novel insights into the neurodynamic correlates of chronic war-related stress, this study has several limitations. First, the sample size was relatively modest and restricted to university students within a narrow age range and cultural background, which may limit the generalizability of the findings to broader populations and different age cohorts. Second, although pre-war and wartime groups were compared, the cross-sectional design does not allow for direct causal inferences about individual neurodynamic changes across time. Third, while EEG coherence is a robust measure of large-scale cortical integration, it cannot capture deeper subcortical dynamics or the full spectrum of neurochemical processes that may contribute to stress-related adaptations. Finally, the reliance on a single cognitive stress-inducing paradigm (mental arithmetic) limits the scope of inference about how stress reshapes brain coordination across other domains of cognition and emotion. Future longitudinal and multimodal studies with more diverse populations are needed to extend and validate these findings.

CONCLUSIONS

The present findings indicate that war-related stress is accompanied by a systematic reweighting of functional coupling across large-scale cortical networks. In the pre-war cohort, coherence topographies in both θ and β ranges were dominated by robust homotopic parietal and occipital links, as well as a preserved dorsal midline axis, consistent with efficient posterior association-cortex integration for visuospatial processing and context maintenance. Men additionally showed stronger sensorimotor and long-range midline coupling, whereas women exhibited a mild right-posterior emphasis and comparatively weaker fronto-posterior integration.

During the war, coupling redistributed from posterior hubs toward anterior control and salience territories. In θ, men demonstrated strengthened fronto-temporal links and an accentuated dorsal midline, while women showed diffuse reinforcement with maxima over the right temporoparietal and posterior midline regions, as well as emergent cross-hemispheric fronto-temporal connections; long-range fronto-posterior integration remained relatively attenuated in women. In β, both sexes displayed reduced interhemispheric posterior homotopy, with men retaining residual sensorimotor and posterior midline strength, and women showing a more pronounced attenuation, accompanied by right-weighted anterior strengthening. Taken together, these patterns describe a stress-adapted yet efficiency-limited regime in which anterior networks help preserve reactivity as posterior integration declines, with a more pronounced shift in women.

These topographies outline coherence-based markers for monitoring stress adaptation and suggest concrete intervention targets for further rehabilitation strategies development. Approaches that reinforce fronto-parietal coordination under load, stabilize posterior integrative hubs, and modulate vigilance may help restore more balanced large-scale coordination. Tailoring by sex-leveraging preserved midline and sensorimotor strengths in men and prioritizing long-range fronto-posterior integration in women may optimize outcomes.

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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

CONTENTS

Consideration and analysis of exocrine pancreatic insufficiency in assotiative pathology of the digestive tract, unresolved issues

Olesya M. Horlenko, Lyubomyra B. Prylypko, Bohdan M.Halay, Fedir V. Horlenko, Halyna M.Beley, Lyubov A. Halay, Volodymyr M. Bilak
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ABSTRACT

Aim: To identify and analyze the patterns of exocrine insufficiency development in patients with assotiative pathology of the digestive tract and its relationship with homeostasis disorders depending on the degree of insufficiency.

Materials and Methods: The total number of patients is 135, aged 50.7±6.2 years, with a diagnosis of Chronic pancreatitis (CP), remission phase with exocrine insufficiency in assotiation with Metabolic-associated steatotic liver disease (MASLD) and gastroesophageal reflux disease (GERD). The divided of patients into groups was based on determining the degree of exocrine pancreatic insufficiency according to the results of the level of fecal elastase-1(FE-1):

Results: In the studied children of the first group, significant differences (p1=0.01-<0.001) were observed in the levels of biochemical indicators, except the values of AST and creatinine levels. Significant intergroup differences were found among the indicators of vitamin D3 (p1<0.001; p2=0.001; p3<0.001), folic acid (p1<0.001; p2<0.001; p3<0.001), Zn (p1=0.001; p2=0.001; p3<0.001), Se (p1<0.001; p2<0.001; p3<0.001) and partly, Na (p1=0.02;) Ca (p2=0.002; p3=0.001), Cl(p3=0.04).

Conclusions: The highest communicative correlations in children of the first group were found for the vitamin D3 level with FE-1 (r=0.64) and fibrinogen in a negative direction (r=-0.30). The value of α1-antitrypsin was correlated in the first group with the minerals Ca (-0.30 at p=0.006), FE-1 (r=-0.26 at p=0.02), while in the second group there was a predominance of communications with inflammatory markers ALT (r=-0.30 at p=0.03), AST (r=-0.29 at p=0.04).

KEY WORDS: exocrine pancreatic insufficiency, Metabolic-associated steatotic liver disease, gastroesophageal reflux disease, correlation, patient

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INTRODUCTION

Chronic pancreatitis is caused by recurrent episodes of inflammation that eventually progress to fibrosis. As a result, both endocrine and exocrine pancreatic functions may be impaired.[1] Proposed mechanisms include theories of toxic metabolism, oxidative stress, obstructive and necrotizing-fibrotic infections.[2,3] The pancreas is a unique organ with a dual function, both endocrine and exocrine. Historically, these two functions have often been studied independently. However, emerging evidence suggests that the complex crosstalk between endocrine and exocrine components plays a critical role in maintaining pancreatic function and has significant implications for various diseases.[4]

Normally, when chyme reaches the duodenum, both secretin and cholecystokinin stimulate the secretion of approximately 1.5 liters of pancreatic fluid, which contains pancreatic enzymes (amylase, lipase, and protease), water, and ions (bicarbonate and phosphate). These enzymes are essential for digestion in the small intestine.[5] Pancreatic insufficiency is often results from impair acinar cell function conditions, which reducing the production of digestive enzymes.[6] Exocrine pancreatic insufficiency (EPI) has long been considered to result from a secretory deficiency of pancreatic enzymes and/or bicarbonate.[7]

As a result, EPI has been observed almost exclusively in the context of pancreatic diseases, mainly chronic pancreatitis (CP) and cystic fibrosis (CF), and more recently, pancreatic cancer (PC) or after pancreatic resection. The first evidence-based guidelines using the Oxford System or the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system to address EPI in the context of CP were published in 2012. [8] Following an award from the United European Gastroenterology (UEG), the first European guidelines were developed and published in 2017 [9].

It has recently been noted that there is a paucity of studies on EPI in the general population and in patients

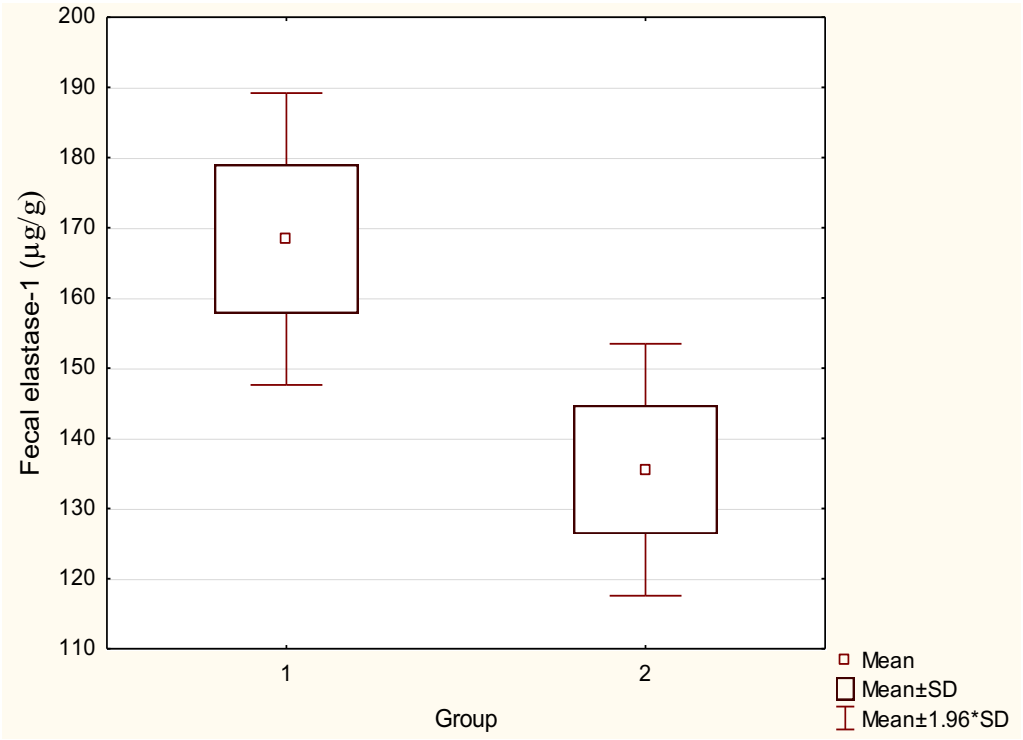


Fig. 1. Gender distribution of patients
Picture taken by the authors

with non-pancreatic diseases, although the number of relevant publications is increasing[10,11].

AIM

The aim is to identify and analyze the patterns of exocrine insufficiency development in patients with assotiative pathology of the digestive tract and its relationship with homeostasis disorders depending on the degree of insufficiency.

MATERIALS AND METHODS

The total number of patients was 135, aged 50.7±6.2 years, diagnosed with Chronic pancreatitis (CP), remission phase with exocrine insufficiency in assotiation with Metabolic-associated steatotic liver disease (MASLD) and gastroesophageal reflux disease (GERD). The divided of patients into groups was based on the determination of the degree of exocrine pancreatic insufficiency according to the results of the level of fecal elastase-1:

Group 1 (n=85) – patients with mild exocrine pancreatic insufficiency (FE-1= 168.40 ± 10.61 µg/g of feces).

Group 2 (n=50) – patients with moderate grade of exocrine pancreatic insufficiency (FE-1 = 135.52 ± 9.16 µg/g of feces) (Fig. 1).

ETHICS

Written informed consent was obtained from all participants. The study protocol was approved by the University Ethics Board of the State Higher Educational Institution "UzhNU". All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki.

RESULTS

Pancreatic exocrine insufficiency (PEI), defined by insufficient secretion of digestive enzymes, can develop in the setting of metabolic dysfunction even in the absence of overt pancreatic pathology [12]. MASLD is now the most common illness of chronic liver disease in the worldwide. Although the consequences of MASLD on the liver are well documented, its impact on pancreatic function remains poorly understood.

Recent studies have demonstrated a pathophysiological crosstalk between pancreatic dysfunction and hepatic steatosis. FE-1) is a widely accepted noninvasive biomarker for the diagnosis of exocrine insufficiency. A level below 200 µg/g is indicative of exocrine dysfunction [13,14].

Understanding the development and prevalence of metabolic correlates of pancreatic dysfunction in patients with MAFLD is important because the aforementioned pancreatic dysfunction may influenced on nutritional status, glycemic profile, and subsequent progression of liver disease. [15]

A serum biochemical study was performed to interpret the relationship between the results and the degree of exocrine insufficiency (EPI) (Table 1).

There are significant differences (p1=0.01-0.001) in the levels of indicators (total protein, total bilirubin, total

Table 1. Serum biochemical study in the patients

Parameters	Control group (n=23)	1 group (n=85)	2 group (n=50)	Statistical significance of the difference
Total protein , g/l	73.62 ± 3.89	68.60 ± 3.49	66.27 ± 2.91	p_1=0.001; p_2<0.001; p_3<0.001
Total Bilirubin, mkmol/l	10.82 ± 3.85	11.84 ± 6.34	16.02±12.11	p_1=0.01; p_2=0.46; p_3=0.05
AST, mkmol/l	0.44 ± 0.11	0.48 ± 0.20	0.56 ± 0.37	p_1=0.09; p_2=0.36; p_3=0.13
ALT, mkmol/l	0.66 ± 0.15	0.93 ± 0.46	1.05 ± 0.55	p_1=0.19; p_2=0.01; p_3=0.001
Creatinin, mkmol/l	93.59 ± 12.53	104.59 ± 27.89	102.88 ± 35.14	p_1=0.76; p_2=0.07; p_3=0.22
Total Cholosterol mlmol/l	4.25 ± 0.48	5.04 ± 1.11	5.60 ± 0.80	p_1=0.002; p_2=0.001; p_3<0.001
Triglycerides mlmol/l	1.31 ± 0.23	1.75 ± 0.44	1.99 ± 0.30	p_1<0.001; p_2<0.001; p_3<0.001

Notes: p_1 – statistical significance of the difference between the indicators of groups 1 and 2; p_2 – statistical significance of the difference between the indicators of group 1 and the control group; p_3 – statistical significance of the difference between the indicators of group 2 and the control group
Source: compiled by the authors of this study

cholesterol, triglycerides), in addition to the values of AST and creatinine, partly in the levels of total bilirubin and ALT, according to the data in table 1. It is noteworthy that there is a significant decrease in the level of total protein (p_2<0.001; p_3 <0.001) and an increase in total bilirubin in the 1st group of patients in 1.1 times and a significant increase in the 2nd group in 1.6 times (p3=0.05). The indicators level of the vitamin-mineral homeostasis is demonstrated on Table 2.

According to the stude results, which are presented on table II, there are significant differences between the indicators of Vitamin D3 (p1=0.001; p2=0.001; p3=0.001), folic acid ((p1=0.001; p2=0.001; p3=0.001), Zn (p1=0.001; p2=0.001; p3=0.001), Se (p1=0.001; p2=0.001; p3=0.001)) and partly Na (p1=0.02;) Ca (p2=0.002; p3=0.001), Cl (p3=0.04). No differences were found in the values of potassium levels. Significant decreases were noted in the levels of Vitamin D3 (1.1 and 1.6 times), folic acid (1.5 and 1.9 times), Zn (1.2 and 1.4), Se (1.3 and 1.6 times),

The study of inflammatory response markers is informative (Table 3).

A significant increase in the levels of all indicators of the organism's inflammatory response is observed, with a more pronounced in group 2, acording to the results of table III. It should be noted that all indicators varied within the reference values, except for IL-6. Also, the highest comparative values of the decrease were noted in the level of IL-6 (1.5 and 1.6 times, respectively, in the groups).

To identify patterns and indicators interdependencies of changes in the secretory function of the pancreas, a correlation analysis was conducted. The correlation communications of indicators in the first group of patients with mild exocrine pancreatic insufficiency are presented in Table 4.

The highest communicative correlations were found for the indicator Vitamin D3 with FE-1 (r=0.64, at p=0.001) and fibrinogen in the negative direction (r=-0.30 at p=0.006), according to the data in table IV. The levels of mineral metabolism components correlated in the following relationships: Ca with α1-antitrypsin (r=-0.30 at p=0.006) and Zn (r=0.41 at p=0.001) and Se (r=0.37 at p=0.001); the level of potassium correlated with the values of folic acid (r=0.30 at p=0.006). Along with this, there were communications of the level of chlorine with the transferrin values (r=0.45 at p=0.001). It should be noted that the severity of clinical symptoms communicated with the studied indicators with a correlation coefficient below 0.30, namely by age categories, pain intensity, disease duration. We present a representative correlogram of the relationships between vitamin D3 in the blood and elastase-1 in feces (Fig. 2) and between the level of Se and Zn in the blood in patients of group 1 (Fig.3).

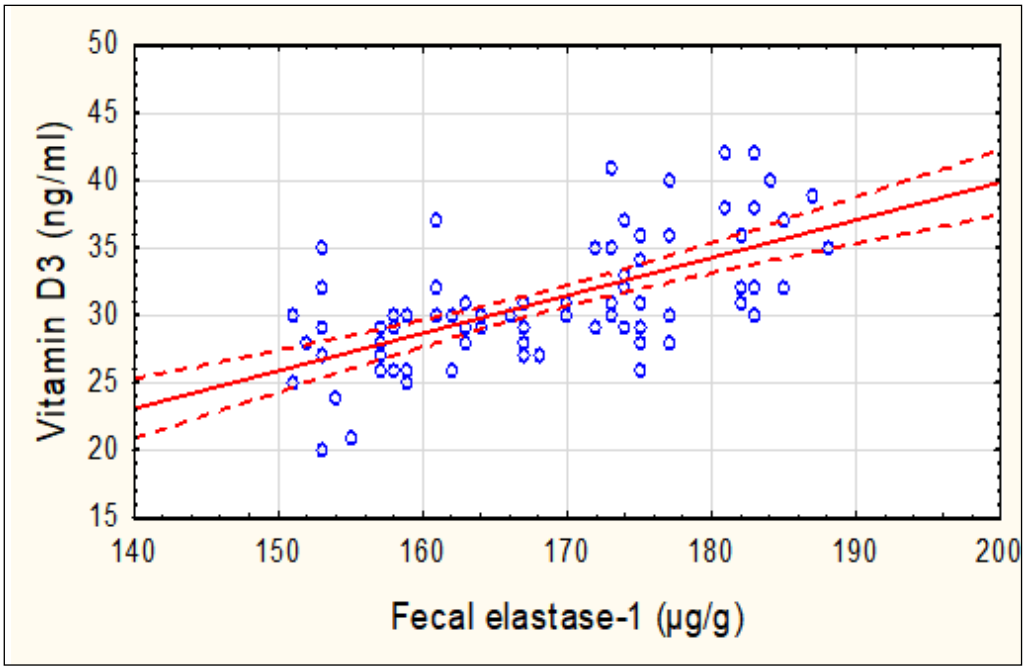


Fig. 2. Correlation between the level of vitamin D3 in the blood and elastase-1 in feces in patients of group 1(r=0,64; p<0,001)
Picture taken by the authors

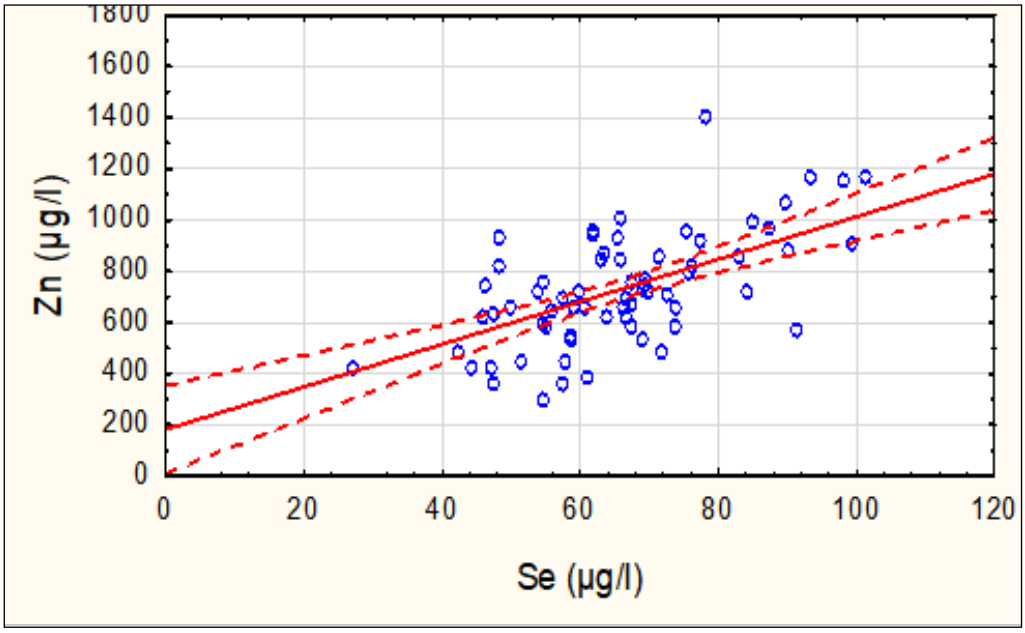


Fig. 3. Correlation between Se and Zn levels in the patients blood of group 1(r=0,58; p<0,001)
Picture taken by the authors

We inform about correlations between the indicators values in the studied contingent of the group 2 (Table 5).

The numerous relationships between the levels of indicators are noted, according to the data in table 5. The correlation coefficient of FE-1 with vitamin D3 is higher than in the first group (r=0.67 at p=0.001) and a new communication with creatinine was detected (r=0.29 at p=0.04). α1-antitrypsin, which is responsible for inhibiting the number of enzymes activity, correlated in the first group with the minerals Ca (-0.30 at p=0.006) and in low correlation coefficient with the levels of K (r=0.24 at p=0.03), Zn (r=0.23 at p=0.03)) and FE-1 (r=0.26 at p=0.02). While in the second group there were a predominance of communications with inflammatory markers ALT (r=0.30 at p=0.03), AST (r=0.29 at p=0.04), which are markers of liver and heart cell damage. Communicative relationships of α1-antitrypsin were also observed with transferrin (r=0.34 at p=0.01) and potassium ions (r=0.47 at p=0.001). Alpha-1 antitrypsin is an acute phase protein, a circulating glycoprotein synthesized primarily by the liver. Its concentration increases in response to acute infection. The accumulation of unfolded or misfolded proteins and their polymers in the lumen of the endoplasmic reticulum has a toxic effect of increasing function, leading to a stress response of cells associated with the endoplasmic reticulum and likewise ultimately causes chronic liver damage in some

Table 2. Investigation of vitamin-mineral cmpositions in the patients

Parameters	Control group (n=23)	1 group (n=85)	2 group (n=50)	Statistical significance of the difference
Vitamin D3 (30-70, ng/ml)	35.52 ± 5.16	31.02 ± 4.61	19.28 ± 3.45	p_1<0.001; p_2=0.001; p_3<0.001
Folic acid (3,89-26,8 ng/ml)	12.07 ± 3.78	7.83 ± 2.06	6.27 ± 2.30	p_1<0.001; p_2<0.001; p_3<0.001
Zn (543-1130 mkg/л)	895.27 ± 189.44	729.05 ± 209.72	620.94 ± 127.44	p_1=0.001; p_2=0.001; p_3<0.001
Sel(23-190 mkg/l)	85.93 ± 12.5	65.81 ± 14.64	53.52 ± 10.70	p_1<0.001; p_2<0.001; p_3<0.001
Na (135-155mmol/l)	143.38 ± 3.27	143.58 ± 3.16	142.26 ± 2.81	p_1=0.02; p_2=0.79; p_3=0.14
Ca (2,1-2,6mmol/l)	2.33 ± 0.11	2.16 ± 0.25	2.14 ± 0.27	p_1=0.75; p_2=0.002; p_3=0.001
K (3,6-5,5 mmol/l)	4.49 ± 0.37	4.43 ± 0.33	4.31 ± 0.43	p_1=0.07; p_2=0.45; p_3=0.09
Cl (95-108 mmol/l)	103.2 ± 2.64	101.83 ± 3.30	101.70 ± 2.90	p_1=0.81; p_2=0.07; p_3=0.04

Notes: p_1 – statistical significance of the difference between the indicators of groups 1 and 2; p_2 – statistical significance of the difference between the indicators of group 1 and the control group; p_3 – statistical significance of the difference between the indicators of group 2 and the control group
Source: compiled by the authors of this study

individuals [16,17]. Scientists believe that there are next interrelated issues with EPI. The diagnosis and treatment of EPI must go beyond the pancreas and require a more holistic approach. Many other EPI conditions are being considered in this direction, some of which have an anatomically intact pancreas but impaired pancreatic enzyme activity in the lumen. For "normal" digestion, food and pancreatic enzymes must meet at the right time, place, and environment [18,19].

DISCUSSION

The common clinical pancreatic secretion scenario is about reduced but sufficient for normal nutrient digestion cannot be defined as insufficiency but as pancreatic exocrine dysfunction. Although its hepatic complications are well documented, emerging evidence suggests that MAFLD may also be associated with EPI, a condition in which the pancreas does not produce or supply sufficient digestive enzymes [12].

The varying probabilities of developing EPI in different clinical settings significantly influence the diagnostic approach and management in clinical practice [20]. EPI always requires treatment, and symptom relief and normalization of nutritional status are therapeutic goals. Other clinical consequences of EPI depend on the disease [21].

The results of our study demonstrated that the presence of comorbidities of the digestive tract are interconnected by common pathophysiological links and influences. These results are consistent with previous studies by Niriella et al. [22] and Yu et al. [23]

Liver function tests in our study showed elevated liver enzymes in the study group. Nguyen et al. [24] and Zdanowicz et al. [25] also found an increase in ALT compared with the control group ($p < 0.001$).The most significant finding of our study was a reliable reduced level of FE-1 in the MAFLD study group, suggesting impaired pancreatic exocrine function. Our findings are consistent with those of Herzig et al. [26], who reported that 21.7% of elderly subjects without gastrointestinal disease or diabetes had fecal elastase levels below 200 μg/g, suggesting subclinical pancreatic dysfunction. Similarly, Naruse et al. [14] confirmed that fecal elastase is a specific marker of severe exocrine pancreatic insufficiency. No significant age, gender, or

Table 3. Inflammatory response markers in the study children

Parameters	Control group (n=23)	1 group (n=85)	2 group (n=50)	Statistical significance of the difference
Fibrinogen (1,8-3,5 g/l)	2.47 ± 0.39	2.85 ± 0.54	3.10 ± 0.55	p_1=0.01; p_2=0.002; p_3<0.001
Transferrin (2,0-3,6 g/l)	2.16 ± 0.27	2.35 ± 0.30	2.76 ± 0.34	p_1<0.001; p_2=0.007; p_3<0.001
α1- antitrypsin (0,9-2,0 g/l)	1.35 ± 0.18	1.49 ± 0.24	1.80 ± 0.22	p_1<0.001; p_2=0.01; p_3<0.001
IL-4 (0-4 pg/ml)	1.41 ± 0.57	1.92 ± 0.20	2.13 ± 0.33	p_1<0.001; p_2<0.001; p_3<0.001
IL-6 (0-10 pg/ml)	7.97 ± 1.84	12.09 ± 1.61	13.08 ± 1.63	p_1=0.001; p_2<0.001; p_3<0.001

Notes: p_1 – statistical significance of the difference between the indicators of groups 1 and 2; p_2 – statistical significance of the difference between the indicators of group 1 and the control group; p_3 – statistical significance of the difference between the indicators of group 2 and the control group
Source: compiled by the authors of this study

comorbidity was found, which is in agreement with Zsóri [27], who suggested that EPI in metabolic liver disease results from complex metabolic dysfunctions rather than traditional risk factors. Numerous studies [28,29] have linked low levels FE-1 to altered glycemic profiles. In particular, Rathmann et al. [30] demonstrated a negative correlation between HbA1c and pancreatic elastase even in nondiabetic patients, suggesting that exocrine pancreatic dysfunction may develop early [31] with metabolic abnormalities. Fatty infiltration of the pancreas may contribute to exocrine dysfunction, linking these two conditions as part of a systemic metabolic disorder, as highlighted in recent studies by Maetzel et al. [32].

Multivariate regression analysis identified MAFLD as an independent predictor of EPI by Boga et al. [12], who similarly found NAFLD to be a major predictor of reduced pancreatic function.

FE-1, although validated as a noninvasive marker of pancreatic exocrine function, is subject to potential confounding factors, namely the presence of diarrhea, which may dilute the enzyme concentration, and technical problems associated with sample collection. These factors may affect the accuracy of the measurement. [33]

CONCLUSIONS

1. Indicative correlations of the levels vitamin D3 with FE-1 (r=0.64, at p=0.001) and fibrinogen in a negative direction were found (r=-0.30 at p=0.006) in ccordance of communicative relationships in the 1st study group.
2. The levels of mineral metabolism components correlated in the following relationships: Ca with α1-antitrypsin (r=-0.30 at p=0.006) and Zn (r=0.41 at p<0.001) and Se (r=0.37 at p<0.001); the potassium level correlated with the values of folic acid (r=0.30 at p=0.006). Along with this, communications of the chlorine level with the values of transferrin (r=0.45 at p=0.001) were noted. It should be noted that the severity of clinical symptoms communicated with the studied indicators in coefficient less then 0.30, namely by age categories, pain intensity, disease duration.
3. In group 2, numerous relationships between the levels of indicators are noted. The correlation coefficient of FE-1 with Vitamin D3 is higher than in the first group (r=0.67 at p<0.001) and a new communication with creatinine was detected (r=0.29 at p=0.04).
4. α-1-antitrypsin, as a substance synthesized in the liver and responsible for inhibiting the activity of a number of enzymes, correlated in the levels of date first group with the minerals Ca (r=-0.30 at p=0.006) and with a low correlation coefficient with the levels of K (r=0.24 at p=0.03), Zn (r=-0.23 at p=0.03)) and FE-1 (r=-0.26 at p=0.02), while in the 2 group there was a communication predominance with inflammatory markers ALT (r=-0.30 at p=0.03), AST (r=-0.29 at p=0.04), which are markers of liver and heart cell damage. Communicative relationships with transferrin (r= 0.34 at p=0.01) and potassium ions (r=0.47 at p=0.001) were also observed.
5. Our results highlight a significant association

Table 4. Date correlations in cildren (1 groupe)

Parameters		r	p
FE-1	Total bilirubin.	-0.25	0.02
	Fibrinogen	-0.26	0.02
	Zn	0.24	0.03
	Vitamin D3	0.64	<0.001
	α1-antitrypsin	-0.26	0.02
	Pain intensity	-0.25	0.02
Transferrin	AST	-0.22	0.04
	Cl	0.45	<0.001
Fibrinogen	Vitamin D3	-0.30	0.006
	FE-1	-0.26	0.02
	Pain intensity	0.28	0.01
α1-antitrypsin	Ca	-0.30	0.006
	K	0.24	0.03
	Zn	-0.23	0.03
	FE-1	-0.26	0.02
IL-4	ALT	-0.24	0.02
IL-6	Ca	0.28	0.01
	K	-0.26	0.02
Folic acid	K	0.29	0.005
Vitamin D3	Fibrinogen	-0.30	0.006
	FE-1	0.64	<0.001
Zn	Ca	0.41	<0.001
	Se	0.58	<0.001
	FE-1	0.24	0.02
	α1-antitrypsin	-0.23	0.03
	Age	-0.23	0.03
Se	Total cholesterol	0.23	0.03
	Ca	0.37	<0.001
	K	-0.27	0.01
	Zn	0.58	<0.001
Ca	Zn	0.41	<0.001
	Se	0.37	<0.001
	α1-antitrypsin	-0.30	0.006
	IL-6	0.28	0.009
	Age	-0.25	0.02
K	-0,30	-0.30	0.006
	Folic acid	0.30	0.006
	Se	-0.27	0.01
	α1-antitrypsin	0.24	0.02
	IL-6	-0.26	0.02
	Pain intensity	0.26	0.02
Cl	Disease duration	0.22	0.05
	Age	-0.22	0.05
	Transferrin	0.45	<0.001

Source: compiled by the authors of this study

Table 5. Date correlations in cildren (2 groupe)

Parameters		r	p
FE-1	Creatinine	0.29	0.04
	Vitamin D3	0.67	<0.001
Transferrin	α1-antitrypsin	0.34	0.01
Fibrinogen	-	-	-
α1-antitrypsin	Transferrin	0.34	0.01
	ACT	-0.29	0.04
	ALT	-0.30	0.03
	K	0.47	0.001
IL-4	Total bilirubin	0.28	0.05
	Ca	0.34	0.01
	Cl	0.32	0.02
IL-6	-	-	-
Folic acid	Na	0.30	0.03
Vitamin D3	FE-1	0.67	<0.001
	Creatinine	0.38	0.006
Zn	AST	0.38	0.006
	Se	0.29	0.04
	Age	-0.38	0.006
Se	Ca	0.35	0.01
	Zn	0.29	0.04
	Total bilirubin	0.34	0.01
Ca	Se	0.35	0.01
	IL-4	0.34	0.01
K	AST	-0.28	0.05
	ALT	-0.35	0.01
	Total cholesyerol	-0.40	0.004
	Triglycerides	-0.60	<0.001
	α1- antitrypsin	0.47	0.001
	Age	0.32	0.02
Na	Folic acid	0.30	0.03
Cl	Total bilirubin	-0.31	0.02
	Il-4	0.32	0.02

Source: compiled by the authors of this study

between GERD, MAFLD, and EPI, with FE-1 levels serving as a reliable marker for detecting pancreatic dysfunction in this population. The definition, pathogenesis, clinical consequences, diagnosis, and treatment of EPI in different clinical conditions require further investigation and proper patient monitoring to reduce the risk of complications and improve the quality of life of patients with EPI.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Development and validation of a non-invasive risk prediction nomogram for metabolic syndrome in young adults: A cross-sectional study based on NHANES 2011–2018

Olga S. Palamarchuk, Oleh O. Moroz, Volodymyr P. Feketa
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ABSTRACT

Aim: To develop and validate non-invasive predictive models for detecting metabolic syndrome in young adults using NHANES 2011–2018 data to enable effective screening without laboratory testing.

Materials and Methods: Using data from the National Health and Nutrition Examination Survey (NHANES 2011–2018), we established a homogeneous cohort of Non-Hispanic White individuals (N=2,911). Gender-specific multivariate logistic regression models were developed to predict MetS risk using strictly non-invasive anthropometric and clinical parameters, including age, waist-to-height ratio (WHtR), and blood pressure.

Results: The resulting algorithms demonstrated robust discriminatory power, achieving an area under the ROC curve (AUC) of 0.87 for males and 0.84 for females. WHtR emerged as the most significant independent predictor across both genders (Adjusted OR 1.10 per 0.01 unit increment; $p < 0.001$). Notably, while chronological age was significantly associated with risk in males (OR 1.07), it lacked statistical significance in the female population ($p = 0.904$). This divergence suggests a dominant role of phenotypic features over chronological aging in shaping MetS risk among young women.

Conclusions: The developed nomograms and risk heatmaps enable precise cardiometabolic risk stratification in primary care without requiring laboratory resources. This non-invasive framework provides a scalable and practical tool for early intervention and personalized health management in young adults.

KEY WORDS: metabolic syndrome, risk prediction, nomogram, young adults, non-invasive diagnostics, NHANES, validation, waist-to-height ratio

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INTRODUCTION

Metabolic syndrome (MetS) is a pressing global health challenge characterized by a cluster of interconnected risk factors, including central obesity, elevated blood pressure, hyperglycemia, hypertriglyceridemia, and reduced high-density lipoprotein (HDL) cholesterol. Recent evidence suggests a sharp rise in the prevalence of this condition not only among older populations but also within younger age groups, transforming MetS into a systemic threat to healthcare stability worldwide [1-3]. Epidemiological data underscore the scale of this crisis: Bayesian modeling of 3,236 data points covering 45.5 million adults estimates that global MetS prevalence increased from 14.7% (95% CI: 13.1–16.7) in women and 9.0% (95% CI: 7.5–10.8) in men in 2000 to 31.0% (28.5–33.9) and 25.8% (23.5–28.5), respectively, by 2023 [1]. This more than doubling in prevalence in less than a quarter-century indicates a critical decline in population metabolic health.

The shift towards younger demographics is particularly concerning. Among adolescents with obesity, the prevalence of MetS averages 31.2% (95% CI: 26.5–36.2), with regional rates peaking in South America at 43.1% [4]. In the United States, MetS frequency among adolescents is closely linked to socioeconomic determinants, specifically household food insecurity. Data from NHANES (2001–2020) indicates that adolescents from food-insecure households face a significantly higher risk of developing MetS compared to those with stable nutrition [5]. This highlights MetS as a societal issue rooted in unequal access to healthy nutrition and preventive medical care.

Conventional diagnosis per NCEP ATP III (National Cholesterol Education Program Adult Treatment Panel III) criteria requires the presence of at least three of five components: increased waist circumference, hypertriglyceridemia (≥150 mg/dL), reduced HDL cholesterol (<40 mg/dL in men, <50 mg/dL in women), hypertension (SBP ≥130 mmHg or DBP ≥85 mmHg), and elevated fasting glucose (≥100 mg/dL) [6,7]. While clinically robust, this approach faces major hurdles in mass screening, especially among young adults, due to

the requirement for laboratory-based fasting tests. The necessity of an 8–12 hour fast is a significant logistical barrier. Young adults typically lead active lifestyles with irregular eating patterns and low rates of routine medical visits. The fasting requirement necessitates visit planning and potential time loss from work or education, leading to poor screening uptake and an underestimation of the actual MetS burden in this demographic.

Furthermore, traditional health assessment tools for obesity have notable drawbacks in the modern epidemiological context, where «metabolically unhealthy» phenotypes are increasingly common even among individuals with normal or slightly elevated body weight. Many diagnostic instruments fail to account for key pathophysiological mechanisms such as insulin resistance, central obesity, or visceral adipose tissue dysfunction, thus underestimating the risk of cardiovascular disease and type 2 diabetes. Body Mass Index (BMI) remains the most widely used tool, yet it lacks the sensitivity to distinguish between muscle and fat mass or account for fat distribution. Consequently, BMI often misses individuals with the «skinny fat» phenotype—those with a normal body weight but high visceral fat and metabolic abnormalities. Research shows that up to 30% of individuals with a normal BMI may exhibit signs of MetS, particularly in the presence of abdominal obesity [5,8]. Moreover, most clinical scales, including those from NCEP ATP III or the Framingham Heart Study, were derived from middle-aged or elderly cohorts and may lack sensitivity for early-stage metabolic shifts in younger groups [5].

The objective of this study was to develop and validate high-precision, non-invasive predictive models for MetS that do not require blood chemistry analysis, specifically tailored for young adults (aged 18–44 years). Unlike universal screening tools, we constructed gender-specific multivariate models to account for the differential impact of risk factors on MetS development. Utilizing a large, representative sample from NHANES (2011–2018), we developed practical diagnostic instruments—nomograms and risk heatmaps—that enable clinicians to instantly assess individual risk during a primary care consultation. This approach facilitates effective mass screening and early identification of high-risk groups without invasive, costly laboratory tests, thereby promoting timely preventive interventions.

AIM

The objective of this study was to develop and validate high-precision, gender-specific predictive models and practical visual tools (nomograms and heatmaps) for detecting metabolic syndrome in young adults (aged 18–44) using strictly non-invasive anthropometric and clinical parameters from the NHANES 2011–2018 dataset, thereby facilitating mass screening without the need for invasive laboratory testing.

MATERIALS AND METHODS

STUDY DESIGN AND DATA SOURCE

This study is based on a cross-sectional analysis of data from the National Health and Nutrition Examination Survey (NHANES) covering the period from 2011 to 2018. NHANES is a nationally representative survey of the United States population conducted by the National Center for Health Statistics (NCHS) at the Centers for Disease Control and Prevention (CDC) [9]. The NHANES protocols were approved by the NCHS Research Ethics Review Board, and all participants provided informed written consent prior to examination. To ensure the results are representative at the national level, sample weights (MEC weights, WTMEC2YR) were incorporated into all statistical calculations [10].

STUDY POPULATION CHARACTERISTICS

The target sample was derived from the Non-Hispanic White cohort.

INCLUSION CRITERIA

1. age between 18 and 44 years;
2. male or female gender;
3. availability of complete information regarding metabolic syndrome components.

EXCLUSION CRITERIA

1. current pregnancy;
2. missing anthropometric or laboratory data;
3. age outside the specified range.

Following a Complete Case Analysis for variables with a missing data rate of less than 5%, the final sample comprised 2,911 individuals.

OUTCOME DEFINITION

The primary outcome (Risk_Status) was the presence or absence of metabolic syndrome (MetS), defined according to NCEP ATP III criteria [11]. A diagnosis of MetS was established if at least three of the following five criteria were met:

1. Abdominal obesity: Waist circumference > 102 cm for men and > 88 cm for women.
2. Hypertriglyceridemia: Triglyceride levels ≥ 150 mg/dL (1.7 mmol/L).
3. Reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) for men and < 50 mg/dL (1.29 mmol/L) for women.

4. Hypertension: Systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg.
5. Fasting hyperglycemia: Plasma glucose ≥ 100 mg/dL (5.6 mmol/L).

PHYSICAL EXAMINATION AND ANTHROPOMETRY

Anthropometric measurements were performed by trained personnel in a Mobile Examination Center (MEC) following standardized protocols [12].

• Waist Circumference: Measured with a horizontal tape measure immediately above the superior border of the iliac crest at the end of a normal expiration.

• Height and Weight: Determined using a digital stadiometer and Mettler-Toledo scales. These data were used to calculate Body Mass Index (BMI) and the primary predictor—waist-to-height ratio (WHtR), defined as waist circumference (cm) divided by height (cm).

• Blood Pressure: Measured three times using the auscultatory method with a Baumanometer mercury sphygmomanometer after a 5-minute rest. The arithmetic mean of the three readings was used for analysis.

LABORATORY PROCEDURES

Blood samples were collected via venipuncture following an 8–9 hour fast.

• Lipid Profile and Glucose: Total cholesterol was determined using enzymatic methods on Roche Modular P or Cobas 6000 analyzers. HDL-C levels were measured via a direct immunoassay. Triglycerides and glucose were measured using enzymatic methods on Roche analyzers.

• Calculated Indices: The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as follows:

The Lipid Accumulation Product (LAP) was calculated using gender-specific formulas incorporating waist circumference and triglycerides.

STATISTICAL ANALYSIS AND MODEL DEVELOPMENT

Data processing was performed in the RStudio environment using the survey, rms, glm, and gtsummary packages (R version 4.3.1).

• Modeling: Two gender-specific binary logistic regression models were constructed to predict MetS risk. Non-invasive predictors included age, WHtR, smoking status, sleep duration, and blood pressure (SBP, DBP). The logit formula (z) was defined as:

$$z = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k$$

• Validation: The dataset was split into training (70%) and testing (30%) sets. Discriminatory power was assessed using the Area Under the ROC Curve (AUC-ROC). Model calibration was verified via Calibration Plots, and clinical utility was evaluated using Decision Curve Analysis (DCA).

• Visualization: For practical implementation, nomograms and risk heatmaps were generated based on the regression coefficients. Statistical significance was set at $p < 0.05$.

DATA AVAILABILITY AND ETHICAL CONSIDERATIONS

The datasets utilized in this study are publicly available on the official NHANES CDC repository website [12]. This study adheres to the TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis) statement for reporting the development of predictive models for medical applications [13].

ETHICS

The NHANES study protocols were reviewed and approved by the NCHS Research Ethics Review Board (ERB). All participants provided informed written consent. As this study is a secondary analysis of publicly available, de-identified data, it was exempt from additional institutional review board (IRB) review. The study was conducted in accordance with the Declaration of Helsinki.

The datasets analyzed during the current study are publicly available in the National Health and Nutrition Examination Survey (NHANES) repository, maintained by the Centers for Disease Control and Prevention (CDC). Data can be accessed via the official website: https://www.cdc.gov/nchs/nhanes/index.htm.

FRAMEWORK

This work is a continuation of previous research conducted within the framework of the research program "Informativeness of indicators of the body composition in the diagnosis, treatment and prevention of diseases of internal organs" (state registration number 0122U201421).

RESULTS

POPULATION CHARACTERISTICS

A homogeneous cohort of Non-Hispanic White young adults (aged 18–44 years) was established for model

Table 1. Anthropometric and biochemical characteristics of the study population (M ± SD or n, %)

Parameter	Total Sample (n=2,911)	Men (n=1,477)	Women (n=1,434)	p-value
Age, years	31.4 ± 7.8	31.2 ± 7.9	31.6 ± 7.7	0.165
WC, cm	96.2 ± 16.4	99.8 ± 15.2	92.5 ± 16.8	<0.001
WHtR	0.56 ± 0.09	0.57 ± 0.08	0.56 ± 0.10	0.004
SBP, mmHg	119.4 ± 13.6	123.2 ± 12.4	115.5 ± 13.8	<0.001
DBP, mmHg	72.8 ± 11.2	74.5 ± 10.8	71.1 ± 11.4	<0.001
Glucose, mg/dL	102.3 ± 24.5	104.8 ± 26.1	99.7 ± 22.4	<0.001
Triglycerides, mg/dL	138.4 ± 98.2	152.6 ± 112.4	123.8 ± 78.5	<0.001
HDL-C, mg/dL	52.6 ± 14.8	47.4 ± 12.6	57.9 ± 15.2	<0.001
Smoking (%)	880 (30.2%)	466 (31.6%)	414 (28.9%)	0.029
Sleep, hours	7.3 ± 2.5	7.1 ± 1.4	7.5 ± 3.6	<0.001
MetS (Diagnosis), n (\%)	652 (22.4%)	318 (21.5%)	334 (23.3%)	0.252

Abbreviations: WC, waist circumference; WHtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; MetS, metabolic syndrome.
Source: compiled by the authors of this study

development and validation. After data cleaning and excluding participants with incomplete anthropometric or laboratory records, the final sample comprised 2,911 individuals. The gender distribution was balanced: 1,477 men (50.7%) and 1,434 women (49.3%). National representativeness was ensured by applying MEC sample weights [10].

METABOLIC SYNDROME PREVALENCE

According to NCEP ATP III criteria, MetS prevalence was 22.4% (n=652). Although prevalence was slightly higher in women (23.3%, n=334) than in men (21.5%, n=318), this difference was not statistically significant (p=0.252), indicating a comparable metabolic burden across sexes in this demographic.

DEMOGRAPHIC AND ANTHROPOMETRIC PARAMETERS

The mean age of the cohort was 31.4 ± 7.8 years, with no significant gender difference (p=0.165). Pronounced sexual dimorphism was observed in key anthropometric measures: men had significantly larger waist circumferences (99.8 ± 15.2 cm vs. 92.5 ± 16.8 cm, p<0.001) and higher WHtR (0.57 ± 0.08 vs. 0.56 ± 0.10, p=0.004).

CLINICAL AND LABORATORY PROFILES

Detailed comparisons of MetS components are presented in Table 1:

• **Hemodynamics:** Men had significantly higher systolic (123.2 ± 12.4 mmHg) and diastolic (74.5 ± 10.8 mmHg) blood pressure than women (115.5 ± 13.8 and 71.1 ± 11.4 mmHg, respectively; p<0.001).

• **Metabolism:** Fasting glucose (104.8 ± 26.1 mg/dL) and triglycerides (152.6 ± 112.4 mg/dL) were elevated in men (p<0.001). Conversely, HDL-C levels were higher in women (57.9 ± 15.2 mg/dL vs. 47.4 ± 12.6 mg/dL, p<0.001), consistent with known physiological lipid variations.

• **Behavioral factors:** Women reported longer sleep duration (7.5 ± 3.6 vs. 7.1 ± 1.4 hours, p<0.001), while active smoking was more frequent among men (31.6% vs. 28.9%, p=0.029).

These baseline data reveal a substantial MetS burden in a relatively young population, necessitating precision screening tools that account for these gender-specific clinical profiles.

PREDICTORS OF METABOLIC SYNDROME (MULTIVARIATE ANALYSIS)

Two gender-specific multivariate logistic regression models were constructed to identify independent determinants of metabolic syndrome (MetS) and develop predictive algorithms. The models integrated a comprehensive set of non-invasive parameters: waist-to-height ratio (WHtR), chronological age, hemodynamic indicators (SBP, DBP), and behavioral factors (smoking status and sleep duration).

THE ROLE OF ANTHROPOMETRIC INDICATORS AND SCALING

Waist-to-height ratio (WHtR) emerged as the most potent shared predictor of MetS across both cohorts. To enhance clinical interpretability, coefficients were linearly scaled by 0.01 units. Each 0.01 increase in WHtR (equivalent to a 1% increment) was associated with a

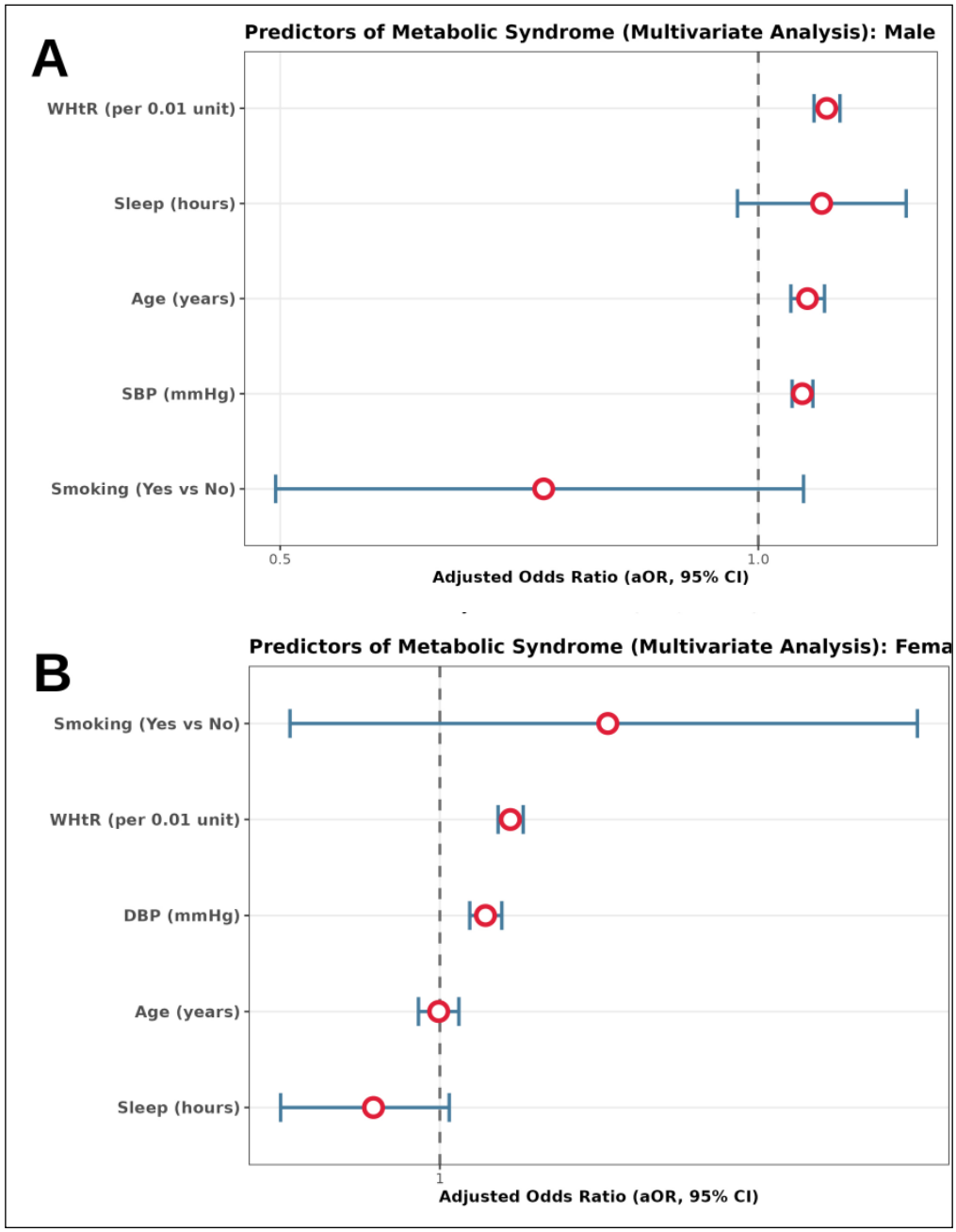


Fig. 1. Multivariate analysis of non-invasive predictors for metabolic syndrome. Forest plots displaying adjusted Odds Ratios (aOR) and 95% confidence intervals (CI) for (A) women and (B) men. Significant predictors ($p < 0.05$) are those with confidence intervals that do not cross the vertical reference line (OR = 1.0). WHtR is calculated per 0.01-unit increment
Picture taken by the authors

10% increase in MetS risk regardless of gender (Adj. OR 1.10; 95% CI: 1.08–1.12; $p < 0.001$).

Multivariate analysis revealed significant sexual dimorphism in the secondary predictor structure (Table 2).

• **Male model (n=1,477):** Beyond WHtR, chronological age (Adj. OR 1.07; 95% CI: 1.05–1.10; $p < 0.001$) and systolic blood pressure (SBP) (Adj. OR 1.06; 95% CI: 1.05–1.08; $p < 0.001$) significantly contributed to the risk profile.

• **Female model (n=1,434):** Age was not a significant predictor (Adj. OR 1.00; $p = 0.904$), suggesting that phenotypic traits dominate over chronological aging in metabolic risk development among women aged 18–45. Diastolic blood pressure (DBP) was identified as the primary hemodynamic predictor for this group (Adj. OR 1.06; 95% CI: 1.04–1.09; $p < 0.001$).

Behavioral factors, including sleep duration and active smoking, demonstrated no independent statistical significance in either model after adjusting for anthropometric and clinical parameters ($p > 0.05$) (Fig. 1).

The derived regression coefficients were used to establish logit (z) equations for calculating individual probability (P):

MODEL PERFORMANCE AND VALIDATION

The predictive reliability of the gender-specific models was evaluated by analyzing their discriminatory power and calibration accuracy in accordance with TRIPOD protocols [13].

Receiver Operating Characteristic (ROC) analysis confirmed the high performance of both non-invasive

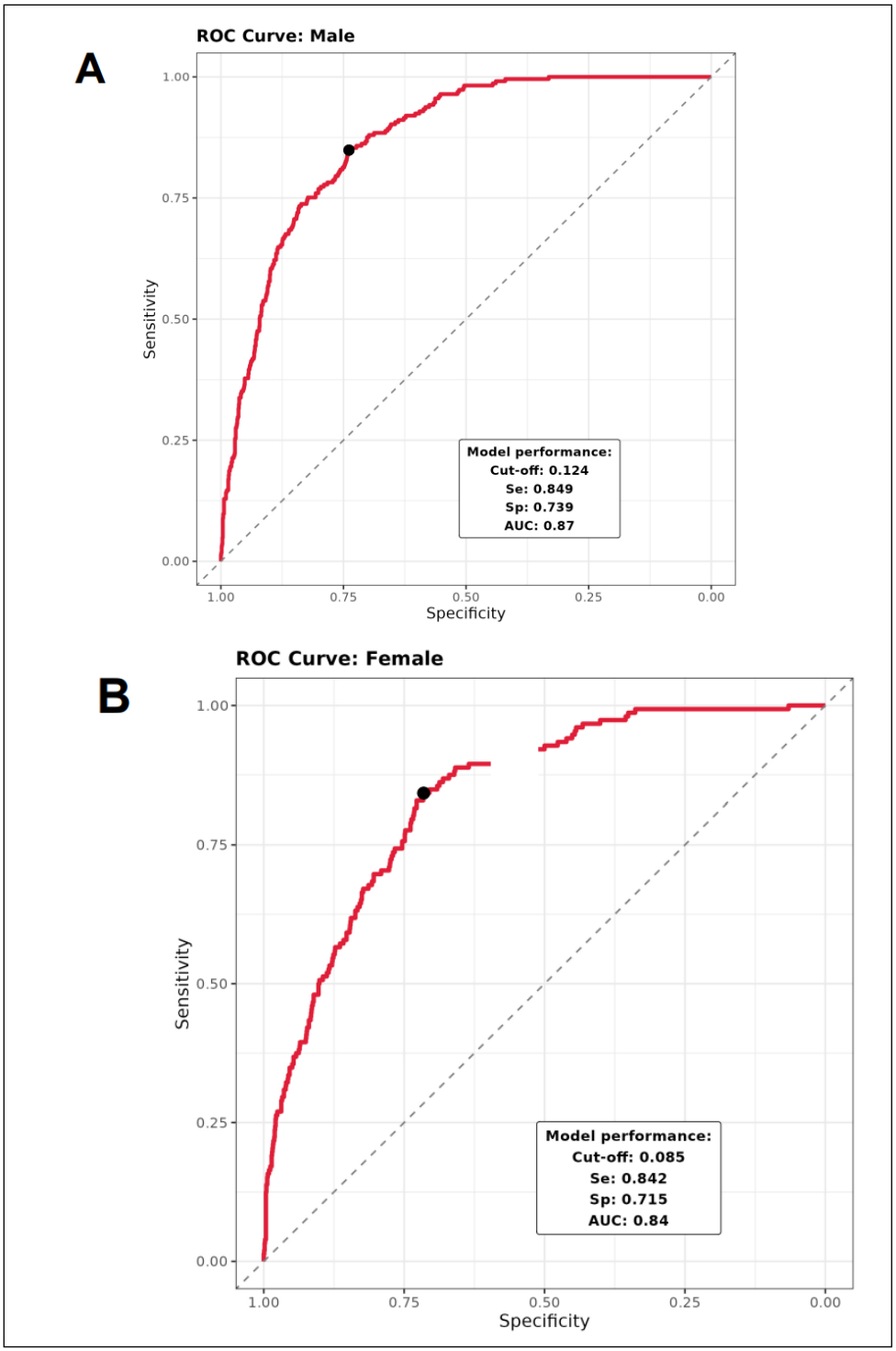


Fig. 2. ROC curves and diagnostic performance of the predictive models. Receiver Operating Characteristic (ROC) analysis for (A) the male cohort and (B) the female cohort. The black dots represent the optimal cut-off points determined by the Youden index. (A) Male model: AUC = 0.87, Cut-off = 0.124. (B) Female model: AUC = 0.84, Cut-off = 0.085
Picture taken by the authors

algorithms in classifying metabolic syndrome (MetS) status among young adults.

• **Male model:** The Area Under the Curve (AUC) was 0.87, indicating «excellent» discriminatory power. The optimal cut-off point, determined by the Youden index, was 0.124, yielding high sensitivity (Se: 0.849) and specificity (Sp: 0.739).

• **Female model:** The AUC was 0.84, representing «very good» predictive accuracy. The optimal cut-off for the female population was 0.085, with a sensitivity of 0.842 and a specificity of 0.715.

High sensitivity values (exceeding 84% for both sexes) justify the use of these models as primary screening tools to minimize false negatives in resource-limited clinical settings (Fig. 2).

Calibration plots were constructed to assess the agreement between predicted probabilities and the actual observed frequency of MetS.

• **Men:** The model trend demonstrates close convergence with the ideal calibration diagonal. Predicted risks show stable agreement with observed data across

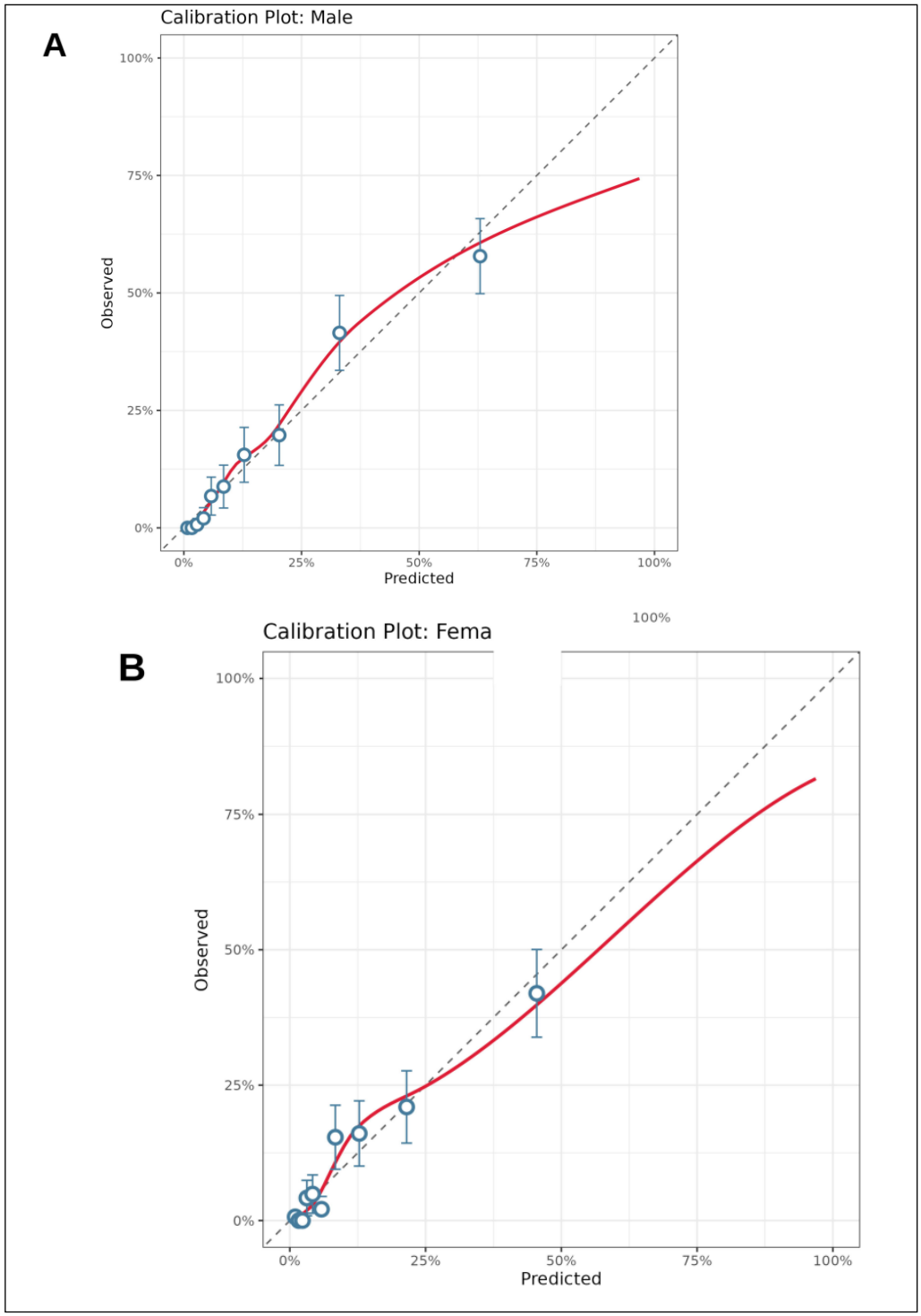


Fig. 3. Calibration plots for the predicted MetS risk. Agreement between the predicted probabilities (X-axis) and the actual observed frequencies (Y-axis) of metabolic syndrome for (A) men and (B) women. The dashed diagonal line represents perfect calibration (Slope = 1, Intercept = 0). The solid line represents the model trend, showing high reliability across the probability range
Picture taken by the authors

the entire probability range, characterized by an intercept near 0 and a slope near 1.

• **Women:** The calibration curve shows high accuracy within the low-to-moderate probability range (up to 0.5). However, at extremely high predicted risks (above 0.6), a slight overestimation occurs. This trend may be attributed to the lower frequency of terminal-stage abdominal obesity observations within this specific age cohort (Fig. 3).

Overall, the validation results indicate that non-invasive models based on WHtR and blood pressure provide clinically acceptable accuracy. These algorithms serve as a reliable alternative to laboratory-based testing for mass screening in young adults.

PREDICTIVE TOOLS AND RISK VISUALIZATION

To implement the statistical analysis into clinical practice, two types of visual tools were developed based on the gender-specific logistic regression models: nomograms and

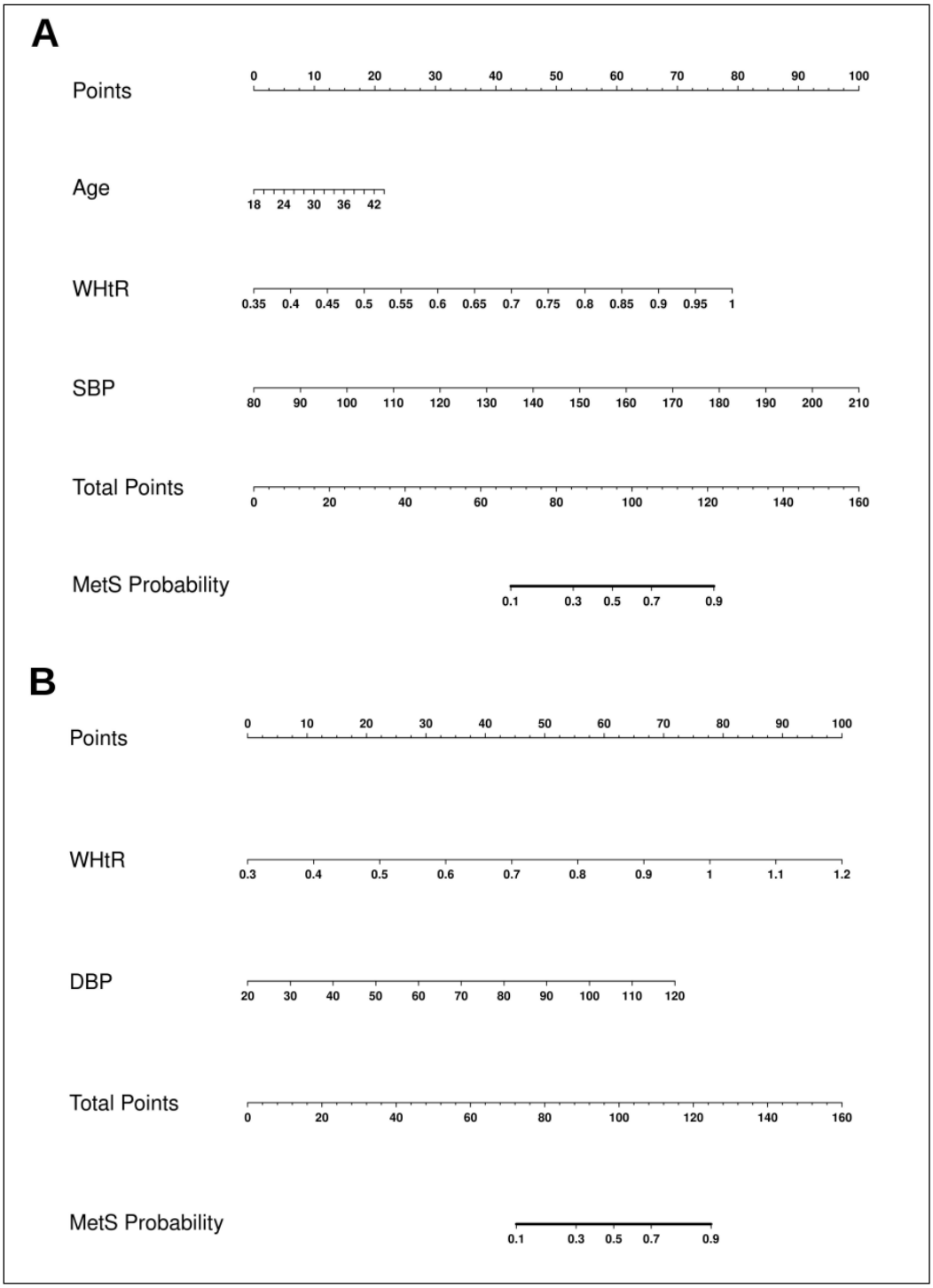


Fig. 4. Clinical nomograms for individual MetS risk assessment. Graphical scoring systems developed for (A) women and (B) men. Points are assigned for age, WHtR, and blood pressure (SBP for men, DBP for women). The «Total Points» sum corresponds to the «Probability of MetS» scale at the bottom of the nomogram
Picture taken by the authors

risk heatmaps. These tools enable clinicians to perform an instantaneous assessment of metabolic syndrome (MetS) probability without requiring complex mathematical logit calculations.

STRATEGIC RISK ZONES AND CLINICAL INTERPRETATION

Based on the Youden threshold analysis and the probability distribution within the young adult sample (N=2,911), three strategic risk zones were identified:

- **Green Zone (Low Risk):** P < 12.4 % for men and P < 8.5 % for women. This range corresponds to the population norm, where the probability of MetS is minimal. Patients in this category require only standard preventive observation.
- **Yellow Zone (Moderate Risk):** Ranging from 12.4 %/8.5 % to 50.0 %. This represents a zone for preventive intervention. Although clinical criteria according to NCEP ATP III may not be fully met, the patient has a high predisposition to their manifestation, necessitating early lifestyle modification.
- **Red Zone (High Risk):** P ≥ 50.0 %. This is a zone of high diagnostic certainty where the probability of MetS pres-

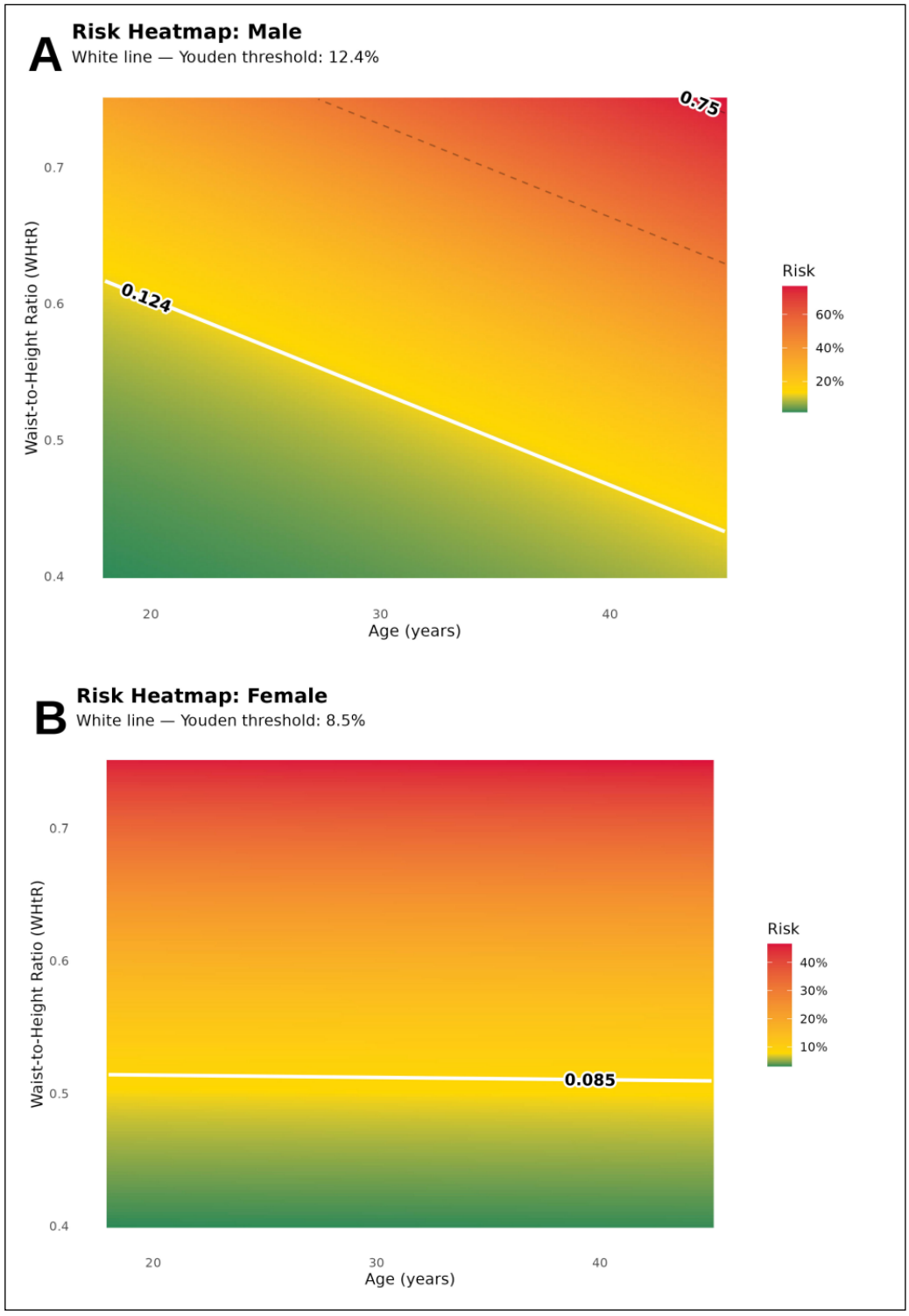


Fig. 5. Risk heatmaps for metabolic syndrome stratification. Visual representation of individual risk based on the interaction between age (X-axis) and WHtR (Y-axis) for (A) women and (B) men. Color-coded zones indicate low (green), moderate (yellow), and high (red) risk levels. The white dashed line represents the Youden index threshold for each gender group
Picture taken by the authors

ence exceeds the probability of its absence. Classification into this zone requires immediate clinical evaluation and invasive laboratory diagnostics (glucose and lipid profiles).

NOMOGRAM CLINICAL WORKFLOW

Nomograms (Fig. 4, Fig. 5) function as graphical calculators where specific values of each predictor correspond to a point score. The clinical workflow includes the following steps:

1. **Identify predictor values:** For each patient parameter (age, WHtR, and SBP for men or DBP for women), the clinician identifies the corresponding point on the predictor scale.
2. **Project to the point scale:** A perpendicular line is drawn upward from the identified value to the «Points» scale (0–100).
3. **Calculate Total Points:** The points for all significant non-invasive parameters are summed.

Table 2. Logistic regression parameters for MetS risk prediction

Predictor	Coefficient (β)	SE	Wald χ2	p-value	Adj. OR (95% CI)
Men (n=1,477)					
Constant	-17.502	1.14	235.6	<0.001	—
Age (years)	0.068	0.012	46233	<0.001	1.07 (1.04–1.10)
WHtR (per 0.01)	0.099	0.009	109.8	<0.001	1.10 (1.08–1.12)
SBP (mmHg)	0.063	0.008	67.7	<0.001	1.06 (1.05–1.08)
Women (n=1,434)					
Constant	-12.304	0.94	171.6	<0.001	—
Age (years)	0.002	0.013	0.02	0.904	1.00 (0.97–1.03)
WHtR (per 0.01)	0.093	0.008	121.0	<0.001	1.10 (1.08–1.12)
DBP (mmHg)	0.060	0.011	46203	<0.001	1.06 (1.04–1.09)

Abbreviations: \beta, regression coefficient; SE, standard error; OR, Odds Ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Source: compiled by the authors of this study

4. **Determine probability:** The total value is located on the «Total Points» scale, from which a line is drawn downward to the «Probability of MetS» scale.

INTERPRETATION OF RISK HEATMAPS

Heatmaps (Fig.5) provide immediate visual risk stratification based on age and WHtR. In the female heatmap, the Youden threshold (represented by a white line at 8.5%) is nearly horizontal. This indicates that for women aged 25 and 40 with the same WHtR (e.g., 0.52), the MetS risk is identical, marking the onset of the moderate risk zone. Conversely, the male Youden threshold (12.4%) exhibits a distinct slope. While a 20-year-old man with a WHtR of 0.60 is situated in the moderate risk zone (~40.0%), by age 45 with the same WHtR, he automatically transitions into the «red» high-risk zone (P > 60.0 %), demonstrating the synergistic effect of age in the male population.

Integrating these tools into primary care facilitates the identification of high-risk patients without invasive procedures, aligning with contemporary principles of personalized medicine.

DISCUSSION

SUMMARY OF MAIN FINDINGS

The primary outcome of this study is the development and internal validation of gender-specific predictive models for metabolic syndrome (MetS) in young adults (aged 18–44 years) using exclusively non-invasive parameters. Leveraging representative data from the NHANES 2011–2018 cycles, we demonstrated that integrating anthropometric, demographic, and clinical indicators achieves high classification accuracy without requiring costly and invasive biochemical blood tests. The models exhibited excellent discriminatory power, with an Area Under the Curve (AUC) of 0.87 for men and 0.84 for women. These results support the hypothesis that, for Non-Hispanic White young adults, a combination of basic physical parameters is sufficient for the precise identification of cardiometabolic risk.

Waist-to-height ratio (WHtR) emerged as the most potent shared predictor of MetS across both cohorts. Every 0.01-unit (1%) increase in WHtR was associated with a 10% elevation in the risk of the pathology, independent of gender (Adj. OR 1.10; 95% CI: 1.08–1.12). This underscores abdominal obesity as a critical marker of metabolic derangement in young individuals, outperforming traditional BMI in predictive value.

Our analysis revealed significant gender-specific differences in MetS predictor structures. For young men, chronological age and systolic blood pressure (SBP) were significant risk-enhancing factors (OR 1.07 and 1.06, respectively). Conversely, in women aged 18–44, chronological age did not significantly influence MetS probability (OR 1.0; p > 0.05), shifting the diagnostic focus for this demographic primarily toward anthropometric parameters and blood pressure.

The derived mathematical models were translated into applied visualization tools—nomograms and risk heatmaps. These instruments convert complex logistic regression equations into a simplified scoring system accessible for use during primary care consultations. This approach facilitates rapid screening and identifies patients in the «high-risk» zone who genuinely require further invasive evaluation according to NCEP ATP III criteria. Consequently, these findings not only validate non-invasive MetS diagnostics but also offer a ready-to-implement algorithm capable of reducing the economic burden on healthcare systems by optimizing laboratory testing in low-risk groups.

ETHNIC HOMOGENEITY AND MODEL VALIDITY

Metabolic syndrome and its components exhibit pronounced ethnic specificity, particularly concerning anthropometric indicators like waist circumference (WC) and BMI. These differences are not solely biologically determined but reflect a complex interplay of genetic, epigenetic, cultural, socioeconomic, and behavioral factors [14]. Populations from South and East Asia, as well as the Pacific region, develop metabolic complications at much lower levels of total fat mass compared to European populations [15-17]. For instance, research by Sigit et al. (2020) [18] indicated that Indonesians have significantly higher levels of abdominal obesity and hypertriglyceridemia at the same BMI as Dutch individuals, explaining their higher MetS risk at lower body weights. Similarly, age-standardized MetS prevalence in Chinese ethnic groups varied from 24.6% in Han populations to 13.7% in Miao groups [19], suggesting intra-national variations linked to genetic predisposition and lifestyle.

Waist circumference remains a pivotal indicator of abdominal obesity. Data from two large New Zealand cohorts (Workforce Diabetes Study and the Diabetes, Heart and Health Survey) showed that the Metabolic Syndrome Severity Score (MetSSS) correlates strongly with glucose-regulatory and cardiovascular status, an association clearly linked to ethnicity: the highest MetSSS levels were found in Māori and Pacific participants compared to Europeans [20]. This necessitates ethnicity-specific diagnostic criteria. Furthermore, research in Suriname [21] established optimal WC and BMI thresholds for predicting hypertension and metabolic risk across six distinct ethnic groups, proving that universal criteria are clinically inadequate. Additionally, Rønn et al. (2017) [22] found that traditional anthropometrics (BMI and WC) have varying degrees of informativeness for quantifying visceral adipose tissue (VAT) across ethnic groups. Identical waist measurements may indicate different volumes of metabolically active VAT, directly impacting risk levels, with South Asian populations being particularly vulnerable.

A systematic review and meta-analysis by Adjei et al. (2024) [14] across 23 high-income countries demonstrated that MetS prevalence varies substantially by ethnicity, often significantly exceeding rates in White populations for groups such as African Americans, Hispanics, and Pacific Islanders. This variability is tied to differing distributions of anthropometric risks. Scientific evidence thus argues against universal anthropometric standards, confirming that ethnic specificity is a fundamental principle in MetS diagnosis and treatment.

LIMITATIONS AND GENERALIZATION

The current results, including the nomograms and heatmaps, have limited applicability to Asian, African American, or Indigenous populations, where different correlation coefficients and risk thresholds are required [23,24]. These models cannot be recommended for non-White populations without additional external validation and coefficient recalibration. However, this narrow specialization contributed to the high accuracy (AUC 0.87 for men) observed. Restricting the study to a White cohort is viewed here as a methodological advantage aligned with personalized medicine principles, avoiding the «averaging» of risks and providing clinicians with a tool tailored to the biological characteristics of a specific population group [22].

The cross-sectional nature of the NHANES data is a significant limitation for establishing causality. While the models effectively predict MetS presence at the time of examination, they do not confirm that predictors such as WHtR or blood pressure are primary etiological factors [25]. Furthermore, data regarding lifestyle, sleep duration, and smoking status are self-reported, which may introduce classification errors due to recall bias or social desirability bias [26]. Such errors may slightly underestimate the weight of behavioral factors in the final logit equations.

In large epidemiological cohorts, it is also challenging to fully account for the influence of antihypertensive, hypoglycemic, or lipid-lowering medications already being taken by participants [20]. Since therapy can artificially normalize MetS components (BP, glucose, or lipids), it may mask the actual prevalence of the syndrome and lead to an underestimation of the strength of certain predictors [18].

Consequently, the clinical applicability of the developed nomograms is strictly limited to individuals not currently receiving antihypertensive therapy. Since the models rely heavily on unmodified blood pressure values, medication-induced normalization would artificially lower the calculated risk score, rendering the prediction inaccurate for treated patients.

DIRECTIONS FOR FURTHER RESEARCH

To enhance accuracy and expand the clinical scope of the diagnostic algorithm, prospective studies using independent international cohorts are a priority next step to evaluate the capacity of non-invasive nomograms to predict long-term cardiometabolic risks, such as type 2 diabetes or cardiovascular events. Subsequent stages should involve developing specific nomograms for other populations (Asian, African American, Hispanic), accounting for their unique anthropometric thresholds and visceral fat accumulation patterns.

The creation of automated clinical decision support tools (web-based calculators) based on the derived mathematical formulas is a promising direction [27, 28]. This would provide clinicians with instantaneous risk

calculations during outpatient visits. Future research must also incorporate factors such as food security, education levels, and income, as these socioeconomic determinants significantly impact MetS prevalence and may explain substantial risk variability even within the same ethnic group.

CONCLUSIONS

This study establishes gender-specific, non-invasive predictive models for metabolic syndrome (MetS) in young adults, demonstrating robust discriminatory power (AUC ≥ 0.84). The findings identify waist-to-height ratio (WHtR) as the most significant independent determinant of MetS across both sexes. A distinct gender dimorphism was observed: chronological age significantly enhances risk in men but lacks predictive value in women, underscoring the dominance of phenotypic features over chronological aging in female cardiometabolic risk stratification.

The resulting nomograms and heatmaps provide evidence-based tools for immediate risk assessment in primary care settings. These instruments enable the identification of high-risk individuals without the need for invasive laboratory diagnostics, offering a scalable solution for early intervention. By streamlining screening protocols and reducing reliance on fasting biochemical profiles, these tools can improve diagnostic uptake and reduce healthcare costs in young populations.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Acute and chronic toxicity of Spinosad in common Carp (*Cyprinus carpio*): Implications for health and environmental safety

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ABSTRACT

Aim: The study aims to analyzes the acute and chronic effects of the Spinosad insecticide on common carp (*Cyprinus carpio Linnaeus, 1758*). Aquatic ecosystems face increasing threats from agricultural runoff, especially pesticides.

Materials and Methods: Acute toxicity tests were conducted over 96 hours with six concentrations of Spinosad (2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L) to determine the median lethal concentration (LC50) and observe behavior. For chronic exposure, carp were exposed to three sublethal concentrations (1.25, 3.75, and 5.0 mg/L) for 20 days. Key physiological indicators, including blood cell counts, hemoglobin levels, liver enzyme activities (AST and ALT), and liver histology, were assessed for any histopathological changes.

Results: Acute toxicity tests show an LC50 of 7.41 mg/L for Spinosad in common carp, with behavioral changes more noticeable at higher concentrations. Chronic exposure caused significant alterations in hematological parameters (RBC, WBC, PCV, and Hb), increased liver enzymes (AST and ALT) with longer exposure times ($P \leq 0.001$ for AST; $P \leq 0.0001$ for ALT). Histological analysis revealed liver damage, including vacuolation, swelling, and necrosis, with severity rising with concentration and exposure duration.

Conclusions: This study shows that Spinosad, labeled as a "reduced-risk" pesticide, causes significant short-term and long-term toxicity in common carp, affecting behavior, blood parameters, liver enzyme activity, and tissue health. These results emphasize the environmental dangers of Spinosad runoff from agricultural fields and underline the importance of careful use and monitoring to protect aquatic ecosystems. More research is needed to explore the long-term impacts on non-target aquatic species.

KEY WORDS: Spinosad, common carp, water pollution, acute exposure, chronic exposure, hematological parameters, and histological examinations

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INTRODUCTION

The application of pesticides can significantly impact fish populations, the health of aquatic ecosystems, and the accumulation of harmful substances in humans. Consequently, this raises considerable concern since fish are particularly vulnerable to the effects of insecticides. Insecticide contamination in water is a common issue, as these chemicals can enter surface water through runoff from agricultural fields and treated soil or be sprayed directly onto the water's surface, as seen in mosquito control efforts. Such contamination can harm aquatic plants, reduce dissolved oxygen levels in the water, lead to toxic algal blooms, and negatively affect fish physiology and behavior. The overuse of fertilizers has also been linked to declines in various fish populations [1].

While using biological products is desirable and highly specific to targeted pests, creating suitable formulations poses challenges. Specifically, the formulated product must maintain the functionality of the biological agent during storage and application while also possessing good physical properties and ease of use. One natural product, Spinosad, has been authorized by various national and international certifying agencies for use in organic agriculture [2]. It is produced through the fermentation of bacteria. According to the U.S. Environmental Protection Agency (U.S. EPA, undated), Spinosad is classified as a "reduced-risk" substance and carries the lowest human hazard signal word, "Caution," underscoring its status as a naturally occurring, low-impact insecticide.

Spinosad is a pesticide within the spinosyn class, derived from the bacterium *Saccharopolyspora spinosa*, and consists of spinosyns A and D. Typically, "Spinosad" refers to a combination of spinosyn A and D, with spinosyn A accounting for between 85% and 90% of the mixture. Spinosyns can affect insects through direct contact, toxicity from body surface exposure, or ingestion via the food chain. The biochemical interaction of the bioinsecticide is associated with the disruption of nicotinic acetylcholine receptors and GABA-gated ion channels in the nervous systems of insects. Notably,

Spinosad specifically stimulates nicotinic acetylcholine receptors in fleas, while it does not activate other nicotinic receptors or GABA receptors [2].

Pesticides are recognized as one of the most detrimental agricultural pollutants, contributing to both mortality and physiological changes in aquatic life. Among aquatic life, Fish eggs, larvae, yearlings, and fingerlings are particularly susceptible to contamination from pesticides and heavy metals, as these aquatic species are not equipped to cope with such pollutants. Injury to the essential organs like liver, kidneys and gills will disrupt important physiological processes, such as survival, buoyancy, osmoregulation and reproduction. These interferences could eventually lead to the loss of population and recruitment challenges to the stock [3].

A perfect example of exceeding adaptability that ensures a lot of success in various environmental conditions is the *Cyprinus carpio*, a very renowned representative of the largest freshwater fish family, *Cyprinidae,* namely, the common carp. Fisheries, whether produced in aquaculture or caught in the wild ecosystem, are a lifeblood of fishing communities and constitute a very substantial portion of the world's food supply [4].

Therefore, since *Cyprinus carpio* (common carp) is a one of the main food sources, the physiological effects of Spinosad exposure could pose risks to humans who eat contaminated fish. By examining these biomarkers, this study provides a critical link between environmental toxicology and public health, emphasizing the need for sustainable farming practices that protect both aquatic life and the human food supply.

The ability of a substance to be harmful, whether through acute or cumulative chronic action is its toxicity. The conditions that affect the toxicity include chemical composition of the substance, its concentration and duration of exposure, and the nature of the organism that is exposed, i.e. species, age, sex, and nutritional status. The exposure route, i.e., oral, inhalation, or dermal, also comes into play since some chemicals also have the potential of causing the skin and the eyes to be irritated [5]. Among the toxic compounds, pesticides are the most toxic chemicals to fish and other creatures in the food chain.

The ecotoxicity testing common to vertebrates is still acute fish toxicity testing. Mortality is normally measured using them: standard carp is normally subjected in a test material in 96 hours, and mortality is monitored after 24, 48, 72, and 96 hours. Based on such observations, the LC50 value is calculated and this is the concentration of the substance that causes 50 percent mortality of test group in the short exposure period.

Besides, the evaluation of long-term effects and regulatory data requirements should be conducted with the help of chronic toxicity tests. Biochemical and hematological indicators are widely used in fish toxicology since they are a simple and cheap mechanism of testing physiological alterations. Among them, the main predictors of immune reactions are differential leukocyte count (DLC) and white blood cell (WBC), whereas the great importance is paid to red blood cells (RBCs) or erythrocytes in providing oxygen. Such indicators are usually determined after exposure to different chemicals [6].

Biomarkers are also useful in biomarkers research, in understanding the impacts of environmental changes to living organisms and the ecosystems. Biomarkers in liver functioning, such as enzymes alanine transaminase (ALT) and aspartate transaminase (AST), are useful as sources of data on the health of organisms and in response to [7]. Histological biomarkers are of particular concern in the process of locating the damage inflicted by toxic substances and carcinogens, since they provide an opportunity to study specific organs required in respiration, excretion, and xenobiotic metabolism. The gills, kidneys and liver histopathological analysis are important data in the monitoring of the environment where the biological impact of a toxic exposure is to be determined [8].

AIM

The study aims to analyze the acute and chronic effects of the *Spinosad* insecticide on common carp (*Cyprinus carpio Linnaeus,* 1758). Aquatic ecosystems face increasing threats from agricultural runoff, especially pesticides. This study assessed the acute and chronic toxic effects of Spinosad, a low-risk, naturally derived bioinsecticide, on common carp (*Cyprinus carpio Linnaeus*, 1758), a bioindicator for water pollution.

MATERIALS AND METHODS

EXPERIMENTAL FISH

The study used common carp (*C. carpio Linnaeus*, 1758), specifically juvenile *Cyprinus carpio* weighing 30 ± 3 grams, measured with a precise scale. These fish samples were collected from the Babylonian reservoirs and transported to

Table 1. Result of physical and chemical properties of aquariums

Physical and Chemical Properties	Range
Temperature (°C)	21- 25
Hydrogen Ion Concentration(pH)	6.9-7.6
Dissolved Oxygen (D.O) (mg/l)	5.5-7.9
Electrical Conductivity (µs/cm)	850- 1220

Table 2. Amount of pesticide added (mL) per basin volume (liters) and effective concentration [mg/L]

Amount of pesticide added [ml]	Basin volume [litres]	Effective concentration [mg/L]
0.10	40	2.5
0.20	40	5.0
0.25	40	6.25
0.30	40	7.5
0.35	40	8.75
0.40	40	10.0

Source: Compiled by the authors of this study

Table 3. Sublethal Concentrations of Spinosad for *C. carpio*

Groups	LC50 value\UI	Spinosad (mg/l)
1	1\50	0.1482
2	1\20	0.3705
3	1\10	0.741
4	control	control

Source: Compiled by the authors of this study

Table 4. Spinosad concentration (mg/L) and mortality percentage in common Carp at 24, 48, 72, and 96 hours

Concentration [mg/L]	Mortality after 24 hours [%]	Mortality after 48 hours [%]	Mortality after 72 hours [%]	Mortality after 96 hours [%]
2.5	0	0	0	0
5.0	0	0	12.5	25
6.25	12.5	25	37.5	50
7.5	25	37.5	62.5	75
8.75	37.5	50	75	87.5
10.0	50	75	87.5	100

Source: Compiled by the authors of this study

the laboratory in plastic bags. Approximately 165 fish were sampled to determine their acute exposure. Any weak or diseased fish were excluded to ensure that only healthy fish were included in the experiment (US Environmental Protection Agency, 1996). To promote acclimatization, the fish were kept under laboratory conditions for 10–14 days before starting the experiments. The control group was kept in experimental water without adding the Spinosad pesticide, keeping all other conditions constant.

GLASS AQUARIUMS

The fish tanks used for acclimatization and experiments measured 40 × 50 × 30 cm and held 40 liters of water. Oxygen was supplied to the tanks *via* a central pump that distributed it through rubber tubing to each tank. During the acclimatization and experimental periods, these tanks received a continuous flow of oxygen around the clock. Dechlorinated water was used; to achieve this, tap water was left for 72 hours to dissipate the chlorine before being added. In both acute and chronic exposure experiments, tank water was replaced every 48 hours with 40 liters, with constant aeration, maintaining a pH of 6.9 to 7.6 and a temperature of 21-25°C (Table 1). The same procedure was followed during the acclimatization and experimental phases [9].

THE CONCENTRATIONS NEEDED FOR THE ACUTE TOXICITY EXPERIMENT

The concentrations utilized in the experiments were calculated using the equation $C1 \times V1 = C2 \times V2$, expressed in mg/L. The test chemical, Spinosad, was administered to common carp over a 96-hour period, during which mortality rates were recorded at 24, 48, 72, and 96 hours. The LC50, defined as the concentration that leads to the death of 50% of the fish, will be determined when applicable. Each experimental group consisted of at least eight fish across various concentrations of Spinosad, in addition to a control group. Furthermore, the threshold concentration for this test was set at 100 mg/L, with observations conducted at specified intervals [10].

In the acute toxicity test, six different concentrations of Spinosad were examined: 2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L, to ascertain the LC50 values (Table 2). Data tables presented

Table 5. The logarithm of the concentration and the probit unit

Log Concentration	Probit Value
0.796	4.33
0.875	5.00
0.942	5.67
1.0	6.28

Source: Compiled by the authors of this study

Table 6. The behavioral changes in common carp exposed to Spinosad

Concentration	Behavioral changes
Control	The fish's behavioral and swimming patterns remained unchanged, with no mortality observed throughout the entire testing period.
2.5 mg/l	No mortality was recorded, and normal fish movement was documented.
5.0 mg/l	After exposure, sudden jumping and darting were observed. All fish settled to the bottom after more than 24 hours.
6.25 mg/l	Fish exhibited increased abnormal swimming behavior, and hyperventilation was observed.
7.2 mg/l	Vertical and downward swimming patterns were observed, and jumping frequency increased. Four fish died within 24 hours.
8.75 mg/l	Swimming problems, loss of balance, and sudden jumping were observed. Increased gill movement was observed, and the fish attempted to breathe air from the surface. Six fish died within the first hours, and over time, all fish died after 24 hours.
10.0 mg/l	The fish exhibited increased gill movement and attempted to breathe air from the surface. They experienced difficulty swimming, loss of balance, and sudden jumps. After less than eight hours, all the fish died.

Source: Compiled by the authors of this study

Table 7. The primary hematological parameters in fish

Parameters	Control	0.1482 [mg/L]	0.3705 [mg/L]	0.741 [mg/L]	LSD (P<0.05)
RBC [10^{12}/L]	3.79 a	2.97 b	2.09 c	1.94 c	0.42
HGB [g/dL]	12.80 a	11.80 b	11.30 b	9.80 c	1.15
HCT [%]	42.20 a	36.10 b	38.70 b	30.90 c	3.8
LYM% [%]	32.04 c	27.02 b	9.07 c	27.06 b	4.95
MON% [%]	4.70 a	6.08 c	27.16 a	18.54 b	2.88
NEU% [%]	57.90a	60.30 a	57.36 a	44.45 b	6.2

Note: a = the group with the highest concentration, and it differs significantly from the groups with the other letters;
b = the group that differs significantly from the groups with the letters a and c;
c = The group with the lowest concentration, and it does not differ from any group with the same letter
Source: Compiled by the authors of this study

the logarithm of the concentrations and the probit units for the 24, 48, 72, and 96-hour periods. At elevated concentrations, Spinosad induced notable behavioral changes in the fish. Throughout the 96 hours, the behavior and condition of the fish were meticulously documented every 24 hours. A control group of fish was concurrently acclimatized and monitored, showing no mortality after 24, 48, 72, or 96 hours.

In contrast, the fish exposed to Spinosad exhibited behaviors such as sudden twitches, loss of balance, disrupted swimming patterns, difficulty breathing, surfacing, and darting movements. It is also important to highlight that many insecticides inflict harm by disrupting biological systems [11]

CHRONIC TOXICITY FOR COMMON CARP

Three groups of common carp, each comprising eight fish per aquarium, were subjected to exposure to the biocide Spinosad (1/10, 1/20, 1/50) for a duration of 20 days, alongside a control group (Table 3). To effectively eliminate waste, the water in the aquariums was refreshed every 48 hours. The fish received daily feedings, and samples were collected after 20 days.

HEMATOLOGICAL PARAMETERS

Hematological indices serve as important indicators of changes in metabolism and physiology, as blood is involved in numerous physiological processes throughout the body.

Table 8. Comparison between different groups in ALT and AST

Group	Means ±SE	
	ALT (IU/L)	AST (IU/L)
Control	30.33 ±1.45 c	32.33 ±2.34 c
Pesticide 0.1482 mg/L	34.00 ±1.15 c	36.00 ±3.05 c
Pesticide 0.3705 mg/L	56.67 ±3.75 b	51.67 ±2.03 b
Pesticide 0.741 mg/L	70.00 ±2.31 a	64.33 ±3.92 a
L.S.D.	7.708 **	9.554 **
P-value	0.0001	0.0002
Means that the different letters in the same column differed significantly. ** (P≤0.01).		

Source: Compiled by the authors of this study

Table 9. Histological changes in the liver of C. carpio cod after chronic exposure to concentrations of 0.1482, 0.3705, and 0.741 mg/L of Spinosad

Concentration	Exposure period (20 days)
Control	Normal at all times.
0.1482 mg/l	Vacuolar degeneration of the cytoplasm in hepatocytes (see Figures 6, 7, 8, and 9).
0.3705 mg/l	Hepatocyte hypertrophy and cytoplasmic vacuolation are observed, along with moderate degeneration and atrophy of the pancreatic acini (see Figures 10 and 11). There is significant thickening of the intestinal villi, which is associated with hyperplasia and aggregation of white blood cells (leukocytes). This indicates inflammation and impaired immune function (refer to Figures 12 and 13).
0.741 mg/l	Severe and widespread histological changes include cytoplasmic vacuolation, hepatocyte hypertrophy, blood congestion in the sinusoids, and necrosis of hepatocytes (Figures 14 and 15). Necrosis indicates significant toxic damage.

Note: a = the group with the highest concentration, and it differs significantly from the groups with the other letters;
b = the group that differs significantly from the groups with the letters a and c;
c = the group with the lowest concentration, and it does not differ from any group with the same letter
Source: Compiled by the authors of this study

Collecting a blood sample from live fish is generally a quick, simple, and minimally invasive procedure. To analyze the blood, researchers utilize a hemocytometer (Neubauer's counting chamber) in conjunction with a cover slip. Hematological examinations play a crucial role in assessing the physiological health of fish. Indeed, the methods employed for blood analysis are widely considered more reliable than those used for other biological or cellular samples [11, 12].

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

The liver functions as the primary metabolic center for most xenobiotics, making it one of the organs most susceptible to harmful chemical exposure. In line with this, [13] the liver is essential for detoxification and biotransformation processes.

The colorimetric determination of alanine aminotransferase (ALT) is based on the following reaction:

L-Alanine + ALT → Pyruvate + L-Glutamate

The pyruvate produced reacts with two molecules of 4-dinitrophenylhydrazine, yielding a colored hydrazone that can be measured at a wavelength of 546 nm (within the range of 530 to 550 nm). For precise results, it is crucial to separate serum from hemolysis from blood cells as soon as possible after collection.

SERUM ASPARTATE AMINOTRANSFERASE (AST)

AST enzymes are essential for developing resistance and enhancing defense mechanisms against pesticides [14]. Given this role, the colorimetric determination of aspartate aminotransferase (AST) involves the following reaction:

L-Aspartate + +oxoglutarate → Oxaloacetate + L-Glutamate

The resulting oxaloacetate reacts with two molecules of 4-dinitrophenylhydrazine, producing a colored hydrazone that can be measured using a spectrophotometer at a wavelength of 546 nm (within the range of 530 to 550 nm). It is crucial to separate serum that is free of hemolysis from blood cells as quickly as possible after collection.

These histopathological changes in common carp biomarkers refer to the signs of major cellular and tissue-levels changes that occur in organisms that are exposed to poisonous chemicals. This kind of information is highly valuable to scientists and health professionals, as they can be used to assess the possibility of a risk that comes with the environmental and industrial exposures [15-18] Fish are also sensitive

to environmental changes, such as xenobiotics, which significantly threaten ecosystem health and non-target species.

Examples of such magnifications through the environment include the concentrations of pesticides, which pose a threat to human health through the food chain. Such chemicals are bioaccumulated in aquatic life and biomagnified as they pass up the food chain to eventually get to the human being through consumption of fish containing the chemicals [19, 20]. Besides the accumulation, the chemical contaminants may lead to colossal amounts of lesions in fish organs, including the gills, liver, and kidneys. The histopathology of such organs is important in offering critical data regarding how much and how much the environment is polluted [21].

THE HISTOPATHOLOGICAL ALTERATION IN THE LIVER OF COMMON CARP

The liver is an important organ in fish toxicology because it plays an important role in the uptake, detoxification and elimination of xenobiotics. It is a highly important organ because morphological changes of the liver to toxic exposure tend to give reproducible patterns, hence serve as an excellent organ to do toxicological studies. The anatomical and physiological properties of the fish liver can also be helpful in determining the potential of metabolism in the liver and the microscopic changes of the liver as a result of the exposure to the toxin. Data of the hepatic reaction to particular toxicants is therefore needed. Some comprehensive reviews have fully addressed these aspects [22-24].

STATISTICAL ANALYSIS

The Statistical Package of Social Sciences was used to analyze data. To determine differences between groups, pair and multiple mean comparisons were done using the Least Significant difference (LSD) test and the Multiple Range Test of Duncan respectively.

RESULTS

The aquarium laboratory has physical and chemical characteristics

The physicochemical properties of the rearing environment and the formulation of the administered pesticide directly affect the organism's behavior and distribution, such as temperature, pH, dissolved oxygen, and electrical conductivity.

Acute exposure test

MEDIAN LETHAL CONCENTRATION (LC_{50}) OF SPINOSAD

This study and others considered the ecological (physical and chemical characteristics) along with experimental factors that significantly affect pesticide toxicity to fish when measuring the LC50 values (Table 4). Accordingly, in the acute toxicity test, six different Spinosad concentrations - 2.5, 5.0, 6.25, 7.5, 8.75, and 10.0 mg/L - were used to determine the LC50 values. The results of this study showed that Spinosad exhibits varying degrees of acute toxicity on common carp, with an LC50 value of 7.41 mg/L (Fig. 1). The logarithm of the concentration and the probit unit (Table 5).

BEHAVIORAL CHANGES

The behavior of fish in test and control tanks was monitored every 24 hours for up to 96 hours. Exposure to different concentrations of Spinosad resulted in significant changes, including anxious swimming and increased movement, particularly at higher concentrations. Fish showed increased sluggishness and agitation, producing excess mucus. Some carp displayed heightened arousal, leading to unpredictable behaviors (Table 6) [25].

CHRONIC EXPOSURE TEST

BLOOD PARAMETERS

Hematological parameters serve as essential indicators of physiological changes in fish, often arising from factors such as stress, pollution, and pesticide exposure. Consequently, analyzing blood samples yields valuable insights into the impact of pesticides on fish in their environment. Notably, exposure to Spinosad has been recognized as a key factor leading to significant reductions in hemoglobin, hematocrit, and red blood cell count, critical indicators of anemia. This decline in hemoglobin levels may result from damage caused by free radicals, impaired gas exchange, and the oxidation of methemoglobin (Table 7).

RED BLOOD CELL (RBC)

The observed hematological condition of the treated fish with Spinosad pulse or continuous was erythropenia (decrease in the number of red corpuscles or anemia), thrombocytopenia (decrease in the number of platelets), and leucocytosis (increase in the number of white corpuscles). The previous studies in fish treated with pesticides exhibited the same kinds of anemia.

HEMOGLOBIN (HB)

Hemoglobin (Hb), the principal protein in red blood cells (erythrocytes), is crucial for transporting oxygen from the

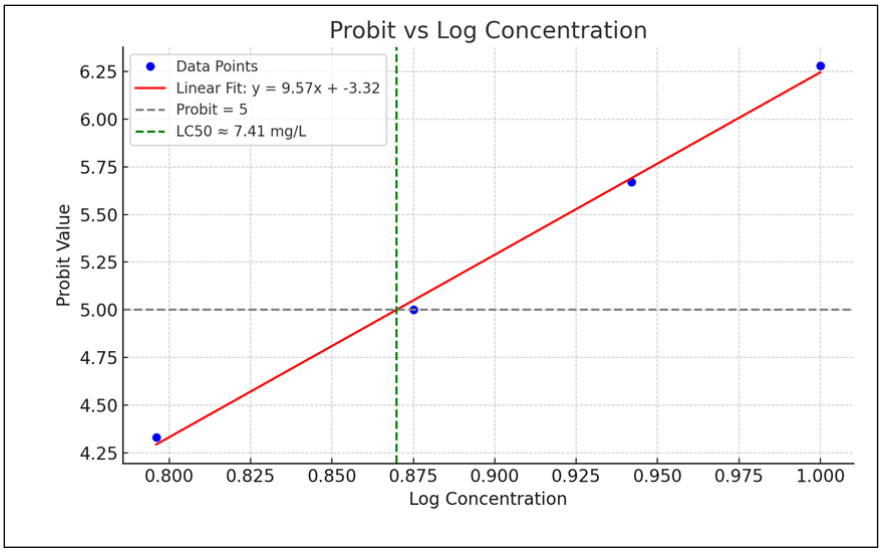


Fig. 1. The logarithm of the concentration and the probit
Source: Own materials

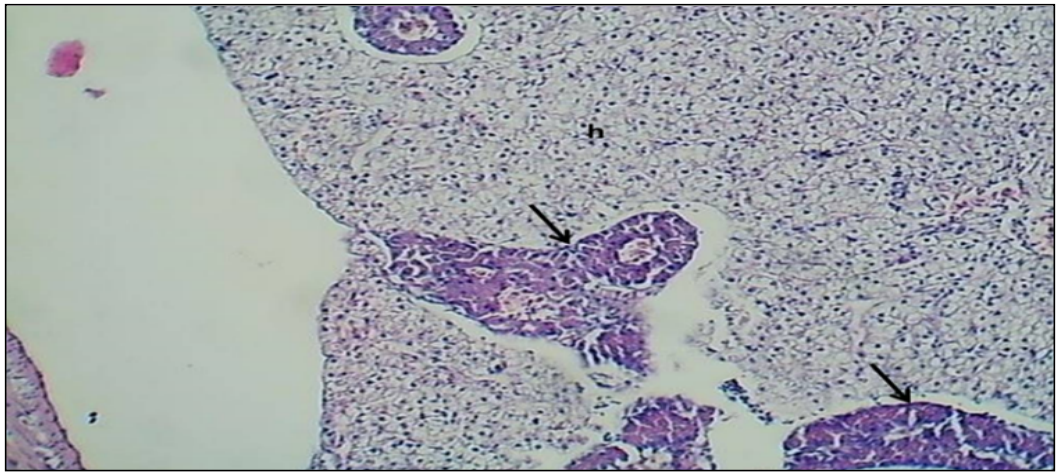


Fig. 2. Section of liver & pancreas (Control) shows normal appearance of pancreatic acini (arrows), normal hepatocytes (h). H&E.100x
Source: Own materials

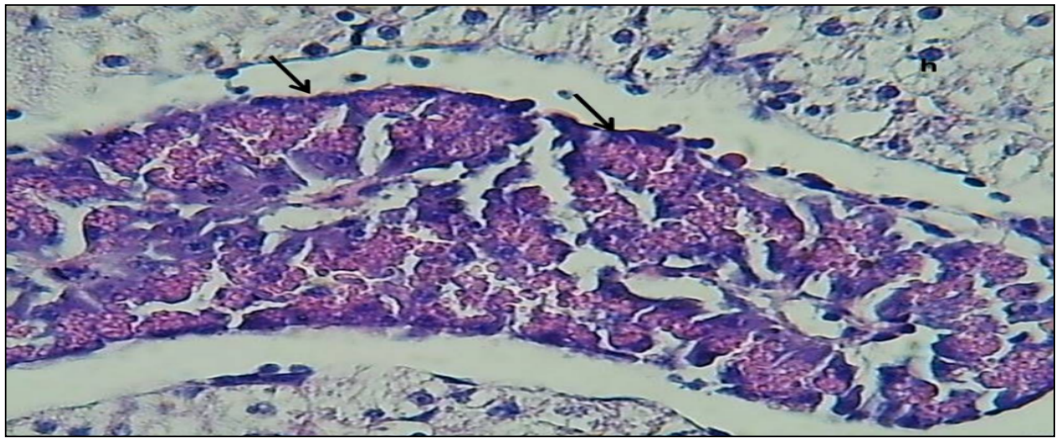


Fig. 3. Section of liver & pancreas (Control) shows normal appearance of pancreatic acini (arrows) & normal hepatocytes (h). H&E.400x
Source: Own materials

environment to body tissues. In fish, it also facilitates the rapid release of oxygen into the swim bladder and the choroid rete of the eye. This distinctive oxygen-transport system in fish possesses characteristics that are typically absent in other vertebrates, underscoring the specialized physiological role of fish hemoglobin.

PACKED CELL VOLUME (PCV)

The hematocrit (PCV) refers to the percentage of blood that is occupied by red blood cells of the total blood volume (100 milliliters). Different health problems may be represented by the abnormal values of hematocrit. In this study, the researcher tested an average packed

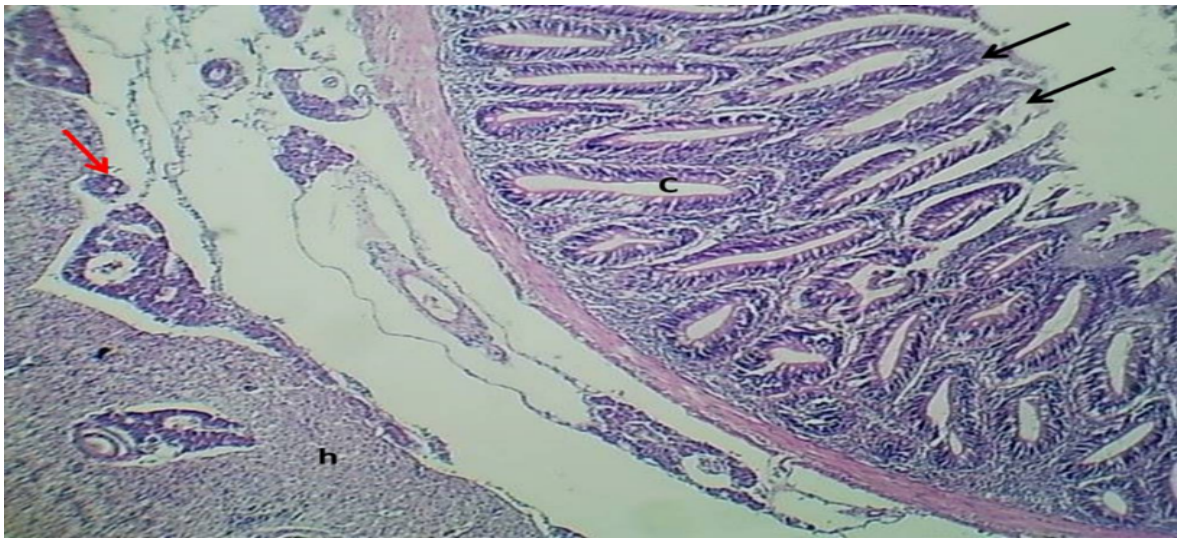


Fig. 4. section of liver with pancreas & intestine (Control) shows normal appearance of pancreatic acini (red arrows), normal hepatocytes (h) & intestinal villi (black arrow) with crypts (C). H&E.100x
Source: Own materials



Fig. 5. A section of intestine (Control) shows) show normal intestinal villi (black arrow) with crypts (C). H&E.200x
Source: Own materials

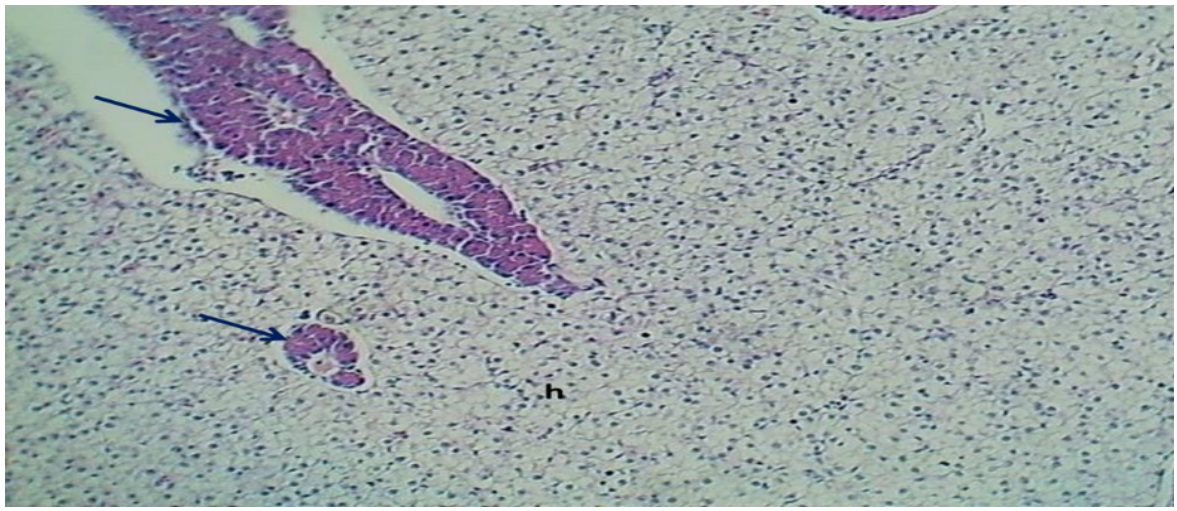


Fig. 6. Section of liver & pancreas (0.1482) shows normal appearance of pancreatic acini (Arrows), normal hepatocytes (h). H&E.100x
Source: Own materials

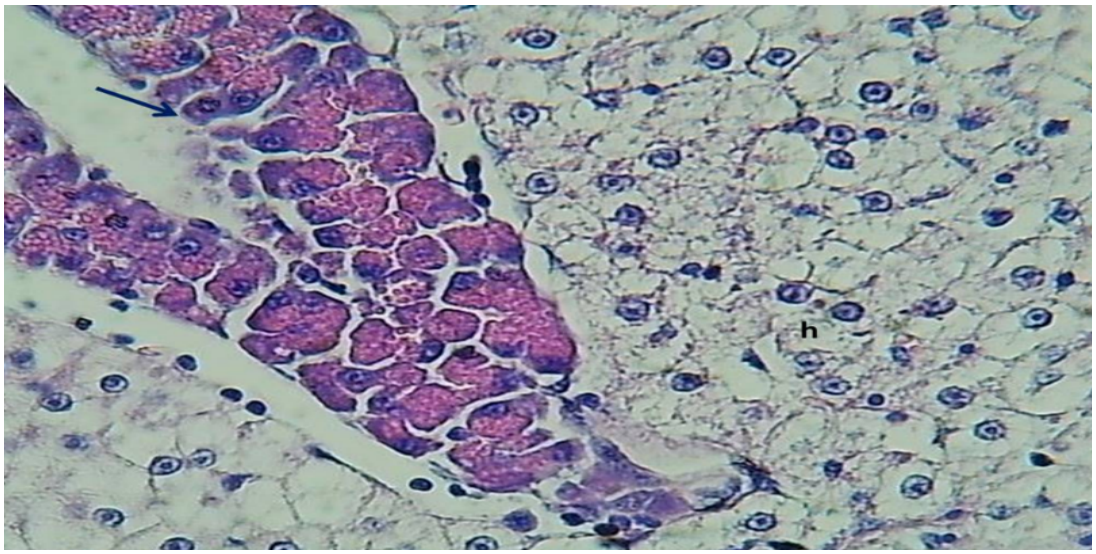


Fig. 7. Section of liver & pancreas (0.1482) shows normal appearance of pancreatic acini (Arrows) & normal hepatocytes (h). H&E.400x
Source: Own materials

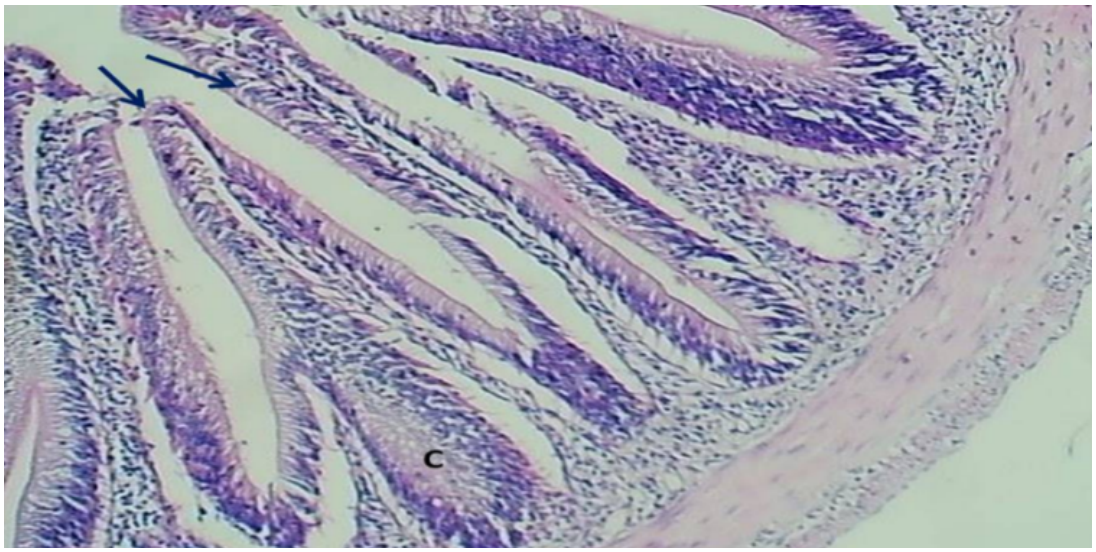


Fig. 8. A section of small intestine (0.1482) shows normal appearance of intestinal villi (black arrow) with crypts (C). H&E.100x
Source: Own materials

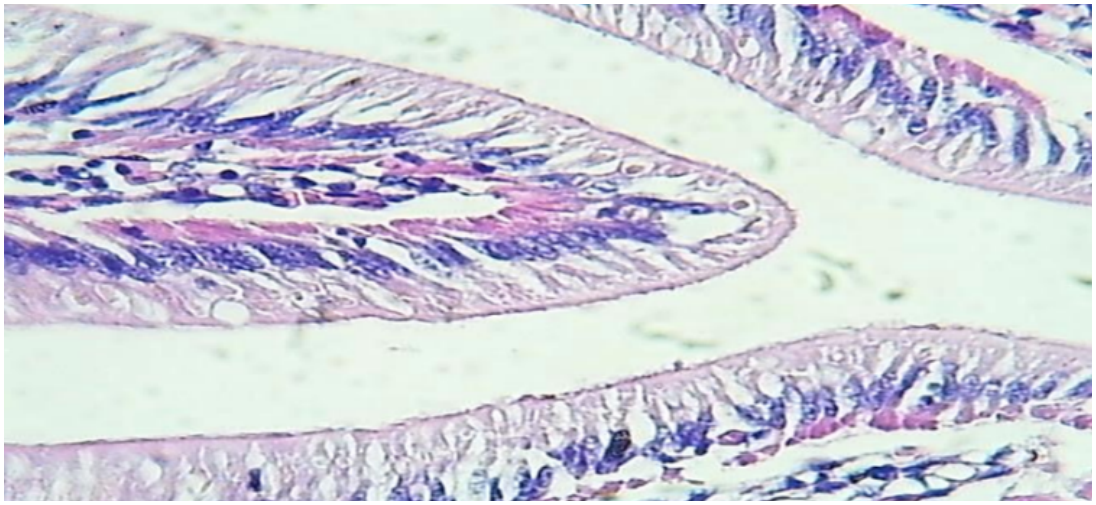


Fig. 9. A section of small intestine (0.1482) shows the normal appearance of enterocytes of the intestinal villi. H&E.400x
Source: Own materials

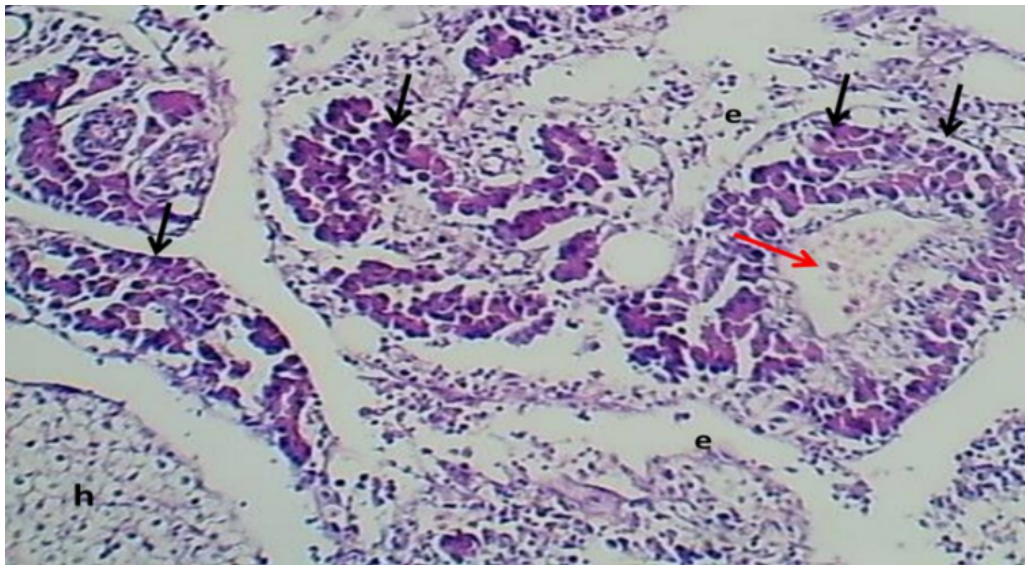


Fig. 10. Section of liver & pancreas (0.3705) shows moderate degeneration with atrophy of pancreatic acini (black arrows), congestion with dilation of central veins (red arrows), with edema (e). H&E.100x
Source: Own materials

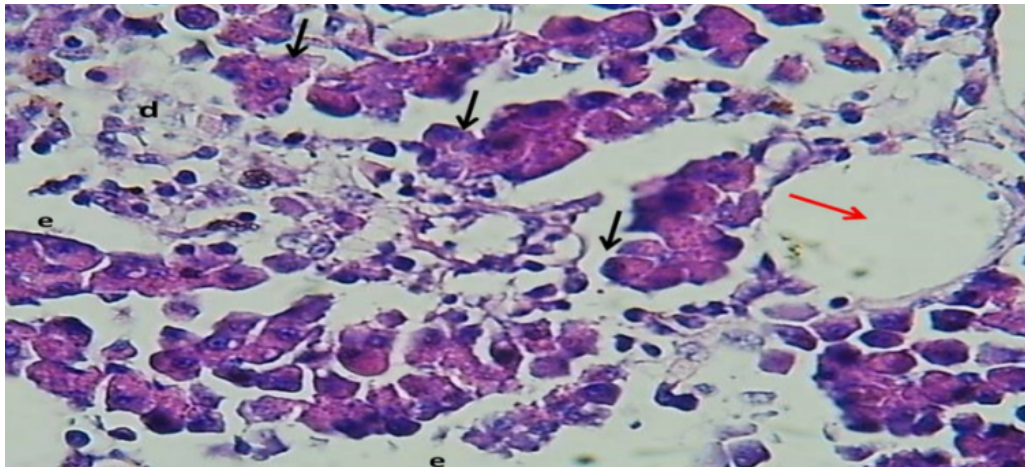


Fig. 11. A section of liver & pancreas (0.3705) shows moderate degeneration with necrosis and atrophy of pancreatic acini (black arrows), dilation of central veins (red arrows) with edema (e) & tissue depletion (d). H&E.400x
Source: Own materials

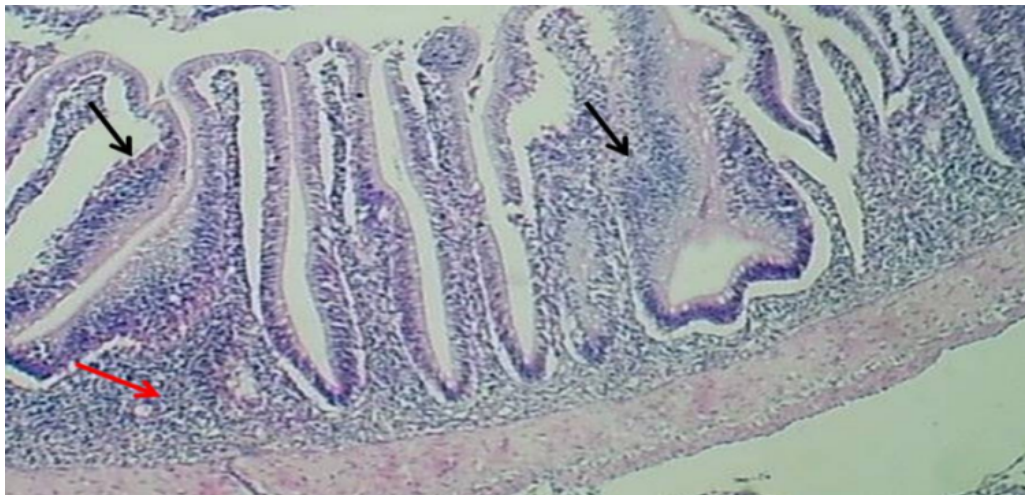


Fig. 12. A section of small intestine (0.3705) shows marked mucosal thickening of intestinal villi associated with hyperplasia (black arrow) & aggregation of leukocytes (red arrows). H&E.40x
Source: Own materials

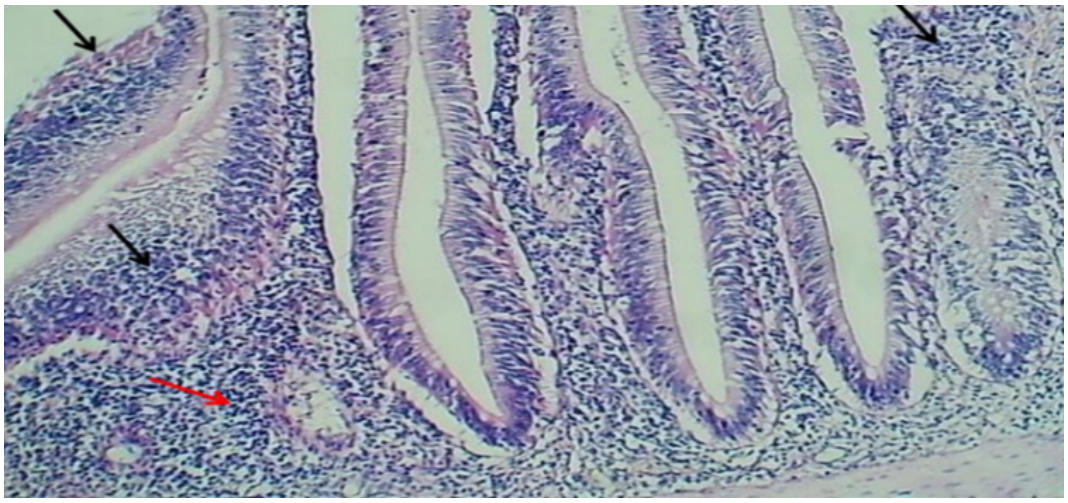


Fig. 13. A section of small intestine (0.3705) shows marked mucosal thickening of intestinal villi associated with hyperplasia (black arrow) & aggregation of leukocytes (red arrows). H&E.100x
Source: Own materials

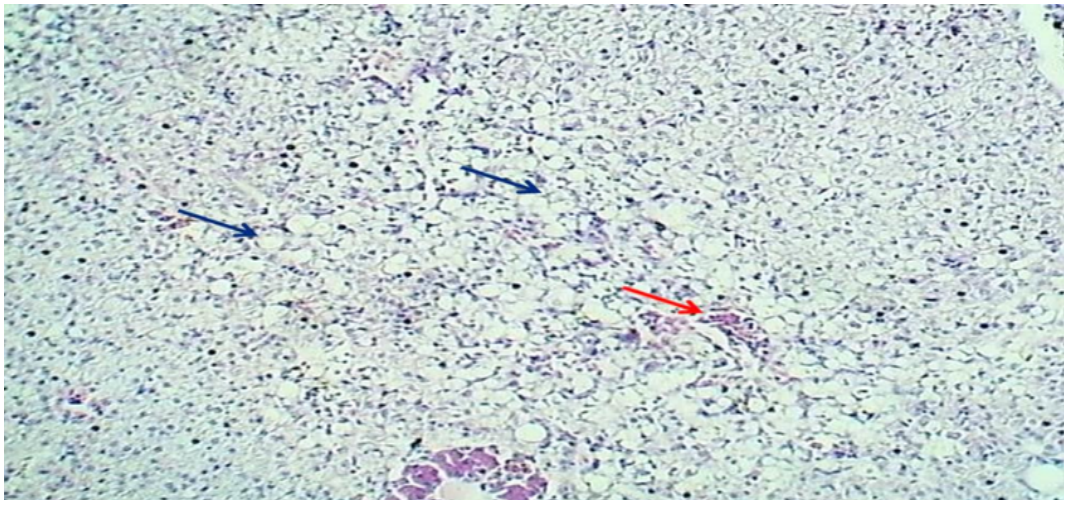


Fig. 14. A section of liver & pancreas (0.741) shows moderate vacuolar degeneration with cellular swelling of hepatocytes (black arrows) & sinusoidal congestion (red arrow). H&E.100x
Source: Own materials

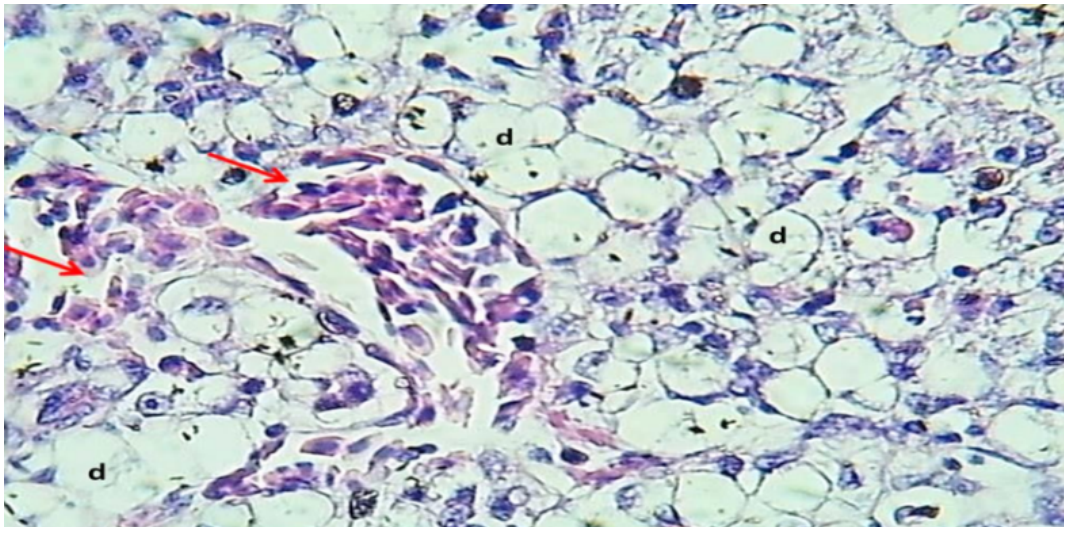


Fig. 15. A section of liver (0.741) shows moderate vacuolar degeneration with cellular swelling of hepatocytes (d) & sinusoidal congestion (red arrow). H&E.400x
Source: Own materials

cell volume (PCV) of fish that were subjected to a series of concentrations of a pesticide.

The PCV values varied considerably, ranging from 30.90 in fish exposed to the highest concentration of 0.741 mg/L to 38.70 in those exposed to the lowest concentration of 0.1482 mg/L. The average PCV at a concentration of 0.741 mg/L was 36.10. In contrast, the control group exhibited a mean PCV of 42.20, significantly higher than the lowest recorded value.

Our analysis of variance showed significant differences ($P \leq 0.001$) among the various pesticide concentrations. Furthermore, the duration of exposure had a notable impact on the results ($P < 0.05$). A least significant difference (LSD) test, which yielded an LSD value of 3.8, confirmed the significant differences across the different exposure periods (Table 6).

WHITE BLOOD CELL (WBC)

Fish immune systems are essential for host defense and act as significant biochemical indicators in environmental assessments. However, these systems can be adversely affected by exposure to various environmental toxins. A decrease in white blood cell count may indicate tissue damage due to exposure to Spinosad, while lower-than-normal lymphocyte levels can signify a compromised immune system. It is important to note that white blood cell counts can exhibit considerable variability.

In our study, this was identified a significant reduction ($p < 0.05$) in the white blood cell count of common carp subjected to sublethal concentrations of Spinosad for 20 days. Specifically, at a concentration of 0.3705 mg/L, the percentage of lymphocytes (LYM%) dropped to 9.07%, compared to 32.04% in the control group. This difference surpassed the least significant difference (LSD) value of 4.95, indicating a suppression of cellular immunity. Conversely, the percentage of monocytes (MON%) increased to 27.16%, reflecting heightened inflammatory activity, which was statistically significant. The percentage of neutrophils (NEU%) decreased at the higher concentration but remained within normal fluctuation limits up to 0.3705 mg/L (Table 6).

Our analysis demonstrated that both the concentration of the pesticide and the duration of exposure had highly significant effects ($P \leq 0.01$) on the distribution of white blood cells (WBC). This finding was further supported by an LSD value of 1.539.

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

The liver's vital role in the body and its anatomical positioning, along with its rich blood supply, make it particularly vulnerable to waterborne pollutants

In the study, a pronounced increase in ALT enzyme activity was noted in groups treated with Spinosad doses of 0.1482 mg, 0.3705 mg, and 0.741 mg, with recorded ALT values of 34.00 ± 1.15 IU/l, 56.67 ± 3.75 IU/l, and 70.00 ± 2.31 IU/l, respectively. In contrast, the control group exhibited an ALT value of 30.33 ± 1.45 IU/l (refer to Table 7).

These results indicate that ALT levels rise with increasing pesticide concentrations and extended exposure durations compared to the control group. Elevated ALT levels may signal liver injury, as they are typically associated with damaged liver cells. This aligns with prior research indicating that exposure to xenobiotics can disrupt an organism's antioxidant defense system

LIVER ENZYMES

SERUM ALANINE AMINOTRANSFERASE (ALT)

Fish liver biomarkers associated with conjugation enzymes, carboxylesterase activity, and antioxidant defenses can indicate exposure to biopesticides [26].In the study, a significant increase in ALT activity was observed in groups treated with Spinosad at doses of 0.1482 mg/L, 0.3705 mg/L, and 0.741 mg/L, with values of 34.00 ± 1.15, 56.67 ± 3.75 IU/L, and 70.00 ± 2.31 IU/L, respectively. The control group had an ALT value of 30.33 ± 1.45 IU/L (Table 8).

These results indicate that ALT levels increase with higher pesticide concentrations and longer exposure, which may suggest liver injury. Furthermore, exposure to xenobiotics can disrupt the antioxidant defense system in organisms [27].

SERUM ASPARTATE AMINOTRANSFERASE (AST)

The liver plays an essential role in maintaining homeostasis and is the primary site for metabolizing xenobiotics. However, the AST test results showed notable variations in average values. Specifically, AST enzyme activity was significantly higher in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. This differs from the control group, which exhibited a value of 32.33 ± 2.34 IU/L (Table 7). Additionally, the analysis of variance indicated highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, highlighting clear distinctions among the groups.

SERUM ASPARTATE AMINOTRANSFERASE (AST)

AST enzymes play a crucial role in developing resistance and enhancing defense mechanisms against pesticides

[28]. The results from the AST test showed significant variations in mean values. Specifically, AST enzyme activity increased notably in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05 IU/L, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. In contrast, the control group exhibited a value of 32.33 ± 2.34 IU/L (see Table 8). Furthermore, the analysis of variance revealed highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, indicating clear distinctions among the groups.

In this study, we observed an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. As noted by [29], these toxic metabolites can interact with intracellular macromolecules, potentially leading to hepatocellular necrosis (Table 8).

BIOMARKERS FOR HISTOPATHOLOGICAL ANALYSIS

Histopathological studies conducted in both laboratory and natural environments are effective in identifying the potentially harmful effects of pesticides on the target organs of fish. The further use of histopathological changes as the main parameter in the environmental monitoring is gaining growing relevance, especially in the determination of specific organs.

HISTOPATHOLOGICAL OBSERVATIONS ON THE COMMON CARP LIVER

The fish liver can be also infected by toxins, as well as is an active part of the metabolism and excretion of foreign substances. Liver injury or dysfunction may result in severe changes in a number of physiological functions that cause inflammation and metabolic dysfunction. The experiments have indicated that the glycol and glutamic acid can be toxic to the fish liver, and other animals as well. The analysis of common carp has found a disturbed antioxidant defense capacity, heightened serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) that are two essential enzymes used to assess liver health. After 20 days of exposure to varying concentrations of Spinosad (0.1482,0.3705, and 0.741 mg/L), histological changes were observed in the liver (cytoplasmic vacuolation, hepatocyte hypertrophy, blood congestion, and cellular necrosis), while the pancreatic glands and hepatocytes in the control group retained a normal appearance (Fig. 2 and 3 and Table 8).

HISTOLOGICAL CHANGES IN THE LIVER OF COMMON CARP (C. CARPIO)

The liver is a key organ in aquatic toxicology, crucial for the uptake, excretion, and detoxification of xenobiotics. Toxicant-induced changes in the liver reflect general findings on fish liver damage, highlighting the need to study hepatotoxic effects of specific toxins [30]. In common carp exposed to Spinosad, liver cells showed light vacuoles, indicating fatty degeneration, and necrosis occurred in some areas, possibly due to the fish's detoxification efforts and inability to produce new hepatocytes [31]. Studies suggest that glycol and glutamic acid may harm liver function in fish and other animals. Research has shown that common carp exhibit reduced antioxidant defenses and elevated serum enzymes GOT and GPT, indicating liver dysfunction [32]. The liver is vital for maintaining metabolic balance [33].

Histological changes in the liver were consistently observed after 20 days of exposure to different concentrations of Spinosad (0.1482, 0.3705, and 0.741 mg/L), as shown in Table 9 and Figure 2, 3, 4 and 5, which illustrate the normal appearance of pancreatic glands and hepatocytes in the control group. Figures 6-15 show

Microscopic analysis of common carp (Cyprinus carpio) tissues exposed to the Spinosad insecticide revealed a series of concentration-dependent changes. Lowest concentration group (0.1482 mg/L) – figures. 6, 7, 8, and 9. In this group, the histological appearance remained within normal limits. Both the pancreatic acini and hepatocytes (liver cells) maintained a normal appearance. Similarly, the small intestine showed normal structures of intestinal villi, crypts, and enterocytes. Medium concentration group (0.3705 mg/L) – figures 10, 11, 12, and 13. Exposure to this dose led to distinct damage. The liver and pancreas showed moderate degeneration, necrosis, and atrophy of the pancreatic acini. Dilation of central veins, edema, and tissue depletion were also noted. In the small intestine, marked mucosal thickening of the villi was observed, associated with hyperplasia and leukocyte aggregation, indicating inflammatory activity. Highest concentration group (0.741 mg/L) – figures 14 and 15. At a concentration of 0.741 mg/L, degenerative changes intensified. Liver tissue exhibited moderate vacuolar degeneration and cellular swelling of hepatocytes. Additionally, sinusoidal congestion was observed, confirming the pesticide's toxic effects on the vascular system and organ parenchyma.

Microscopic analysis of common carp (Cyprinus carpio) tissues exposed to the Spinosad insecticide revealed a series of concentration-dependent changes. Lowest concentration group (0.1482 mg/L) (Fig. 6-9). In this group, the histological appearance remained within normal limits. Both the pancreatic acini and hepatocytes (liver cells) maintained a normal appearance. Similarly, the small intestine showed normal structures of intestinal villi, crypts, and enterocytes. Medium con-

centration group (0.3705 mg/L) (Fig. 10-13). Exposure to this dose led to distinct damage. The liver and pancreas showed moderate degeneration, necrosis, and atrophy of the pancreatic acini. Dilation of central veins, edema, and tissue depletion were also noted. In the small intestine, marked mucosal thickening of the villi was observed, associated with hyperplasia and leukocyte aggregation, indicating inflammatory activity. Highest concentration group (0.741 mg/L) (Fig. 14 and 15). At a concentration of 0.741 mg/L, degenerative changes intensified. Liver tissue exhibited moderate vacuolar degeneration and cellular swelling of hepatocytes. Additionally, sinusoidal congestion was observed, confirming the pesticide's toxic effects on the vascular system and organ parenchyma.

DISCUSSION

The condition and behavior of fish in various test and control tanks were assessed every 24 hours for a period of up to 96 hours. When exposed to different concentrations of Spinosad, the fish exhibited notable changes in behavior, particularly at elevated concentrations. Accelerated movement patterns and anxious swimming behaviors have been observed [34]. Significantly, these behavioral alterations may serve as indicators of water pollution resulting from pesticide exposure [35]. The excessive mucus secretion acts as a nonspecific defense against toxins, likely reducing their interaction with harmful substances. Specifically, mucus aids in scavenging toxins through the epidermis or establishes a barrier between the fish's body and the surrounding medium, thereby mitigating irritating effects.

Rao (2006) reported comparable findings when fish were exposed to RPR-V, a novel phosphorothionate insecticide (2-butenoic acid-3[diethoxy phosphinothionyl] ethyl ester). Blood analysis can effectively identify and assess stressful conditions and diseases affecting fish production performance. This comprehensive evaluation enhances our understanding of the physiological status and overall health of fish [36]. What was observed in this case is that the maximum mean red blood cell (RBC) level of 2.97 million cells per microliter was measured at a concentration of 0.1482 mg/L that was observed in 20 days of exposure. Notably, this count was significantly lower than that of the control sample, which had a mean RBC count of 3.79 million cells per microliter.

Conversely, the lowest mean RBC counts recorded were 2.09 million cells per microliter and 1.94 million cells per microliter, noted at concentrations of 0.3705 mg/L and 0.741 mg/L, respectively, after the same exposure duration (see Table 6). These results suggest that increasing pesticide concentrations, along with extended exposure times, have a significant impact on red blood cell counts in the examined common carp. Analysis of variance indicated significant differences in red blood cell counts among the various pesticide doses ($P \leq 0.001$) and exposure durations. Furthermore, the least significant difference (LSD) value of 0.42 reinforces these findings. Research has indicated that hematopoietic dysfunction in fish exposed to insecticides can result in significant reductions in hemoglobin and red blood cell levels [37].

Indeed, the findings of the current study corroborate this, reporting diminished levels of both red blood cells and hemoglobin. Among the hemoglobin level explored, the maximum mean of 11.80 g/dL was attained among those fish exposed to pesticide concentration of 0.1482 mg/L during 20 days. The average of the level of 0.3705 mg/L of exposure was 11.30 g/dL and the lowest average of 9.80 g/dL was of those exposed to the level of 0.741 mg/L of the pesticide (Table 6). All these were significantly lower than the control sample which had an average hemoglobin concentration of 12.80g/dL.

According to [38] anemia can arise from various disorders that lead to a decrease in either the quantity or size of red blood cells. These disorders may include excessive bleeding, dietary deficiencies, cell death due to a transfusion reaction, or complications with hemoglobin formation. A lower hematocrit level reflects a reduction in both the number and size of red blood cells, consequently decreasing the space they occupy. The diagnosis of anemia is reinforced by abnormal blood test results that indicate a low hematocrit level. There was a significant statistical variance difference of both doses of the pesticide and exposure time on the level of hemoglobin in fish blood (P 0.001). The lowest significant difference (LSD) calculated was 1.15 which indicated that there were significant differences in some of the measured samples especially in the 0.1482 mg/L and 0.741 mg/L dosage.

These results indicate that ALT levels increase with higher pesticide concentrations and longer exposure, which may suggest liver injury. Furthermore, exposure to xenobiotics can disrupt the antioxidant defense system in organisms [27].

In this study, the researcher noted an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. These toxic metabolites can interact with intracellular macromolecules, potentially resulting in hepatocellular necrosis.

Extensive application of systemic insecticides in agriculture has led to contamination of waters and soils of the treated crops with pesticide residues. As a result,

human contact with stored pesticides is achieved by consuming contaminated fish. A major concern is the bioaccumulation of Spinosad residues in fish tissues, which may lead to biomagnification in humans via the food chain. As *Cyprinus carpio* (common carp) is a major protein source, consuming contaminated fish poses public health risks and may lead to long-term physiological disruptions, as these compounds are not easily eliminated by the human body. However, the initial symptoms of pollution can manifest as altered fish behavior or physical appearance, which indicates that both the consequences of pollution and the effects of pollution can be observed at both cellular and structural levels. Spinosad can consequently react with common carp on its entry into the water system.

The results from the AST test showed significant variations in mean values. Specifically, AST enzyme activity increased notably in groups exposed to pesticide concentrations of 0.1482 to 0.3705 mg/L and 0.741 mg/L, with values of 36.00 ± 3.05 IU/L, 51.67 ± 2.03 IU/L, and 64.33 ± 3.92 IU/L, respectively. In contrast, the control group exhibited a value of 32.33 ± 2.34 IU/L (see Table 8). Furthermore, the analysis of variance revealed highly significant effects of both pesticide concentration and duration of exposure ($P \leq 0.001$) on the results, with a least significant difference of 9.554, indicating clear distinctions among the groups.

In this study, we observed an increase in mean AST values compared to the control group as both pesticide concentrations and exposure durations increased. As noted by [29], these toxic metabolites can interact with intracellular macromolecules, potentially leading to hepatocellular necrosis.

CONCLUSIONS

This experiment comprehensively evaluated the effects of Spinosad exposure on common carp. Our results revealed significant dose-dependent toxicity, manifested by severe histopathological damage to the liver, along with significant changes in hematological and biochemical parameters. Relevant observations would involve those that shed light on the role of Spinosad in the natural freshwater environment and the impacts this runoff may have should it run off farms. These dynamics are important to understand in an attempt to enhance the water management practices and reduce the environmental risks. Furthermore, given that common carp represent a vital global food source, the observed toxicological impairments raise concerns about potential risks to human consumers through the food chain. Although Spinosad is a natural genetic option in terms of natural origin, the present study emphasizes the fact that, albeit low in concentration, it may have immense negative effects on aquatic life, including common carp. This emphasizes the highest need to use the environment wisely and monitor its use in order to achieve a balance between agricultural demands and the conservation of aquatic organisms. The longitudinal effects of Spinosad toxicity and molecular machineries on non-target aquatic organisms should be highlighted in future studies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

CONTENTS

Dysbiosis of the large intestine and impaired barrier function of the intestine in patients with autoimmune thyroiditis

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ABSTRACT

Aim: To determine the relationship between changes in serum and fecal zonulin levels depending on the severity of colonic dysbiosis in patients with autoimmune thyroiditis.

Materials and Methods: A total of 143 patients with autoimmune thyroiditis (AIT) were included in the study. An assessment of the species and quantitative composition of the colonic microbiota (CM) was performed in the examined patients. The zonulin level was determined in blood serum and feces using enzyme-linked immunosorbent assay. The α1-antitrypsin (α1-AT) level was also determined in blood serum and feces using ELISA. Based on the results obtained, the α1-AT clearance was calculated. The level of fecal calprotectin (FC) was also assessed using the ELISA method.

Results: The data obtained indicate that the vast majority of patients had reduced thyroid hormone levels (hypothyroidism) – in 70 (49.0% of those examined). Patients with thyroid hypofunction in AIT were assigned to group 1 of the examined patients. Euthyroidism was diagnosed in 47 (32.9%) patients, who were classified as group 2. Group 3 consisted of 26 (18.1%) patients with AIT who were diagnosed with elevated thyroid hormone levels in their blood serum.

As the data show, patients with AIT and thyroid hypofunction are significantly more likely to have grade 3 colonic dysbiosis ($p<0.001$). It is in patients with AIT and hypothyroidism that grade 4 colonic dysbiosis is significantly more common (in 11.4% of cases – $p<0.01$), which is 5.0% and 7.6% more than in patients in groups 2 and 3.

Conclusions: Patients with AIT have been found to have colonic dysbiosis, the severity of which depends on the functional state of the thyroid gland. At the same time, patients with hypothyroidism in AIT are mainly diagnosed with grade 3 CD (in 57.1% – $p<0.001$), while patients with AIT and euthyroidism and hyperthyroidism are mainly diagnosed with grade 2 CD (in 55.3% ($p<0.001$) and 46.2% ($p<0.01$) of patients, respectively). CD (in 55.3% ($p<0.001$) and 46.2% ($p<0.01$) of the examined patients, respectively). In patients with AIT, impaired intestinal barrier function was established based on the levels of biomarkers such as zonulin, α1-AT in feces and blood serum, regardless of thyroid function. However, the maximum values of zonulin and α1-AT in feces were diagnosed in patients with AIT with pancreatic hypofunction (an increase in their levels to 168.73±0.51 ng/ml ($p<0.001$) and to 41.07±0.37 mg/dl($p<0.01$), respectively), which directly correlates with the severity of CD in these patients.

KEY WORDS: autoimmune thyroiditis, thyroid hormones, intestinal dysbiosis, impaired intestinal barrier function, zonulin, α1-antitrypsin, fecal calprotectin

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INTRODUCTION

Autoimmune thyroid disease (AITD) is the most common organ-specific autoimmune disease, characterized primarily by thyroid dysfunction and immune imbalance. Normal thyroid function is vital for growth, development, reproduction, and metabolism in the body. As a serious endocrine disorder, thyroid dysfunction disrupts glucose homeostasis, kidney function, and reproductive health, posing significant health risks [1-3].

Environmental factors such as radiation, smoking, and iodine intake, as well as certain endocrine disruptors such as mercury and vanadium, are considered triggers for AITD, and certain comorbidities may further increase the risk of developing AITD [4].

AITD primarily includes Graves' disease (GD) and Hashimoto's thyroiditis (HT). GD is the most common cause of hyperthyroidism in Western countries and mainly affects people between the ages of 30 and 60. In contrast, HT is the leading cause of hypothyroidism in regions with sufficient iodine intake, with a total prevalence of approximately 7.5% and a prevalence among women of 17.5% [5-7].

Recent studies show that AITD is associated with diseases such as vitiligo, alopecia areata, and celiac disease, as well as an increased risk of miscarriage and infertility in women, and may contribute to various neuropsychiatric symptoms and changes in brain function [8, 9].

The human gut microbiota consists of billions of microorganisms, such as bacteria, viruses, fungi, and pro-

tozoa. Four families of bacteria predominate in healthy gut flora, namely Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. These subpopulations of microorganisms regulate the function of gut-associated lymphoid tissue (GALT) and show a close relationship between their representatives and diet. GALT works closely with gut microbes. This is necessary to maintain proper nutrient absorption and protect the mucous membrane from harmful pathogens. Changes in the gut microbiota can weaken or destroy the integrity of the intestinal barrier. As a result, intestinal permeability increases, leading to activation of the immune system, for example, through molecular mimicry. GALT effector cells are activated, and the pro-inflammatory factors produced at this time cause subclinical inflammation. Initially, the entire process develops only in situ, but it can spread and turn into persistent generalized inflammation [10-14].

The gut microbiome ferments indigestible food components into absorbable metabolites. It is also responsible for synthesizing essential vitamins and removing toxic compounds. Short-chain fatty acids (SCFA), which are products of microbial fermentation, act as substrates for enterocytes. Therefore, they act as modulatory factors for immune cells. SCFAs form a direct link between the gut microbiome and the immune system due to their ability to induce regulatory T cells (Tregs) and regulate the balance of Tregs and Th17 cells. Thus, any state of gut microbiome dysbiosis can induce increased intestinal permeability with subsequent disruption of SCFA production and then dysregulation of the immune system [7, 15].

Therefore, identifying the factors that influence the development of AITD is extremely important for clinicians and researchers, as it helps to develop individual prevention and treatment strategies.

AIM

The aim to determine the relationship between changes in serum and fecal zonulin levels depending on the severity of colonic dysbiosis in patients with autoimmune thyroiditis.

MATERIALS AND METHODS

The study was conducted at the clinical base of the Department of Propaedeutics of Internal Diseases of the Medical Faculty of Uzhhorod National University. A total of 143 patients with autoimmune thyroiditis (AIT) were included in the study.

The examined patients with AIT were hospitalized in the endocrinology and gastroenterology departments of the Municipal Non-Commercial Enterprise "Andrii Novak Transcarpathian Regional Clinical Hospital" of the Transcarpathian Regional Council or were under outpatient follow-up by a family physician during the period from 2023 to 2026.

Among the examined women, there were 97 (67.8%), and among men, there were 46 (32.2%), aged 47.8±5.4 years. The control group consisted of 30 practically healthy individuals. Among them were 19 women (63.3%) and 11 men (36.7%), with an average age of 47.2±6.2 years.

All studies were conducted with the consent of the patients, and their methodology complied with the 1975 Helsinki Declaration on Human Rights, as amended in 1983, the Council of Europe Convention on Human Rights and Biomedicine, as well as the legislation of Ukraine and the local bioethics committee of UzhNU.

All patients included in the scientific observation were examined using general clinical examination methods. To verify the diagnosis, attention was paid to the nature of the complaints and the medical history of the disease. During the anthropometric examination, height and weight were measured, and body mass index (BMI) was calculated. According to WHO recommendations, patients were classified according to their BMI.

All patients underwent an ultrasound examination (US) of the thyroid gland (TG). Standard general and biochemical tests were performed on blood serum. All patients (for the diagnosis of AIT) had their serum thyroid hormone levels determined (free triiodothyronine (T4), triiodothyronine (T3)) and thyrotropin (TSH), and antibodies to thyroglobulin and thyroid peroxidase were also evaluated.

The diagnosis of AIT was verified taking into account the criteria of clinical guideline 00512 "Autoimmune thyroiditis", the protocol for the management of patients with AIT (E06.3).

The criteria for excluding patients from the study were: thyrotoxicosis, hypothyroidism, thyroid cancer, type 1 diabetes mellitus (DM), type 2 DM (decompensation stage), oncological diseases, psychiatric diseases that do not allow adequate assessment of the condition of patients, acute infectious diseases (including acute intestinal infections), inflammatory bowel diseases (ulcerative colitis, Crohn's disease), celiac disease, lactose intolerance, pseudomembranous colitis, intestinal lesions Clos.

The criteria for inclusion of patients in this scientific study were: patients with autoimmune thyroiditis.

An assessment of the species and quantitative composition of the colonic microbiota (CM) was performed in the examined patients. The material was cultured on a standardized set of selective and differential diagnostic nutrient media to identify aerobic and anaerobic microor-

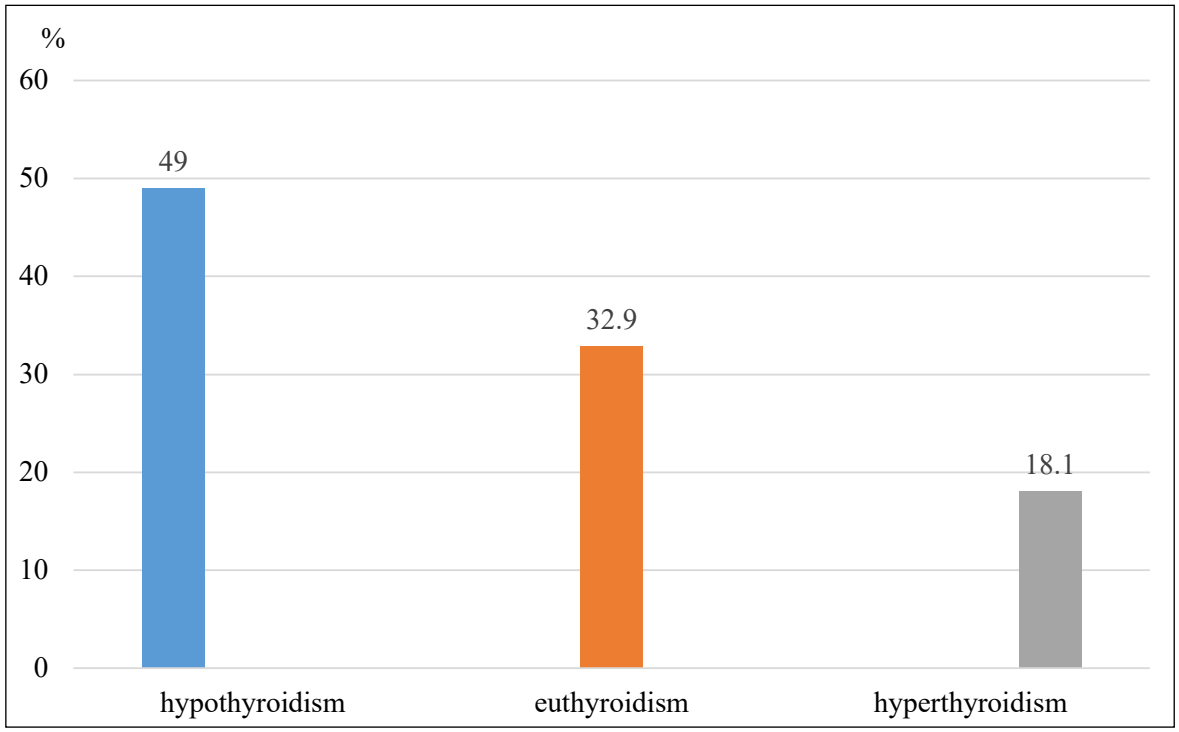


Fig. 1. Distribution of patients with AIT according to the functional state of the thyroid gland
Picture taken by the authors

ganisms using the serial tenfold dilution method (10^{-1}-10^{-9}). To determine the hemolytic forms of intestinal and coccal microflora, 5% blood agar was used; for the cultivation of bifidobacteria; identification of fungi of the genus Candida and other pathogenic fungi was carried out on Sabouraud medium; for the isolation of enterobacteria. Endo medium was used. The assessment of quantitative and qualitative composition disorders of the CM was carried out in accordance with the unified working classification of intestinal dysbiosis according to Kuvayeva-Ladodo (1991).

The zonulin level was determined in blood serum and feces using enzyme-linked immunosorbent assay (ELISA) (test systems from Elabscience, USA). The α1-antitrypsin (α1-AT) level was also determined in blood serum and feces using ELISA (test systems from Immundiagnostic AG, Germany). Based on the results obtained, the α1-AT clearance was calculated. The level of fecal calprotectin (FC) was also assessed using the ELISA method (Tecan Sunrise test system, ImmunDiagnostic, Germany).

The analysis and processing of the examination results were performed using the Statistics for Windows v.10.0 computer program (StatSoft Inc, USA) with parametric and nonparametric methods of evaluating the results obtained.

FRAMEWORK

The study was performed within the framework of the scientific topics "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University".

RESULTS

After assessing the level of thyroid hormones in the blood serum of patients with AIG, they were divided into groups depending on the functional activity of the gland – Fig. 1.

The data obtained indicate that the vast majority of patients had reduced thyroid hormone levels (hypothyroidism) – in 70 (49.0% of those examined). Patients with thyroid hypofunction in AIT were assigned to group 1 of the examined patients. Euthyroidism was diagnosed in 47 (32.9%) patients, who were classified as group 2. Group 3 consisted of 26 (18.1%) patients with AIT who were diagnosed with elevated thyroid hormone levels in their blood serum.

We determined the quantitative and qualitative composition of the colon microflora in patients with AIT. At the same time, patients with AIT were diagnosed with a decrease in the number of bifidobacteria, lactobacilli, and E. coli with normal enzymatic properties compared to the control group. These changes were accompanied by an increase in the number of pathogenic and

Table 1. Distribution of subjects by severity of colonic dysbiosis

Severity of colon dysbiosis	Group of examined patients with AIT, %		
	Group 1 (n=70)	Group 2 (n=47)	Group 3 (n=26)
Grade 1	4.4 %	12.8 % *	26.9 % ** +
Grade 2	27.1 %	55.3 % ** +	46.2 % *
Grade 3	57.1 %	25.5 % **	23.1 % **
Grade 4	11.4 %	6.4 % *	3.8 %

Note: the difference between the indicators in patients in group 1 and groups 2-3 is significant: * – p<0.01; ** – p<0.001; the difference between the indicators in patients in groups 2 and 3 is significant: + – p<0.05; ++ – p<0.01
Source: compiled by the authors of this study

Table 2. Indicators of biomarkers of intestinal damage in the examined patients

Parameter	Patients			
	Control group (n=30)	Group 1 (n=70)	Group 2 (n=47)	Group 3 (n=26)
FC, µg/L	21,07±0,21	112.15±0.76 **	73.75±0.24 *++	84.55±0.18 *+#
Zonulin:				
in blood serum, ng/mL	13.78±0.15	118.12±0,62 **	87.23±0.41 **+	101.33±0.15 **#
in fecal, ng/mL	17.01±0.35	168.73±0.51 **	112.34±0.51 **+	134.26±0.39 **+#
α1-AT:				
in blood serum , mg/dL	115.12±0.98	241.15±2.44 *	176.15±1.71 *++	216.51±1.32 *+##
in fecal, mg/dL	13.89±0.17	41.07±0.37 *	29.09±0.18 *+	38.56±0.40 *#
clearance α1-AT, mL/day	17.28±0.41	76.18±0.45 **	47.42±0.53 *+	69.13±0.26 **+##

Note: the difference between the control group and the examined patients is statistically significant: * – p<0.01; ** – p<0.001;
The difference between the indicators in patients in group 1 and groups 2-3 is significant: + – p<0.01; ** – p<0.001;
The difference between the in dicators in patients in groups 2 and is significant: # – p<0.05; ## – p<0.01.
Source: compiled by the authors of this study

conditionally pathogenic microflora. Microbiological examination revealed hemolytic *E. coli*, fungi of the genus *Candida*, *Enterobacter*, *Citrobacter*, *Staphylococcus*, *Klebsiella*, and *Clostridium* in increased concentrations in feces compared to the control group.

After summarizing the results obtained, patients with AIT were classified according to the severity of colonic dysbiosis, as shown in Table 1.

As the data show, patients with AIT and thyroid hypofunction are significantly more likely to have grade 3 colonic dysbiosis (p<0.001). It is in patients with AIT and hypothyroidism that grade 4 colonic dysbiosis is significantly more common (in 11.4% of cases – p<0.01), which is 5.0% and 7.6% more than in patients in groups 2 and 3. In the examined patients with AIT and euthyroidism, grade 2 colonic dysbiosis was significantly more common (in 55.3% of cases – p<0.001), as well as grade 3 colonic dysbiosis (in 25.5% of cases – p<0.001). In patients of group 3 , grade 2 colon dysbiosis was also significantly more common (in 46.2% of patients), but it should be noted that it was in patients with hyperthyroidism that grade 1 colon dysbiosis was most commonly found (in 26.9% of patients).

We evaluated biomarkers of intestinal barrier dysfunction in the patients we examined (Table 2).

The data obtained indicate an increase in the level of biomarkers in dicating a violation of the intestinal barrier function (levels of zonulin, α1-AT in blood serum and feces). The maximum increase in zonulin levels in serum and feces was found in patients with AIT group 1 (patients with hypothyroidism), while the minimum deviation from the norm was found in patients with group 2 (patients with AIT, whose thyroid hormone levels indicated euthyroidism). The same dynamics can be observed in the analysis of α1-AT levels in blood serum and feces, as well as its clearance in the patients we examined.

Table 3. The relationship between the severity of colonic dysbiosis and biomarkers indicating impaired intestinal permeability

Parameter	Groups of examined patients with AIT					
	Group 1 (n=70)		Group 2 (n=47)		Group 3 (n=26)	
	Grade of colonic dysbiosis					
	Grade 2	Grade 2	Grade 2	Grade 3	Grade 2	Grade 3
Zonulin (blood)	r=0.90; p<0.01	r=0.96; p<0.01	r=0.80; p<0.01	_	r=0.84; p<0.01	r=0.76; p<0.01
Zonulin (fecal)	r=0.94; p<0.01	r=0.98; p<0.01	r=0.82; p<0.01	r=0.78; p<0.01	r=0.90; p<0.01	r=0.80; p<0.01
α1-AT (blood)	r=0.70; p<0.05	r=0.74; p<0.05	r=0.68; p<0.05	_	r=0.70; p<0.05	_
α1-AT (fecal)	r=0.92; p<0.01	r=0.96; p<0.01	r=0.72; p<0.05	r=0.70; p<0.05	r=0.76; p<0.01	r=0.68; p<0.05
FC	r=0.80; p<0.01	r=0.82; p<0.01	r=0.78; p<0.01	_	r=0.90; p<0.01	_

Source: compiled by the authors of this study

Analysis of the studies conducted also indicates a tendency toward an increase in FC levels, but the results obtained in patients in groups 2 and 3 with AIT did not exceed 5 times the norm (compared with the data obtained in the control group). The maximum increase in FC levels was observed in patients with thyroid hypofunction in AIG.

The relationship between the severity of colonic dysbiosis and changes in the levels of biomarkers indicating impaired intestinal barrier function was analyzed (Table 3).

A correlation between changes in zonulin and α1-AT levels was established mainly in the feces of patients with grade 2 and 3 CD. At the same time, a strong correlation was established mainly in patients of group I with AIT. In patients with hypothyroidism in AIT (group I), the severity of CD affects the increased levels of zonulin and α1-AT in blood serum. According to our research, the increased FC index also depends on the degree of colon dysbiosis in patients with AIT.

Thus, changes in the quantitative and qualitative composition of the colon have been established in patients with AIT, and the severity of intestinal dysbiosis affects intestinal permeability in these patients.

DISCUSSION

According to Cayres LCF et al. (2021), individuals diagnosed with TH have intestinal dysbiosis. Patient samples showed a significant increase in Bacteroides species with a concomitant decrease in Bifidobacterium among the gut microbiota [16]. Intestinal dysbiosis is associated with increased intestinal permeability [17]. Recent studies indicate that zonulin is a highly sensitive biomarker of leaky gut syndrome. Zonulin is a protein that is a potential modulator of tight junctions in the intestine [18].

Demir E. et al. indicate elevated zonulin levels in chronic thyroiditis [19]. Küçükemre Aydın B et al. (2020) examined 30 children and adolescents with HT and 30 patients with congenital hypothyroidism (CH) selected by age, sex, and body mass index (BMI). Serum zonulin, free thyroxine (fT4), thyroid-stimulating hormone (TSH), thyroglobulin antibodies, and thyroid peroxidase antibodies were measured. Zonulin levels were significantly higher in patients with TH than in patients with GHD (59.1±22.9 ng/ml vs. 43.3±32.9 ng/ml, p=0.035). In patients with HT, zonulin levels positively correlated with weight (r=0.406, p=0.03), BMI (r=0.486, p=0.006), and levothyroxine dose (r=0.463, p=0.02). In patients with CH, zonulin levels were positively correlated with age (r=0.475, p=0.008), weight (r=0.707, p<0.001), BMI (r=0.872, p<0.001), and levothyroxine dose (r=0.485, p=0.007). After adjusting for age, weight, TSH and fT4 levels, serum zonulin was associated only with levothyroxine dose in patients with HT (R2=0.36, p=0.05). In patients with CH, only weight was associated with zonulin levels (R2=0.62, p<0.001). The authors concluded that higher zonulin levels in children and adolescents with HT indicated increased intestinal permeability in these patients. In addition, the relationship between zonulin levels and levothyroxine dose may indicate an association between serum zonulin and disease severity [20].

The exact mechanism that causes autoimmunity in TH is not fully understood. Many pathologies are considered to be the cause, in particular, disruption of the intestinal epithelial barrier, which causes an inadequate immune response due to the interaction of immune cells of the submucosa with antigens. An abnormal increase in zonulin levels causes increased intestinal permeability and plays a key role in the development of autoimmune diseases. According to İşleyen ZS et al. (2022), the correlation between anti-thyroperoxidase levels, an indicator of the autoimmune process, and serum zonulin levels suggests that this molecule plays a role in the pathogenesis of TH [21].

Therefore, the gut microbiota has an undeniable impact on the integrity of the intestinal mucosa and the function of the immune system. Attempts are being made to modify the microbiome in several autoimmune

diseases, including TH. However, research findings suggest that regular use of probiotics, prebiotics, or synbiotics may have very limited benefits for patients with primary hypothyroidism [22]. Treatment with probiotics or short-chain fatty acids (SCFAs) is not commonly used in autoimmune diseases. However, studies have shown that human-derived probiotics increase SCFA production by modulating the gut microbiome in mice and humans [23]. This may indicate that this specific type of therapy could improve the gut microbiome and indirectly affect the immune system.

The results of our studies also indicate intestinal dysbiosis in patients with AIG, and the relationship between zonulin levels and thyroid dysfunction and hormone levels suggests a possible role for intestinal permeability in the development of autoimmune reactions in these patients. Further research is needed to determine the effectiveness of correcting dysbiotic changes and their possible positive effect on the functional state of the thyroid gland.

CONCLUSIONS

1. Patients with AIT have been found to have colonic dysbiosis, the severity of which depends on the functional state of the thyroid gland. At the same time, patients with hypothyroidism in AIT are mainly diagnosed with grade 3 CD (in 57.1% – $p<0.001$), while patients with AIT and euthyroidism and hyperthyroidism are mainly diagnosed with grade 2 CD (in 55.3% ($p<0.001$) and 46.2% ($p<0.01$) of patients, respectively). CD (in 55.3% ($p<0.001$) and 46.2% ($p<0.01$) of the examined patients, respectively).
2. In patients with AIT, impaired intestinal barrier function was established based on the levels of biomarkers such as zonulin, α1-AT in feces and blood serum, regardless of thyroid function. However, the maximum values of zonulin and α1-AT in feces were diagnosed in patients with AIT with pancreatic hypofunction (an increase in their levels to 168.73±0.51 ng/ml ($p<0.001$) and to 41.07±0.37 mg/dl($p<0.01$), respectively), which directly correlates with the severity of CD in these patients.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Surrogacy and child trafficking: Problems of correlation and delineation of categories

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ABSTRACT

Aim: to distinguish such outwardly similar legal phenomena in the form of irreversible transfer of a child from one person to another, such as child trafficking and surrogacy, primarily paid (commercial).

Materials and Methods: In order to achieve the stated objective, the study employed a set of general scientific and specialized methods of cognition, including comparative law, the formal-logical method, historical method etc. More than 3 thousand records of the Unified Register of Pre-Trial Investigations on the registration and results of pre-trial investigation of criminal proceedings on the facts of human trafficking, including on the facts of illegal surrogacy, were processed. 5 court verdicts from the Unified Register of Court Decisions were analyzed, and 6 criminal proceedings on such facts, in which the investigator or prosecutor made a decision to close them, were also studied.

Conclusions: Due to significant differences from both the objective and subjective sides, surrogacy and child trafficking are different legal agreements, which makes it necessary to assess all the factual circumstances in each specific case when qualifying the act.

KEY WORDS: human rights, natural rights, right to continue of the genus, reproductive rights, state reproductive policy, motherhood, surrogate mother, human trafficking, child trafficking

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INTRODUCTION

Many countries are experiencing a decline in birth rates. One of the reasons is a significant proportion of those who cannot have their own child, but still want to have one. The absence of children may be associated with fertility problems, reluctance to have a child with other people, same-sex marriages/partnerships, etc.

However, modern reproductive technologies have reached a level that can help solve the issue of one's own reproductive reproduction, and among them is the surrogacy method. At the same time, there is a tendency to significantly increase the use of these technologies, their improvement, including when using surrogacy. For example, in just 20 years, starting in 1999, the number of surrogacy procedures used in the USA has increased fourfold [1]. In the USA in 2022, 4.7% of assisted reproductive embryo transplantation cycles used gestational surrogacy [2].

At the same time, although this method is effective, it is also one of the most controversial from both a moral and legal perspective. After all, the point is that the person who carried and gave birth to a child actually has no legal rights to it.

As rightly pointed out, for the first time in the history of mankind it became possible to "separate the functions of a woman as a person who produces a female gamete and a person who carries a child" [3].

Therefore, in world practice there are no unified approaches to this method of reproduction. Moreover, state policy on surrogacy, depending on many factors, is at opposite poles. In some states, surrogacy is a crime, in others it is a legal procedure. Some of them introduce additional increased requirements for surrogacy, while in others there is a tendency to simplify the grounds and conditions for its application.

Supporters and opponents of surrogacy often use the same facts, but with different arguments, trying to prove the need for liberalization or, conversely, the prohibition of surrogacy.

In recent years, the discussion of commercial and altruistic surrogacy has become the subject of wide debate. This plays a special role in the discussion of cross-border surrogacy (surrogacy tourism) [4].

As a result, some call surrogacy a business or human trafficking, others - a miracle and a last chance [5]. One

of the main arguments of opponents, in addition to the moral side, is the argument that surrogacy by its nature is trafficking in a newborn child, which is recognized as one of the most serious crimes. F.M. Abdullah believes that carrying and giving birth to a child for another man or woman solely for financial gain is immoral, illegal, and offends the fundamental values of a democratic society, such as the value of protecting women from exploitation and protecting the child born in this way [3].

Thus, challenging the registration and birth certificates of children of French citizens from a surrogate mother (a procedure carried out in the USA), the prosecutor of the city of Creteil emphasized that the agreement under which a woman undertakes to conceive and bear a child, which she must leave at birth, is null and void, since it contradicts the principles of public law - the inviolability of the human body and the inviolability of the person [5].

Ukraine belongs to the states with a liberal approach to surrogacy, this method is not only legal and widespread, but is also officially permitted on a commercial basis, that is, with appropriate payments from interested parties to surrogate mothers.

At the same time, a number of criminal prosecutions have been initiated in Ukraine for illegal surrogacy and human trafficking (exploitation). Several such criminal cases have been considered by the court and the perpetrators have been convicted, which makes the study of this topic even more relevant. After all, even at this time, the line of demarcation between the legality and illegality of the use of this technique is very narrow.

AIM

The aim of the article is to study and distinguish such outwardly similar legal phenomena in the form of the irreversible transfer of a child from one person to another, such as child trafficking and surrogacy, primarily paid.

In addition, recently, law enforcement agencies of many states, including Georgia, Ukraine, and France, have initiated criminal prosecutions for human trafficking, fraud, etc. against persons who organized or facilitated the implementation of surrogacy.

Therefore, this study has not only a theoretical goal, but also practical significance: to identify the features that distinguish child trafficking from its legal transfer when using the specified medical procedure of reproductive technologies.

MATERIALS AND METHODS

The research methodology uses a systematic approach that corresponds to the interdisciplinary nature of the research topic in order to study the legality of the transfer of a child from a surrogate mother to a third party through the prism of child trafficking.

To do this, the authors used the methodological potential available in legal science, primarily comparative law and formal legal methods, methods of legal analysis and modeling.

The comparative law method allowed us to study the legislation of a significant number of states from different legal families (Great Britain, India, Iran, Italy, Spain, China, Germany, the USA, Poland, Ukraine, etc.) regarding the features of regulating the surrogacy procedure.

The formal-logical method allowed us to investigate how the concept of reproductive technologies is interpreted at the legislative level, and the place of surrogacy in them.

The historical method allowed us to assess the legal development of surrogacy regulation in chronological order, including the emergence of new methods of reproductive reproduction, the influence of historical, religious and other factors on the modern legal regulation of reproductive technologies in different countries and the features of the regulation of surrogacy.

The use of a combination of statistical and analytical methods made it possible to identify and determine the place in the crime structure of Ukraine of those crimes related to human trafficking, including those directly related to illegal surrogacy.

It should be noted that many scientists have thoroughly investigated the issues of legal regulation of surrogacy, including comparing its regulation in different countries, studying the causes of cross-border surrogacy tourism, state policy in this area, moral, ethical and other problems associated with the use of this methodology.

However, there are few publications that specifically study the similarities and differences between surrogacy and child trafficking, as this requires not only theoretical knowledge, but also practical access to materials that are mostly not publicly available. As a rule, the relevant state registers can be analyzed by a very narrow circle of practitioners, and the materials of criminal proceedings - exclusively by those authorized by law (as a rule, only individual prosecutors and judges are granted such powers).

To fill this knowledge gap, this article conducts an in-depth analysis of data contained in the Unified Register of Pre-Trial Investigations (this state register of Ukraine with limited access contains information on all registered criminal offenses since 2012 and the results of their pre-trial investigation), from which more than 3 thousand plots of criminal proceedings registered by law enforcement agencies of Ukraine were processed,

as well as the Unified Register of Court Decisions (this register contains court decisions of courts of Ukraine, including on criminal proceedings of the studied category, some of which are closed for public access), from which 5 court verdicts on the facts of illegal surrogacy were studied. In addition, using the above-mentioned methods, 6 criminal proceedings were studied in which the investigator or prosecutor made a decision to close them due to the absence of a crime - child trafficking when using the surrogacy method.

REVIEW AND DISCUSSION

The study identified key objective and subjective criteria distinguishing commercial surrogacy from child trafficking, including the absence of genetic ties between the surrogate mother and the child, the timing of contract execution prior to embryo implantation, and the presence or absence of direct criminal intent to unlawfully transfer a child for financial gain.

Each country tries to develop its own approach to resolving the issue of the legality of surrogacy, taking into account its own historical experience, development conditions, and the ideology and morality prevailing in society. In some states, this reproductive technique is liberalized (primarily by expanding the grounds and the range of subjects who can use it), while in others, on the contrary, they try to restrict it (including in order to protect their own citizens from surrogacy tourism).

For example, in the Swiss Confederation, the use of surrogacy is regulated at the constitutional level (Article 119 of the Federal Constitution of the Swiss Confederation, 1999) [6].

In France, surrogacy is prohibited in accordance with the decision of the Constitutional Council of the French Republic from 1991, according to which any agreement, even if it does not provide for remuneration, under which a woman agrees to conceive, carry and give birth to a child, and then abandon it, is contrary to public order, the principle of the inviolability of the human body and the personal status of an individual. The same provisions are enshrined in the Law "On Respect for the Human Body" from 29.07.1994. In Art. 16-7 of the French Civil Code it is stated: "Any contract concluded for the purpose of conceiving or carrying a child for the benefit of a third party is null and void" (Article 16-7 of the French Civil Code). Violation of these norms provides for criminal liability in the form of imprisonment and a fine, including for mediation in the implementation of surrogacy (Article 227-12 of the French Criminal Code).

As a result, the world community has encountered a new phenomenon – reproductive tourism, since it is not only about surrogacy.

Every year, thousands of people travel abroad from their countries to circumvent restrictive legislation or take advantage of lower prices. In this context, surrogacy raises many bioethical and legal issues [7].

The countries where surrogacy is permitted and widely practiced include: some states of Australia and the USA, Great Britain, Canada, Portugal, Uganda, Ukraine, etc.

In some countries, surrogacy is permitted only on a gratuitous basis, that is, the surrogate mother cannot receive financial compensation for carrying and giving birth to a child (Australia, Belgium, Great Britain, Greece, Canada, etc.). However, it should be borne in mind that in some of them, reimbursement of expenses incurred by the surrogate mother is not considered payment for the procedure itself.

In other countries, paid surrogacy is permitted (Armenia, Georgia, Kazakhstan, Cyprus, Kyrgyzstan, Ukraine, etc.).

Similar heterogeneity in relation to surrogacy is also observed in South America [9].

Another difference in access to surrogacy is due to the fact that the regulations of a number of countries require both partners to provide gametes when using surrogates, while in others only one of the biological parents is sufficient. Therefore, in the first case, single people (singles), as a rule, actually lose the right to have a child due to the specified method.

According to Article 12 of the Law of the Republic of Armenia "On Reproductive Health and Human Reproductive Rights", a person who has the right to use assisted reproductive technologies may also be one of the biological parents (Article 12 of the Law of the Republic of Armenia "On Reproductive Health and Human Reproductive Rights").

Article 146 of the Code of the Republic of Kazakhstan on Public Health and the Health System declares that a woman or a man who is not married has the right to use assisted reproductive methods and technologies if she (he) has informed consent to medical intervention (Article 146 of the Code Republic of Kazakhstan on Public Health and the Health System). However, departmental acts stipulate that only spouses have the right to surrogacy.

In international practice, there are more and more precedents for the birth of a child by a surrogate mother not only for married couples, but also for single individuals under certain conditions.

Such conditions include the death of their loved one, after whom genetic material remained, and the deceased expressed his will for its use, the reluctance of single men to marry in the presence of a natural need for procreation, etc. [17]. For example, Spanish actress

Ana Obregon used her deceased son's frozen sperm and a donor egg to give birth to his daughter in 2023 via surrogate mother in the United States [10].

A completely different approach has been taken at the international and national levels regarding human trafficking, which is recognized as illegal and carries strict legal liability. The prohibition of human trafficking, including child trafficking, is a global practice and is enshrined in international law, such as the Convention on the Rights of the Child and its Optional Protocol of 01.01.2000, as well as in national legislation.

Article 149 of the Criminal Code of Ukraine provides that human trafficking, as well as the recruitment, movement, harboring, transfer or receipt of a person, committed for the purpose of exploitation, using coercion, abduction, fraud, blackmail, material or other dependence of the victim, his vulnerable state or bribery of a third party who controls the victim, to obtain consent to his exploitation, is a criminal offense.

Responsibility for the recruitment, movement, concealment, transfer or receipt of a minor or minor arises regardless of whether such actions are committed using coercion, abduction, fraud, blackmail or the vulnerable state of the said persons or using or threatening to use violence, using official position, or by a person on whom the victim was in material or other dependence, or bribing a third person who controls the victim, in order to obtain his consent to the exploitation of a person (Article 149 of the Criminal code of Ukraine).

Similar provisions are contained in the Law of Ukraine "On Combating Trafficking in Human Beings", which provides a definition of trafficking in minors (minors).

According to the Unified Register of Pre-Trial Investigations in Ukraine, over the past ten years (2015-2025), 1,121 criminal proceedings have been registered on 3,094 criminal offenses on the facts of human trafficking, of which 467 have been closed, 479 have been sent to court for consideration with charges. The absolute majority of these relate to human trafficking for the purpose of their labor or sexual exploitation, use for begging, involvement in criminal activity, etc.

During this period, 33 criminal proceedings have been registered on 106 criminal offenses on the facts of human trafficking or exploitation when using reproductive technologies, primarily surrogacy. Of these, 13 criminal proceedings have been closed due to the absence of a crime, 8 (of which 4 are interconnected) have been sent to court with charges, and the rest are being investigated.

However, an analysis of court verdicts indicates that the basis for the accusation was not the fact of buying and selling a child, but the falsification of documents by the surrogate mother about the birth of the child, the exploitation of women who were surrogate mothers.

For example, according to the investigation materials, a resident of Kharkiv was suspected of having participated in the recruitment of women, mainly from the Kharkiv region, to participate in surrogacy programs, while working as an administrator at a medical clinic. The women were misled, being told that they would give birth to children for married couples, and that their participation was limited only to carrying and giving birth to the child. After giving birth, surrogate mothers were forced to register the newborns in their own names and give consent for the child to be taken abroad by a foreign father. In case of disagreement, they were threatened with incomplete payment of the promised remuneration [11].

In the absolute majority of cases, criminal prosecution was initiated on the facts of surrogacy for the benefit of foreign citizens, where such a procedure is either complicated or prohibited altogether. Kirsty Horsey [12] discussed the reasons for international reproductive tourism in considerable detail, and a specific example of India is given by Saran J, Padubidri JR [8].

At the same time, the facts of buying and selling a child also occur and outwardly they are quite similar to the transfer of a child through paid surrogacy.

For example, the Sumy District Prosecutor's Office sent an indictment to the court on the fact of human trafficking against a mother who tried to sell her own newborn child for 30 thousand US dollars in May 2025.

Therefore, there is a need to distinguish between the legality and illegality of a transaction whose object is a child, as well as the presence of a crime in the actions of individuals precisely in the case of the transfer of a child by the person who gave birth to him or her to a third party.

We emphasize that the object of such trade should be a child, and not a person's gametes, zygote, embryo or fetus. If there are grounds, illegal transactions with the latter may be qualified under other articles of the criminal law.

Surrogacy requires that a civil contract be concluded before the embryo is implanted in the woman who assumes the role of surrogate mother, unlike child trafficking, where a written or oral contract can be concluded at any stage (from the decision to become pregnant, to insemination, to carry the fetus, or after the birth of the child).

When a child is trafficked in the form of its sale, from an objective point of view, it is precisely its illegal, irreversible transfer from one person to another.

The legal transfer of a child in states where surrogacy is permitted is not a crime. In legal surrogacy agreements, the legal transfer of a child occurs, since the object of such a contract concluded between the

surrogate mother and the future parents is not the child, but the services related to the implantation of the embryo, carrying the fetus, and the birth of the child, i.e. a long process.

At the same time, it is necessary to distinguish between the payment of surrogacy services and child trafficking for remuneration (payment).

In the first case, we are talking about the provision of paid services by a surrogate mother and the child must be given (returned) to her parents who have genetic kinship, where the transfer of the child is a logical legal completion of the entire medical procedure related to ensuring reproductive rights. In the second case, we are talking about the initially illegal transfer of a child from one person to another for remuneration, where the main goal is precisely the illegal transfer of the child, and the goal is to provide (receive) an illegal benefit.

Receiving remuneration for surrogacy services should also be distinguished from receiving compensation for services provided, which are only externally similar in content. At the same time, they are of no fundamental importance for Ukrainian legislation. Instead, in some countries (for example, Great Britain) only the possibility of compensation for inconveniences and expenses incurred is provided, otherwise the surrogacy service is illegal and may entail liability.

Therefore, from an objective point of view, the mere fact of payment for surrogacy services cannot indicate child trafficking.

And here an important element is the issue of the presence of a genetic connection in persons who ordered the surrogacy service, as well as in the surrogate mother.

Modern medical science distinguishes two types of surrogacy:

- full or gestational surrogacy - the transfer of a human embryo conceived by a spouse and a donor into the body of a surrogate mother. In this case, the surrogate mother has no genetic connection with the child;

- partial or gender surrogacy implies a genetic connection with the child, since the surrogate mother's egg is used.

Taking into account the norm of Art. 123 of the Family Code of Ukraine, the method of surrogacy involves the transfer of a human embryo conceived by the spouses (genetic parents) into the body of the surrogate mother. That is, Ukrainian legislation provides for only one method - full (gestational) surrogacy (Article 123 of the Family Code of Ukraine). This condition is provided for by the vast majority of other national legislations.

We believe that a mandatory condition for legal paid surrogacy, which does not contain signs of child trafficking, is the absence of genetic kinship between the child and the surrogate mother.

At the same time, the assessment of the actions of a surrogate mother who was previously an egg donor, other persons should be given taking into account all the circumstances of the case and does not exclude child trafficking.

A separate solution is required for the issue of the mandatory genetic connection between the child and the persons who resorted to the surrogacy method.

The ideal option is cases when both spouses are the biological parents of the future child. However, sometimes only one of the customers of this medical procedure has a genetic connection. Thus, it is resorted to by spouses in which only the husband is fertile, single people who used donor gametes, etc.

Therefore, in the absence of a genetic connection of the child with both parents at once, we can talk about other types and combinations of artificial insemination, including the use of embryo or gamete donation, which in itself does not entail the illegality of surrogacy, refusal to recognize paternity and automatic establishment of the fact of human trafficking.

The exception is the case when such a newborn child is "sold" by the genetic parents (one of them) to other persons. However, in this case, the situation is similar to the usual illegal sale of a baby, regardless of the method of conception.

We believe that the combination of surrogacy and the use of fully donor gametes is actually a "hidden form of adoption" and is not related to the realization of one's own natural right to reproduction. However, while agreeing with the illegality of the use of the method itself, it cannot be stated that "child trafficking" definitely takes place, that is, a crime.

Thus, if a person used this hybrid of methods due to reluctance to adopt a child (including to prevent future risks of property claims from relatives of the adopted child, or the child being taken away by its parents, or for other objective or subjective reasons (quite often it is used to hide one's own infertility) but for the purpose of paternity, then the chance of proving child trafficking in court is minimal. Moreover, in this case, there is no agreement on the transfer of one's own child to another person (i.e., the trafficking itself), since formally it does not belong to the surrogate mother (who has no genetic relationship), who transfers the born child under the relevant legal contract.

At the same time, there are facts when pregnant women sell their children, falsifying surrogacy. In this case, such actions should be qualified as human trafficking.

For example, in February 2021, Kyiv Local Prosecutor's Office No. 5 sent an indictment to court against a citizen on charges of trafficking in a minor child. She, participating in the surrogacy program, contrary to the

terms of the contract and knowing for sure that she was the biological mother of a newborn child, in order to receive 15 thousand US dollars illegally gave her to foreign customers who did not suspect fraud and believed that the baby was their genetically native child.

In addition to the objective side, the subjective side plays a significant role in the qualification, that is, the person's own attitude to the act committed by her.

The subjective side assumes the presence of direct intent to unlawfully "alienate" the child, primarily for payment.

The perpetrator must be aware that he is illegally transferring the child to third parties, including on a paid basis (for example, a surrogate mother is aware that she is the genetic mother, but sells her for money under a false surrogacy agreement), or the surrogacy program is carried out for the purpose of further trafficking in the child (i.e., the birth of a child is not intended to create paternity in the persons who used this method, primarily genetically related parents, but for another purpose, for example, selling the child to persons for begging, using its stem cells or organs, etc.).

At the same time, if a person used artificial insemination techniques for the purpose of his own paternity and paid for artificial insemination services, then we believe that his actions do not constitute human trafficking.

Among the exceptions are cases when one of the genetic parents consciously renounces paternity by "alienating" the child for a certain fee to another person. For example, a mother sells a newborn child, or one of the genetic parents, for payment, renounces it and recognizes his own genetic paternity in favor of another person. Child trafficking will also occur if the genetic mother (egg donor) when using surrogacy services knowingly received funds not only for a fictitious marriage, but also for transferring her own child to another person, that is, a conscious act of trafficking in a newborn child.

At the same time, no person, including the surrogate mother, officials who provided the surrogacy program, can be held liable for the subsequent actions of the parents who used this method and their treatment ("order") of the child, if such actions were not covered by the common criminal intent to commit this crime.

CONCLUSIONS

Today, there is no consensus in the international community on the legality of using the surrogacy method and its relationship with such a particularly serious crime as child trafficking. However, none of the states can be a universal example of solving this problem.

The problems that arise when implementing commercial surrogacy in Ukraine and other states, the existing abuses in this area are a reason to improve the system, not to ban this medical procedure.

Paid surrogacy, regardless of whether it is recognized by the state as a crime, should be distinguished from trafficking in humans, including children. Surrogacy has significant differences from both the objective and subjective sides from human trafficking, and therefore these components should be investigated by law enforcement agencies in each individual case for the correct qualification of the committed act.

In the criminal law aspect, it is necessary to prove the fact of a deliberate violation by the subjects participating in the program of the procedure and grounds for conducting surrogacy for the subsequent trafficking of a newborn child.

Investigators, prosecutors, judges must investigate all the circumstances that were related to the surrogacy method used in order to establish whether child trafficking took place in each specific case.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Lifestyle interventions for diabetic retinopathy during pregnancy: Current evidence from clinical studies

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ABSTRACT

Aim: To summarize current evidence on lifestyle interventions, metabolic stabilization and screening strategies relevant to diabetic retinopathy during pregnancy.

Materials and Methods: A narrative review of clinical studies, systematic reviews, meta-analyses and guideline documents was performed using PubMed/MEDLINE, Scopus and Google Scholar.

Baseline retinal status in early pregnancy is the strongest predictor of progression. Additional risk is associated with longer diabetes duration, elevated glycated hemoglobin, glycemic variability, nephropathy and hypertensive disorders, including preeclampsia. Women without retinopathy in early pregnancy generally have a low risk of sight-threatening disease during the same gestation, whereas women with pre-existing retinopathy require close surveillance. Lifestyle interventions do not directly treat retinal lesions, but they improve glycemic control, support appropriate gestational weight gain and reduce vascular burden. Evidence also suggests that continuous glucose monitoring and automated insulin delivery may indirectly support retinal protection by improving metabolic stability.

Conclusions: In pregnant women with diabetes, ophthalmic screening remains the main clinical priority. However, lifestyle-based metabolic support is a meaningful complementary strategy and should be integrated into antenatal care alongside risk-stratified retinal follow-up.

KEY WORDS: pregestational diabetes, metabolic control, preeclampsia, glycemic variability, retinal screening

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INTRODUCTION

Pregnancy is associated with several ocular changes, most of which are transient, but diabetic retinopathy remains one of the most clinically relevant microvascular complications in pregnant women with diabetes [1-3]. The risk of retinal deterioration is highest in women with pregestational diabetes, particularly when retinopathy is already present before conception or is detected in early pregnancy [2-4]. Pregnancy may modify the course of retinal disease through hormonal, inflammatory, hemodynamic and metabolic shifts, yet progression is not driven by pregnancy alone. The most consistent determinants are baseline retinal status, duration of diabetes, glycemic control, hypertension and renal involvement [2-4].

Current recommendations therefore emphasize systematic ophthalmic screening before conception or in the first trimester, followed by risk-adjusted surveillance throughout pregnancy [5, 6]. At the same time, supportive systemic management deserves more attention. Diet quality, physical activity, gestational weight control, blood pressure optimization and modern diabetes technologies do not directly reverse retinal lesions, but they can improve the metabolic environment in which retinopathy progresses [7-12]. The available evidence is strongest for women with type 1 or type 2 pregestational diabetes; in gestational diabetes, data are more indirect and mainly reflect broader vascular risk rather than established retinal disease [13, 14].

AIM

The aim of this review was to summarize current clinical evidence on diabetic retinopathy progression during pregnancy, with particular emphasis on lifestyle interventions, metabolic stabilization and screening strategies that may help reduce retinal risk in routine antenatal care.

MATERIALS AND METHODS

This study was designed as a narrative review with a practice-oriented synthesis. Literature was searched in PubMed/MEDLINE, Scopus and Google Scholar. The search strategy included combinations of the following terms: diabetic retinopathy, pregnancy, pregestational diabetes, type 1 diabetes, type 2 diabetes, gestational diabetes,

screening, retinal photography, lifestyle intervention, diet, Mediterranean diet, DASH, exercise, glycemic control, continuous glucose monitoring and metabolic stabilization.

Priority was given to publications from 2018-2025, together with selected earlier landmark studies needed to contextualize established clinical risk factors. Eligible sources included cohort studies, randomized trials, systematic reviews, meta-analyses and guideline documents if they addressed prevalence or progression of diabetic retinopathy in pregnancy, screening strategies, baseline risk factors, diet, physical activity, behavioral support or technology-assisted metabolic management relevant to maternal retinal outcomes. The evidence was synthesized qualitatively.

The research was made based on 23 research papers such as 13 original clinical studies 9 of them were observational studies and other 4 interventional studies, 2 systematic reviews and meta-analyses, 4 narrative reviews and 3 topical reviews, and 1 guideline document.

REVIEW

PATHOPHYSIOLOGICAL BACKGROUND AND EPIDEMIOLOGY

Diabetic retinopathy is a microvascular complication of diabetes driven by chronic hyperglycemia, endothelial dysfunction, inflammatory activation and breakdown of the blood-retinal barrier [3, 15]. These processes increase vascular permeability, promote capillary non-perfusion and, in advanced disease, lead to neovascularization and vision-threatening complications. Pregnancy may amplify this instability because of rapid shifts in metabolism, circulation and hormonal signaling, especially in women with pre-existing retinal vulnerability [3, 15].

Contemporary clinical data show that the burden of diabetic retinopathy in pregnancy is concentrated in women with pregestational diabetes [2, 14, 17-19]. A substantial proportion already have retinopathy at baseline, and progression during pregnancy occurs mainly in those with established disease. In contrast, women without retinopathy in early pregnancy appear to have a relatively low risk of developing sight-threatening retinal disease during the same gestation [17-19]. These observations support early retinal staging as the basis of clinical risk stratification.

RISK FACTORS FOR PROGRESSION

Across recent cohort studies and systematic reviews, baseline severity of retinopathy at conception or in early pregnancy is the strongest predictor of worsening [2, 16-19]. Women with moderate or severe non-proliferative changes are much more likely to progress than women with no retinal lesions, and progression to proliferative disease occurs predominantly in those with pre-existing abnormalities.

Longer duration of diabetes, higher early-pregnancy glycated hemoglobin (HbA1c), marked glycemic variability and rapid changes in glycemia are additional risk factors [2, 4, 16-18]. This is clinically relevant because tighter glucose control remains beneficial overall, but it should be accompanied by closer ophthalmic monitoring in women with established microvascular disease. Hypertensive disorders of pregnancy, chronic hypertension, higher systolic blood pressure, nephropathy and microalbuminuria also identify women at increased retinal risk [4, 16-18]. Moreover, retinal worsening documented during pregnancy may persist after delivery rather than regress spontaneously, which strengthens the rationale for postpartum reassessment [17].

LIFESTYLE INTERVENTIONS AND METABOLIC STABILIZATION

Direct interventional studies that use diabetic retinopathy progression as a predefined endpoint are scarce. Nevertheless, evidence from pregnancy studies focused on metabolic control suggests that lifestyle measures are clinically relevant because they influence the same systemic pathways consistently linked with retinal worsening. In women with gestational diabetes, structured lifestyle intervention achieved satisfactory glycemic control in most participants within a short period and reduced the need for treatment escalation in many cases [10].

Diet quality appears particularly important. Mediterranean-style and DASH-like dietary patterns have been associated with lower rates of gestational diabetes, hypertensive disorders of pregnancy and less adverse metabolic profiles [7-9]. In women with type 1 diabetes, the ENDIA study showed that a dietary pattern characterized by higher intake of fresh foods was associated with lower odds of preeclampsia and preterm birth, with part of the effect mediated by body mass index and HbA1c [11]. Although these studies did not evaluate retinal endpoints directly, they support the concept that nutritional quality influences vascular risk through measurable metabolic mechanisms.

The broader evidence base also supports physical activity and structured behavioral care. A recent systematic review and Bayesian network meta-analysis found that both dietary and exercise interventions improved outcomes in gestational diabetes, with

some strategies reducing insulin use and improving metabolic control [12]. This is relevant to retinal protection because poor glycemic control, blood pressure burden and systemic vascular stress are repeatedly associated with progression of diabetic retinopathy during pregnancy [2, 4, 16-18].

Modern diabetes technologies can be interpreted within the same framework. Automated insulin delivery, continuous glucose monitoring and personalized technology-supported management improve glucose awareness and support day-to-day decisions that favor glycemic stability [20-22]. They are not a retinal treatment in themselves, but they may strengthen lifestyle-based self-management and indirectly lower retinal risk, especially in women with pregestational diabetes who require tight control throughout pregnancy [20-22].

PRACTICAL IMPLICATIONS FOR CARE

The available evidence supports a prevention-oriented model in which screening and metabolic support are integrated rather than treated as competing priorities. Women with pre-existing diabetes should undergo retinal assessment before conception or, if already pregnant, in the first trimester [5, 6]. Those with any baseline retinopathy, long diabetes duration, hypertension or renal disease require closer follow-up than women with normal early pregnancy retinal findings [17-19].

Lifestyle counseling should begin early and target diet quality, safe physical activity, appropriate gestational weight gain, blood pressure control and glycemic stability. Postpartum retinal reassessment is important in women with baseline disease or progression during pregnancy [17]. This integrated pathway is especially important because real-world adherence to recommended retinal screening remains suboptimal [23].

DISCUSSION

The main practical message from the current literature is that diabetic retinopathy in pregnancy should be managed through a risk-stratified and multidisciplinary model. Ophthalmic screening identifies which women are at highest immediate risk, while lifestyle and metabolic interventions address the systemic exposures that shape progression over time [2, 5, 6, 17, 18]. These approaches are complementary. Screening remains the central clinical priority, but supportive metabolic care should not be treated as secondary or optional.

The strongest argument for lifestyle intervention is indirect but consistent. Studies on diabetic retinopathy progression repeatedly implicate poor glycemic control, hypertensive disease and nephropathy as major drivers of retinal worsening [2, 4, 16-18]. At the same time, studies on diet, exercise and technology-assisted diabetes management during pregnancy show measurable improvement in exactly these variables [7-12, 20-22]. This does not prove a retina-specific treatment effect, yet it provides a biologically plausible and clinically useful basis for prevention-focused care.

Several limitations should be acknowledged. Most available evidence on retinal progression is observational, and many intervention studies report metabolic or obstetric outcomes rather than ophthalmic endpoints [13, 14]. The literature also mixes women with type 1 diabetes, type 2 diabetes and gestational diabetes despite major differences in baseline retinal risk. Postpartum follow-up remains inconsistent, even though progression during pregnancy may persist beyond delivery [17]. Future prospective studies should therefore include standardized retinal outcomes, clearer separation of diabetes subtypes and longer postpartum follow-up. Until such data are available, the most defensible strategy is to combine early retinal staging with intensive support for glycemic stability, blood pressure control and screening adherence [5, 6, 23].

CONCLUSIONS

Pregnancy is a period of increased vulnerability to diabetic retinopathy progression, especially in women with pregestational diabetes and in those with retinal disease already present at conception or in early pregnancy. Baseline retinal status is the strongest predictor of worsening, while longer diabetes duration, higher HbA1c, glycemic variability, hypertension and renal involvement further increase risk.

Retinal screening remains the primary clinical priority and should ideally be performed before conception or in the first trimester, with follow-up tailored to baseline risk. Lifestyle intervention does not directly treat retinal lesions, but by improving metabolic stability, blood pressure control and gestational weight trajectories, it is a meaningful component of prevention. The most appropriate care model therefore combines early ophthalmic staging, risk-stratified surveillance, structured lifestyle-based support and postpartum reassessment.

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REVIEW ARTICLE

CONTENTS

Igf-1 in acne pathogenesis and inositol as possible adjuvant treatment of acne

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ABSTRACT

Aim: To provide a comprehensive review of acne pathogenesis and data regarding inositol efficacy in targeting IGF-1 pathway and decreasing IGF-1 levels and find pathophysiological substantiation (rationale, reasoning) of inositol therapy feasibility in acne treatment.

Materials and Methods: Search of literature was performed on PubMed, Scopus, ResearchGate to identify scientific publications that aimed to research the role of inositol in acne and IGF-1 in acne pathogenesis and treatment and were published between 1999 and 2024 yielding information about possible therapeutic approach targeting IGF-1 induced increased sebum production. Peer-reviewed full-text articles, systematic reviews and evidence-based guidelines were included.

Conclusions: We provided a comprehensive review of acne pathogenesis and data regarding inositol efficacy in targeting IGF-1 pathway.

KEY WORDS: insulin-like growth factor-1, inositol, acne

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INTRODUCTION

Acne is a chronic, inflammatory disease of the pilosebaceous unit. According to one of the most influential epidemiological studies, The Global Burden of Disease Study, it is known that acne is the eighth most common disease in the world with a total prevalence (for all age groups) of 9.38% [1]. As we know from the literature, the pathogenesis of acne is explained by four key factors: excess production of sebum, hyperproliferation of bacteria *Cutibacterium acnes* (*C. acnes*, formerly called *Propionibacterium acnes*), hyperkeratinization of the sebaceous follicle, and inflammatory mechanisms [2]. Excessive sebum production occurs due to increased activity of androgenic hormones and insulin-like growth factor 1 (IGF-1) [2]. Most textbooks describe acne as a hormone- dependent dermatosis [3]. Without a doubt, the participation of male sex hormones is an important link in the pathogenesis of acne. However, serum androgen levels increase during puberty and may remain high for decades, whereas acne physiologically resolves spontaneously after puberty. From the paper of Deplewski and Roseneld it is known that it is not serum androgens, but the level of IGF-1 that correlates with the clinical manifestation of acne. After all, the peak incidence usually falls on the teenage period, when the level of insulin-like growth factor - 1 in the blood serum reaches its maximum. This happens during a growth spurt [4]. After the culmination of puberty, the level of Somatomedin-C (IGF-1), the main hormone of puberty, is known to decrease continuously [5]. There are scientific publications that confirm that the increase in the level of IGF-1 in addition to androgens correlates with worsening of acne in adult men and women [6]. There is increasing evidence that interactions between growth hormone, insulin, and IGF-1 signaling during puberty may play a causative role in acne pathogenesis by influencing adrenal and gonadal androgen metabolism [7]. This opinion is also supported by the fact that acne is absent in adolescents with Laron syndrome and in people with a hereditary predisposition to dwarfism resulting from congenital deficiency of IGF-1 due to growth hormone receptor (GHR) mutations. Conversely, in some patients, the development of acne is observed against the background of treatment with insulin-like growth factor (IGF-1) drugs. And also regresses when IGF-1 dosage is reduced or stopped. [2] This data can imply that new therapeutic strategies that target IGF-1 levels may be implied in acne treatment.

AIM

We aimed to provide a comprehensive review of acne pathogenesis and data regarding inositol efficacy in targeting IGF-1 pathway and decreasing IGF-1 levels and find pathophysiological substantiation (rationale, reasoning) of inositol therapy feasibility in acne treatment.

MATERIALS AND METHODS

Search of literature was performed on PubMed, Scopus, ResearchGate to identify scientific publications that aimed to research the role of inositol in acne and IGF-1 in acne pathogenesis and treatment and were published between 1999 and 2024 yielding information about possible therapeutic approach targeting IGF-1 induced increased sebum production. Peer-reviewed full-text articles, systematic reviews and evidence-based guidelines were included.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

Growth hormone and IGF-1 are important components of epidermal homeostasis Somatotropin is produced by the adenohypophysis. It binds to growth hormone receptors (GHR), which are expressed on most peripheral cells of the body. Somatotropic hormone induces hepatic synthesis and secretion of IGF-1 [8]. Somatotropin, insulin and insulin-like growth factor-1 have a clear effect on the growth and differentiation of sebocytes [4]. According to the literature, it is known that IGF-1 signaling is a central endocrine pathway of puberty and is a key mechanism through which nutrition influences the development of acne [2], which is closely associated with the Western diet [9].

Three main components of the Western diet have been identified as driving forces in the pathogenesis of acne: 1) hyperglycemic carbohydrates (high glycemic load); 2) insulinotropic milk/ dairy products, which promote secretion insulin/insulin-like growth factor (IGF)-1; 3) saturated fats, including trans fats and a deficiency of polyunsaturated fatty acids [5].

Epidemiological and clinical data evidence confirms that milk and other insulinotropic dairy products have an acne-promoting or acne-aggravating effect. Also, according to data from placebo- controlled studies, it is known that diets with a high glycemic load can exacerbate acne, increase levels of free IGF-1 in blood serum and stimulation of postprandial hyperinsulinemia [5].

INSULIN-IGF-1 PATHWAY AND ACNE

As it was mentioned before growth hormone and Western diet induce production of insulin and IGF-I in the liver and many other target tissues. Which, in turn, on the one hand activates the excess production of sebum, and on the other - androgens, which stimulate abnormal keratinization of the sebaceous glands. As a result, all these processes lead to the development of acne [5,8,9]. For a better understanding of all links of pathogenesis, let's take a closer look at the processes that occur after the activation of the insulin-insulin-like growth factor pathway. Insulin and IGF-1 activate insulin and insulin-like growth factor receptors [5]. These receptors by their structure are very similar to the insulin receptor, as it is a member of the insulin gene family. However, it is known that functionally they have differences: insulin plays a more metabolic role and IGF-I is more involved in cell growth [8]. In this way, it is a possible explanation of the adjacent overlapping of signal transduction between receptors.

Stimulation of insulin receptors and insulin-like growth factor receptors promotes cell growth and proliferation through activation of the phosphoinositol-3-kinase (PI3K)–protein kinase B (AKT) signaling cascade [5]. Activation of the PI3K/Akt cascade affects the regulation of FoxO1 and its nuclear export by insulin, insulin-like growth factor-1 (IGF-1). In the end this process has impact on the transcriptional activity of key target genes and nuclear receptors involved in the pathogenesis of acne [10].

IGF-1 downregulates FoxO1 and activates mTORC1. According to the literature, insulin-like growth factor-1 is known to reduce the nuclear levels of the Fork head box class O transcription factor 1 (FoxO1) and lead to the activation of the mammalian target of rapamycin complex 1 (mTORC1) [2] FoxO1 modulates the expression of genes involved in cell cycle control, DNA damage repair, apoptosis, oxidative stress, cell differentiation, glucose and lipid metabolism, inflammation, and innate and adaptive immune functions. FoxO1 is expressed in all mammalian tissues, including human sebaceous glands [11]. The main way of regulating FoxO1 transcription factors is their transport either to the nucleus or to the cytosol. Inhibition of FoxO1 occurs through its export from the nucleus to the cytoplasm. As it was mentioned before, insulin and insulin-like growth factor by specific phosphorylation of FoxO1 due to the activation of Akt kinase initiates its nuclear export. This process leads to the activation of the mammalian target of rapamycin complex 1 (mTORC1) [10]. This protein complex is involved in cell proliferation and metabolism. In acne, mTORC1 mediates sebaceous gland hyperproliferation, lipid synthesis, and keratinocyte hyperplasia [12]. Insu-

lin-like growth factor-1 also increases androgen levels, which in turn increases endogenous IGF-1, creating a positive feedback loop that increases lipogenesis [2].

IGF-1 AND INFUNDIBULAR KERATINOCYTES LOCAL PROLIFERATION

IGF-1 possesses the stimulatory effects on sebofollicular androgen signaling, by regulatory mechanisms that enhance androgen receptor (AR) transcriptional activity [13]. AR activate the expression of genes which are responsible for androgen-dependent growth and proliferation. For initiation of this nuclear transcription factors require two major aspects: 1) binding of its hormonal ligand (androgen) and 2) depression of its inhibitory nuclear coregulator FoxO1. Activation of AR depends on how it is related to androgen binding. Dihydrotestosterone (DHT), which is ten times more active than testosterone, has the highest ability to bind with AR. IGF-1 is also known to be a powerful inductor of the production of gonadal testosterone and adrenal dehydroepiandrosterone (DHEA) and also promotes the conversion of testosterone to DHT in the skin by increasing the activity of 5α-reductase [5,13].

IGF-1 AND LIPOGENESIS IN SEBACEOUS GLANDS

IGF-1 plays a key role in the lipogenesis of sebaceous glands. According to research it is known that, the level of concentration of IGF-I, has effect on increase in the accumulation of lipids in the sebocyte [14]. Signaling pathway of IGF-1/PI3K/AKT affects four key lipogenic transcription factors: the AR, peroxisome proliferator-activated receptor-γ (PPARγ), liver X receptor-α (LXRα), and sterol response element binding protein-1c (SREBP-1c). They are negatively regulated by FoxO1 [5, 13, 15]. Thus, activation of the insulin/IGF-1 pathway reduces nuclear FoxO1 and derepresses all major transcription factors of sebaceous gland lipogenesis [5]. High levels of IGF-1 induced by glycemic load result in both increased sebum production in sebocytes and upregulation of inflammatory cytokines such as IL-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α [25]. Besides, there is a positive correlation between serum IGF-1 levels and amount of facial sebum excretion [26].

NEW TRAJECTORIES IN THE TREATMENT OF ACNE

The treatment of acne is regulated by the European guidelines for local and systemic therapy. At the moment, oral isotretinoin is the most effective drug for acne treatment. It reduces sebum secretion in humans through sebocyte-mediated apoptosis. However, isotretinoin is associated with the risk of teratogenicity and other serious side effects. It is also possible to use antiandrogens in women without contraindications as an alternative method of treatment [16]. Thus, there is a need to develop alternative methods to suppress sebum production with fewer side effects. Therefore, according to modern scientists, several key development trajectories have been outlined:

- Medicines that increase insulin sensitivity (insulin sensitizers) such as MYO and D-Chiro- inositol (DCI)
- Probiotics are an important component in the complex treatment of acne and correction dysbiosis
- Transition from symptom control to a holistic patient-centered approach and stress control are further directions for future acne treatments [17]

INOSITOL AND ACNE

In the literature there is increasing information about the relationship between acne and insulin resistance [18,19]. Both pathologies have shared signaling pathways of mammalian target of rapamycin complex 1(mTORC1) and insulin-like growth factor-1 (IGF-1). Insulinemia, hyperandrogenism and elevated levels of insulin-like growth factor are important components in the development of the pathogenesis of acne [20]. Increased insulin levels can stimulate IGF-1 receptors boost, which leads to the proliferation and dysfunction of keratinocytes. On the other hand, hypersecretion of IGF-1 can produce abnormal production of sebum, hyperproliferation of sebocytes, and lipogenesis [1]. Also, insulin and insulin-like growth factor activate the androgen signaling pathway, and as a result - increase level of androgens. [2,6].

The results of the review of modern literature gave an understanding that inositol is a new player in the complex treatment of mild acne [21-23]. Chemically it can be characterized as hexahydroxycyclohexane and belongs to vitamin B group. Epimerization of the six hydroxyl groups leads to the formation of nine stereoisomers, including myo - (MYO) and D-chiro-inositol (DCI). [24] This vitamin-like substance mediates cellular signal transmission in response to various hormones, neurotransmitters, and growth factors [25]. It has been reported that an alteration of inositol levels plays a pivotal role in the pathogenesis of some metabolic diseases, such as metabolic syndrome (MetS), type 2 diabetes mellitus (T2D), and polycystic ovary syndrome (PCOS), which are conditions all related to altered insulin sensitivity it has been reported that an alteration of inositol levels plays a pivotal role in the pathogenesis of some

metabolic diseases, such as metabolic syndrome (MetS), type 2 diabetes mellitus (T2D), and polycystic ovary syndrome (PCOS), which are conditions all related to altered insulin sensitivity Both myo- and D-chiro-inositol can regulate insulin signal transduction by increasing its sensitivity and improving glucose metabolism. Inositol was also examined in patients with glucose intolerance, given its properties as an insulin-sensitizer and second messenger, thus exerting an insulin-like effect on as an insulin-sensitizer and second messenger, thus exerting an insulin-like effect on metabolic enzymes [27]. In type-1 diabetes (T1D), inositol was tested to replace metformin metabolic enzymes [27]. For this reason, inositol can be used as a complementary therapy for improving the cellular response to metabolic cascades following the binding of insulin to its receptor, specifically be reducing insulin resistance and improving glucose metabolism [6].

CONCLUSIONS

Recent studies have shown that inositol treatment improves tissue sensitivity to insulin and reduces circulating androgen levels. As a result, inositol preparations contribute to the reduction of manifestations of skin diseases in hyperandrogenic conditions; reduce the rate of hirsutism and acne manifestations. Inositol may be beneficial in treating female patients with acne as an adjunct therapy. Short-term treatments with low dosages of d-chiro-Ins, on the other hand, can be considered safe in man with normal testosterone levels.

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REVIEW ARTICLE

CONTENTS

Analysis of artificial intelligence errors in practical medicine and rehabilitation

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ABSTRACT

Aim: To structure the types of errors that occur when using artificial intelligence in healthcare, as well as assess their impact on the accuracy of diagnostics and therapeutic decisions. Identify ways to minimize errors and increase the effectiveness of the use of AI in practical healthcare and rehabilitation.

Materials and Methods: Publications published from January 2021 until December 2025 were processed and analyzed according to the keywords of the topic of work "Pathology", "research on the results of diagnostics and treatment", "artificial intelligence", "machine learning", "deep learning", "federated learning", "use of AI in rehabilitation", "structuring of AI errors" in the databases of PubMed, MEDLINE, Web of Science. Articles were selected based on the presence of: quantifiable results and usage of AI as the main or secondary evaluation method. A total 57 articles were reviewed, out of which 38 were excluded based on eligibility criteria and 2 were excluded as duplicates.

Conclusions: Artificial intelligence is becoming an integral part of modern medical diagnostics and therapeutic solutions. Its implementation significantly increases the accuracy of diagnostic processes and allows for personalized treatment, but today there is no universal solution for the practical use of AI. A lot of errors are still recorded when using AI in diagnostic and prognostic processes, ethical issues have not been resolved, integration of all molecular information available to the patient is not always ensured, there are no uniform standards for collecting and processing medical data, a unified medical language, etc. To ensure professional machine learning, widespread implementation of an open healthcare system, balanced and unified guiding principles is necessary. The implementation of AI technologies depends on the training of doctors and the availability of technologies.

KEY WORDS: Pathology, artificial intelligence, use of AI in rehabilitation, structuring of AI errors

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INTRODUCTION

Artificial intelligence (AI) is becoming increasingly prevalent in healthcare. There is ample evidence of the potential of AI in the diagnosis, treatment, and management of patients. However, with its widespread use, numerous errors arise that can have serious consequences for patients. More and more AI tools are being implemented in clinical practice in histopathology and other areas of medicine. The application of AI in oncology includes the use of machine learning and deep learning algorithms that are able to analyze huge data sets and provide predictions and recommendations for patient treatment. Modern artificial intelligence models provide diagnostic accuracy ranging from 85% to 91% depending on the type of illness and the data being processed. In the case of melanoma for instance, one study by Andre Esteva et al showed a diagnostic accuracy of 91%, which is equal or higher than the diagnostic accuracy of experienced dermatologists [1]. In the case of lung and breast cancer diagnostic accuracy was in the range of 87-89% when using AI [2].

Histopathology, along with other imaging-based diagnostic specialties such as radiology, has seen significant development in AI tools. From a diagnostic perspective, AI can improve the accuracy and efficiency of diagnostics. However, AI tools and the machine learning (ML) algorithms on which they are based are not foolproof, and achieving perfect accuracy is unlikely. Therefore, the implementation of AI will bring not only the above-mentioned benefits, but also the general problem of errors in AI tools [3].

Artificial intelligence is revolutionizing rehabilitation, enabling personalized and effective data-driven patient care. AI analyzes patterns, predicts outcomes, and tailors treatments to individual needs, allowing doctors to provide more targeted and timely care [4].

AI is also used to process unstructured medical data, such as text-based patient reports and electronic medical records (EMR), which can increase decision-making accuracy by up to 90% in complex clinical cases [5]. In telemedicine systems that use large language models (LLM), diagnostic accuracy is also improved through

efficient processing of patient text data. Thus, the use of such tools will bring a number of benefits to diagnostic specialties - increased diagnostic accuracy and efficiency in particular.

However, despite significant advances, AI faces a number of challenges and its use will inevitably lead to new errors occurring, thus achieving perfect accuracy is unlikely [6]. A report by the European Parliamentary Research Service identified harm to patients caused by AI errors as one of the main risks arising from the introduction of AI in healthcare [7].

One of the main concerns is bias in the data which the models use for training, which can lead to incorrect diagnoses or treatment recommendations. In addition, there are ethical issues related to the confidentiality of patients' medical data and the transparency of AI algorithms. These errors made by AI tools are essentially misclassifications made by a computational algorithm. There is often no understanding of how these errors affect patients' clinical outcomes, which means that data on the safety of AI tools is incomplete. But in reality, the problem is much more complex.

AIM

To structure the types of errors that occur when using artificial intelligence in healthcare, as well as assess their impact on the accuracy of diagnostics and therapeutic decisions. Identify ways to minimize errors and increase the effectiveness of the use of AI in practical healthcare and rehabilitation.

MATERIALS AND METHODS

Publications published from January 2021 until December 2025 were processed and analyzed according to the keywords of the topic of work "Pathology", "research on the results of diagnostics and treatment", "artificial intelligence", "machine learning", "deep learning", "federated learning", "use of AI in rehabilitation", "structuring of AI errors" in the databases of PubMed, MEDLINE, Web of Science. Articles were selected based on the presence of: quantifiable results and usage of AI as the main or secondary evaluation method. A total 57 articles were reviewed, out of which 38 were excluded based on eligibility criteria and 2 were excluded as duplicates.

ETHICS

All sources used for review are publicly available.

REVIEW AND DISCUSSION

First, let's define what diagnostic errors are. According to the WHO definition, "A diagnostic error emerges when a diagnosis is missed, inappropriately delayed or is wrong. Diagnoses can be completely missed (cancer missed despite symptoms), wrong (patients told they have one diagnosis when there is evidence of another) or delayed (abnormal test result suggestive of cancer, but no one has told the patient). There may be overlaps in these classifications. Diagnoses often occur over time, rather than at one point in time, including initial assessment, performing and interpreting diagnostic tests, follow-up and tracking" [8].

It is important to emphasize that, despite the importance of such a definition, it does not fully correspond to the problems of our study. Indeed, the greatest difficulties are the inability to substantiate and identify the degree of detail of the diagnosis in specific clinical cases. The effectiveness of AI may be sufficient with a lack of pathologic details, but each new step can be catastrophic. It should also be noted that studies assessing the effectiveness of AI in clinical practice are limited by retrospective designs and sample sizes. Such designs potentially include characteristics of dispersion, spectrum shift, and other confounders. In other words, models are developed according to a specific data set. In practice, they are not repeated in other data sets. At the same time, comparing artificial intelligence with doctors, although well represented in the scientific literature, is probably not the best way to address the issue of differing effectiveness. Moreover, some studies now approach the interaction of doctors and algorithms as a combination of human and artificial intelligence, and the possible comparison of diagnoses when monitoring the pathological process is especially important. But this is practically impossible in emergency medicine, and it is extremely difficult to determine the thresholds for decision-making.

Errors in image recognition (diagnosis, patient assessment) are one of the most common types of errors of artificial intelligence in medicine. In particular, machine learning algorithms, such as deep learning, are used to analyze medical images in various fields, including oncology. For example, a systematic review done by Alina Cornelia Pacurari et al found that the accuracy of AI for diagnosing lung cancer ranged from 77.8% to 100%, with sensitivity (which is known to measure the ability of a test to correctly identify patients with the disease) ranging from 0.81 to 0.99. Despite this, specificity (the test's ability to correctly identify healthy patients who do not have the disease) ranged from 0.46 to 1.00, indicating the possibility of significant false-positive results in some cases. Despite high sensitivity rates, specificity remains problematic and requires further research [9]. However, the use of AI in medicine does not always produce such conclusions. For example,

artificial intelligence is increasingly used in oncology for medical image analysis, biomarker assessment, molecular profiling, and treatment outcome prediction. AI errors are recorded along with correct conclusions and this can affect clinical decisions. For example, some studies show that despite significant advances in AI in personalizing treatment, oncological processes remain extremely heterogeneous. This complicates the work of AI due to the variability of clinical data and the complexity of the molecular characteristics of tumors [10].

So, the question we must ask is – if artificial intelligence and machine learning algorithms can be reduced to clinically useful "apps", will they be able to break through mountains of clinical, genomic, metabolomic, and environmental data to aid in accurate diagnosis? On the one hand, the implementation of multiomic approaches that combine various biomedical data (genetic, proteomic, metabolomic) allows for increased prediction accuracy and more personalized treatment recommendations. Quantitative indicators from a number of studies demonstrate that when molecular profiling is used, the accuracy of therapeutic decision-making increases by 15-20%. On the other hand, it remains a challenge for AI to fully integrate this data due to its variability and complexity. The choice of the wrong treatment is often related to the complexity of the molecular characteristics of tumors and individual patient responses. The aforementioned study showed that AI sometimes cannot correctly differentiate different types of cancer cells due to the complexity of the histological and molecular characteristics of tumors. The accuracy of such systems can reach 87-89%, but in cases with complex tumor heterogeneity this indicator may decrease [10].

Further evidence that AI does not always integrate all available molecular information to make therapeutic decisions was established in a study by Yousaku Ozaki et al [11]. It was found that a multiomic approach (genetic, proteomic, and metabolic data) can increase the accuracy of treatment predictions by 12%. This demonstrates the importance of developing algorithms that take into account different types of data to improve treatment outcomes.

Artificial intelligence is widely used to analyze medical images such as computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). For example, research done by Diego Ardila et al showed that using AI achieves an accuracy of 77.8% to 100% in diagnosing lung cancer with a sensitivity of 0.81 to 0.99 and a specificity of 0.46 to 1.00 [12].

In the field of breast cancer diagnosis, one study showed that artificial intelligence improves diagnostic accuracy by 5-15% when using dual assessment of mammograms. However, up to 30% of breast cancers may be missed due to thick parenchyma or interpretation errors [13]. The aformentioned systematic review by Alina Cornelia Pacurari et al demonstrated that using artificial intelligence to analyze mammograms improved diagnostic accuracy by 5-15%, reducing the number of false negatives to 2% compared to traditional methods. The algorithms also decrease false positives, reducing unnecessary biopsies by 5% [9]. Scott Mayer McKinney et al Research highlights that the accuracy of breast cancer diagnosis using AI reaches 94.6%, which exceeds the performance of radiologists by 6-7% [14].

The study by Diego Ardila et al also showed that deep learning algorithms can detect lung cancer with 94.4% accuracy, especially in the early stages of the disease. This significantly improves the possibility of early diagnosis, which is critical for successful treatment. The use of AI helps reduce the number of false positives by 12% [12]. At the same time, the question of quantitative assessment of the severity of the pathological process remains unclear. Almost the same applies to the article by Jasper Twilt et al, where the authors characterize the accuracy of prostate cancer diagnosis using MRI and AI algorithms as reaching 90%, which is 10-15% higher than traditional methods. This significantly reduces the number of false-positive results and increases the efficiency of biopsies [15].

The question of using AI in the early stages of a disease is extremely important. But we cannot answer this question without establishing a normal baseline. This simple question shows one of the weaknesses of the use of artificial intelligence and machine learning in medicine in the form in which it is widely used today.

Disease prediction is an important application of AI, with the potential to enhance our ability to predict the spread of infectious diseases and subsequently inform and guide public health interventions. It is an essential component of public health because it allows experts to prevent outbreaks and act quickly if they do occur. Considering that mathematical methods for diagnosing and predicting specific outcomes as a direct result of medical interventions are available, one should have expected some success in using AI. However, it has been shown that AI can provide inaccurate predictions of patient treatment efficacy, particularly in radiotherapy and chemotherapy. Just by integrating multiomic data, such as genetic and proteomic profiles of tumors, the accuracy of predicting treatment responses can be increased by 15–20% [5]. However, the complexity of mutational variability in tumors still poses challenges for AI in determining the most effective treatment strategies. With technological development, artificial intelligence algorithms have shown significant improvements in

the accuracy of predictions and diagnostic decisions. For instance, it was demonstrated that the accuracy of predicting the response to radiotherapy using AI increased to 90%, which significantly exceeds the accuracy of traditional methods (65-75%). The integration of multiomic data allowed to increase the efficiency of treatment prediction and take into account the genetic and molecular profiles of tumors [5].

AI is also being actively used to provide treatment recommendations where treatment decisions are complex and require personalization. For example, in the study by William Lotter et al, AI was used to individualize therapy based on the molecular characteristics of tumors [10]. However, the technology still needs to be improved, as some treatment recommendations do not take into account specific patient characteristics, which can lead to erroneous decisions. These difficulties can be overcome in the era of accessible healthcare, which is slowly being implemented in various countries today.

Let us now focus on the results of using AI in rehabilitation. The work by Seyyedeh Fatemeh Mousavi Baigi et al summarizes the opinions of university teachers and students. Most teachers considered the main problem to be the inability to make decisions in unforeseen circumstances that go beyond their planning (57.1%) [16]. Lack of practical knowledge was cited as a barrier to implementing AI in rehabilitation among students and faculty. In addition, the most common barriers noted by experts and students were the cost and availability of AI resources in rehabilitation. Another study noted that while AI has many advantages in predicting patient diagnosis and prognosis, there is no clear evidence on the current understanding of physiotherapists' views and willingness to use AI in their practice, raising the question of the need for further research. Nevertheless, some respondents support the positive impact of AI-enabled applications on rehabilitation management [17].

In clinical setting, practical application is an important factor in increasing the interest of therapists and physicians in learning about AI and its implementation in clinical practice. Only 5% of the total sample reported practical usage of AI applications at work [18]. This is consistent with other studies that have shown that less than 10% of surgeons currently use robotic surgical techniques in hospitals, and 60% of surgeons documented the absence of AI and robotic technologies in their clinical practice [19]. These results emphasize the need to accelerate the implementation and adoption of AI through training courses and seminars among physical therapists.

Finally, research by Francesco Lanotte et al highlights that there are still challenges, especially regarding data privacy, physician training, and technology availability, that could hinder the widespread adoption of AI in rehabilitation settings [18]. Ethical considerations regarding fairness, transparency, and security of AI-based systems must also be considered to ensure appropriate integration of these technologies into clinical practice. Efforts to reduce risks, ensure patient safety, and promote ethical use of AI will be crucial for the further development of these technologies.

Looking ahead, artificial intelligence opens up enormous opportunities for the rehabilitation development. Innovative approaches such as multimodal rehabilitation, AI-based virtual therapeutic aids, and advanced predictive analytics are designed to increase the accuracy of treatment and increase the accessibility of services, especially in regions with insufficient levels of health care. By continuing to explore and overcome existing barriers, we can open new horizons in rehabilitation, which will lead to more effective, patient-centered interventions and improved long-term outcomes.

In this regard, the implementation of AI in rehabilitation has the potential to change the landscape of patient care. With careful consideration of the challenges and ethical aspects, AI can play a key role in improving rehabilitation practices, providing patients with the best possible care tailored to their unique needs.

To increase the results of AI in oncology, it is recommended to improve and integrate multiomic models, which, according to previously mentioned study by Yousaku Ozaki et al, can increase the accuracy of therapeutic predictions by up to 12% [11]. The important implementation of a single medical language (such as UMLS), uniform standards for collecting and processing medical data is a key step to improve the accuracy of AI results [20]. Research highlights the need to improve the infrastructure for AI (medical institutions should invest in infrastructure to fully integrate AI with electronic health records (EHR), which will help improve the quality of diagnosis and treatment). At the same time, lack of consistency of data from different medical institutions makes it difficult to standardize results and implement unified approaches to treatment using with AI assistance.

A separate issue is the need for guidelines. They are needed to support and standardize this process, but as noted in an article by Axel Wismüller and Larry Stockmaster, there is currently no guidance on "objective evaluation of AI systems for clinically relevant performance measures" [21]. There are only a few guides specifically designed for AI to evaluate different types of studies, including CONSORT-AI (Consolidated Standards of Reporting Trials – Artificial Intelligence) for randomized controlled trials, SPIRIT-AI (Standard Protocol Items: Recommendations for Interventional

Trials – Artificial Intelligence) Diagnostic Accuracy Study for studies of diagnostic tests [22]. These guidelines provide checklists of minimal requirements to be included in reports of AI interventions and indicate that researchers should analyze error cases. However, there is currently no indication that this includes clinical error assessment, although this would be an ideal place to introduce such requirements and could be included in future versions.

Other important concerns about AI in healthcare include ethical issues and data privacy. A study conducted under the auspices of the National Academy of Medicine notes that patient data protection can be compromised due to errors in algorithms or due to cyberattacks. In addition, bias in training data can lead to inconsistency in diagnosis and treatment of patients of different demographic groups [20]. Clear ethical norms and standards need to be established to protect the privacy of patient data and reduce potential biases in AI algorithms and issues of fairness in access to technology.

Ensuring data standardization remains an important issue. Available research indicates that the lack of uniform standards for collecting and processing medical data is a major obstacle to the accurate and effective use of AI [9]. Inconsistency of data formats across medical institutions complicates the integration of AI into clinical practice. Improving the infrastructure for implementing AI in healthcare settings, particularly through interoperability of EHR, is essential to ensure more accurate diagnostic decisions and personalized treatment. Investments in this area will help avoid technical difficulties and increase the efficiency of healthcare. To improve diagnostic quality, it is necessary to develop common standards for medical systems, which will reduce the number of errors and provide more accurate results. In addition, the integration of AI with a single electronic health system remains technologically difficult due to the lack of unified data transfer protocols. This makes it difficult to ensure the accuracy of diagnostic predictions and the effectiveness of therapeutic decisions.

Thus, further development of artificial intelligence in oncology and other medical fields should focus on improving algorithms, standardizing and classifying data, and addressing ethical challenges to ensure maximum benefit for patients and physicians.

CONCLUSIONS

1. Artificial intelligence is becoming an integral part of modern medical diagnostics and therapeutic solutions. Its implementation significantly increases the accuracy of diagnostic processes and allows for personalized treatment, but today there is no universal solution for the practical use of AI. A lot of errors are still recorded when using AI in diagnostic and prognostic processes, ethical issues have not been resolved, integration of all molecular information available to the patient is not always ensured, there are no uniform standards for collecting and processing medical data, a unified medical language, etc.
2. To ensure professional machine learning, widespread implementation of an open healthcare system, balanced and unified guiding principles is necessary.
3. The implementation of AI technologies depends on the training of doctors and the availability of technologies.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

CONTENTS

Photodynamic therapy in modern dentistry: Mechanisms, clinical applications, and future perspectives - a narrative review

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ABSTRACT

Aim: To review the mechanisms of action, clinical applications, current evidence, challenges, and future directions of photodynamic therapy in modern dental practice.

Materials and Methods: A narrative synthesis of recent experimental, clinical, and review literature was conducted. The review focused on PDT mechanisms, commonly used photosensitizers and light delivery systems, clinical outcomes across dental specialties, and emerging technological developments aimed at improving therapeutic performance.

Photodynamic therapy demonstrates strong antimicrobial efficacy, effective biofilm disruption, and selective tissue action without inducing microbial resistance. Clinical evidence supports its use as an adjunctive therapy in periodontology, endodontics, peri-implant therapy, caries management, oral infections, and early oral malignancies. PDT is associated with a favorable safety profile and minimal invasiveness. However, variability in treatment protocols, differences in photosensitizer properties, limited light penetration, and cost considerations contribute to inconsistent clinical outcomes.

Conclusions: Photodynamic therapy represents a valuable adjunct in minimally invasive dentistry, offering targeted therapeutic benefits across multiple clinical applications. Despite promising evidence, barriers to routine clinical adoption remain. Ongoing advances in photosensitizer design, nanotechnology-based delivery systems, and light technologies are expected to enhance standardization, clinical effectiveness, and wider implementation.

KEY WORDS: biofilms, dentistry, oral infections, periodontitis, photodynamic therapy, photosensitizing agents

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INTRODUCTION

The pursuit of minimally invasive dental treatments has accelerated in recent years, driven by increasing patient expectations for therapeutic approaches that limit discomfort, preserve healthy structures, and promote rapid functional recovery [1, 2]. These preferences align with broader trends in contemporary healthcare, where conservative strategies are prioritized to reduce postoperative complications and enhance patient satisfaction [2, 3]. Within dentistry, this shift is especially apparent in the management of periodontal disease, endodontic infections, peri-implant conditions, mucosal lesions, and early-stage malignancies. Traditional interventions in these areas often rely on mechanical debridement, surgical excision, or systemic antibiotics, each of which may be associated with significant morbidity or contribute to the global challenge of antimicrobial resistance [3-5]. As the limitations of these conventional methods become more evident, clinicians and researchers have increasingly explored alternative modalities that maintain or improve therapeutic efficacy while reducing biological and procedural burdens.

Photodynamic therapy (PDT) has emerged as a promising candidate within this context. PDT is a non-invasive photochemical treatment that combines a photosensitizing agent, a light source of an appropriate wavelength, and molecular oxygen to produce reactive oxygen species (ROS) capable of inducing targeted cytotoxic effects [1, 4, 6]. ROS, which include singlet oxygen and free radicals, interact with cellular components to disrupt microbial membranes and initiate apoptosis in dysplastic or neoplastic tissues. The principles underlying PDT involve the selective accumulation or application of a photosensitizer, followed by controlled irradiation of the affected site. Because the photochemical reactions occur only within illuminated regions and require oxygen for activation, PDT can confine its effects to specific tissues with minimal collateral damage [4, 7]. This high degree of spatial control distinguishes PDT from many conventional therapies and supports its use in anatomically sensitive areas of the oral cavity.

The growing attention toward PDT in dentistry reflects a combination of scientific, clinical, and practical advantages. From a microbiological perspective, PDT demonstrates broad-spectrum antimicrobial activity against bacteria, fungi, and viruses, including strains resistant to commonly used drugs [3, 6]. The ability to disrupt biofilms, which represent a major challenge in periodontal and endodontic therapies, further enhances its clinical usefulness. Moreover, PDT avoids the selective pressures that drive antimicrobial resistance, making it a sustainable long-term strategy in infection control [8, 9]. Clinically, its safety profile is highly favorable, with minimal evidence of genotoxicity, mutagenicity, or adverse tissue reactions. These properties have supported the integration of PDT into a wide range of dental applications, spanning periodontics, endodontics, implantology, pediatric dentistry, oral surgery, and the management of mucosal disorders [1, 7, 10-12]. Emerging evidence also suggests synergistic benefits when PDT is combined with conventional mechanical or pharmacologic interventions, enhancing overall treatment outcomes.

AIM

The scope of this review is to provide a comprehensive and critical examination of the role of PDT in modern dentistry. It will explore the underlying mechanisms that drive its therapeutic action, discuss its diverse applications across dental specialties, and evaluate the existing clinical evidence regarding its efficacy and safety. In addition, the review aims to identify current limitations and technological barriers. Finally, it will highlight future perspectives, research directions, and strategies for integrating PDT more effectively into routine dental practice. Through this analysis, the review seeks to clarify the importance of PDT as both an established and evolving tool within minimally invasive dentistry.

MATERIALS AND METHODS

This review was developed based on a targeted search of the scientific literature concerning the use of photodynamic therapy in dental practice. Publications were retrieved from PubMed, Scopus, and Embase, covering the period from January 2000 to April 2025. The search incorporated terms related to PDT mechanisms and dental applications, including "photodynamic therapy", "photosensitizers", "photosensitizing agents", "light-activated antimicrobial therapy", "dental infections", "biofilm control", "periodontal treatment", "endodontic disinfection", "oral lesions" and "oral potentially malignant disorders".

Studies were considered eligible if they presented experimental data, clinical evidence, or comprehensive reviews addressing the role, performance, or clinical value of PDT within dentistry. Papers not directly related to dental applications or those lacking accessible full text in English were excluded. Additional relevant works were identified through manual screening of bibliographies.

Publications that met the selection criteria and were included in the synthesis. Because of variations in study methodologies and reported outcomes, a narrative approach was used to organize and interpret the findings. The analysis focused on the foundational principles of PDT, photosensitizer and light-system characteristics, established and emerging dental indications, clinical benefits, safety considerations, and current challenges limiting broader implementation.

REVIEW AND DISCUSSION

PHOTODYNAMIC THERAPY MECHANISM

Photodynamic therapy relies on three essential components: a photosensitizer, a light of a specific length, and molecular oxygen. The photosensitizer, often a dye such as methylene blue, toluidine blue, or indocyanine green, is administered topically or systemically and selectively accumulates in target tissues or microorganisms. Upon irradiation with light of a wavelength matching the absorption spectrum of the photosensitizer (commonly in the red or near-infrared range), the

photosensitizer transitions to an excited state [1, 4, 13]. In the presence of oxygen, the excited photosensitizer transfers energy to molecular oxygen, generating reactive oxygen species (ROS), primarily singlet oxygen ($^1O_2$) and free radicals (superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals (HO^-), and hydrogen peroxide (H_2O_2), which induce oxidative damage to microbial cell membranes, proteins, lipids, and nucleic acids, leading to cell death and biofilm disruption. In addition to these direct antimicrobial effects, ROS can modulate local immune responses and contribute to tissue healing [1, 4, 13, 14].

Several factors influence the efficacy of PDT, including the photosensitizer type and concentration, as different agents vary in microbial affinity and photochemical efficiency [9, 15]. Light source parameters such as wavelength, energy density, and exposure time must be optimized to achieve effective activation and adequate tissue penetration [1]. Oxygen availability is also critical, as hypoxic conditions can impair ROS generation and reduce therapeutic success [6, 16]. Biofilm architecture and microbial composition further affect treatment outcomes, with dense biofilms and certain pathogens requiring higher doses or repeated applications [9]. Compared to conventional antimicrobial therapies, PDT provides broad-spectrum activity without promoting resistance, delivers rapid microbial inactivation, and effectively disrupts biofilms. Unlike systemic antibiotics, its action is localized and independent of microbial metabolic state, enabling effectiveness against dormant or resistant organisms [9, 14]. In contrast to surgical debridement, PDT is minimally invasive, preserves healthy tissue, and can be safely repeated without cumulative toxicity [16, 17]. Nonetheless, standardized protocols and additional clinical validation are needed to support its routine clinical integration.

PHOTOSENSITIZERS AND LIGHT SOURCES AS KEY COMPONENTS INFLUENCING THE EFFECTIVENESS OF PHOTODYNAMIC THERAPY IN DENTISTRY

Common photosensitizers used in dental photodynamic therapy include methylene blue, toluidine blue, indocyanine green, malachite green, erythrosine, rose bengal, eosin-Y, curcumin, and phenalen-1-one derivatives, selected for their strong ability to generate reactive oxygen species upon light activation, leading to antimicrobial and cytotoxic effects against oral pathogens and complex biofilms [18-22]. Ideal characteristics for dental applications include high photostability, selective uptake into microbial cells or diseased tissues, low toxicity toward host structures, good water solubility, and chemical compatibility with saliva and oral surfaces. Effective photosensitizers also require absorption peaks that match wavelengths capable of penetrating oral tissues, most commonly in the red or near-infrared region between 600 and 800 nm [3-5].

Light systems used to photosensitizing agents include lasers such as diode and Nd:YAG units, light-emitting diodes, and fiber-optic devices that allow precise illumination of challenging anatomical sites. Lasers provide narrow wavelength specificity and high energy density for deep activation, LEDs offer low-cost and flexible illumination for surface or shallow applications, and fiber-optic systems enable targeted delivery into periodontal pockets, root canals, and peri-implant defects, making them especially useful in minimally invasive dentistry. The choice of light source depends on the absorption profile of the photosensitizer, the clinical site, and the desired depth of tissue interaction [3, 8, 13].

Recent progress in formulation science has introduced nanocarrier-based delivery platforms such as liposomes, polymeric nanoparticles, and dendrimers, which enhance photosensitizer solubility, stability, penetration into biofilms, and retention within target tissues, while protecting the drugs from premature degradation. Innovations in light devices now focus on optimizing dosimetry, integrating real-time monitoring systems, and miniaturizing intraoral applicators to expand accessibility and improve ease of clinical use [18, 23, 24]. Collectively, these advances have significantly strengthened the efficacy, safety, and versatility of photodynamic therapy in modern dental practice.

DENTAL APPLICATIONS OF PDT

Photodynamic therapy has gained significant traction across multiple areas of dental practice due to its antimicrobial efficacy, tissue selectivity, and minimally invasive nature [1, 2]. Its ability to target microbial biofilms, disrupt pathogenic communities, and enhance healing makes it a valuable adjunct or alternative to conventional therapies [4]. The following subsections summarize key clinical applications in contemporary dentistry (Table 1).

PERIODONTAL THERAPY

PDT has been widely investigated as an adjunctive treatment for chronic periodontitis and peri-implantitis, conditions that involve complex biofilms and inflammatory destruction of periodontal or peri-implant tissues [2, 11, 15-18, 20, 24-26]. By delivering targeted antimicrobial action, PDT enhances the reduction of periodontal pathogens beyond what is achievable

Table 1. Summary of main clinical indications for photodynamic therapy in dentistry

Indication	Description / Target	Key Citations
Periodontal therapy	Adjunct for chronic periodontitis and peri-implantitis; reduces pathogens, inflammation, and pocket depth	[2, 11, 15-18, 20, 24-26]
Endodontic disinfection	Eliminates biofilms in root canals and dentinal tubules; effective against resistant pathogens such as *E. faecalis* and *Candida spp.*	[3, 4, 9, 21, 27]
Caries management	Targets cariogenic bacteria (*S. mutans, Lactobacillus*); supports minimally invasive control of early lesions	[3, 4, 6, 7, 13, 14, 23]
Oral and maxillofacial infections	Treats fungal, viral, and mixed infections (e.g., candidiasis, recurrent herpes) without systemic drug effects	[3, 8, 9, 19, 28, 29, 30]
Oral cancer and precancerous lesions	Selective destruction of dysplasia and early oral malignancy with preservation of surrounding tissue	[4, 5, 21, 22, 31]
Aesthetic and restorative uses	Enhances bleaching; disinfects cavities; reduces postoperative sensitivity; improves restoration longevity	[2, 4, 13, 14, 23, 24]

with scaling and root planning alone and can improve clinical parameters such as pocket depth reduction, bleeding on probing, and microbial load, while preserving surrounding healthy structures [11, 15, 17, 24]. Its minimally invasive nature makes PDT a suitable option for maintenance therapy and for patients who cannot tolerate more invasive surgical interventions [2, 18, 25].

ENDODONTICS

In endodontic therapy, PDT offers a powerful approach for eliminating biofilms that persist in the complex anatomy of root canal systems [3, 4, 9, 27]. Conventional irrigants often fail to penetrate deeply into dentinal tubules, accessory canals, and anatomical irregularities. PDT enables deeper antimicrobial action by generating reactive oxygen species that diffuse into inaccessible areas and is effective against resistant pathogens, including *Enterococcus faecalis*, *Candida* species, and other organisms commonly associated with persistent infections [4, 9]. When used to complement standard chemical irrigation protocols, PDT can improve disinfection and reduce the risk of reinfection [27].

CARIES MANAGEMENT

PDT has demonstrated strong antibacterial effects against cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus species* [4, 6, 23]. Its application in caries management supports minimally invasive and preventive strategies by reducing microbial load in early lesions, fissures, and enamel surfaces [3, 4, 13]. When combined with remineralization therapies or sealants, PDT can help control initial carious activity without the need for extensive mechanical removal of tooth structure, which is particularly valuable in pediatric, geriatric, and medically compromised patients [6, 7, 13].

ORAL AND MAXILLOFACIAL INFECTIONS

PDT shows therapeutic potential for a variety of oral and maxillofacial infections, including fungal, viral, and mixed microbial conditions [3, 8, 28, 29]. It has been used in the management of oral candidiasis, recurrent herpes simplex lesions, and other mucosal infections [9, 19, 28, 30]. Its antimicrobial activity without systemic side effects makes it advantageous for immunocompromised individuals who may not tolerate conventional antifungals or antivirals, or who are at heightened risk for drug interactions [3, 28, 30]. PDT can accelerate symptom relief and support tissue healing while minimizing recurrence [28, 30].

ORAL CANCER AND PRECANCEROUS LESIONS

PDT plays an important role in the management of precancerous lesions such as leukoplakia and dysplasia, as well as early-stage oral malignancies [4, 5, 21, 22, 31]. Its mechanism allows selective destruction of dysplastic or neoplastic tissue with minimal impact on surrounding normal structures [4]. Compared to surgical excision, PDT offers reduced morbidity, preservation of function and aesthetics, and the possibility of repeated treatments without cumulative damage. It is especially useful for lesions in anatomically delicate areas or for patients who are poor surgical candidates [5, 21, 22, 31].

AESTHETIC DENTISTRY

In aesthetic dentistry, PDT is applied as an adjunct to tooth whitening procedures, where it can enhance the oxidation of chromogenic molecules and improve whitening outcomes while reducing the need for high concentrations of bleaching agents [2, 4, 13]. Additionally, photodynamic disinfection is used in restorative dentistry to sterilize cavity preparations, reduce postop-

erative sensitivity, and improve longevity by minimizing bacterial contamination at the tooth-restoration interface [2, 14, 23]. Its gentle and localized action supports better clinical outcomes without compromising healthy tissues [2, 24].

CLINICAL EVIDENCE AND OUTCOMES

A growing body of clinical research has evaluated the effectiveness of photodynamic therapy across various dental specialties, with numerous randomized controlled trials, split-mouth designs, and longitudinal studies contributing to the evidence base. Major clinical trials in periodontology have reported significant reductions in periodontal pocket depth, bleeding on probing, and microbial load when PDT is used as an adjunct to scaling and root planing [11, 15, 17, 18, 20, 25]. In endodontics, clinical studies demonstrate enhanced disinfection of root canals, particularly against persistent pathogens such as *Enterococcus faecalis*, although complete sterility remains difficult to achieve with any single modality [4, 9, 27]. Trials focused on peri-implantitis have shown variable but promising outcomes, with some reports indicating improved implant survival and decreased inflammatory markers following PDT-assisted therapy [24, 26]. Clinical evidence in caries management, pediatric dentistry, and oral mucosal infections supports PDT's role as an antimicrobial adjunct, though outcome measures vary widely across studies [3, 7, 14, 23, 28, 30].

Success rates reported in clinical settings generally reflect improvements in microbial reduction, accelerated healing, and patient-reported comfort [11, 14, 15, 17, 18, 24, 25]. Many studies emphasize that PDT enhances treatment efficacy when used alongside conventional mechanical or chemical methods rather than as a standalone therapy [2, 11, 15, 17, 18, 24, 25]. Patient outcomes frequently include reduced postoperative discomfort, lower recurrence rates of infection, shorter healing times, and improved aesthetic and functional results in soft-tissue applications [2, 15, 18, 22, 25]. In oncology-related uses, PDT has shown high lesion-resolution rates in early-stage cancers and precancerous conditions, with notable preservation of tissue integrity and function [5, 21, 22, 31].

The safety profile of PDT is considered highly favorable, with adverse effects typically limited to transient erythema, mild swelling, temporary sensitivity, or local irritation at the treatment site [1, 2, 4, 13]. Systemic complications are rare because photosensitizers used in dental applications are often rapidly cleared, used in low concentrations, or applied topically [11, 19, 22, 30]. Importantly, PDT does not contribute to antimicrobial resistance, a significant advantage over traditional pharmacologic therapies [2, 6, 14, 23, 24, 28]. Long-term safety data indicate minimal risk of cumulative toxicity, even after repeated sessions [1, 2, 4].

Despite encouraging results, the existing clinical data have important limitations. Sample sizes in many trials are small, follow-up periods are often short, and methodologies vary substantially across studies, making direct comparison difficult [11, 15, 18, 25]. Differences in photosensitizer types, concentrations, light parameters, and delivery methods also contribute to inconsistent outcomes [11, 15]. Many studies rely on surrogate laboratory markers rather than robust clinical endpoints, and high-quality evidence from large multicentre trials remains limited [20, 30]. These gaps underscore the need for standardized protocols and more rigorous study designs to establish long-term efficacy and optimal therapeutic strategies.

CHALLENGES AND BARRIERS

Despite its promising clinical potential, photodynamic therapy faces several challenges that hinder widespread adoption in routine dental practice. One major barrier is the significant variability in treatment protocols, including inconsistencies in photosensitizer concentration, incubation time, light wavelength, power settings, exposure duration, and delivery methods. This lack of uniformity leads to mixed outcomes across clinical studies and makes it difficult for practitioners to follow evidence-based guidelines [11, 15, 18, 20, 25, 30].

Another challenge involves differences in photosensitizer penetration and activation. The ability of an agent to infiltrate biofilms, penetrate dentinal tubules, or selectively accumulate in diseased tissues varies significantly among photosensitizers [11, 19, 30, 31]. Suboptimal penetration can reduce the therapeutic impact, while incomplete activation due to mismatched light parameters can further limit effectiveness [20-22].

Light itself presents inherent limitations. The depth of penetration for visible and near-infrared wavelengths is restricted by tissue absorption and scattering, which affects the treatment of deep pockets, complex root canal systems, or lesions located beneath dense tissues. These constraints sometimes require repeated applications or combined approaches to achieve adequate disinfection or tissue response [5, 15, 25, 27].

Cost and accessibility also pose barriers. High-quality laser systems and specialized fiber-optic delivery devices may be expensive for small or resource-limited practices [8, 9]. While LEDs offer a more affordable option, they may not provide the same level of specificity or power density required for certain photosensitizers

[18]. Maintenance costs and the need for practitioner training further contribute to adoption challenges [4].

EMERGING DEVELOPMENTS AND FUTURE DIRECTIONS

Rapid scientific and technological advancements continue to expand the potential of photodynamic therapy in dentistry, paving the way for more precise, efficient, and biologically integrated treatment strategies. Several promising directions are currently shaping the evolution of PDT and are expected to enhance its clinical impact in the coming years.

Next-generation photosensitizers represent one of the most significant areas of development [18, 20, 30]. Researchers are designing molecules with improved photostability, stronger ROS-generating capacity, enhanced selectivity for target cells or pathogens, and reduced toxicity to host tissues. Novel agents with tunable absorption spectra aim to allow activation by wavelengths that penetrate deeper into oral tissues, thus overcoming some of the limitations of conventional formulations. Additionally, new photosensitizers with intrinsic antimicrobial or anti-inflammatory properties may offer multifunctional therapeutic effects [28, 32]. Targeted nanoparticles and smart delivery systems are transforming the way photosensitizers interact with oral tissues and biofilms. Nanocarriers such as liposomes, polymeric nanoparticles, silica nanostructures, and dendrimers can improve solubility, stability, and controlled release of photosensitizers [1, 33]. Smart systems equipped with stimuli-responsive behavior, such as pH- or enzyme-activated carriers, allow selective delivery to diseased sites while minimizing exposure of healthy tissues. These approaches also enhance penetration into dense biofilms and deep periodontal pockets, addressing a key challenge in conventional PDT [23, 28, 30, 33, 34].

Advances in light technology are further improving clinical usability. Modern devices include compact diode lasers with fine-tuned wavelength specificity, high-power LEDs optimized for dental spectra, and wireless light-delivery tools designed for better access in confined oral spaces [1, 35]. Some emerging systems incorporate real-time feedback mechanisms that monitor tissue response or dosimetry to ensure optimal activation. The development of flexible fiber-optic tips, miniaturized applicators, and wearable intraoral light systems may significantly enhance precision and patient comfort [24, 35, 36].

Combination therapies are gaining attention as a means of maximizing treatment efficacy. PDT has shown synergistic effects when combined with antibiotics, allowing lower drug doses and helping to counteract antimicrobial resistance [17, 23, 28]. Integration with biomaterials, such as antimicrobial scaffolds, hydrogels, or implant coatings, may enable sustained antimicrobial activity and improved healing outcomes. Combining PDT with regenerative materials, growth factors, or stem cell-based therapies could also amplify tissue repair and promote healthier long-term outcomes [1, 24, 26, 37, 38].

One of the most exciting frontiers is the potential expansion of PDT into regenerative dentistry. Beyond its antimicrobial benefits, PDT can modulate immune responses, stimulate angiogenesis, and influence cellular signalling pathways involved in tissue regeneration [2, 15, 25]. These properties may support applications such as periodontal regeneration, enhanced wound healing after oral surgery, biomaterial integration, and improvement of the peri-implant environment [17, 24, 31, 39]. Continued research in photobiomodulation and hybrid PDT-regenerative approaches could establish new paradigms in minimally invasive restorative care.

Collectively, these emerging innovations demonstrate that the future of PDT in dentistry extends far beyond its current antimicrobial applications. The integration of advanced photosensitizers, targeted nanotechnology, next-generation light devices, and regenerative strategies is likely to position PDT as a key component of modern, biologically oriented dental practice.

CONCLUSIONS

Photodynamic therapy has become an important addition to modern dentistry, offering targeted antimicrobial action, effective biofilm disruption, and selective tissue effects with minimal invasiveness. Evidence from multiple clinical fields, including periodontology, endodontics, implantology, caries management, oral infections, and early-stage oncology, shows that PDT can enhance treatment outcomes, improve patient comfort, and reduce reliance on systemic antimicrobials. Its favourable safety profile and lack of resistance development further support its relevance in contemporary practice.

Despite these advantages, challenges remain. Variability in treatment protocols, differences in photosensitizer performance, and limitations in light penetration contribute to inconsistent results. Cost, equipment accessibility, and the absence of standardized guidelines also hinder broader adoption. Addressing these issues through rigorous research and unified clinical recommendations is essential.

Future innovations, including advanced photosensitizers, nanoparticle delivery systems, improved light devices, and combination therapies, are likely to expand PDT's utility and integration into routine care. As these developments progress, PDT is positioned to play an increasingly significant role in minimally invasive, biologically oriented dental treatment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Possibilities of using artificial intelligence in orthodontics (literature review)

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ABSTRACT

Aim: to review and analyze scientific and medical information on artificial intelligence in orthodontic dentistry.

Materials and Methods: A review and analysis of scientific and medical literature on the application of artificial intelligence in orthodontic dentistry was conducted. The literature search was carried out in the Scopus, Web of Science, and Google Scholar databases. The following combinations of keywords were used: "artificial intelligence in orthodontics", "3D planning orthodontics", and "machine learning in orthodontics". Inclusion criteria: publications from 2020–2025; original studies (clinical and experimental); the use of AI in diagnosis, planning, or treatment in orthodontics. Exclusion criteria: systematic reviews (considered only in the introduction), commentaries, expert opinions, and studies without a description of the practical use of AI. Search results: 312 articles were found; after removing duplicates — 238; after analyzing titles and abstracts — 82; a total of 12 articles were included in the final analysis.

Conclusions: Artificial intelligence represents a promising tool in orthodontics, enhancing diagnostic accuracy, optimizing treatment planning, and improving outcome prediction. The integration of multimodal data supports comprehensive patient assessment and individualized treatment strategies.

KEY WORDS: artificial intelligence, orthodontics, 3D planning, treatment planning

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INTRODUCTION

The use of innovative technologies in orthodontics includes the potential of software to optimize various processes, transforming the appearance of medical care and ensuring its rapid integration into dentistry. The ability to quickly and accurately analyze large amounts of data is one of the most significant advantages of artificial intelligence, which, in turn, facilitates clinical decision-making for dental practitioners in various clinical situations.

Orthodontists, with the aid of artificial intelligence, can provide customized solutions for each patient. This is the foundation of treatment prediction. However, this does not change the fact that specialists in this field, using their acquired knowledge and experience, remain responsible for establishing an accurate diagnosis and selecting the optimal treatment plan [1-8].

In connection with the active implementation of digital technologies in healthcare, machine learning tools are increasingly used as effective means for processing large-scale patient data. These algorithms provide automated classification, interpretation, and prediction based on both structured and unstructured medical data, thereby accelerating clinical decision-making. An essential advantage of such technologies is their ability to be integrated into medical documentation systems — either as fully autonomous solutions or with varying degrees of user involvement. This provides physicians with flexibility in selecting the optimal tool that best suits the specifics of their clinical practice, ensuring seamless integration into their existing workflow.

The further development of cloud computing has opened new perspectives for organizing medical documentation in an online environment. Cloud platforms enable the storage, processing, and analysis of medical data on remote servers with high computational capacity and cybersecurity. The main advantages of these systems include: (1) no need for expensive local equipment, (2) high scalability and performance, and (3) the possibility of cross-platform access from various devices — including desktop computers, laptops, tablets, and smartphones — which is particularly relevant for

multidisciplinary clinics with extensive networks. Collectively, this allows healthcare institutions to optimize document management, reduce costs, and improve the accessibility of medical information for all participants in the care delivery process [1, 4, 6].

Furthermore, intelligent algorithms enable the prediction of treatment duration, assessment of the likelihood of achieving planned outcomes, modeling of the long-term stability of treatment results, and identification of potential risks and complications even before therapy initiation. This level of personalization contributes to the development of individualized orthodontic protocols based on objective data. As a result, the implementation of AI-based approaches in clinical practice increases patient engagement in decision-making, improves their understanding of treatment measures and expected outcomes, and overall contributes to achieving predictable and long-term results [1, 3, 5, 8].

AIM

To review and analyze scientific and medical information regarding the application of artificial intelligence in orthodontic dentistry.

MATERIALS AND METHODS

A review and analysis of scientific and medical literature on the application of artificial intelligence in orthodontic dentistry was conducted. The literature search was carried out in the Scopus, Web of Science, and Google Scholar databases. The following combinations of keywords were used: "artificial intelligence in orthodontics", "3D planning orthodontics", and "machine learning in orthodontics".

The selected keywords encompassed both general AI applications and specific areas, including prediction, 3D modeling, treatment planning, and patient interaction. This ensured the selection of relevant studies corresponding to the topic of the review.

Inclusion criteria: publications from 2020–2025; original studies (clinical and experimental); the use of AI in diagnosis, planning, or treatment in orthodontics.

Exclusion criteria: systematic reviews (considered only in the introduction), commentaries, expert opinions, and studies without a description of the practical use of AI.

Search results: 312 articles were found; after removing duplicates — 238; after analyzing titles and abstracts — 82; a total of 12 articles were included in the final analysis.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The application of AI in orthodontics can be divided into several main areas:

1. Diagnosis and Prediction

• Baxi et al. (2022, India): demonstrated that AI algorithms can improve diagnostic accuracy of dentoalveolar anomalies, reducing subjective clinician bias.

• Gupta (2020, India): explored AI capabilities for X-ray and CT image analysis; found accuracy challenges in complex cases.

• Tsolakis et al. (2022, Greece): confirmed in a systematic review the effectiveness of AI in CBCT airway analysis, improving measurement accuracy and reducing diagnostic time.

2. Treatment Planning

• Elnagar et al. (2020, USA): developed an AI-assisted digital workflow for orthognathic surgery, which reduced inaccuracies and accelerated planning.

• Caruso et al. (2021, Italy): automated treatment monitoring allowed timely protocol adjustments.

3. Patient Interaction

• Thurzo et al. (2022, Slovakia): investigated the use of smartphones for 3D facial scanning with AI algorithms, making the procedure accessible outside the clinic.

• Gandedkar et al. (2021, Australia): studied AR/VR simulations with AI elements that improve patient understanding of treatment prognosis and enhance motivation.

4. Innovative Approaches

• Prasad et al. (2023, USA): integration of wearable sensors with AI algorithms for monitoring muscle activity during orthodontic treatment.

PREDICTIVE ANALYTICS AND OUTCOME ASSESSMENT

Prediction of treatment duration. AI algorithms consider both clinical and individual patient characteristics, including tooth movement dynamics and compliance levels, enabling more accurate predictions of treatment duration. This enhances patient satisfaction by reducing overall treatment time and allowing for the visualization of results in 3D models [1, 2, 4].

ASSESSMENT OF TREATMENT SUCCESS

The success of orthodontic treatment is determined by multiple factors. Comparing actual outcomes with predicted ones (final tooth position, occlusion, dura-

tion) allows for an objective assessment of treatment effectiveness. In cases of significant deviations, AI assists the clinician in identifying their causes and making necessary adjustments to future protocols [1, 6, 9].

ANALYSIS OF LONG-TERM STABILITY

AI enables the prediction of relapse risk after treatment completion, taking into account bone density, anatomical features, and previous results. This facilitates the development of individualized retention programs and the timely detection of changes affecting treatment stability [7, 11].

PATIENT INTERACTION AND ENGAGEMENT

VIRTUAL CONSULTATIONS AND FOLLOW-UP

Modern telemedicine platforms, integrated with AI, provide remote monitoring, video consultations, and seamless data exchange. This reduces the number of in-office visits while maintaining treatment quality [1, 10].

PERSONALIZED PROGRESS TRACKING

Mobile applications allow patients to visualize treatment dynamics in 3D models and receive reminders about appointments, improving motivation and adherence to treatment protocols [1, 10, 12].

GAMIFICATION AND AR/VR

Game-based elements (bonuses, tasks) in mobile applications encourage compliance with recommendations. VR/AR technologies demonstrate predicted dentoalveolar changes, help reduce anxiety, and increase patient engagement [10, 12].

The results of the study demonstrate that artificial intelligence is a reliable tool that makes orthodontic treatment more predictable — specifically, faster, more cost-effective, and more convenient. In recent years, AI has become increasingly popular in modern dentistry. Artificial intelligence is now widely applied in diagnostics, treatment planning, and various orthodontic procedures.

Currently, AI technologies have not yet become a leading instrument in everyday practice. However, it is worth noting that although AI cannot fully replace human intelligence, it can significantly enhance treatment outcomes and clinical efficiency in this field. Nevertheless, artificial intelligence in orthodontics still requires further refinement, especially in diagnostic processes, to achieve higher precision and effectiveness in daily orthodontic practice [1, 3, 9, 10].

CONCLUSIONS

Artificial intelligence in orthodontics is applied for diagnostics, prediction, and treatment planning. Its use enhances the accuracy and speed of clinical decision-making but does not replace the physician. The most promising directions include automated diagnostics, 3D planning, and remote monitoring.

The main advantages are accuracy, speed, and personalization; the main limitations include cost and the need for algorithm standardization.

Future research should focus on clinical validation, cost optimization, and the integration of AI into daily orthodontic practice.

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CONFLICT OF INTEREST
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REVIEW ARTICLE

CONTENTS

Mechanisms of nephroprotective action of sodium–glucose cotransporter type 2 inhibitors

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ABSTRACT

Aim: To study the potential mechanisms underlying the beneficial nephroprotective effects of sodium-glucose cotransporter 2 (SGLT-2) inhibitors.

Materials and Methods: An analysis of literature sources was conducted regarding the clinical, epidemiological, and fundamental aspects of the nephroprotective effects of SGLT-2 inhibitors. For this purpose, articles were searched for and selected in the PubMed database using the following keywords: "sodium-glucose cotransporter-2 inhibitors," "heart failure," "chronic kidney disease," and "cardiorenal syndrome," with a primary focus on studies published in the last 5 years. To provide context and explain the underlying mechanisms, several classical and fundamental studies relevant to the aim of the review were also included.

Conclusions: SGLT-2 inhibitors exert nephroprotective effects through a complex of interrelated hemodynamic, metabolic, and cellular-molecular mechanisms that are largely independent of their hypoglycemic action. The combination of these mechanisms explains the clinically proven nephroprotective effect of SGLT-2 inhibitors in both patients with and without DM and justifies considering them not only as glucose-lowering agents but also as fundamental agents of pathogenetic therapy for chronic kidney disease.

KEY WORDS: sodium-glucose cotransporter 2 (SGLT-2) inhibitors, chronic kidney disease, nephroprotection, cardiorenal syndrome

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INTRODUCTION

Currently, chronic kidney disease and heart failure are epidemics of chronic diseases that may coexist and are based on shared risk factors, causing the development of systemic disorders. The presence of one disease accelerates the onset and progression of the other. Conversely, coexistence of both conditions increases the risk of primary and recurrent hospitalizations, the need for therapy intensification, progression to end-stage kidney disease, and death [1-3]. The spread of CKD has increased significantly over recent decades, mainly due to the substantial rise in DM, which has become the leading cause of CKD. When CKD develops in the presence of cardiovascular diseases (CVD), chronic HF occurs more frequently and has a more severe course. The incidence of newly diagnosed HF in patients with established CKD ranges from 17% to 21% [4]. A decrease in glomerular filtration rate (GFR) is associated with an increased risk of all-cause mortality, cardiovascular mortality, and hospitalization in patients with HF [5-7]. It should also be noted that elevated urinary albumin levels are a prognostic factor for adverse HF outcomes [8].

In recent years, the arsenal of medications for HF treatment has expanded significantly, and therapeutic options for CKD have also increased, particularly due to the introduction of SGLT-2 inhibitors into routine clinical practice. Their effectiveness in patients with HF and CKD is of particular scientific interest. These medications, which entered clinical practice relatively recently and were originally developed for the treatment of type 2 DM, have demonstrated significant benefits in reducing cardiovascular mortality in patients with carbohydrate metabolism disorders in several studies, while also positively affecting the decline in renal function in this patient group [9-13]. Furthermore, several randomized controlled trials subsequently demonstrated significant efficiency of SGLT-2 inhibitors in the treatment of HF and CKD regardless of the presence of type 2 DM [14-17]. These studies reported a substantial nephroprotective effect of SGLT-2 inhibitors, manifested through combined secondary renal endpoints and determining the frequency of renal outcomes in these patients. These unexpected and surprisingly favorable renal effects proved to be highly significant. According to the conducted studies, SGLT-2 inhibitors significantly slowed CKD progression and reduced the risk of acute

kidney injury. Although some differences in endpoint definitions prevent demonstration of absolute identity of results among different representatives of gliflozins, overall outcomes were similar and very promising. The results regarding the risk of onset and progression of albuminuria were also particularly impressive [18-20].

Owing to these striking results, SGLT-2 inhibitors have evolved from a class of glucose-lowering agents into a cornerstone therapeutic option for the prevention, slowing of progression, and improvement of prognosis in cardiorenal syndrome. The mechanisms underlying the nephroprotective effects of gliflozins are therefore of exceptional interest for thorough investigation.

AIM

The aim is to investigate the potential mechanisms underlying the beneficial nephroprotective effects of SGLT-2 inhibitors.

MATERIALS AND METHODS

An analysis of literature sources was conducted regarding the clinical, epidemiological, and fundamental aspects of the nephroprotective effects of SGLT-2 inhibitors. For this purpose, articles were searched for and selected in the PubMed database using the following keywords: "sodium-glucose cotransporter-2 inhibitors," "heart failure,""chronic kidney disease," and "cardiorenal syndrome," with a primary focus on studies published in the last 5 years. To provide context and explain the underlying mechanisms, several classical and fundamental studies relevant to the aim of the review were also included.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The primary mechanism of the hypoglycemic action of gliflozins in humans is inhibition of sodium–glucose cotransporter 2 (SGLT-2), which is localized in the early segments of the proximal renal tubules and is responsible for the reabsorption of more than 90% of glucose filtered by the glomeruli. The remaining glucose is reabsorbed in the terminal segments of the proximal tubules by another sodium-glucose cotransporter, SGLT-1. SGLT-2 inhibitors reduce glucose reabsorption by approximately 30-50%, resulting in decreased blood glucose levels, glucosuria, and osmotic diuresis [21]. A distinctive feature of these agents is that their glucose-lowering effect is independent of insulin secretion or pancreatic β-cell function; therefore, they do not increase the risk of hypoglycemia. Consequently, SGLT-2 inhibitors provide a mild reduction in both fasting and postprandial glycemia [22].

Under physiological conditions, SGLT-2 is also responsible for the reabsorption of approximately 5% of sodium in tubular fluid. In the presence of diabetes mellitus, sodium reabsorption increases due to upregulation of SGLT-2 and SGLT-1 expression in the proximal tubular epithelium. Accordingly, SGLT-2 blockade is accompanied by natriuresis [23, 24]. It should be noted that another sodium reabsorption mechanism is present in the proximal tubules – the sodium-hydrogen exchanger isoform 3 (NHE3), which mediates reabsorption of up to 30% of filtered sodium [25]. Experimental knockout of NHE3 has been shown to suppress SGLT-2 activity, while inhibition of SGLT-2 also affects NHE3 function [25-27]. As a result, one of the earliest effects of gliflozins observed in clinical studies is increased natriuresis due to inhibition of SGLT-2 activity in the proximal renal tubules. In addition, SGLT-2 inhibitors have been shown to inhibit sodium reabsorption through direct suppression of NHE3 in this nephron segment, likely via phosphorylation mechanisms [28, 29].

Unreabsorbed glucose, which reaches the distal nephron, induces moderate osmotic diuresis. Diuresis associated with SGLT-2 inhibitors occurs both under euglycemic conditions and, to a greater extent, during hyperglycemia, and remains enhanced even in patients with CKD stages 3-4, as well as in chronic and acute heart failure [30, 31]. Notably, the natriuretic and diuretic effects of gliflozins differ substantially from those of conventional diuretics. The diuretic effect of SGLT-2 inhibitors is more short-lived, not accompanied by significant changes in plasma electrolyte concentrations, and does not disturb acid-base balance [32-34]. Moreover, the long-term use of gliflozins does not lead to severe metabolic disturbances such as hyperglycemia or hyperuricemia and, importantly, does not activate the sympathetic nervous system (SNS). The commonly used diuretics, such as loop and thiazide diuretics, act on more distal nephron segments by inhibiting electrolyte reabsorption in the loop of Henle and distal convoluted tubules, respectively. Gliflozins act in the proximal tubule, where sodium reabsorption is coupled with the reabsorption of glucose, uric acid, and other metabolites. By directly inhibiting their reabsorption, SGLT-2 inhibitors induce glucosuria and uricosuria, thereby reducing hyperglycemia and hyperuricemia, unlike other diuretics [35]. This combined diuretic effect – osmotic diuresis due to glucosuria and moderate natriuresis – promotes redistribution of fluid

between intracellular and extracellular, as well as intravascular and extravascular compartments, unlike the effects of thiazide and loop diuretics. Thus, gliflozins primarily reduce interstitial rather than intravascular fluid volume. On the one hand, it contributes to a reduction in sodium content within internal organs, which may be beneficial in cardiovascular diseases, including heart failure and arterial hypertension. Indirect evidence supporting this hypothesis is the ability of dapagliflozin to significantly reduce skin sodium concentration in patients with type 2 DM during long-term use [36]. Earlier studies demonstrated that sodium concentration in the skin and muscles positively correlates with cardiovascular risk in patients with CKD [37, 38]. On the other hand, the absence of significant changes in intravascular volume prevents abrupt alterations in organ perfusion and limits activation of the SNS and the renin–angiotensin system (RAS) [39-42]. Finally, traditional diuretics do not exhibit the pronounced cardioprotective effects observed with SGLT-2 inhibitors and displayed in reduced cardiovascular mortality and fewer hospitalizations for heart failure [43].

One of the key effects of SGLT-2 inhibitors explaining their nephroprotective action is their influence on glomerular filtration and restoration of tubuloglomerular feedback within the nephron [44]. According to the hyperfiltration theory formulated by B. Brenner et al. in 1996 to explain the development and progression of CKD [45], agents that reduce intraglomerular pressure should exhibit nephroprotective properties. The theory posits that a universal renal response to nephron loss, regardless of its etiology, is increased pressure and hyperfiltration in the remaining intact nephrons, allowing temporary maintenance of filtration capacity. Indeed, in early stages, this mechanism normalizes or even increases GFR. Although the pathogenesis of hypertension and hyperfiltration is complex and varies among different diseases, in most cases it is driven by afferent arteriole dilation and efferent arteriole constriction via tubuloglomerular feedback and activation of the RAS [45, 46]. However, prolonged maintenance of renal excretory function through nephron overload is detrimental. Hyperfiltration increases mechanical stress on glomerular capillaries and enhances filtration of tubulotoxic factors such as albumin, growth hormones, and advanced glycation end products. Their interaction with the tubular system increases energy demand, promotes hypoxia, impairs autophagy, and triggers oxidative stress, inflammation, and fibrosis. Over time, nephron loss progresses, and despite single-nephron hyperfiltration, global renal function declines [44].

As noted above, SGLT-2 mediates reabsorption of both glucose and sodium. Therefore, SGLT-2 blockade increases sodium concentration in the tubular fluid by reducing proximal sodium reabsorption, thereby stimulating the juxtaglomerular apparatus (macula densa) in the distal tubule. This leads to adenosine triphosphate (ATP) release from juxtaglomerular cells, which is subsequently degraded to adenosine. Activation of A1 adenosine receptors causes afferent arteriole constriction, resulting in reduced intraglomerular pressure and suppression of hyperfiltration [47, 48]. An additional mechanism increasing sodium delivery in distal parts of the nephron (near the macula densa) is the inhibitory effect of gliflozins on NHE3 activity [49]. To a lesser extent, adenosine also affects A2 receptors in efferent arterioles, leading to the vasodilation [47-49].

It should be emphasized that while a reduction in intraglomerular pressure confers long-term benefits, the initiation of SGLT-2 inhibitors may be associated with a transient increase in serum creatinine or a decrease in GFR. However, GFR gradually recovers within 3-9 months and subsequently remains significantly higher in patients receiving SGLT-2 inhibitors compared with the placebo group. Interestingly, early GFR responses vary. A recent subanalysis of the EMPA-REG OUTCOME trial (patients with type 2 DM and established atherosclerosis) identified three patient subgroups based on GFR dynamics during the first 4 weeks of therapy [50]: those with a significant (>10%) decline in estimated GFR ("dippers," 28%), those with a mild decline (0-10%, 41%), and those with no decline or even an increase in estimated GFR (31%). Multivariate analysis showed that predictors of significant early estimated GFR decline included concomitant diuretic therapy and higher KDIGO risk category (CKD stage/proteinuria degree). Importantly, the magnitude of early estimated GFR decline did not affect long-term safety or efficacy in preventing cardiovascular and renal outcomes. Recent studies further indicate that nephroprotective effects are independent of the extent of initial GFR reduction at therapy initiation [50, 51]. Thus, transient suppression of hyperfiltration is a class effect of gliflozins, followed by a slower long-term decline in GFR compared with placebo. The initial reduction in GFR during SGLT-2 inhibitor initiation is transient and fully reversible. Reduction in albuminuria following decreased intraglomerular pressure is a critically important beneficial effect that lowers the risk of end-stage kidney disease. Long-term SGLT-2 inhibitor therapy reduces the risk of developing microalbuminuria and progression to more severe albuminuria, while increasing the likelihood of regression of established albuminuria during treatment with gliflozins. Accordingly, the urinary albumin-to-creatinine ratio (UACR) significantly decreases in patients with both micro- and macroalbuminuria [18, 52-54]. A meta-analysis of 48 randomized clinical trials involving

more than 50,000 patients treated with gliflozins for over 12 weeks demonstrated a significant reduction in UACR (weighted mean difference – 14.6 mg/g, p = 0.006), with a more pronounced effect in patients with higher baseline UACR [52]. Specifically, the risk of microalbuminuria (RR 0.69, p = 0.032), macroalbuminuria (RR 0.49, p < 0.001), nephropathy progression (RR 0.73, p = 0.012), and end-stage kidney disease (RR 0.70, p = 0.001) was significantly reduced. Thus, SGLT-2 inhibitors exert a beneficial renal effect by reducing the risk of development or progression of albuminuria and lowering the risk of end-stage renal disease compared with placebo or other antidiabetic agents [52].

Under SGLT-2 inhibitor therapy, reabsorptive workload increases in downstream tubular segments, which traditionally receive less oxygen supply. Together with a metabolic shift from glucose to free fatty acids induced by gliflozins, this state mimics renal ischemia. Consequently, hypoxia-inducible factor (HIF)-dependent adaptive mechanisms are activated, leading to increased erythropoietin production and improved oxygen delivery to renal tissue. This represents an indirect antihypoxic mechanism of gliflozins [55, 56]. SGLT-2 inhibitors also increase nitric oxide bioavailability, further contributing to their antihypoxic properties [57]. A direct nephroprotective effect on tubular epithelium has also been demonstrated, manifested by inhibition of mitochondrial lipid peroxidation in proximal tubular epithelial cells. Therapy is associated with reduced markers of inflammation and fibrosis [58-60]. However, distinguishing direct anti-inflammatory and antifibrotic effects from secondary effects mediated by hemodynamic changes and improved carbohydrate metabolism remains challenging and requires further investigation.

Beyond their effects on glomerular hemodynamics, SGLT-2 inhibitors possess additional properties that explain their beneficial impact on CKD development and progression. One key mechanism is metabolic reprogramming: SGLT-2 inhibitors promote lipolysis, induce a negative energy balance, and stimulate ketogenesis by increasing free fatty acid delivery to the liver and lowering the insulin-to-glucagon ratio in systemic circulation. Ketone bodies serve as an efficient energy source for tissues under ischemic conditions. The kidneys and heart exhibit the greatest capacity to utilize ketone bodies (particularly β-hydroxybutyrate) under oxidative stress, which is crucial for maintaining adequate energy supply during impaired perfusion. Beyond energetic benefits, this shift reduces lipotoxic cellular injury and prevents fibrosis [61].

Treatment with SGLT-2 inhibitors leads to weight loss. The body-weight-reducing effect of SGLT-2 inhibitor therapy may be explained by the fact that inhibition of SGLT-2 induces renal glucose excretion, and glucose is a high-calorie substrate. Therapy with SGLT-2 inhibitors is accompanied by reduced leptin production and decreased fat deposition in the perivisceral, pericardial, and perivascular spaces, which contributes to a reduction in insulin resistance, may play a role in improving metabolic processes, has a positive effect on renal function, and is critically important for reducing cardiometabolic risk [61-65].

The use of SGLT-2 inhibitors is also associated with a reduction in serum uric acid levels by an average of 5.9-17.8%, due to decreased urate reabsorption by the epithelium of the proximal tubules via GLUT9b [20, 65]. In particular, a meta-analysis (43 studies, 31,921 patients) demonstrated that SGLT-2 inhibitor therapy is associated with a reduction in uric acid levels both in patients with diabetes mellitus [-31.48 μmol/L; 95% confidence interval (CI): -37.35 to -25.60] and in those without diabetes [-91.38 μmol/L; 95% CI: -126.53 to -56.24]. At the same time, the uricosuric effect of these drugs decreases with the progression of renal dysfunction [66]. The exact mechanism of the hypouricemic effect of gliflozins is unknown. It is assumed that the reduction in serum uric acid occurs in the setting of pronounced glucosuria due to activation of the type 2 isoform of the cotransporter, GLUT9, which increases uric acid excretion into the tubular lumen. Notably, the uricosuric effect of SGLT-2 inhibitors is not accompanied by an increased incidence of nephrolithiasis [67]. Hyperuricemia, through the induction of inflammation, oxidative stress, endothelial dysfunction, and activation of the renin-angiotensin system (RAS), contributes to the progression of tubulointerstitial fibrosis. Therefore, by lowering serum uric acid levels, SGLT-2 inhibitors may indirectly promote protection of the renal tubules.

Numerous studies and meta-analyses have demonstrated a beneficial effect of gliflozins on blood pressure levels, which is not accompanied by an effect on heart rate (HR). On average, these drugs reduce systolic blood pressure by 2-4 mmHg [20]. Moreover, this effect is fully preserved in patients despite reduced renal function. Possible mechanisms underlying this effect include a reduction in plasma volume, weight loss, improved glycemic control (in patients with diabetes or prediabetes), reduced plasma renin production and inhibition of the systemic RAS, decreased inflammation and arterial stiffness, and improved endothelial function. The most important mechanism is considered to be the reduction in plasma volume due to a combined diuretic effect (osmotic diuresis and natriuresis) [61, 68-71].

An important advantage of SGLT-2 inhibitors is the reduction in systolic blood pressure without an increase in

heart rate (HR), which contradicts the conventional concept linking natriuresis with tachycardia [61]. This observation led to the hypothesis that, in addition to natriuresis, SGLT-2 inhibitors may attenuate aberrant SNS stimulation, since a low HR is associated with low sympathetic tone. Evidence supporting this assumption comes from animal studies in which SGLT-2 inhibitors reduced levels of SNS activity markers – norepinephrine, neuropeptide Y, and tyrosine hydroxylase [72]. These data indicate that drugs of this class may contribute to cardioprotective and renoprotective effects by attenuating sympathetic hyperactivity.

Recently, the theory of nutrient deprivation signaling, or the autophagy hypothesis formulated by M. Parker [73], has gained popularity in explaining the mechanisms of action of SGLT-2 inhibitors (including their nephroprotective effects). This theory is based on the assumption that the loss of some calories due to urinary glucose excretion induced by SGLT-2 inhibitors triggers a universal cellular response similar to that observed during fasting, namely activation of autophagy processes (the process by which a cell digests its own organelles and cytoplasmic components via lysosomes to eliminate old and damaged structures) [73-75]. At the cellular level, autophagy results in enhancement of antioxidant mechanisms, reduction of endoplasmic reticulum stress, restoration of mitochondrial function, and an increase in mitochondrial number; at the tissue level it prevents apoptosis and cellular aging, and reduces inflammation and fibrosis. Studies in recent years have shown that similar cellular reprogramming during treatment with gliflozins is also observed in isolated cell cultures, indicating a direct (glucosuria-independent) effect of these drugs [73-75].

Thus, at present, the following are considered the main probable mechanisms of the nephroprotective action of SGLT-2 inhibitors: reduction of hyperfiltration and intraglomerular pressure through constriction of the afferent and dilation of the efferent arterioles, reduction of albuminuria, an antihypoxic effect (both direct, mediated by decreased oxygen demand, and indirect, mediated by increased erythropoietin production), anti-inflammatory and antifibrotic effects, a metabolic shift with stimulation of ketogenesis, leading to increased energy efficiency and reduced lipotoxic cellular damage, reduction of pressure and volume overload, and other pleiotropic effects, including lowering of glycemia, uric acid levels, and body weight, among others. It is likely that the use of gliflozins induces a universal cellular response (autophagy) that improves energy efficiency, reduces cellular stress, and increases cellular resistance to overload in the development of type 2 diabetes mellitus, chronic HF, and CKD. The realization of these mechanisms makes it possible to prevent the development and progression of renal failure and prolong patients' lives. Since patient management focused on cardiorenal protection is crucial for prognosis and quality of life, SGLT-2 inhibitors may contribute to improved treatment strategies for a vast number of patients with cardiorenal-metabolic syndrome. Further studies are needed to evaluate the use of this drug class in patients without carbohydrate metabolism disorders and with various spectra of cardiovascular diseases, as well as CKD, particularly to assess the efficacy and safety of SGLT-2 inhibitors in populations excluded from previous randomized clinical trials, such as very elderly individuals (age >85 years) and/or very frail patients.

The significant nephroprotective effect of gliflozins identified in clinical trials has led to updated CKD guidelines that, for the first time, stipulate the possibility of using SGLT-2 inhibitors (dapagliflozin and empagliflozin) in patients without concomitant diabetes mellitus as an adjunct to therapy with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, in order to reduce the risk of CKD progression, prevent the development of acute kidney injury, and decrease cardiovascular mortality [76].

CONCLUSIONS

SGLT-2 inhibitors exert nephroprotective effects through a complex of interrelated hemodynamic, metabolic, and cellular-molecular mechanisms that are largely independent of their hypoglycemic action. The combination of these mechanisms explains the clinically proven nephroprotective effect of SGLT-2 inhibitors in patients both with and without diabetes mellitus and justifies their consideration not only as glucose-lowering agents but also as fundamental agents of pathogenetic therapy for chronic kidney disease.

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A complete list of references can be requested from the authors

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

It is everybody's right to be heard: Social exclusion and mental health

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ABSTRACT

Aim: This paper aims to reveal the essence of social exclusion and examine its impact on mental health.

Materials and Methods: The authors used interpretive research paradigm, integrative anthropological approach, and hermeneutical approach.

The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to search terms: "social exclusion", "social inclusion", "mental health", "well-being", "discrimination", "prejudice", "social injustice", "racial discrimination", "poverty", "values", "Other", "belonging", "depression", "anxiety", "anger", "hopelessness", "intersectionality". From the entire available literature, 65 sources were selected according to specified criteria, such as "an intersectional approach to social exclusion" and "the negative consequences of social exclusion through the lens of mental health and well-being". A limitation was that the authors used only sources available in English during study selection due to the time-saving factor, as well as human resources factor.

Conclusions: Tackling social exclusion, which has a detrimental effect on physical and mental health and undermines human dignity, requires concerted efforts at all levels of society. Today, it is especially important to develop a long-term transdisciplinary strategy, which addresses the multiple and overlapping disadvantages experienced by excluded groups. Social institutions, which guide people's behavior through norms, are key to strategies for combating social exclusion. It is necessary to consider the actual mechanisms at work in terms of social exclusion, as well as interventions at the level of groups or individuals. Addressing discriminatory behavior alone or one type of inequality can lead to certain changes, however, these changes will not be decisive for underprivileged groups, as quite often negative stereotypes and prejudices, which impede social inclusion, are expressed in subtle ways. At the same time, addressing values that underlie exclusion and discrimination may be more effective and have more far-reaching consequences. The goal and outcome of these extensive responses to the acute problem of social exclusion is to create a global inclusive society where all voices are heard, and the voice of the "Other" is as significant as yours.

KEY WORDS: social exclusion, mental health, well-being, discrimination, social injustice

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INTRODUCTION

Many spears have been broken in the struggle for human equality. This struggle has lasted for hundreds of years, and each epoch has responded to it in its own way. For example, the United States Declaration of Independence claims: "We hold these truths to be self-evident, that all men are created equal, that they are endowed by their Creator with certain unalienable Rights, that among these are Life, Liberty and the pursuit of Happiness" [1]. According to the Constitution of Ukraine, "All people are free and equal in their dignity and rights. Human rights and freedoms are inalienable and inviolable" [2]. We should remember that "equality per se does not exist in nature; it is rather a mental construct that arises within the world of culture, not within the world of nature. A long-lasting struggle for equality contributed to a significant improvement in the living standards of people and their happiness, although, like any ideal, full equality is unattainable. Today, however, the denial of the need for equal rights is considered mauvais ton. The contemporary world declares that all people are equal (or should be)" [3].

Today, on the way to reaching the closely interrelated Sustainable Development Goals, efforts to reduce all forms of inequality in society is a priority. However, on this pathway to a better future, the global society faces a number of challenges. Still now, inequalities based on income, sex, age, disability, sexual orientation, race, class, ethnicity, religion, etc. continue to exist across the world, which "threatens long-term social and economic development, harms poverty reduction, and destroys people's sense of fulfillment and self-worth. This, in turn, can breed crime, disease and environmental degradation" [4].

The concept of inequality is closely related to the concept of social exclusion [5]. Inequality in society is dangerous because it further marginalizes and discriminates against underprivileged groups, which ultimately leads to social exclusion, thereby further increasing inequality. Such inequality affects groups of people. It is a multifaceted phenomenon that includes social, economic, and political forms of exclusion. Social exclusion leads to unequal access to the opportunities and services needed to live a dignified and happy life. Even the most basic means of survival may become inaccessible, not to mention the freedom to express one's opinion and influence the life of society [6].

Combating social exclusion goes beyond overcoming poverty and reducing inequality. It must challenge ethnic, racial, gender, etc. discrimination by considering representatives of socially excluded groups at the intersection of their identities. According to the latest estimates, between 2.33 and 2.43 billion people (roughly 32 per cent of the global population) are at high risk of social exclusion [7]. The concept of social exclusion, although often used as a synonym for poverty and inequality, encompasses a broader dimension of the social world. It is associated with a specific understanding that varies depending on cultural norms, values, politics, national contexts, etc. Social exclusion is not only the result of the interaction of disadvantageous factors, but also a process by which individuals or groups become or remain systematically disadvantaged [8]. M. Niño-Zarazúa emphasizes that "women, children, and poor men are the primary groups at risk of exclusion, representing 85% to 90% of excluded population… Other vulnerable groups include members of the LGBTI community, indigenous people, Afro-descendants, and religious minorities. These groups face heightened exclusion because of their identities, circumstances and socioeconomic disadvantages" [9]. The situation is further aggravated when multiple identities come into play. These identities often intersect and reinforce exclusion, and their bearers are subject to increased negative attitudes and discrimination resulting from multiple identities. Social exclusion undermines the foundations of any society negatively impacting all areas of human life; it harms mental health, triggering pain, cognitive impairment, and long-term disorders [10]. Social exclusion is not just a personal problem; it is a societal challenge with far-reaching consequences for human well-being and global future.

AIM

This paper aims to reveal the essence of social exclusion and examine its impact on mental health.

MATERIALS AND METHODS

The authors used interpretive research paradigm, integrative anthropological approach, and hermeneutical approach.

The data collection was carried out using PubMed, Scopus, Web of Science, Google Scholar databases. Research papers were identified according to search terms: "social exclusion", "social inclusion", "mental health", "well-being", "discrimination", "prejudice", "social injustice", "racial discrimination", "poverty", "values", "Other", "belonging", "depression", "anxiety", "anger", "hopelessness", "intersectionality".

From the entire available literature, 65 sources were selected according to specified criteria, such as "an intersectional approach to social exclusion" and "the negative consequences of social exclusion through the lens of mental health and well-being". A limitation was that the authors used only sources available in English during study selection due to the time-saving factor, as well as human resources factor.

ETHICS

This review article is based on an analysis of publicly available scientific data published in peer-reviewed journals, clinical guidelines and databases. No patient-identifying data was used during the work, nor was there a need to obtain approval from an ethics committee, as the study did not include new clinical interventions or initial collection of patient information.

REVIEW AND DISCUSSION

The exclusion discourse emerged in the 1960s in France, where it resonated with the national ideology of republicanism and refers to a rupture of the "social bonds" or "solidarity" [11]. This discourse became the focus of social policy in the 1980s-2000s in EU, the United Kingdom, and Australia. "The social exclusion concept is regarded as a multi-dimensional process that indicates inequality at different levels, including access to resources, capabilities, and rights. The solution to the social exclusion challenge is the inclusion of individuals into society, which is referred to as social inclusion" [12]. While the concept initially focused on economic exclusion, it is necessary to take into account a whole range of issues beyond purely economic parameters.

Indeed, social exclusion describes a number of structures and dynamic processes of inequality among different groups in society. It refers to the inability of certain groups to participate fully in the life of society because, within the framework of structural inequalities, these groups are denied access or have very limited access to economic, political, social, and cultural resources [13]. Such restrictions are determined by social class,

educational level, race, ethnicity, religion, gender, disability, age, minority status, migration status, economic status, HIV positivity, identity and even individual history, that is, anything that distinguishes members of a given group from the mainstream and from what is considered the norm in this or that society. Such ideas vary from epoch to epoch and from culture to culture. This was not always the case for underprivileged groups only. Sometimes, during revolutionary transformations, former upper classes were subjected to downward social mobility being forcibly stripped of their privileges and pushed to the margins of history, thereby being subjected to forced social exclusion. But what remains unchanged is the existence of groups that are being targeted for exclusion from the grand narrative.

Moreover, it is not simply an exclusion due to a lack of resources on the part of a certain group; it is a deliberate marginalization of a group that is denied access not only to the benefits of society, but also to the very recognition of their dignity, which, as Fukuyama noted, has always been a deep-seated human need, but has never been achieved [14]. Such dehumanization makes underprivileged groups even more vulnerable that can lead to the internalization of imposed negative attitudes by this group or to protest movements and violent actions, which destabilize society.

Contemporary researchers aim to identify and explore overarching dimensions of social exclusion. According to Cuesta, López-Noval and Niño-Zarazúa, deprivations caused by social exclusion result from three dimensions:

• Identity – gender, age, race, ethnicity, religion, political affiliation, etc. Identities that differ from established norms and customs can lead to exclusion.

• Circumstances – forced displacement due to conflict, poverty, gender violence. There is a high risk of social exclusion, especially in the context of discriminatory norms, laws, and institutions.

• Disadvantaged socioeconomic position – the risk factors that contribute to exclusion in specific contexts are poverty, unemployment, and low levels of education [7].

Lakhani, Sacks and Heltberg [15] introduced three clusters of negative attitudes, which lead to social exclusion, that can be traced back to three sets of values:

• Intolerance for the poor and for different lifecycle stages.

• Intolerance toward stigmatized attributes and behaviors.

• Intolerance to specific identity groups.

As researchers stated, "variation in the presence of exclusionary values is, in part, related to country-specific factors. In contrast, the associations between the socio-economic characteristics of households and individuals' exclusionary attitudes are weaker than the associations between country-specific factors (history, culture, and other factors that do not vary across households from the same country) and exclusionary attitudes. These results are novel in indicating that three distinct attitude types may drive exclusionary behavior and that country-specific factors are more important in explaining the prevalence of these attitudes than individual-level characteristics" [15].

In any case, being a representative of any of the above groups does not necessarily mean social exclusion, but it does warn of a high risk of finding oneself in such a situation that erodes society at its base. Social exclusion is the culmination of a whole series of specific exceptions to fundamental rights, such as the right to education, work, decent housing, rest and leisure, access to social protection, etc. Excluded people and groups are seen as having no rights, they are effectively deprived of the right to vote. The problems of victims of marginalization and social exclusion are usually not covered in official statistics and mainstream media – they are invisible to society. The final stage of the process of marginalization and social exclusion can be withdrawal from the game of life – suicide is one of the leading causes of death; and its number is growing, especially among young people, the lonely people, and the elderly [16].

Social exclusion is closely linked to income distribution at the international and national levels. According to Xiberras, exclusion accompanies the new stage of globalization, starting from territorial exclusion at both the global and local levels, which ultimately leads to exclusion at the level of social groups [17]: "within societies there are social groups that find themselves excluded and in which income differentiation implies a gradual disintegration of the ties by which they were bound to the rest of society. There are minority groups and indigenous peoples for whom the processes of globalization have led to severely accentuated phenomena of exclusion" [18]. This exclusion from social life leads to the absence of participation, segregation, neglect and being forgotten, and this evokes very specific feelings within the sectors that are not excluded. Freund put it this way: "It would appear that exclusion is now part of normality in societies, and does not arouse any special moral or political conscience but instead evokes pity in the guise of the virtue of charity" [17], concluding that the spread of many forms of exclusion and pseudo-exclusion in society is a sign of the times.

Social injustice fuels the fire of social exclusion, which reinforces unequal access to rights, resources, and opportunities based on factors such as race, gender, socioeconomic status, ethnicity, etc. Rooted in systemic inequality, it favors some groups while marginalizing

others and views basic human needs as privileges rather than rights. Social injustice is very difficult to combat because it is perpetuated by biased policies, cultural norms, and historical inequalities that continue to shape societies. An estimated 5 billion people have unmet justice needs globally, including people who cannot obtain justice for everyday problems, people who are excluded from the opportunity the law provides, and people who live in extreme conditions of injustice. This "justice gap" undermines human development, reinforces the poverty trap, and imposes high societal costs. Closing the justice gap is therefore vital to realizing the broader development agenda and its vision of a "just, equitable, tolerant, open and socially inclusive world in which the needs of the most vulnerable are met" [19].

Social underdevelopment also contributes to social exclusion. Certain groups of people are unable to integrate into the process of socioeconomic development due to lack of education, high levels of malnutrition, disease, and poor housing conditions. Therefore, they may be located territorially close to the main actors of socioeconomic change, but not be connected with them or be pushed by them to the margins of the social world. In societies with complex class systems, segregation, which is perceived there as a natural phenomenon and leads to discrimination in favor of some groups over others, underlies social exclusion.

A significant risk factor for social exclusion is homelessness, which affects both people without a roof over their heads and those living in unstable conditions. Homelessness also represents the absence or weakening of the connections that bind people together in a network of interconnected social structures. This ultimately leads to marginalization, including housing and financial difficulties, as well as non-participation in social and political life. Men usually become homeless due to financial difficulties, while women become homeless due to difficulties in family relationships [20].

A number of factors aggravate social exclusion, and among them (but not limited to them) are:

• Restrictive social groups – communities with strict rules, regulations, or barriers to membership. They limit the rights of non-members of their group: studies indicate that "that around 38% of countries have laws or policies that restrict the rights of certain social groups. Additionally, research shows that marginalized communities, such as ethnic minorities, face greater exclusion, with approximately 70% of countries having discriminatory practices against these groups" [21].

• Racial discrimination – any exclusion, restriction or preference based on race, color, ethnic origin which nullifies or impairs the recognition and exercise of human rights and fundamental freedoms. Racism is generally a tool used by certain groups to strengthen their political and economic power [22].

• Extreme poverty – poverty levels vary and are determined not only by income levels, but also by psychological state, the quality of family relationships, social status, access to social measures and support, the ability to seek help from public organizations. According to the World Bank and the United Nations, "roughly 1.3 billion people live in a state of absolute poverty with nearly 25 million being added to their numbers each year" [23].

• Economic exclusion – social exclusion and discrimination are associated with uneven income distribution at global and local levels. It is associated with both territorial isolation and the presence of discriminated social groups, especially on the basis of gender, ethnicity or race.

• Discrimination against minorities – this discrimination is based on racial, religious, ideological, political, economic, etc. grounds and can manifest itself in education, employment, housing, public services.

• Cultural deprivation is the denial of the right to participate in the cultural life of society or the loss of cultural heritage. This leads to inequality of opportunities, a lack of social and individual development, and social and ethnic disintegration.

• Involuntary mass resettlement is often caused by economic and social inequality, violation of fundamental human rights, terrorism, foreign interference in internal affairs, acts of aggression, and development issues. Today, more and more people are forced to flee their homes as a result of armed conflicts, internal conflicts, and systemic human rights violations: "whereas refugees crossing national borders benefit from an established system of international protection and assistance, those who are displaced internally suffer from an absence of legal or institutional bases for their protection and assistance from the international community" [24]. Researchers stress that climate change will exacerbate existing problems in the regions and could lead to population displacement.

• Disability makes it difficult for people to function in the community which can lead to social exclusion. People with disabilities face an increased risk of social exclusion. For instance, "29.7% of the EU population aged 16 or more with a disability (activity limitation) was at risk of poverty or social exclusion compared with 18.8% of those with no disability" [25].

Social exclusion is rooted in the attitudes and behaviors of certain groups toward other groups that they perceive as different from their own groups. But this is not just a difference, it is an assessment on a value scale – we are better than them. Every group is a bearer of certain values that are cultivated, supported, and transmitted. Group members must share these values. Values

give meaning and purpose to all elements of culture. Values are the basis of cultural reality – they are the core of culture. If we imagine culture as an onion consisting of different layers (the "onion" model of culture) [26], then values are its center. Values give rise to our beliefs and assumptions, which are not always explicit, but they shape both the structure of our personality and group culture. They are taken for granted and support the group culture: "core values can be regarded as forming one of the most fundamental components of a group culture. They generally represent the heartland of the ideological system and act as identifying values which are symbolic of the group and its membership. Rejection of core values carries with it the threat of exclusion from the group… Core values are singled out for special attention because they provide the indispensable link between the group's cultural and social systems; in their absence both systems would suffer eventual disintegration. Indeed, it is through core values that social groups can be identified as distinctive ethnic, religious, scientific or other cultural communities" [27].

Values manifest themselves explicitly in goals, strategies, and philosophies that motivate us and shape our reality. Thus, values underlie people's views and behavior and, when representatives of different groups meet or clash, they can lead to exclusion. As Lakhani, Sacks and Heltberg emphasize, negative attitudes towards another group are unlikely to be limited to social context [15]. According to Schwartz, attitudes toward certain groups are likely to reflect deeper values that transcend specific situations and contexts [28]. Rokeach, the creator of one of the first universal and empirically substantiated theories of values, argued that value orientations, which he understood as a steadfast conviction in the fundamental superiority of certain goals or ways of existence over others, influenced all social phenomena in one way or another [29]. Therefore, the central role of values in defining oneself and others on the social map is crucial in the process of social exclusion.

Why do different value systems clash rather than strive to expand horizons and enrich each other? Is social exclusion inevitable in society? It is quite possible that this opposition to the Other and rejection of the Other is deeply rooted in human history. Ancient people mastered the world by splitting it into two parts: the developed and the undeveloped worlds, the friendly (or at least predictable) world, i.e., known world, Our world, and the hostile, unknown world – Their world (the world of Others). Such the binary opposition is, in essence, a conventional division of the world into two opposing parts. Literally, all objects and phenomena are components of this system. Universal binary oppositions underlie all worldviews, and components of each opposition are not equal in value. Positive or negative values are attributed to all binary oppositions. Osgood came to the conclusion that human consciousness is characterized not only by bipolarity, but also by the fact that one of the poles is assessed as positive and the other as negative [30]. According to Levi-Strauss, binarity has a universal nature, and our thinking is still based on this scheme. Ambivalence in perceiving the world is also determined by physiological factors. The human brain is divided into two hemispheres, which are functionally asymmetrical. Thus, an eternal contradiction arises; and binarity gets the status of a fundamental principle of consciousness, which leads to ontological dissonance perceived as a fact of human existence [31]. Therefore, binarity and disparity in assessment permeate human life from ancient times to today's societies that speak of humanism, the value of each life, equal rights, and inclusion.

Today, in the world of a large number of interacting social groups, people still live in the "We – Other" paradigm due to primordial fear and demonstrate fundamental emotional reactions to danger, namely, stigma and prejudice that lead to social exclusion of the "Other" groups. By projecting controllable risk and, therefore, blame onto out-groups, people feel safer: "people's mental lives are launched within relationships with primary caregivers. Representational life emerges from these early relationships. The unconscious representational structures which infants form there construct the bases of subsequent patterns of thought. Early representations tend to be orientated towards protection of the self from anxiety. In order to accomplish this, 'the other' becomes the repository of all that the infant seeks to push out from its own space for the purpose of protection. These early building blocks of what is to be associated with the self, versus with others, leave their mark on the developing individual. Political and social ideas acquired in the course of the lifespan augment the individual's notion of what can comfortably be associated with self and in-group, and what is unacceptable and must be flung out beyond their walls. Individuals learn which qualities and actions are acceptable from the norms of the societies in which they live. At times of potential threat, when levels of anxiety are particularly high, the early mechanism of defense reappears, and the 'other' becomes the target of a rich array of projections which contain those aspects of experience from which individuals seek to distance their selves" [32]. Thus, responsibility for threats is shifted to the Other, who is then "demonized". This mechanism can also protect the positive image of the in-group and self-identity.

Hence, binarity manifests itself in a social world, in

which "individuals are often classified, by themselves and by others, as belonging to a group or groups. This 'grouping' plays an important role in the normative process of social exclusion… Yet social groups are not natural phenomena; social groups are constructed by social processes and, as such, are often the product of dominant power relations in society. Defining differences and similarities between people is at the heart of 'grouping'. This sorting of people into 'similar' and 'different' exists across all societies and is innate in people" [15].

A characteristic feature of social behavior is that representatives of different groups interact with each other not on the basis of their individual characteristics or personal preferences, but as members of we-groups (in-groups) entering into relationships with members of they-groups (out-groups); and representatives of they-groups are seen as "undifferentiated items in a unified social category, rather than in terms of their individual characteristics" [33]. For stability and cohesion of any group, recognition of their values is paramount. The assessment of one's own group is made through social comparisons with other groups from the standpoint of value-oriented characteristics. Even "the mere perception of belonging to two distinct groups – that is, social categorization per se – is sufficient to trigger intergroup discrimination favoring the in-group. In other words, the mere awareness of the presence of an out-group is sufficient to provoke intergroup competitive or discriminatory responses on the part of the in-group" [33]. Intergroup discrimination tends to increase group self-esteem which leads to positive social identity [34].

In general, people perceive and interpret the world within their own models of reality, which were created by languages and other symbolic systems. At the level of communication, people explain the world around them, including the social world, in oppositions inherent in the culture in which they were socialized. Each of these patterns requires them to consider certain information the most important and absolutely true and respect only a certain class of signals, which pass through their filters [35]. Everything else can be not only ignored, but denied. If person's concepts, attitudes, and ideas do not correspond to reality, he or she often adjusts the reality to match the stereotypes. This form of protection and internal resistance is very stable and almost insurmountable, people do not listen to themselves, but to "the voice of mother, father, to the voice of government, authorities, power, tradition, etc." [36]. This "always right" game may be applicable to all spheres of human life. In general, people protect their concepts, attitudes, and beliefs from the checking by reality, considering this reality as unreliable or interpreting it in their own way [37].

Such a reaction indicates that in the field of human relations there is not only one objective reality, but also a variety of them, which we perceive through the lens of our concepts and attitudes. Classification helps people make sense of and organize the vast array of information, because without it, people would not be able to make judgments to understand the world. When classifying, people are often identified on the base of common characteristics, which may be real or not. However, in social contexts, group classification often plays an important role: group characteristics are attributed to individual members based on shared characteristics. Through this stereotyping, individuals are reduced to a few, often easily identifiable and exaggerated markers of difference, and placed into a group of people who share these characteristics [15]. The situation becomes critical and even dangerous when our concepts and stereotyping are not controlled by reality and are turned into a goal in itself, since people find it very difficult to abandon their individual mythologies, despite the fact that all this does not help them communicate effectively and solve problems constructively. This abandonment seems identical to self-denial [38]. And a vicious circle, which strengthens discriminatory attitudes and beliefs towards other groups that need to be "pushed out" from the center of social space to the margins, arises. These largely subconscious discriminatory attitudes are fueled by social institutions that assign value and allocate opportunities in the way that "systematically discriminates against particular groups of people, denying them the opportunities, resources, and recognition that would allow them to participate fully in social, economic and political life" [15].

Social exclusion has far-reaching negative consequences, detrimentally affecting all areas of lives of socially excluded people, thereby undermining the entire global social organism. Not to mention that social exclusion makes it difficult to achieve many social goals, such as a society of equal opportunities, social justice for all, the eradication of poverty, etc., it poses a threat to the basic need for security, which has an evolutionary basis [39], as well as the need for belonging – one of the strongest human motivations [40; 41]. DeWall stated that "humans have a fundamental need to belong. Just as we have needs for food and water, we also have needs for positive and lasting relationships... This need is deeply rooted in our evolutionary history and has all sorts of consequences for modern psychological processes" [42].

The feeling of being rejected causes pain. Eisenberger, Lieberman, and Williams found that social rejection activates many of the same brain regions involved in physical pain: "the experience and regulation of social

and physical pain share a common neuroanatomical basis. Activity in dorsal ACC (*anterior cingulate cortex*), previously linked to the experience of pain distress, was associated with increased distress after social exclusion. Furthermore, activity in RVPFC (*right ventral prefrontal cortex*), previously linked to the regulation of pain distress, was associated with diminished distress after social exclusion" [43].

Nowadays, increasing attention is being paid to the impact of social exclusion on physical and mental health and well-being of members of socially excluded or marginalized groups who tend to perceive prejudice against themselves in a wider range of contexts than members of privileged groups. Black Americans and ethnic minorities are more likely to report physical and mental health problems. They have very high rates of morbidity and mortality, high disability rates, shorter life expectancy, and high level of infant mortality [44, 45]. A 2015 meta-analysis demonstrated an association of racism and poor mental health including depression, anxiety, psychological stress and various other outcomes. Racism was also associated with poorer general and physical health [46].

Differences in health status are due to differences in material wealth, lifestyle, educational level, housing conditions, access to health services, as well as to discrimination that leads to social exclusion, since experienced or perceived racial prejudice or discrimination can be as important as objective life circumstances. Experience of discrimination is correlated with self-reported poor health, depression, lower life satisfaction, post-traumatic stress disorder. Perception of discrimination is correlated with increased anger, anxiety, hopelessness, feelings of worthlessness, resentment, and fear, and may be associated with increased alcohol consumption and smoking [47]. Persistent perception of prejudice and discrimination can create feeling of depression in disadvantaged groups and reduce the sense of control over their lives.

These negative consequences are also observed among other groups. Perception of in-group disadvantage is negatively correlated with psychological well-being among women, Jews, African Americans, the LGBT+ community, other non-mainstream groups, etc. Members of these groups are more likely to perceive discrimination as a reflection of systemic devaluation and rejection by a dominant culture, which is damaging to self-esteem and detrimental to psychological well-being [48].

For instance, LGBT+ people are more likely to develop mental health issues than the general population. Dealing with stigma, prejudice, and discrimination, they are at more risk of suicidal behavior and self-harm; more likely to develop depression and anxiety disorder; experience conflict and harassment at work, verbal, physical, and sexual abuse; show symptoms of eating disorders, experience shame due to their body image; become victims of hate crime. Heavy drinking or drug use can make their mental health problems worse and potentially trigger new ones [49-51].

Another example is unequal treatment of individuals based on gender discrimination that leads to negative consequences. Gender inequality is harmful and has a negative impact on health including psychological distress, low self-esteem, depression, anxiety, concealed anger, somatic disorders, etc. [52]. Perceived prejudice against women is inversely related to self-reported psychological well-being [48]. Women who experienced gender discrimination at work reported higher levels of work-related stress.

When age discrimination comes into play, the situation becomes even worse. This is especially true for very young girls and older women. While the former are perceived as lacking experience and knowledge and expected to be obedient dolls, women over forty are more likely than older men to be perceived as having outdated skills or being poorly adapted to the changing world [53]. The social environment may in various ways induce age stereotypes and perpetuate internalized ageism, which can have detrimental effects on health and wellbeing. Age discrimination not only "removes" women from the public space rendering them invisible to the world, but also seriously undermines their health downplaying the value of wisdom, knowledge and life experience and eroding women's self-esteem. Though both genders are being devalued as they reach older age, women are under particular pressure [54]. Facing prejudice and discrimination, many women are at risk for mental health issues, such as anxiety, depression, psychological distress, obesity, substance abuse, cognitive decline. Ageism leads to social exclusion and loneliness, increases risk of experiencing violence and abuse. Ageism is associated with earlier death; poorer physical health; risky health behaviors; poor sexual and reproductive health and increase in rates of sexually transmitted infections; inappropriate medication use, including inappropriate prescribing, polypharmacy, and medication nonadherence [55; 56]. Different aspects of our identities intersect and affect each other. Women, in particular women of color, face barriers to economic security, health care, educational opportunities, employment prospects, housing, as well as a decline in mental health and wellbeing, especially when they are aging [57]. As 2021 "Mirror/Mirror" survey showed, African American women 50+ reported the highest levels of discrimination, with 70 percent saying

they experienced discrimination regularly, while 59 percent of Latinas and 60 percent of Asian American/ Pacific Islanders age 50+ said they were regularly discriminated against [58].

Age discrimination affects not only women but also men. Most countries are currently experiencing a demographic transition with a proportional increase in the elderly population, who have higher rates of illness, disability, and partial or total financial dependence. Ageism makes older people more prone to social exclusion. Age-related difficulties, inequality, lack of participation in social and political life, disrupted social relationships, service exclusion increase the likelihood of depression, cognitive impairment, and decreased overall well-being among older adults [59, 60].

Multiple forms of disadvantage and discrimination exacerbate negative impacts on mental health and well-being and are associated with significantly higher rates of distress, suicidal ideation, and substance use [61]. A study on interrelationships between social exclusion, mental health and well-being in adolescents conducted in Australia showed that "young people who identified as gender diverse, Indigenous, living in economically disadvantaged areas and spoke a language other than English at home" were more vulnerable to social exclusion. They reported increased loneliness, negative feelings about the future, lack of control over their lives, poor overall perceived mental health, a mental health condition [62].

Social exclusion has a particularly strong impact on children and adolescents with detrimental consequences for their emotional and behavioral health; it causes academic difficulties, decreased prosocial behavior, and low self-esteem: "youth who report bias-based discrimination such as exclusion and rejection also display higher incidences of substance use/abuse, risky behaviors, mental health concerns (such as depression), and negative school-related outcomes in terms of achievement and truancy... adolescents who experience intergroup bullying that is intersectional… are more likely to engage in self-harm and suicidal ideation, and to experience higher rates of depressive symptoms" [63]. Children and adolescents are a particularly vulnerable group, and adverse factors such as racial, religious, ethnic or other discrimination, as well as forced migration, are associated with increased anxiety, depression, personal insecurity, feelings of threat, and psychotic disorders [64]. Thus, the negative consequences of social exclusion in children and adolescents, who will shape our future, represent a serious challenge to the entire global community.

CONCLUSIONS

Tackling social exclusion, which has a detrimental effect on physical and mental health and undermines human dignity, requires concerted efforts at all levels of society. Today, it is especially important to develop a long-term transdisciplinary strategy, which addresses the multiple and overlapping disadvantages experienced by excluded groups: "social policies can enhance or moderate group consciousness and can exacerbate or reduce exclusion. Most states now have legislation to ban overt discrimination. In some countries, governments have introduced targeting through various forms of affirmative action" [65]. Social institutions, which guide people's behavior through norms, are key to strategies for combating social exclusion. It is necessary to consider the actual mechanisms at work in terms of social exclusion, as well as interventions at the level of groups or individuals. Addressing discriminatory behavior alone or one type of inequality can lead to certain changes, however, these changes will not be decisive for underprivileged groups, as quite often negative stereotypes and prejudices, which impede social inclusion, are expressed in subtle ways. At the same time, addressing values that underlie exclusion and discrimination may be more effective and have more far-reaching consequences. The goal and outcome of these extensive responses to the acute problem of social exclusion is to create a global inclusive society where all voices are heard, and the voice of the "Other" is as significant as yours.

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REVIEW ARTICLE

CONTENTS

A disciplinary alliance between obstetrics and infectious diseases: Building integrated perinatal safety systems

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ABSTRACT

Aim: To summarize contemporary evidence and guideline-informed approaches for integrating infectious diseases expertise into perinatal care, to describe practical alliance models that improve maternal–neonatal infectious outcomes while supporting antimicrobial stewardship, and to propose measurable endpoints for audit and quality improvement.

Materials and Methods: A narrative review was conducted using structured searches in PubMed/MEDLINE and the Cochrane Library (2015–2026; emphasis 2021–2026) and hand-searching key guidance repositories (WHO, ACOG, CDC, RCOG, NIH). Search terms included combinations of maternal sepsis, intraamniotic infection, congenital syphilis, group B streptococcus, perinatal HIV, cytomegalovirus, antimicrobial stewardship, and maternal immunization. Evidence was synthesized with attention to implementation feasibility, workflow triggers, and measurable quality metrics. Sources were included if they provided clinical definitions, diagnostic/therapeutic pathways, stewardship frameworks, or prevention cascades relevant to prenatal, intrapartum, or postpartum care. A total of 30 sources were included in the final synthesis.

Conclusions: Integrating infectious diseases expertise into perinatal systems improves diagnostic precision, accelerates appropriate therapy for true infection, strengthens prevention cascades, and reduces unnecessary antibiotic exposure. Standardized triggers, shared protocols, microbiology support, and audit-feedback are the core implementation ingredients.

KEY WORDS: Maternal sepsis; intraamniotic infection; antimicrobial stewardship; vertical transmission; maternal immunization

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INTRODUCTION

Maternal and neonatal infections remain a major contributor to preventable morbidity, yet many maternity services still treat infection expertise as an "on-call add-on" rather than a built-in safety function. The WHO consensus definition frames maternal sepsis as life-threatening organ dysfunction from infection during pregnancy, childbirth, post-abortion, or postpartum, emphasizing that timely recognition and system readiness are decisive. Maternal outcomes and neonatal consequences are tightly linked, so delays or imprecision affect two patients simultaneously [1].

The consensus definition of maternal sepsis was developed through systematic review and expert consultation and underpins current obstetric sepsis terminology [2]. Pregnancy alters physiology and laboratory baselines, which can mask early deterioration and complicate standard sepsis screening; therefore, obstetric services often adapt general sepsis guidance to pregnancy-specific contexts and escalation pathways [3]. In parallel, obstetric infection syndromes such as suspected intraamniotic infection (IAI) sit on a spectrum that includes both microbial invasion and sterile inflammation, meaning that "fever equals infection" is clinically unsafe [4,5]. These realities make a stable obstetrics–infectious diseases (OB–ID) alliance a practical necessity rather than an academic preference.

AIM

The aim of this review is to synthesize current evidence and guidance on integrating infectious diseases expertise into perinatal care across antenatal, intrapartum, and postpartum settings. A second aim is to describe operational alliance models that balance rapid treatment for likely infection with antimicrobial stewardship tailored to pregnancy. A final aim is to propose measurable endpoints that allow maternity services to audit safety gains and stewardship performance over time.

MATERIALS AND METHODS

A narrative review design was selected to integrate heterogeneous evidence spanning obstetric guidance, critical care sepsis frameworks, antimicrobial stewardship policy, and prevention cascades for vertical transmission. We searched PubMed/MEDLINE and the Cochrane Library for English-language publications from 2015 to 2026, prioritizing 2021–2026 to reflect contemporary practice. We additionally hand-searched WHO, ACOG, CDC, RCOG, and NIH perinatal HIV guideline repositories for updated definitions and pathways, because clinical implementation depends heavily on authoritative guidance cycles. A total of 30 sources were included in the final synthesis.

Search terms included "maternal sepsis," "obstetric sepsis," "intraamniotic infection," "chorioamnionitis," "congenital syphilis,""group B streptococcus,""perinatal HIV,""cytomegalovirus pregnancy,""antimicrobial stewardship," and "maternal immunization." We prioritized guidelines, systematic reviews, and high-quality observational studies that explicitly informed integrated workflows, microbiologic sampling strategies, antibiotic selection/duration, or measurable prevention cascades.

ETHICS

This review used publicly available sources and did not involve collection of new patient data.

REVIEW AND DISCUSSION

The OB–ID alliance should be understood as a perinatal safety system that embeds infectious diseases reasoning into everyday obstetric workflow. In a mature model, infectious diseases specialists co-design protocols, interpret "grey-zone" microbiology and serology, and lead stewardship processes, while obstetric teams integrate these outputs into triage, fetal surveillance, delivery decision-making, and postpartum follow-up. This structure reduces both under-treatment of true infection and over-treatment driven by uncertainty. Persistent missed opportunities for preventing congenital syphilis illustrate how system reliability and follow-through can be more decisive than episodic consultation [6,7]. The balance is especially important in perinatal settings because antibiotic choices are constrained by fetal safety and exposure has downstream effects on maternal and neonatal microbiota.

Maternal sepsis is the clearest domain where integration changes outcomes, because time-critical deterioration coexists with pregnancy-specific diagnostic ambiguity. The Surviving Sepsis Campaign recommends early recognition, prompt cultures when feasible, and timely antimicrobials when clinical likelihood is high, while recognizing that treatment must be adapted to context and source control. In obstetrics, source control may involve uterine evacuation or delivery decisions, which require coordinated planning across OB, ID, anesthesia, and neonatology [3]. A shared pathway prevents "parallel practice," where antibiotics are started without a coordinated plan for evaluation and stop rules, or where escalation is delayed because findings are misattributed to pregnancy physiology.

RCOG Green-top Guideline No. 64 operationalizes maternal sepsis management across antenatal, intrapartum, and postpartum periods, supporting pregnancy-specific recognition and escalation standards. Its value in an alliance model is not only clinical content but also governance: it legitimizes standing triggers for ID involvement and structured reassessment rather than ad hoc consultation. When such guidance is translated into triage prompts, early warning workflows, and shared documentation, the alliance becomes measurable and teachable rather than person-dependent [8].

Suspected intraamniotic infection is another high-impact integration domain because diagnostic criteria influence both maternal treatment and neonatal evaluation. ACOG updated criteria for suspected IAI in 2024, reflecting the need for consistent thresholds and associated findings, and explicitly building on earlier committee guidance. This matters because inconsistent labeling of "chorioamnionitis" can drive unnecessary broad-spectrum antibiotics or, conversely, delayed therapy when infection is likely. An OB–ID pathway improves precision by aligning clinical criteria, sampling strategy, antibiotic selection, and postpartum stop rules with microbiologic and epidemiologic reality [4,5].

The earlier ACOG Committee Opinion No. 712 emphasized that recognition and implementation of intrapartum treatment recommendations can reduce morbidity, but real-world practice often struggles with heterogeneity in fever etiologies. Epidural-associated maternal fever, viral illness, dehydration, and sterile inflammation can mimic infection, and without an alliance, "defensive prescribing" becomes common [5]. The OB–ID partnership adds disciplined reassessment and diagnostic anchoring, reducing the risk that an initial empirical decision becomes an unexamined multi-day course. In this context, reassessment is not a luxury but part of safe obstetric care.

Prevention of vertical transmission provides a complementary demonstration of alliance value because it depends on system reliability more than diagnostic brilliance. Congenital syphilis is preventable with timely screening and treatment, yet CDC analyses of U.S. cases highlight that missed opportunities-late or absent

testing, inadequate or delayed treatment, and gaps in prenatal access-dominate case pathways. In a functioning alliance, obstetrics owns the screening touchpoints while infectious diseases and public health expertise strengthens linkage to treatment, partner management logic, and cascade monitoring. The goal is not simply "test once," but to build a repeatable, auditable cascade that closes the gap between detection and cure [6]. CDC reports show missed prevention opportunities persist despite effective therapy, indicating an implementation failure. Alliances can address this with retesting triggers, fast-track treatment, and simple cascade dashboards for accountability [7].

Group B streptococcus (GBS) prevention illustrates how a standardized OB-led protocol supported by microbiology and stewardship can deliver population-level benefit. ACOG recommends universal screening at 36 0/7–37 6/7 weeks and defines intrapartum prophylaxis indications, creating a workflow that is predictable and teachable [9]. In an alliance, infectious diseases stewardship oversight helps distinguish appropriate prophylaxis from unnecessary treatment and supports regimen selection for penicillin allergy scenarios. The broader lesson is that perinatal infection prevention works best when embedded in routine workflow rather than triggered by crisis.

Perinatal HIV demonstrates a different integration challenge: decisions are scenario-dependent and must synchronize maternal intrapartum management with neonatal prophylaxis and follow-up. NIH perinatal guidance provides detailed intrapartum recommendations based on maternal viral load, antiretroviral history, and timing of diagnosis. The alliance matters because obstetrics controls labor and delivery decisions, while HIV/ID specialists ensure regimen appropriateness, drug interactions, resistance considerations, and continuity into postpartum care. When guideline updates occur, a governance structure that translates them into local protocols prevents drift between evidence and practice [10]. "Update awareness" is a safety issue because perinatal HIV guidance evolves with evidence and policy [11]. NIH "What's New" summaries support rapid protocol updates, with ID leads translating changes and OB teams implementing them across clinics and labor wards.

Antimicrobial stewardship is the connective tissue that allows the alliance to improve outcomes without amplifying resistance and iatrogenic harm. IDSA/SHEA stewardship guidelines emphasize that optimal antibiotic use requires systems, leadership, and measurement, not only prescriber education. In perinatal care, stewardship must incorporate fetal safety, lactation compatibility, and the microbiome consequences of exposure, while still supporting rapid therapy when severe infection is likely [12]. A practical alliance standard is a documented antibiotic plan that states indication, intended duration, and a mandated reassessment point, typically within 48–72 hours.

The WHO AWaRe classification provides a widely adopted framework to monitor antibiotic consumption and set targets that encourage use of "Access" agents when appropriate. AWaRe fits maternity services because it converts prescribing patterns into measurable stewardship metrics that can be audited alongside clinical outcomes. When combined with local susceptibility data, AWaRe-informed dashboards can identify drift toward broad-spectrum "Watch" agents without clear indication. This is especially relevant for postpartum prescribing patterns, where unnecessary continuation after prophylaxis is a common stewardship failure [13].

Policy-to-practice translation is strengthened by explanatory literature linking essential medicines policy and AWaRe stewardship goals. Contemporary reviews describe AWaRe as a tool not only for classification but also for setting system targets and monitoring stewardship intervention effects [14]. For perinatal services, the alliance can operationalize these concepts as monthly reports: antibiotic-days per 100 deliveries, proportion of Access agents, and the rate of documented 48–72 hour reassessments.

STI management in pregnancy is another area where integration reduces both overtreatment and undertreatment. CDC STI treatment guidelines provide pregnancy-specific considerations, including syphilis screening logic, recommended regimens for common infections, and management of pathogens with emerging resistance. The alliance is helpful because STI management frequently requires partner therapy planning, retesting intervals, and interpretation of complex serology or NAAT results. Integrating ID expertise into antenatal pathways can reduce recurrences and prevent downstream obstetric complications associated with untreated or inadequately treated infections [15].

Prevention extends beyond screening into immunization, where the OB–ID alliance has increasing relevance due to vaccine hesitancy, evolving schedules, and the need for consistent counseling. ACOG's 2026 committee statement on maternal immunizations emphasizes routine assessment and recommends vaccines such as influenza, COVID-19, and Tdap during pregnancy, positioning prenatal care as the primary platform for protection of both mother and infant [16]. Systematically delivering these recommendations requires workflow design, standing orders, and consistent messaging across clinicians. In an alliance model, ID/public health expertise supports risk communication and program evaluation, while obstetrics ensures delivery at point of care.

CDC guidance for vaccinating pregnant women provides practical scheduling considerations and supports incorporation of vaccination into prenatal care workflows [17]. Surveillance data also show gaps in coverage and measurable hesitancy for influenza, Tdap, and COVID-19 vaccination among pregnant women, indicating that aligned provider recommendations and consistent messaging are key implementation determinants [18]. Practical alliance deliverables include standardized counseling scripts, staff training, and monitoring of coverage gaps by clinic site or demographic group.

Understanding microbiome-linked obstetric risk strengthens the alliance because it explains why indiscriminate antibiotic use can be harmful. Network meta-analysis evidence links low-lactobacilli vaginal community states with increased preterm birth risk, suggesting that microbial ecology is clinically relevant, not merely descriptive. More recent systematic reviews continue to evaluate associations between vaginal microbiota composition and preterm birth outcomes, reinforcing that microbial states during pregnancy can carry prognostic information [19,20]. These findings support stewardship discipline in pregnancy, because altering microbiota without clear indication can plausibly worsen dysbiosis-associated risks.

Cytomegalovirus (CMV) illustrates alliance value in uncertainty management and prevention counseling rather than routine screening [21,22]. ACOG notes that routine serologic screening of all pregnant individuals is not recommended and emphasizes hygiene-based prevention counseling to reduce acquisition risk. CDC similarly provides practical prevention advice focused on reducing contact with saliva and urine from young children, which is central to real-world risk reduction. In alliance terms, obstetrics delivers counseling and surveillance, while ID expertise supports serology interpretation (including false positives) and diagnostic pathway design when primary infection is suspected.

Guideline synthesis and appraisal literature on congenital CMV management highlights variability across recommendations and the need for structured pathways that integrate fetal surveillance, neonatal evaluation, and follow-up. Such variability is exactly where an alliance improves care, because it can translate heterogeneous guidance into a single local pathway with explicit decision points and referral triggers. This avoids the common pattern where CMV testing is performed but results are not followed by consistent counseling, imaging surveillance, or neonatal planning. In practice, the alliance defines who owns each step and when escalation is mandatory [23].

The alliance's stewardship arm is supported by conceptual frameworks that define stewardship as a clinical and ethical practice rather than a restrictive policy [24]. Core stewardship concepts emphasize that optimal antibiotic use improves outcomes, reduces adverse events, and preserves antibiotic effectiveness. In perinatal care, this is amplified because the same antibiotic course can influence maternal health, fetal exposure, and neonatal colonization. A stewardship-literate alliance therefore treats reassessment and de-escalation as clinical care, not administrative oversight.

WHO's AWaRe antibiotic book extends the stewardship concept by providing evidence-based guidance on drug choice, dose, route, and duration for common infections, reinforcing the "right drug, right duration" principle in an implementable format. The companion Lancet Infectious Diseases commentary further positions the AWaRe book as a policy and practice tool for improving empiric antibiotic prescribing aligned with essential medicines logic. In maternity services, these resources help standardize empiric choices while still allowing escalation when severe infection or resistance risk is high. The alliance can adapt these resources to local susceptibility patterns and obstetric safety constraints [25,26].

Several maternal sepsis reviews emphasize diagnostic difficulty and the need for obstetric-specific frameworks, supporting the argument that alliance design matters. The sepsis literature also reinforces that "one-size-fits-all" screening tools may misclassify pregnant patients if obstetric physiology is not considered [27]. In the WHO Global Maternal Sepsis Study, multiple early warning systems (EWS) were evaluated in women with suspected or confirmed infection across many countries, and obstetric- and sepsis-specific approaches generally performed better than non-obstetric tools; importantly, no single score demonstrated sufficient diagnostic accuracy to be used alone, which argues for framework-based escalation rather than score-only decisions [28].

A practical implication is that maternity services should pre-define (and repeatedly train) escalation thresholds, microbiology sampling sets, and early antibiotic decision rules so that care is teachable, repeatable, and consistent across shifts. The AIM "Sepsis in Obstetric Care" bundle explicitly frames preventable maternal deaths as being driven by delays in recognition, treatment, and escalation, and it recommends unit-level readiness plus facility-wide standard protocols for assessment, treatment, and escalation, supported by tools such as standardized order sets, "sepsis alert" workflows that mobilize teams, and processes that prioritize laboratory turnaround and timely antimicrobial administration [29]. In parallel, antibiotic selection in maternal sepsis requires attention to pregnancy-related

physiologic changes that alter pharmacokinetics and complicate diagnosis; contemporary reviews emphasize common pathogens in pregnancy (e.g., E. coli and Group A Streptococcus) and summarize empiric regimens and dosing considerations, reinforcing the need for pre-agreed antibiotic decision rules embedded into the obstetric sepsis pathway [30].

CONCLUSIONS

The disciplinary alliance between obstetrics and infectious diseases is a modern requirement for perinatal safety because infection care in pregnancy requires both speed and precision under physiologic and therapeutic constraints. The alliance has greatest impact in maternal sepsis and suspected intraamniotic infection, where pregnancy-adapted pathways can reduce delays while stewardship prevents unnecessary exposure. Prevention of vertical transmission, especially for syphilis, HIV, and GBS-demonstrates that system design and follow-through can outperform episodic decision-making. A standardized, team-based model with defined triggers, microbiology support, 48–72 hour reassessment discipline, and audit-feedback linked to measurable endpoints is the most reproducible pathway to sustained improvement.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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REVIEW ARTICLE

CONTENTS

Patient autonomy and the right to refuse medical intervention: Medical and legal aspects

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ABSTRACT

Aim: To examine the right to refuse medical treatment and resuscitation as a component of somatic rights, to analyze comparative legal frameworks in the USA, EU member states and Ukraine, and to identify criteria for distinguishing lawful refusal of treatment from violations of medical and legal obligations.

Materials and Methods: The study employs a comparative legal method to analyze legislation across multiple jurisdictions, formal legal analysis of national and international normative acts, and case-law analysis of key ECtHR decisions, including *Arskaya v. Ukraine*, *Pindo Mulla v. Spain*, and *Lambert v. France*. Doctrinal legal sources on patient autonomy, informed consent, and advance directives were also examined.

Conclusions: The right to refuse medical treatment constitutes a fundamental somatic right grounded in personal autonomy and human dignity. Ukraine requires systemic legislative reform to introduce legally binding advance directives and DNR orders, accompanied by safeguards including competency verification, centralized registries, and guaranteed access to palliative care.

KEY WORDS: patient autonomy, advance directives, do-not-resuscitate order, somatic rights, informed consent

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INTRODUCTION

The right to refuse medical treatment and resuscitation is one of the key elements of somatic human rights, which concern control over one's own body and physical integrity. This right stems from the principles of personal autonomy, respect for dignity and freedom from unwanted interventions. In today's world, where medical technologies allow for the prolongation of life even in critical conditions, this right is becoming the subject of intense debate at the intersection of jurisprudence, ethics and morality.

Somatic rights, or the right to the physical integrity of the body, are a component of fundamental human rights enshrined in international instruments such as the Universal Declaration of Human Rights and the European Convention on Human Rights. The right to refuse medical treatment stems from the principle of autonomy, which provides that a competent adult has the right to make decisions about his or her body without coercion. This right is considered a "negative" right – that is, a right to non-interference, in contrast to the "positive" right to receive medical care [1].

In the context of resuscitation, the right to refuse encompasses situations where a patient may refuse measures such as cardiopulmonary resuscitation, ventilation, or other life-sustaining measures. It is based on the doctrine of informed consent, where the patient must be fully informed of the risks and alternatives. However, somatic rights are not absolute: they are limited if the refusal threatens public health, for example in cases of infectious diseases, or if the patient is incompetent, for example due to mental disorders.

In a somatic rights system, this right is related to the right to die with dignity, but differs from euthanasia or assisted suicide in that it focuses on passive refusal rather than active action. In many jurisdictions, such as the United States, it is protected by the Fourteenth Amendment, which guarantees freedom from unwanted medical intervention. In Europe, the ECHR interprets this as part of the right to private life, emphasizing that the freedom to accept or refuse treatment is key to self-determination [2].

AIM

The aim of this study is to examine the right to refuse medical treatment and resuscitation as a component of somatic rights, to analyze comparative legal frameworks in the USA, EU member states and Ukraine, and to identify criteria for distinguishing lawful refusal of treatment from violations of medical and legal obligations.

MATERIALS AND METHODS

The study employs a comparative legal method to analyze legislation across multiple jurisdictions, formal legal analysis of national and international normative acts, and case-law analysis of key ECtHR decisions, including *Arskaya v. Ukraine*, *Pindo Mulla v. Spain*, and *Lambert v. France*. Doctrinal legal sources on patient autonomy, informed consent, and advance directives were also examined.

Number of acts analyzed. The following regulatory acts were directly analyzed: Patient Self-Determination Act 1990 (USA); the decision of the US Supreme Court in the case of Cruzan v. Director (1990); Advance Directives Act 2009 (Germany); Mental Capacity Act 2005 (Great Britain); Medical Assistance in Dying Act 2016 (Canada); relevant legislation of the Netherlands, Belgium and Turkey; profile acts of the legislation of Ukraine on health care and medical assistance; Constitution of Ukraine; ECHR and the practice of the ECtHR in the cases of Arskaya v. Ukraine, Pindo Mulla v. Spain, Lambert v. France.

Excluded acts. The following acts were deliberately excluded from the scope of analysis: acts regulating exclusively active euthanasia (since the study focuses on passive refusal of treatment, not active assistance in death); general codified acts (civil codes, criminal codes) without special norms on advance directives; acts of countries outside the EU/USA/Canada, where the legal mechanism is either underdeveloped or unavailable for verification.

ETHICS

All sources used in this literature review are publicly available.

REVIEW AND DISCUSSION

The analysis revealed a significant asymmetry in the legal protection of patient autonomy across jurisdictions. The USA has the most formalized system of advance directives and DNR orders, the EU demonstrates progressive but variable implementation, while Ukraine lacks a statutory mechanism for legally binding advance medical directives, creating legal uncertainty for patients, physicians, and families of incapacitated persons.

The right to refuse medical treatment is enshrined in law in many countries, with emphasis on advance directives - legal documents that allow a person to specify in advance the medical care they want or do not want if they become incapacitated - and advance directives about not resuscitating. In the United States, since 1990, after the case of Cruzan v. Director [3], Missouri Department of Health, the Supreme Court has recognized the constitutional right to refuse life-sustaining measures, including nutrition and hydration, provided that the will is clearly expressed. Each state has laws on advance directives such as living wills and durable powers of attorney for medical decisions [4].

In the EU, approaches vary. In Germany, the 2009 Advance Directives Act allows patients to record a refusal of treatment, including refusal of resuscitation, with a mandatory competency test. In the UK, the Mental Capacity Act regulates advance decisions to refuse treatment, with a focus on best interests for incompetent patients. In the Netherlands and Belgium, the right has been extended to euthanasia for terminally ill patients, but the basic right to refuse is enshrined in patient rights laws. In Canada, the 2016 Medical Assistance in Dying Act allows for refusal and active assistance in dying for terminally ill patients, with criteria to be expanded in 2021. In Turkey, the right to refuse exists, but with limitations, and needs reform to better protect [5].

Refusal of resuscitation measures. The right of a person to refuse medical intervention, including resuscitation measures, is a complex moral and legal issue, in which the principles of autonomy, humanity and the professional duty of a doctor intersect. In most legal systems of the world, resuscitation measures are considered urgent and aimed at preserving life under all conditions. However, the right of a person to self-determination also includes the ability to refuse measures that continue biological existence without hope of restoring vital functions or consciousness. From a moral point of view, forced resuscitation of a patient who has consciously refused it can be considered a violation of his dignity. This is especially true in cases where such actions only prolong suffering or maintain life in a state incompatible with human consciousness [6].

Legally, the problem lies in the lack of clear procedures that allow recording a person's will to refuse resuscitation. In many countries, this is resolved through a document known as a Do Not Resuscitate Order, a written statement of the patient's will that obliges medical personnel to refrain from resuscitation in the event of clinical death. In Ukraine, a similar mechanism is not enshrined in law, so a doctor who does

not perform resuscitation may be held liable, even if he acted in accordance with the patient's wishes. The ethical dilemma in this case lies in the contradiction between the doctor's duty to save life and respect for the person's will [7].

Recognition of the right to refuse resuscitation does not mean the devaluation of life, on the contrary – it is the recognition that life has meaning only when it retains dignity and a conscious dimension. If medical intervention only prolongs the process of dying, and does not restore health, it can turn into a form of violence against a person. Therefore, in a state governed by the rule of law, it is necessary to provide a mechanism that will allow the patient to determine in advance the limits of permissible intervention. This does not deny the humanism of medicine, but on the contrary – gives it a moral meaning, turning the doctor into a partner in preserving human dignity even at the moment of death [8].

Patient refusal of treatment for fatal diseases. The patient's refusal to treat an incurable or terminal illness is a manifestation of the autonomy of the individual, who recognizes the right to make decisions about his or her own body, suffering, and life expectancy. From a moral point of view, such a decision can be perceived as a desire to preserve dignity in a situation where medicine is no longer able to cure, but only prolongs agony. Legally, this issue is related to the right to informed consent, because a person can consciously refuse treatment only when he or she fully understands the consequences of his or her choice. The doctor is obliged to provide the patient with all information about his or her health condition, treatment options, prognosis, and potential suffering that both treatment and its absence can cause. Such transparency ensures freedom of choice and relieves the doctor of some of his or her moral responsibility. However, in practice, conflicts often arise when doctors, guided by professional ethics or fear of legal liability, continue treatment against the patient's wishes. From a legal perspective, this is a violation of personal autonomy, as no one can be forced into medical intervention without their own consent [2].

The ethical side of the problem is that refusing treatment is not always a manifestation of hopelessness. It is often a conscious choice of a person who wants to live the rest of his life peacefully, next to his loved ones, without pain and forced procedures. Such a decision requires great inner courage and should not be perceived as a denial of the value of life. On the contrary, it can be an expression of respect for it - an understanding that life is not measured by duration, but by dignity and meaning. It is important to establish mechanisms in the legal system that would allow a person to officially refuse treatment so that medical personnel are not faced with a choice between the law and conscience. At the same time, the state must guarantee access to palliative care, psychological support and pain relief so that refusal of treatment is not forced due to pain or hopelessness. This approach combines humanism, freedom and respect for human dignity [8].

Relatives' decisions regarding the treatment of incapacitated persons. Situations where the decision to treat or terminate treatment is made by the relatives of an incapacitated person are among the most complex in both legal and moral terms. The lack of willpower on the part of the patient creates the risk that the relatives' decision will be determined not by the interests of the patient, but by their own beliefs, emotions, or even material motives. From a legal point of view, such decisions should be based solely on the principle of "the patient's best interests" – that is, on what corresponds to his dignity, state of health, and real chances for improvement [9].

The moral dilemma is that even close people cannot fully feel the line between the struggle for life and the continuation of suffering. Often, relatives seek to "keep" a person at any cost, because they are not ready to accept the loss. However, this position may be controversial if the patient is in a vegetative state or in a state of irreversible brain damage. From an ethical point of view, continuing treatment in such cases can be considered a violation of the human right to a dignified death [6].

Legally, it is necessary to establish control mechanisms that would ensure that the decisions of relatives are made objectively, with the involvement of doctors, ethics committees or the court. Ideally, each person should be able to designate a trusted person during life who will make decisions about medical intervention in the event of their loss of capacity. This practice, common in developed countries, allows for the avoidance of conflicts and ensures respect for the will of a person even when they cannot express it. Morally, society must cultivate respect for the dignity of even helpless individuals, recognizing that prolonging life at any cost is not always a manifestation of love or care. Sometimes the most humane decision is to allow a natural end to life without pain and humiliation. The balance between compassion, law and morality in such situations determines the true maturity of society and its ability to respect not only life, but also the humanity in its end.

The United States has a well-developed system of advance directives and DNRs, which allow a person to determine medical decisions in advance in the event of loss of capacity. For example, the federal Patient Self Determination Act (PSDA) of 1990 requires medical institutions to inform a patient of their right to draw up such docu-

ments when they are hospitalized. Advance directives include a "living will", a "health-care proxy" or other forms of document. Specifically, a DNR is a written order that states that a patient does not want to be resuscitated in the event of cardiac or respiratory arrest. Different states have their own forms and rules; for example, the state of California has a regulation that when hospitalized, a medical institution must allow a patient to draw up a "Request to Forego Resuscitative Measures / Advance Health Care Directive / DNR". Legally, all of these documents are valid, but their implementation depends on whether all formal requirements have been met, whether the document is in the medical record, and whether the doctor or medical staff has received a copy of it. Thus, in the USA, the patient has wide opportunities to record his will in terms of medical decisions, and state/medical legislation gives this status [10].

In the European Union, the development of the advance directive mechanism is also underway, but with a lower level of unification at the European level. Studies show that in the EU countries there are different approaches to "advance medical directives" - some states have fully recognized them legally, while others leave them at the level of recommendations or ethical standards. The European Court of Human Rights has recognized in a number of decisions that the patient's right to express his/her preferences regarding medical intervention should be taken into account as an element of autonomy, but member states independently determine the forms of execution. For example, directive studies show that the "advance directive" document should be clear, understandable, and intended for a situation when a person has already lost the ability to make decisions. At the same time, in many cases, the legal force of such documents depends on whether there is an appropriate legal mechanism in a particular country, how the document is recorded, whether it is included in the medical record, and whether there is a possibility of revision or cancellation. So, in the EU, the mechanism exists, but with great variability: sometimes it is legally binding, sometimes it is recommendatory, and sometimes it is applied only in the context of ethics committees. [5].

In Ukraine, the situation is significantly different. Currently, there is no legally established mechanism that would allow a patient to record in advance in writing their wishes regarding medical intervention when they lose their ability to make decisions. Ukrainian legislation establishes that decisions regarding medical intervention are made by the patient himself or by a legal representative in the event of loss of legal capacity, but there are no clear rules for "advance directives". For example, legislative acts on healthcare do not contain provisions that allow a patient to fill out a document in advance that obliges doctors to unconditionally fulfill his wishes in the future. At the same time, the Constitution and laws establish the right to medical care, voluntariness of treatment, and protection of dignity, but the mechanism for exercising the will regarding refusal of treatment or resuscitation is significantly limited. For example, the legislation on healthcare and medical care contains general provisions, but does not specifically define the form and legal force of such a declaration of will. In practice, this means that doctors, medical institutions, or relatives may face legal uncertainty in cases where a patient has left a "medical will." Scientific and legal research in Ukraine emphasizes that the institution of a "living will" (medical will) in the country has theoretical discussion, but does not have clear legal regulation [11].

Comparatively, several key differences between the approaches can be identified: first, in the USA, the mechanism for formalizing the expression of will is highly developed, is drawn up in writing, often has special forms or templates, is valid in medical institutions, includes DNR and advance directive. For example, a doctor can enter a DNR order into the medical record, which significantly increases the practical implementation of the patient's will. In EU countries, there is a movement in this direction, but the variability is great - it depends on the jurisdiction, and even where the document is legally recognized, the practice can be, for example, voluntary and not forced. In Ukraine, the lack of a regulatory framework means that the patient has rights, but the mechanism for implementing them regarding a pre-formulated expression of will is limited and less protected.

Secondly, from the point of view of legal force: in the USA, advance directives and DNR have significant legal force, although depending on the state. Medical institutions are obliged to inform, accept documents, and take them into account during treatment. In the EU, the legal force varies: where it is enshrined in law, the effect is stronger, but a situation is possible when the document is of a recommendatory nature. In Ukraine, the legal consequences of such documents are absent or not defined, which creates legal uncertainty.

Thirdly, from the point of view of practice and implementation: the USA has a wide practice of consulting patients, using such forms, and including them in the healthcare system. The EU emphasizes autonomy and ethics, but implementation depends on the member state. Ukraine does not yet have an established practical mechanism: the patient can express a wish, but the medical institution or doctor is not obliged to follow a written directive formed in advance [4].

This situation has important consequences. On the one hand, the high level of development of the mechanism in the USA means that the patient has a significant degree of control over his medical future. On the other hand, the

absence of such a mechanism in Ukraine means that the patient's will may remain legally unrecorded and will not be guaranteed to be fulfilled. This creates risks: medical decisions may be made without taking into account the wishes of the person, ethical dilemmas arise for doctors, relatives and the healthcare system. In conclusion, the analysis shows that the mechanisms for recording the patient's will in the USA, the EU and Ukraine differ significantly: the USA has the most formalized and practically implemented approach, the EU is intermediate, with great variability, Ukraine is poorly formed legislatively and practically. If the goal is to ensure patient autonomy and protect the will of the person in critical situations, then the Ukrainian legislation and medical system should develop mechanisms that would ensure the legal force of previously drawn up medical orders, as well as their practical application [12].

In this context, it will be appropriate to consider several judicial precedents.

Arskaya v. Ukraine (application no. 45076/05). The case raises a classic problem of the State's positive obligations to protect life in the field of medical care and the procedure for investigating a death resulting from possible medical negligence. The facts of the case indicate that the applicant's son was admitted to hospital with a severe lung infection and abscess, and was offered surgical and diagnostic interventions, but he refused many of the procedures. During the treatment, there were operational and organisational shortcomings – inadequate supervision, errors in the interpretation of diagnostic data and late transfer to intensive care, which together gave rise to well-founded suspicions of inadequate care. The family initiated a criminal and disciplinary investigation, but the domestic authorities failed, in the applicant's view, to ensure an effective investigation of the circumstances and the prosecution of those responsible. The ECtHR in this case emphasizes the two-component nature of the state obligations under Article 2 of the Convention: first, the state must refrain from arbitrary deprivation of life, and second, it has positive obligations to ensure an effective medical system and mechanisms for investigating deaths. The Court carefully examined the medical materials and found that the national investigation practice in this case did not meet the requirement of effectiveness: there was no proper, independent and prompt clarification of the causes of death and an assessment of the actions of medical personnel. On this basis, the ECtHR found a violation of the procedural aspect of Article 2, since the victims could not obtain a real legal answer to the question of possible negligence in the provision of medical care. The decision emphasizes that in the health care system, the state not only guarantees the formal right to life, but also has organizational and procedural responsibilities: proper clinical supervision, diagnostic standards, protocols for transferring patients to intensive care units and, in the event of a fatal outcome, effective investigation procedures and the possibility of prosecution. In practice, the ECHR decision called for an update of approaches in national practice: to improve medical control systems, standardize death investigation processes in medical institutions and ensure victims effective access to justice in order to prevent the recurrence of such tragedies [13].

Pindo Mulla v. Spain (application no. 15541/20, Grand Chamber, 17.09.2024). This case raises a difficult balance between the State interest in protecting life and the guarantees of the autonomy and freedom of conscience of the individual; the alleged issues were advance written refusals to specific medical procedures (in particular blood transfusions) and the ways in which they were taken into account by doctors in emergency situations. In the case, the applicant, motivated by religious beliefs, issued a written and documented refusal to receive a blood transfusion, which was placed in her medical file and had to be taken into account by the medical staff. In a subsequent emergency, when a blood transfusion was administered due to a threat to life, the doctors did not follow the fixed written refusal or, at least, did not establish a procedure for adequately verifying and documenting the reason for not complying with the wishes. The Grand Chamber examined the issue under Article 8 (right to private life) in conjunction with Article 9 (freedom of thought, conscience and religion) and concluded that, while the State has a duty to protect life, it must also ensure that the religious beliefs and wishes of the patient are respected by means of reliable procedures that allow for the timely and accurate identification and compliance with advance directives. The Court emphasised the State's procedural obligation: it is required to establish such mechanisms, organisational algorithms and instructions for staff (including control over the availability of documents, marking in the medical record, algorithms for action in emergency situations) to avoid situations where religious or written refusals are ignored. The Grand Chamber judgment sets the standard: a state cannot reasonably rely solely on the "need to save life" without simultaneously providing guarantees that the providers of medical care acted in accordance with clear procedures for verifying voluntary refusals and documenting them. The Court recalled that the possibility of restricting freedom of conscience stems from a legitimate and proportionate aim (protection of life), but such restrictions must be minimally invasive and compensated by mechanisms ensuring respect for autonomy. The ECtHR judgment recognized a violation of the applicant's rights and set the task for the state to improve national procedures so that in future the will of patients in matters of critical treatment would be reliably protected and could be implemented even in emergency conditions [14].

Lambert v. France (application no. 46043/14, Grand Chamber, 05.06.2015). The case of Vincent Lambert was pivotal in interpreting the relationship between the right to life and the right to a dignified death in situations of a patient's long-term and irreversible condition, highlighting the "margin of appreciation" of States in regulating medical decisions to withdraw artificial support. Following a severe brain injury, Mr Lambert was in a stable state of dependence on long-term artificial nutrition; the clinical team and the French national authorities, guided by domestic legislative and ethical approaches, concluded that the continuation of mechanical life support was "obstination déraisonnable" – unreasonable obstinacy – and decided to withdraw life support in accordance with the established procedure. The relatives objected, relying on the patient's previously stated wishes, and the case underwent complex national and international proceedings. The Grand Chamber, examining the case under Article 2 of the Convention, found that France had not violated the Convention: the national legal framework and procedure, which provided for the participation of doctors, ethical consultations and consideration of the opinions of relatives, were sufficiently clear and provided guarantees to protect the patient's interests. The Court emphasised that the resolution of such issues fell within the margin of appreciation of the Member State, as it was rooted in national ethical, legal and medical traditions; however, such a margin of appreciation was not absolute – national procedures had to be thorough, transparent, include medical justification, the involvement of independent experts and due consideration of the patient's expressed wishes. The Lambert judgment confirmed that the termination of "futile" treatment could be compatible with the Convention provided there was a well-founded, documented clinical argument and sufficient procedural guarantees; This position has since become a guideline for states regulating the termination of artificial life support, combining the protection of life with respect for human dignity and clinical common sense [15].

CONCLUSIONS

The study shows that the right to refuse medical treatment and resuscitation is an integral element of the system of somatic human rights, based on the principles of personal autonomy, respect for human dignity and freedom from unwanted interference. An analysis of international legal standards, in particular the practice of the ECHR in the cases of Arskaya v. Ukraine, Pindo Mulla v. Spain and Lambert v. France, convincingly demonstrates that the right to refuse treatment is not absolute, but its implementation must be ensured by appropriate procedural guarantees from the state. The above decisions have formed a clear standard: the state is obliged not only to formally recognize the autonomy of the patient, but also to create organizational mechanisms that allow for reliable recording, verification and implementation of advance medical directives even in emergency conditions. A comparative analysis of the legislation of the USA, EU countries and Ukraine revealed a significant asymmetry in the level of legal protection of patient autonomy: if in the USA the system of advance directives and DNR orders is legislatively developed and practically implemented, and in EU countries there is a gradual unification of approaches, provided that significant variability between jurisdictions remains, then in Ukraine the corresponding institution is at the initial stage of theoretical understanding and is devoid of clear regulatory regulation. The absence of a legally enshrined mechanism in Ukraine for the implementation of the right to refuse medical intervention creates serious legal and ethical problems for both patients and medical personnel. Doctors find themselves in a situation of legal uncertainty, when compliance with the patient's will may contradict their legal obligation to take all measures to preserve life, and relatives of incapacitated persons are deprived of a clear regulatory basis for making decisions on the termination or limitation of treatment. The problem is exacerbated by the fact that the right to informed consent, enshrined in healthcare legislation, is not supported by effective mechanisms for recording and legal recognition of advance medical directives. On the other hand, the ECHR decision in the Pindo Mulla v. Spain case clearly indicates that the state cannot legitimately refer to the protection of life as a basis for ignoring the patient's documented will without simultaneously introducing appropriate organizational standards and algorithms for verifying such documents. This indicates an urgent need for systemic reform of Ukrainian legislation in this area, taking into account both international standards and domestic legal traditions.

In view of the above, the introduction in Ukraine of the institution of advance medical directives and DNR-directives is not only theoretically justified, but also a practically necessary step towards establishing real patient autonomy and compliance with European standards for the protection of human rights. At the same time, the introduction of an appropriate mechanism should be accompanied by appropriate safeguards against abuse: mandatory verification of a person's capacity when drawing up a directive, the creation of a centralized register of such documents, the involvement of independent medical and ethical consultants in controversial cases, as well as ensuring access to quality palliative care so that refusal of treatment is not forced due to pain or lack of alternatives. The implementation of these measures will allow balancing the state interest in protecting life with respect for the dignity and freedom of each person, turning the Ukrainian medical system into a partner in preserving humanity even in the most difficult moments - up to the dignified end of life.

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CREATIVE COMMONS 4.0

CASE STUDY

CONTENTS

Mucous membrane pemphigoid with oral involvement: Two cases reports

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ABSTRACT

These cases present a fifty-four- and sixty-four-year-old females, one of whom had the second episode of the disease. Clinical and cytological examinations of the oral lesions were performed, and the Oral Health Impact Profile-14 (OHIP-14) questionnaire was administered. Additionally, histopathological examination and direct immunofluorescence microscopy were conducted on the patient's biopsied lesions.

This article presents two case reports of patients with mucous membrane pemphigoid involving the oral cavity. As an autoimmune bullous disease, mucous membrane pemphigoid is often characterized by oral manifestations without skin lesions and typically follows a chronic course. In addition to standard clinical and cytological observations, histopathology and direct immunofluorescence are essential for confirming the diagnosis. Because recurrence is common, identifying potential triggers is vital to minimizing flare-ups. Clinical management includes meticulous oral hygiene, topical anesthetics, corticosteroids, and antimicrobial mouthwashes.

The authors believe these cases will provide valuable insights for dentists and encourage the early diagnosis and treatment of this condition in routine practice.

KEY WORDS: oral mucosa, pemphigoid, direct immunofluorescence, oral health

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INTRODUCTION

Autoimmune bullous diseases can be localized on the skin and mucous membranes of various organs or exclusively on the oral mucosa. In this regard, these pathologies are collectively referred to as dermatostomatitis or dermatostomatosis. Diagnosis of oral mucosa lesions is complicated due to the low prevalence of bullous lesions worldwide (0.3–13%) [1]; different clinical manifestations, course of disease, and similarities to lesions of other origins, posing a challenge for both dentists and family physicians. Systemic autoimmune bullous disorders can be dangerous not only to health but also to life; therefore, early diagnosis of their clinical manifestations in the oral cavity facilitates the timely initiation of treatment.

One of the bullous diseases is pemphigoid, which is classified into gestational, bullous, and mucous membrane pemphigoid (MMP). The oral mucosa is usually the initial site of MMP, with gingivitis being the most common oral manifestation [2]. In the International Classification of Diseases (ICD-11), MMP of the oral cavity is coded as EB41.1: Mucous membrane pemphigoid with oral or esophageal involvement [3].

The aim of this study is to present two cases of MMP with oral involvement. Written informed consent for the publication of these case reports was obtained from the patients.

CASE REPORT

CASE 1

A 54-year-old female presented to the to the Communal Enterprise "Poltava Regional Center of Dentistry – Dental Clinical Polyclinic" of the Poltava Regional Council with mildly painful lesions on oral mucosa. The discomfort is interfering with her eating and general well-being. The patient's score on the OHIP-14 (Oral Health Impact Profile-14) questionnaire was 25, indicating a negative impact of oral health issues on daily activities and quality of life (Table 1).

The oral mucosal lesions first appeared one month ago (Fig. 1, Fig.2, Fig.3), and self-treatment provided no relief. The patient initially consulted a family physician, but the prescribed treatment was ineffective. Subsequently, she was referred to an oncology center,

Table.1. Impact of oral health status on quality of life

No	Category	Symptom/Impacts	Sum of scores of the first patient	Sum of scores of the second patient
1	Functional restrications	Trouble pronouncing words Worsened sense of taste	3 -	4 2
2	Physical pain	Aching pain (in the mouth) Discomfort when eating any food	2 4	4 4
3	Psychological discomfort	Feeling self-conscious Feeling tense	- 3	- 4
4	Physical disability	Unsatisfied with your diet Having to interrupt meals	2 2	4 4
5	Psychological disability	Difficulty relaxing Feeling slightly embarrassed	- 2	4 4
6	Social disability	Irritability with people or situations Difficulty performing usual jobs/tasks	- 3	- 3
7	Handicaps	Life in general is less satisfying Total inability to function	4 -	4 -
	In total		25	41

Source: compiled by the authors of this study



Fig. 1. Erosion on the mucosa of the lower alveolar process; above it, the roof of a bulla is lifted with tweezers (Case 1)
Picture taken by the authors



Fig. 2. Erosions on the mucosa of the lower alveolar process, covered with fibrin exudate and bullae roofs (Case 1)
Picture taken by the authors

where additional diagnostic tests ruled out oncological pathology. The patient had a comorbid condition – hypothyroidism.

The skin was pale pink with decreased turgor and no loss of integrity. The patient's general condition was satisfactory. The corners of the mouth were downturned, and the lower third of the face was reduced due to tooth loss. No visible lesions were found on the skin, eyes, or the vermilion border of the lips. Regional lymph nodes were not palpable. Mouth opening was unrestricted. Body temperature was 36,5°C. Examination of the oral mucosa revealed eight erosions and ulcers of various sizes on the lips, low alveolar process, and sublingual area. These were situated on a bright erythematous,



Fig. 3. Erosions on the mucosa of the lower alveolar process, covered with dense fibrin exudate and bullae roofs (Case 1)
Picture taken by the authors



Fig. 4. Erosions on the buccal mucosa, covered with dense fibrin exudate and bullae remnants (Case 2)
Picture taken by the authors

mildly painful base and were covered with a dense fibrinous coating and blister roofs. Nikolsky's sign was negative. No other cutaneous or mucosal sites were involved, such as the conjunctiva or genital or nasopharyngeal mucosae.

The results of the complete blood count (CBC), urinalysis, and blood glucose levels were within normal limits. Cytological examination showed negative Tzanck test. We biopsied the patient's buccal mucosal erosion lesions and nearby normal mucosa tissue. Histopathological examination revealed signs of subepithelial splitting without evidence of acantholysis. The underlying connective tissue showed moderate inflammation, characterized by the presence of eosinophils, neutrophils, lymphocytes, and histiocytes. Direct immunofluorescence (IF) microscopy showed linear deposits of IgG and C3 along the epithelial basement membrane.

Differential diagnosis was performed with Bechet's disease, lichen planus, erosive-ulcerative leukoplakia, erythema multiforme, chronic recurrent herpetic stomatitis, pemphigus vulgaris, and bullous pemphigoid. Bullous Pemphigoid Disease Area Index (BPDA) was used for the assessment of pemphigoid severity. The final diagnosis was mucous membrane pemphigoid with oral involvement, mild degree.

Careful tooth brushing with a soft toothbrush and 0.0015% benzydamine hydrochloride spray for painful erosions were recommended. We also administered 0,12% chlorhexidine gluconate oral rinses followed by topical applications of 0.064% betamethasone cream. This therapy resulted in the gradual healing of the oral mucosal erosions. The patient's oral lesions had not recurred at the most recent follow-up (4 months).

CASE 2

A 64-year-old female patient presented to the same clinic complaining of painful oral ulcers that made eating impossible, sleep disturbances, deterioration of general well-being, anxiety, and a weight loss of 7 kg. The total OHIP score was 41 (Table 1), indicating a significant negative impact of oral health problems on daily activities and overall quality of life.

Oral mucosal lesions appeared 3 weeks ago. She initially consulted a family physician, but the prescribed treatment was ineffective. Subsequently, she was referred to an oncologist, whose evaluation ruled out oncological pathology. Medical history and comorbidities: a severe course of acute respiratory viral infection two months ago, and esophageal reflux, for which she periodically takes Omeprazole.

The patient's general condition was poor, with noted weakness and fatigue. Similar symptoms had been observed in the patient 7 years ago. Body temperature was 36.7°C. The skin was pale pink with decreased tur-



Fig. 5. Erosions on the mucosa of the lateral surface of the tongue, covered with dense fibrin exudate, bullae remnants, and bullae roofs (Case 2)
Picture taken by the authors

gor and no loss of integrity. The corners of the mouth were downturned, and the lower third of the face was reduced due to tooth loss. No visible lesions were detected on the skin, eyes, or the vermilion border of the lips. Regional lymph nodes were not palpable. Mouth opening was unrestricted. Examination of the oral mucosa revealed approximately 20 erosions and ulcers of various sizes on a bright erythematous, painful base, covered with a dense fibrinous coating and blister roofs (Fig. 4, Fig. 5). Nikolsky's sign was negative. No other cutaneous or mucosal sites were involved, such as the conjunctiva or genital or nasopharyngeal mucosae.

The CBC revealed thrombocytopenia. Urinalysis results and blood glucose levels were within normal limits. The Tzanck test was negative. Histological examination showed subepithelial splitting (formation of a bulla beneath the epithelium) with no signs of acantholysis. Direct immunofluorescence indicated the linear basement membrane zone deposition of IgG and C3, while IgA and IgM were rarely found.

Differential diagnosis was performed with lichen planus, erosive-ulcerative leukoplakia, erythema multiforme, chronic recurrent herpetic stomatitis, pemphigus vulgaris, and bullous pemphigoid.

The final diagnosis was MMP with oral involvement, moderate degree with BPDA index.

Careful tooth brushing with a soft toothbrush and 0.0015% benzydamine hydrochloride spray for painful erosions were recommended. We also administered 0,12% chlorhexidine gluconate oral rinses followed by topical applications of 0.064% betamethasone cream. Given the fact that this patient was experiencing a second episode in the oral mucosa after a long remission, the authors focused on the medications she was taking. We noted from the literature [4] that omeprazole is associated with pemphigus; therefore, we speculated that this medicine might be a trigger for MMP due to its short-term use. Consequently, we consulted with the family physician and agreed to discontinue omeprazole for three weeks, replacing it with pantoprazole tablets. Topical therapy gradually healed the oral mucosal erosions. The patient's oral lesions had not recurred at the most recent follow-up (4 months).

PATHOGENESIS OF MMP

The basis of the disease is an autoimmune aggression against proteins of the basement membrane zone (BMZ), leading to subepithelial detachment of the mucosa. In cases of isolated oral involvement, the primary targets for autoantibodies are BP180 (type XVII collagen) and laminin 332. A distinctive feature is that antibodies more frequently attack the C-terminal domain of BP180, whereas in classic bullous pemphigoid, they target the NC16A domain. Autoantibodies (primarily IgG and IgA) bind to antigens within the hemidesmosomes.

This binding directly disrupts protein-protein interactions (e.g., the bond between collagen XVII and collagen IV), which weakens epithelial adhesion to the underlying tissue without necessarily requiring protein internalization. An inflammatory cascade is then triggered. The binding of antibodies activates the complement system and recruits inflammatory cells (neutrophils, eosinophils). This leads to the release of proteolytic enzymes that destroy the outer basement membrane, resulting in the formation of a subepithelial bulla [2,5,6]. A significant infiltration of T-lymphocytes is observed at the lesion sites, which also participate in the pathogenetic chain.

IL-26 was considered as a perspective marker to detect the inflammation level in lung tissue of COPD patients [7]. IL-26–DNA complexes enhanced the production of inflammatory cytokines in monocytes and neutrophils, and augmented the production and activity of proteases from co-cultured monocytes and neutrophils, which induced BP180 cleavage in keratinocytes and dermal-epidermal separation in a modified human cryosection model [8]. Complement activation begins when IgG autoantibodies bind to target antigens, such as BP180. This binding activates both the classical and alternative pathways, leading to the release of anaphylatoxins C3a and C5a. These

mediators recruit neutrophils to the site, ultimately resulting in proteolytic tissue damage and subepidermal separation.

DIAGNOSIS OF MMP

Clinically, MMP is divided into "low-risk" and "high-risk" progression subtypes based on the anatomical distribution of lesions. Low-risk cases involve lesions limited to the oral mucosa, with or without skin involvement. High-risk cases involve critical mucous membranes, such as the ocular, genital, nasopharyngeal, esophageal, or laryngeal epithelium [2]. Therefore, MMP in our patients presented a low risk of progression.

MMP with oral involvement primarily affects patients over 50 years of age, predominantly women. The disease course, duration of remission, lesion area, and epithelialization period vary and depend on the patient's overall health. Our patients exhibited increased oncological alertness and anxiety, seeking consultation after seeing an oncologist; however, such patients typically first present to a dentist with oral lesions. MMP is significantly associated with hypothyroidism [9]. Medications such as Captopril, Enalapril, Furosemide, Ibuprofen, and Omeprazole have been implicated in inducing bullous pemphigoid [4,10]. Thus, the first case might be associated with hypothyroidism, while the second was likely induced by omeprazole, triggering a recurrent episode after 7 years.

The most common manifestation of MMP with oral involvement is desquamative gingivitis (up to 97%), characterized by marked erythema and gingival desquamation. In the oral cavity, the gingiva is most frequently affected (70%), followed by the buccal mucosa (60%), palate (27%), and the tongue and lips (13%) [5].

The diagnosis of oral MMP is based on clinical and laboratory data. Clinical methods include subjective assessment (complaints, medical and life history, questionnaires) and objective examination (inspection, palpation, laboratory tests, and photo protocols). To assess the clinical and psycho-emotional state of patients, questionnaires are utilized as key tools to measure the impact of bullous diseases on daily life, such as the ABQOL (Autoimmune Bullous Disease Quality of Life), TABQOL, or OHIP-14 (Oral Health Impact Profile-14) [11]. Assessment of quality of life in these patients aids in prescribing patient-oriented therapy [1].

The BPDAI (Bullous Pemphigoid Disease Area Index) is used for the objective assessment of bullous pemphigoid severity [12]. One component of the BPDAI evaluates mucosal severity based on the total number of erosions/blisters: mild (<10), moderate (10–24), and severe (>24 lesions).

Direct immunofluorescence (DIF) and serological testing are critical for confirming the diagnosis. Laboratory methods include cytological, histological, and direct immunofluorescence studies. Punch biopsy is considered the most effective sampling method compared to scalpel biopsy for oral bullous lesions. The gingiva has been established as the optimal site for sampling. Histological analysis typically shows subepithelial splitting with a non-specific mixed infiltrate consisting of lymphocytes, histiocytes, plasma cells, neutrophils, and eosinophils. To confirm the diagnosis, DIF of the mucosal biopsy is used to detect fixed autoantibodies (IgG, IgA, IgM) and complement components (C3) directly in the patient's tissues [13,14].

The variability in disease course–severity, duration, and epithelialization time–requires a multidisciplinary approach involving consultations with specialists from other fields. Such patients require dynamic and long-term follow-up.

DIFFERENTIAL DIAGNOSIS

Pemphigus vulgaris is characterized by acantholysis and the presence of Tzanck cells. In pemphigoid patients, Nikolsky's sign is negative and Tzanck cells are absent. Bechet's syndrome and lichen planus are typically characterized by extraoral manifestations. Erythema multiforme may present only in the oral cavity but primarily affects younger individuals; erosions are located on erythematous, edematous mucosa, covered with a difficult-to-remove fibrinous coating, are highly painful, and bleed upon trauma. Hemorrhagic crusts on the lips and enlarged regional lymph nodes are also common for erythema multiform. In chronic recurrent herpetic stomatitis, regional lymph nodes are enlarged, and cytological examination in the first 5–7 days reveals giant multinucleated herpes cells (ballooning degeneration). In our patients, the erosions were only mildly painful, with no lip involvement or lymphadenopathy.

Mucosal involvement can occur in 10% to 30% of bullous pemphigoid patients, most commonly affecting the oral mucosa, including the buccal mucosa or soft palate, followed by the gingival or labial mucosa. These patients are usually younger, have more extensive skin disease, and require more intensive therapy [15]. In contrast to the more commonly multifocal MMP, mucosal involvement in bullous pemphigoid is typically limited to the oral mucosa and associated with more extensive cutaneous involvement. In standard indirect immunofluorescence (IIF), oral pemphigoid typically shows lower antibody titers compared to cutaneous forms [16].

PROPOSED CLASSIFICATION BY LOCALIZATION

Based on clinical experience, the authors suggest categorizing MMP with oral involvement into the following forms: localized-fixed, generalized, and gingival.

I – Localized-fixed. Symptoms are mild and do not affect the patient's general condition. Small, painless, solitary vesicles/bullae (3–10 mm) with transparent content appear and persist for 2–3 days. They have a firm consistency and do not rupture under instrument pressure. Eventually, they burst to form erosions covered by a dense roof, located on a mildly erythematous or unchanged base, usually epithelializing within 6–10 days. Recurrence may occur after weeks or years at the same site.

II – Generalized. Numerous bullae are distributed across the entire oral mucosa. They rupture, forming erosions on a mildly erythematous base; pain arises due to the large surface area of the lesions. Blister remnants remain tightly adherent to the erosion. Patients often have a long history of the disease. While epithelialization may initially occur spontaneously, medical intervention eventually becomes necessary. Treatment is prolonged, and relapses are possible. At this stage, oral hygiene, nutrition, denture use, and dental treatment are severely compromised. Secondary infection of erosions often leads to anxiety and decreased quality of life.

III – Gingival. This form occurs predominantly in women. Patients complain about pain during eating (especially firm foods). Difficulties arise with oral hygiene and dental procedures. Examination reveals painful areas of desquamation on a mildly erythematous gingival base.

Our patients presented with the generalized form, with mild to moderate severity and a low risk of progression.

TREATMENT

Mild disease may respond to high-potency topical corticosteroids (clobetasol or betamethasone). Treatment of chronic lesions may include triamcinolone acetonide [2,7]. Considering the risk of secondary candidiasis, the authors recommend antifungal agents, so our patients were advised to use chlorhexidine mouthwashes. Since MMP is a low-risk bullous lesion, the appearance of new erosions or recurrence requires a search for potential triggers (medications, comorbidities). Alternatively, a dermatologist or family physician may prescribe systemic corticosteroids or immunosuppressants for moderate to severe cases to achieve remission. The efficacy of doxycycline [7,17] and monoclonal antibodies [7,18] in treating bullous pemphigoid has also been established.

CONCLUSIONS

The described cases emphasize that a definitive diagnosis of MMP with oral involvement should be established through a combination of histopathology and DIF. Because recurrence is common, identifying potential triggers is vital to minimizing flare-ups. Clinical management includes meticulous oral hygiene, topical anesthetics, corticosteroids, and antimicrobial mouthwashes. Systemic medications are prescribed in cases of severe manifestations or recurrent episodes of MMP under the supervision of a dermatologist or family physician. The authors believe these cases will provide valuable insights for dentists and encourage the early diagnosis and treatment of this condition in routine practice.

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CONFLICT OF INTEREST

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CREATIVE COMMONS 4.0

CASE STUDY

CONTENTS

Dynamics of ophthalmological symptoms due to vascular pathology of the central nervous system

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ABSTRACT

An analysis of the patient's medical history with changes in the organ of vision due to TBI was performed. The difficulties in making the correct diagnosis in our case were associated with the delayed onset of the first symptoms affecting the organ of vision and their slow progression, the presence of other concomitant diseases, and the absence of classic complaints (such as constant noise in the head). More than two months passed from the first signs of the disease to the correct diagnosis.

With the start of the full-scale invasion of Ukraine by the Russian Federation, the number of traumatic brain injuries among the civilian population and military personnel in Ukraine has increased significantly, and the likelihood of KCS also increases. In all cases of traumatic exophthalmos, it is recommended to be vigilant in terms of the carotid-cavernous connection.

KEY WORDS: exophthalmos, carotid-cavernous fistula, ocular hypertension, angiography

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INTRODUCTION

The vagueness of the clinical picture in routine medical practice can lead to misdiagnosis and untimely treatment of patients. Diagnostic errors are more common in pathological conditions that occur at the borderline between two specialties. Carotid-cavernous fistula (CCF) is such a borderline disease between ophthalmology and neurosurgery. Carotid-cavernous fistula forms when a fistula develops in the cavernous part of the internal carotid artery (ICA). In this case, blood from the ICA enters the cavernous sinus cavity. Blood pressure in the sinus increases, which causes difficulty in the outflow of blood into the cavernous sinus from the ophthalmic vein and other veins. A characteristic clinical syndrome of pulsating exophthalmos develops.

The first observation of the carotid-cavernous fistula was made by Travers (1813). In 1856, Henry reported a patient with pulsating exophthalmos and suggested that the cause of the disease was a rupture of the ICA into the cavernous sinus cavity. After the death of this patient from nasal bleeding, the assumption was confirmed by autopsy. A complete rupture of the BCA in the cavernous sinus (after trauma) was found [1].

Different authors refer to this disease differently. The most commonly used terms are pulsating exophthalmos and arteriovenous carotid-cavernous (carotid-cavernous) fistula. Most often, CCS develops as a result of trauma in 75% of cases, but it can also occur spontaneously.

There are several working classifications of carotid-cavernous fistula (CCF). In 1985, Barrow et al. proposed a classification based on angiography data [2, 3]. The following types are distinguished: Type A (high-flow) CCS is defined as the presence of a direct connection between the cavernous segment of the internal carotid artery and the cavernous sinus. Type B (low flow) CCS is defined as the presence of an abnormal connection between the cavernous sinus and the meningeal branches of the internal carotid artery. Type C (low flow) CCS is defined as the presence of an abnormal connection between the cavernous sinus and the meningeal branches of the external carotid artery.

Type D (low flow) CCS is defined as the presence of an abnormal connection between the cavernous sinus and one or more meningeal branches of the internal carotid artery and external carotid artery.

The clinical picture of CCS is characterized by: pulsating exophthalmos; unilateral constant noise in the patient's head (mainly above the eye), synchronous with the pulse, of various nature; on auscultation, such noise

can be heard above the eye socket, temporal region, and mastoid process. Exophthalmos in CCS is most often unilateral, rarely bilateral. The degree of exophthalmos varies from slightly noticeable to 15-20 mm. Severe protrusion of the eyeball may be accompanied by its downward and outward displacement, since the largest orbital veins are located in the upper inner corner of the eye socket. [1]; chemosis, congestive injection of the conjunctival vessels, and eyelid edema occur as a result of impaired blood outflow; lagophthalmos, paralytic strabismus, and the loss of sensitivity are the result of compression by the enlarged cavernous sinus of the oculomotor, trochlear, abducens nerves, and the first branch of the trigeminal nerve (most often, damage to the abducens nerve is noted, followed by damage to the oculomotor nerve); Anisocoria may occur due to irritation of the sympathetic plexus; ocular hypertension is a common symptom.

Changes in the posterior segment of the eyeball in CCS can manifest as congestive neuro- and retinopathy (edema of the optic disc, retina, and dilation of the retinal veins), which are clearly recorded and tracked using optical coherence tomography (OCT) and angi-OCT-OCT, and photoregistration [4, 5]; OCT and angio-OCT also reveal an increase in choroidal thickness as a result of venous stasis [6, 7], possible central serous chorioretinopathy [8], glaucomatous or ischemic optic neuropathy; possible occlusion of the central retinal artery, Terson's syndrome (Terson's syndrome — the appearance of hemophthalmos due to subarachnoid hemorrhage. Hemophthalmos can also occur due to intracranial hemorrhage and increased intracranial pressure. Intraocular hemorrhage may be subretinal, retinal, preretinal, subhyaloid, or vitreous hemorrhage; combined retinal and choroidal detachment is possible; neovascular glaucoma [9].

According to the literature, the most common sign, regardless of the strength of blood flow in the CCS (low or high), was dilation of the retinal veins.

Alam, M.S., Jain, M., Mukherjee, B. et al., studying changes in the fundus in various types of CCS, identified the "3-point sign." Although none of the three signs (disc. hyperemia, retinal vein dilation, and intraretinal hemorrhage) individually predicted vision loss, their combined presence led to vision loss. The authors indicate that the "3-point sign" should be detected as early as possible to prevent functional deterioration [10].

The prognosis for spontaneous disease progression in CCS is poor. Recovery from spontaneous thrombosis of the choroid is observed in only 5-10% of cases, 10-15% of patients die from intracranial or nasal bleeding, and 50-60% are disabled due to vision loss and mental disorders [1].

Conservative and surgical methods are used to treat CCS. Conservative methods designed to clot CCS are not effective enough.

Until 1970, surgical treatment of CCS was performed as ligation or clipping of the BCA in the cranial cavity or on the neck. In 1971, the Burdenko Research Institute of Neurosurgery of the USSR Academy of Medical Sciences developed a new method for treating CCS—endovascular occlusion of the shunting opening in the cavernous section of the BCA with a balloon catheter (F.A. Serbinenko, 1971) [1].

Currently, direct CCS is treated using the endovascular method or with the use of detachable balloons or platinum coils, transarterially and transvenously. The most promising method is intravascular occlusion of the ostium using a balloon catheter, which is inserted into the internal carotid artery and advanced to the ostium. The balloon is filled with a rapidly hardening mass—silicone—and then deflated. In this way, the ostium is excluded from the blood circulation while maintaining the patency of the vessel [11, 12].

Early diagnosis and treatment of carotid-cavernous fistula increase the chances of complete regression of exophthalmos, restoration of eye muscle function, and visual function [1, 12].

To report a case of carotid-cavernous fistula (CCF) as an interesting clinical issue.

Purpose of the study

To report a case of carotid-cavernous fistula (CCF) as an interesting clinical issue.

CASE REPORT

Patient L., born in 1961, visited her local ophthalmologist on February 9, 2024, complaining of moderate pain and redness in her left eye. According to the patient, the symptoms appeared on February 6, 2024. The doctor diagnosed «acute conjunctivitis of the left eye» and prescribed local anti-inflammatory therapy. The treatment did not improve the condition, and during a follow-up examination (February 16, 2024), a significant increase in intraocular pressure (IOP) in the left eye (32 mm Hg) was detected. The doctor diagnosed «Open-angle I/C glaucoma of the left eye. Suspected glaucoma of the right eye, hyperopia of both eyes.» Hypotensive therapy (Lanotan, Mardozia) was prescribed. Against the background of hypotensive therapy, IOP was compensated, but the patient developed eyelid edema and an injection in the right eye. Due to the negative dynamics of the disease and the lack of effect from anti-inflammatory therapy, the patient was referred for consultation to the A. Novak Regional Clinical Hospital of the Zaporizhzhia Regional Council. On March 7, 2024, the patient



Fig. 1. Photo of the patient during examination on 03/28/2024 Bilateral exophthalmos, congestive injection, chemosis of the conjunctiva in both eyes
Picture taken by the authors



Fig. 2. Fundus photograph (March 28, 2024) of patient L., born in 1961 before surgery. Left eye: optic disc hyperemia, blurred contours, and dilated retinal veins
Picture taken by the authors

was consulted at the head and neck department with ophthalmology. At that time, the patient complained of redness in both eyes and swelling of the eyelids. Upon closer examination, it was found that on 06.12.2023, the patient had suffered a concussion and was treated on an outpatient basis. The patient also noted a history of thyroid disease. When collecting the medical history, it was noted that the patient was unable to concentrate on her answers and provide an accurate chronology of events. Visual acuity of the right eye = 0.3 with correction sph +2.0D=1.0, left eye = 0.3 with correction sph +2.0D=1.0. When measuring IOP with a pneumotonometer, 16 was determined in the right eye and 21 mm Hg in the left eye on hypotensive therapy. In kinetic perimetry, the boundaries of the visual field are within normal limits in both eyes. Objectively, on external examination, there is moderate swelling of the eyelids, congestive injection of both eyes, moderate chemosis in the left eye, moderate proptosis of the left eye, and full mobility of the eyeballs. Biomicroscopy showed that the cornea was transparent in both eyes, the anterior chamber was of medium depth, the iris was structural, the pupil reacted to light, the lenses were transparent, the vitreous body was transparent, the fundus of the optic disc was pale pink, the boundaries were clear, and in the right eye, there is hyperemia of the disc and significant dilation of the veins in the left eye. No hemorrhages or focal changes were found. The diagnosis was made:



Fig. 3. 11.04.2024 CT – angiography of the head and neck vessels with intravenous contrast, sagittal section. Signs of carotid-cavernous fistula
Picture taken by the authors

«congestive optic disc, exophthalmos, open-angle I C glaucoma of the left eye. Hyperopia of the second degree in both eyes. To clarify the diagnosis, an MRI of the orbits was prescribed to determine the size of the eye muscles (to rule out endocrine ophthalmopathy).

25.03.2024 — MRI of the orbits and eyeballs was performed on a SIMENS MAGNETOM AERA tomograph, magnetic field strength 1.5 T. Without contrast enhancement. The series of MRI tomograms of the orbits shows an enlargement of the lumen of the superior ophthalmic vein with a diameter of 0.47 cm on the right and 0.35 cm on the left, with patency preserved. The muscle cone of the right and left orbits - the extraocular muscles are not thickened, with no pathological changes in the MR signal, the fat- d tissue of the retrobulbar region shows no visible pathological changes. The optic nerves are not deformed or thinned, and visualization of the subarachnoid space along the optic nerves. Visually, on the left in the polar part of the temporal lobe, an area of cystic-gliotic changes measuring 5.8 by 2.1 cm is determined, which is probably an area of brain contusion as a result of a previous traumatic brain injury. Conclusion: MR signs of enlargement of the superior ophthalmic vein on the right. Cystic-gliotic changes in the left temporal lobe. Fig. 1.

At the next examination on March 28, 2024, the patient complained of a «protrusion» of the left eye and diplopia when looking to the left. Visual acuity of the right eye = 0.3 with correction sph +2.0D=1.0, left eye = 0.3 with correction sph +2.0D=1.0. Intraocular pressure on hypotensive therapy on a pneumotonometer was 15 in the right eye and 19 mm Hg in the left eye. The visual field boundaries were within normal limits on the kinetic perimeter. Objectively, on external examination, there is moderate swelling of the eyelids, congestive injection of both eyes, moderate chemosis in the left eye, insignificant exophthalmos of the right eye, and moderate exophthalmos of the left eye, with limitation of outward movement of the left eye. Biomicroscopy

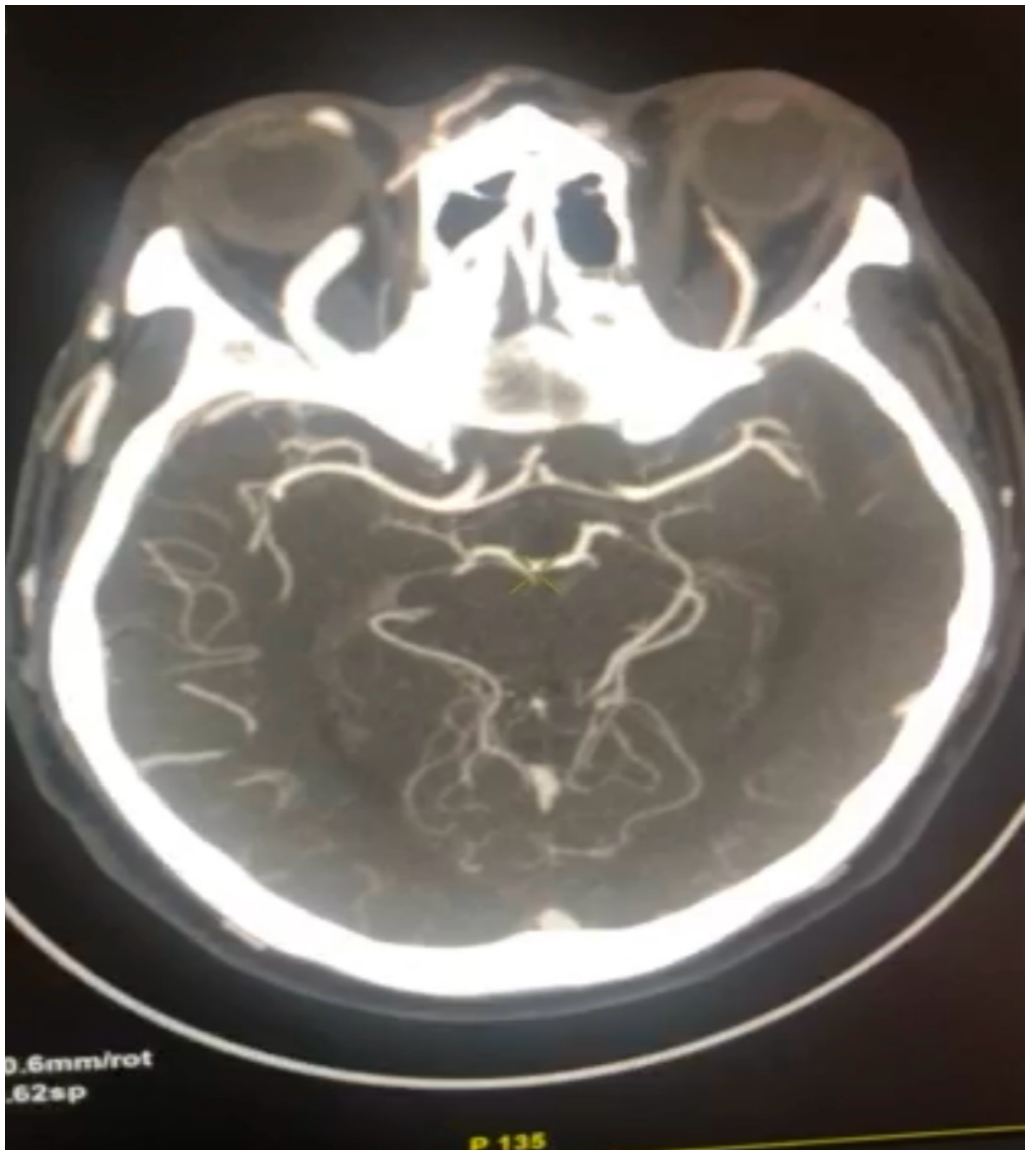


Fig. 4. 11.04.2024 CT angiography of the head and neck vessels with intravenous contrast, axial section. Signs of bilateral carotid-cavernous fistula include visualization of enlargement of the superior ophthalmic veins with a diameter of 4.8 mm and 2.4 mm on the left, which branch off from the enlarged cavernous sinus
Picture taken by the authors

of both eyes shows a transparent cornea, an anterior chamber of medium depth, a structural iris, a pupil reacting to light, transparent lenses, a transparent vitreous body, pale pink fundus, clear boundaries, dilated veins, and tortuous in the right eye. Noteworthy are hyperemia, blurred optic disc margins in the left eye, significantly dilated and twisted veins, clear macular reflex, no hemorrhages or focal changes (Fig. 2).

After ruling out a diagnosis of endocrine ophthalmopathy and worsening symptoms (increased exophthalmos in the left eye and its appearance in the right eye, stagnant phenomena in the fundus, diplopia, stagnant injection and chemosis of the conjunctiva in both eyes), MR signs of enlargement of the superior ophthalmic vein on the right, the patient was referred for angiography of the head and neck vessels to rule out the presence of CCS (Fig. 3, Fig. 4).

At place of residence 11.04.2024 Performed: CT angiography of the head and neck vessels with intravenous contrast Ultravist 370 80 ml. On a General Electric 64 tomograph, a series of MSCT scans showed no sharp narrowing of the lumen of the arterial vessels of the carotid and vertebral basins. In the bifurcation area of the right common carotid artery, a soft semi-concentric atherosclerotic plaque was detected, causing stenosis of the initial section of the right internal carotid artery within 20%. Both carotid arteries are visible throughout their entire length. The circle of Willis is closed. There is visualisation of the upper ophthalmic veins with a diameter of 4.8 mm and 2.4 mm on the left, which branch off from the enlarged cavernous sinus. CTA - no signs of organic pathological damage to other anatomical structures of the circulus arteriosum cerebri. Conclusion: MSCT shows signs of bilateral carotid-cavernous fistula.

It is recommended to continue treatment at the Scientific and Practical Center for Endovascular Neurosurgery of the National Academy of Medical Sciences of Ukraine, where the patient was hospitalized on 17.04.2025. At that



Fig. 5. Photo of patient L., born in 1961 (May 23, 2024), after successful surgery for CCS - eyes are calm, position is correct, no exophthalmos
Picture taken by the authors

time, the patient's general condition was relatively satisfactory. Consciousness — 15 points. No meningeal signs. Exophthalmos on both sides, photoreactions preserved. Tongue in the midline. Swallowing is not impaired. Complete blood count and biochemical analysis within normal limits. On the same day, total selective digital cerebral angiography was performed — a carotid-cavernous fistula on the left was diagnosed. No other pathological changes in the extra- and intracranial sections of the cerebral arteries were found. On 04/22/2024, endovascular total disconnection of the carotid-cavernous fistula on the left side was performed using microcoils with balloon assistance. There were no complications. In the early postoperative period, there were no negative dynamics. Significant regression of exophthalmos and chemosis. The pulsating noise in the head disappeared. Consciousness according to the Glasgow Coma Scale — 15 points.

April 25, 2024 — the patient's condition deteriorated sharply — complaints of sharp protrusion of the left eye and hyperemia of the sclera of the left eye, increased blood pressure.

April 25, 2024 — selective cerebral subtractive digital angiography — recurrence of carotid-cavernous fistula on the left. On the same day, surgery was performed: endovascular total separation of the carotid-cavernous fistula on the left using a micro-spiral with balloon assistance and an adhesive composition. There were no complications. Drug therapy was performed: Plasmovene, Asparkam, Ketolong, Co-Prenesa, Prenesa, Concor, Pangastro, Detralex, Azarga, Amlodipine. Against the background of drug therapy, the patient's condition showed positive dynamics: significant regression of exophthalmos, chemosis, and injection of both eyes. Consciousness according to the GCS — 15 points. The patient was discharged on 04/30/2024.

On May 23, 2024, the patient's condition is satisfactory. No complaints. Visual acuity of the right eye = 0.3 with correction sph +2.0D=1.0, left eye = 0.3 with correction sph +2.0D=1.0. Intraocular pressure without hypotensive therapy on a pneumotonometer is 13.5 in the right eye and 17.8 mm Hg in the left eye. Objectively, the position of the eyeballs is symmetrical, correct, there is no exophthalmos, full mobility of the eyeballs, no congestive injection of the eyeballs. (Fig. 5).

In both eyes, the cornea is transparent, the anterior chamber is of medium depth, the iris is structural, the pupil reacts to light, the lenses are transparent, the vitreous body is transparent, the fundus of the optic disc is pale pink, the borders are clear, the caliber and course of the vessels are normal, no hemorrhages or focal changes were found. The patient is under dispensary supervision at her place of residence (Fig. 6).

The difficulty in making a correct diagnosis was associated with the delayed onset of the first symptoms affecting the organ of vision and their slow progression, the presence of other concomitant diseases, and the absence of classic complaints (such as constant noise in the head). The first complaints of redness in the left eye appeared on February 6, 2024 (two months after the traumatic brain injury), and 10 days later (February 16, 2024), an increase in IOP in the left eye was recorded. On 07.03.2024, after another 3 weeks, swelling of the eyelids and injection of both eyes were recorded. After another 3 weeks, during the next examination on 28.03.2024, an increase in exophthalmos in the left eye and its appearance in the right eye, conjunctival chemosis and injection in both eyes, restricted outward movement of the left eye, and changes in the fundus during ophthalmoscopy—edema of the optic disc and significant dilation of the retinal veins. Considering



Fig. 6. Fundus photograph (May 23, 2024) of patient L., born in 1961, after surgery for CCS, left eye optic disc pale pink, clear borders, vein course and caliber within normal limits
Picture taken by the authors

the bilateral exophthalmos and the patient's history of thyroid disease, an MRI of the orbits was performed on March 25, 2024. Considering the study data, the eye muscles were not thickened without pathological changes in the MR signal, the retrobulbar area fat tissue had no visible pathological changes, and the presumed diagnosis of endocrine ophthalmopathy was rejected, but the MR signs of enlargement of the superior vena cava on the right were alarming.

11.04.2024 Performed: CT angiography of the head and neck vessels with intravenous contrast. Conclusion: MSCT — signs of carotid-cavernous fistula bilaterally. More than two months passed from the first signs of the disease to the correct diagnosis. 25.04.2024 Last surgical intervention performed — endovascular total disconnection of the carotid-cavernous fistula on the left side using microcoils with balloon assistance and an adhesive composition, which led to the patient's recovery with complete regression of ocular manifestations and preservation of visual functions.

CONCLUSIONS

1. In cases of atypical clinical presentation, unclear symptoms, and increasing dynamics of the pathological process, modern technologies should be used. In our case, diagnosis and targeted treatment yielded positive results in rehabilitation and restoration of visual functions.
2. With the start of the full-scale invasion of Ukraine by the Russian Federation, the number of traumatic brain injuries among the civilian population and military personnel in Ukraine has increased significantly, and the likelihood of TBI also increases. In all cases of traumatic exophthalmos, it is recommended to be vigilant in terms of carotid-cavernous connection.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CASE STUDY

CONTENTS

Cephalic tetanus progressing to generalized tetanus after facial trauma in an unvaccinated elderly patient: A case report

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ABSTRACT

A 67-year-old unvaccinated male patient was analyzed. Clinical manifestations, laboratory and imaging findings, treatment, and disease progression were evaluated using hospital records.

Eight days after facial trauma from a bicycle accident, the patient developed progressive trismus, dysphagia, neck stiffness, and respiratory discomfort. He was hospitalized on day 10 post-injury. Initial treatment included 500 IU of tetanus immunoglobulin. Two days later, his condition deteriorated with progression to generalized tetanus, requiring ICU admission. Additional 2000 IU of tetanus immunoglobulin, sedation, and oxygen therapy were administered. The course was complicated by pneumonia. Gradual improvement occurred, and he was discharged in stable condition on day 35.

Cephalic tetanus is a rare but severe form that may progress to generalized disease. Early recognition and prompt administration of tetanus immunoglobulin are crucial for favorable outcomes.

KEY WORDS: tetanus; cephalic tetanus; trismus; case report

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INTRODUCTION

Tetanus is an acute, potentially fatal disease caused by the neurotoxin tetanospasmin produced by *Clostridium tetani* [1,2]. The incidence has dramatically decreased due to vaccination programs; however, sporadic cases continue to occur, particularly in elderly populations with incomplete immunization [3].

Cephalic tetanus, a rare variant, involves cranial nerves and is usually associated with head and neck injuries or infections [4,5]. Its early presentation may include trismus, dysphagia, facial muscle spasms, and cranial nerve palsies [6]. Despite early recognition, cephalic tetanus often progresses to generalized disease, which carries significant risk of respiratory failure and death [7,8].

Given its rarity and potential severity, reporting detailed cases contributes to improved recognition, management, and outcomes, particularly in vulnerable populations. This report describes a case of cephalic tetanus following facial trauma that progressed to generalized tetanus, highlighting clinical course, management, and literature comparison.

CASE REPORT

A 67-year-old male patient was admitted to the hospital with complaints of inability to open the mouth, difficulty chewing and swallowing, neck muscle rigidity, headache, generalized weakness, and dyspnea that worsened in the supine position.

Clinical evaluation: Neurological and physical examination, vital signs, laboratory tests (CBC, liver/kidney function, CRP, urinalysis), and imaging (CT brain/cervical spine).

TREATMENT INTERVENTIONS:

Immunotherapy: 500 IU tetanus immunoglobulin (Day 10 post-trauma), additional 2000 IU after deterioration (Day 13);

Pharmacotherapy: Metronidazole, cefazolin, moxifloxacin, fluconazole, baclofen, magnesium sulfate, sedatives;

Supportive care: Oxygen therapy, ICU monitoring, nasogastric feeding, airway management.

Follow-up: Monitoring until Day 35 post-trauma for neurological recovery, trismus resolution, respiratory function.

ETHICS

Written informed consent was obtained from the patient for publication of this case report and accompanying clinical information. All procedures performed in this study were conducted in accordance with the ethical standards of the institutional and national research committees and with the principles of the Declaration of Helsinki. Patient have sighned an ethics statement that ensures informed consent, confidentiality, and respect for autonomy. It confirms that the patient voluntarily agreed to participate in research or treatment, understands the risks/benefits, and has consented to the use of their data or images, adhering to ethical standards

CASE

Ten days before hospitalization the patient sustained facial trauma after falling from a bicycle. The wound was treated at home with antiseptic solutions without medical consultation.

Eight days after the injury the patient developed progressive trismus, difficulty chewing food, dysphagia, and neck stiffness. Because of worsening symptoms, the patient sought medical care and was hospitalized.

The patient had no documented history of tetanus vaccination.

At admission the patient's condition was assessed as moderate. Neurological examination revealed marked trismus, increased tone of the cervical muscles, and difficulty swallowing.

Laboratory examination showed leukocyte count of 9.8×10^9/L with elevated inflammatory markers. Biochemical analysis demonstrated increased liver enzyme levels (ALT 860 U/L, AST 950 U/L).

Computed tomography of the brain revealed signs of chronic dyscirculatory encephalopathy. Chest radiography showed features consistent with chronic bronchitis.

Tetanus immunoglobulin (500 IU) was administered after admission.

However, three days later the patient's condition deteriorated with increasing generalized muscle rigidity and respiratory distress. The diagnosis was revised to generalized tetanus.

The patient was transferred to the intensive care unit where additional tetanus immunoglobulin (2000 IU) was administered. Sedation therapy and oxygen support were initiated.

On the 17th day after trauma the clinical course was complicated by pneumonia.

Table 1. Timeline of disease progression

Day	Clinical event
Day 0	Facial trauma
Day 8	Trismus
Day 10	Hospitalization
Day 11	500 IU tetanus immunoglobulin
Day 13	ICU admission
Day 13	Additional 2000 IU immunoglobulin
Day 17	Pneumonia
Day 35	Clinical improvement

Source: compiled by the authors of this study

Gradual clinical improvement was observed during further treatment, and the patient was discharged in stable condition on day 35 after trauma.

The chronological course of the disease is summarized in Table 1.

DISCUSSION

Cephalic tetanus is one of the rarest clinical forms of tetanus and typically develops after injuries involving the head or face or as a complication of otitis media and other craniofacial infections. This variant accounts for less than 3% of all reported tetanus cases worldwide [1,4]. It is characterized primarily by cranial nerve dysfunction, most commonly involving the facial nerve, and may present with trismus, facial muscle spasms, dysphagia, and neck stiffness [4,6].

In the present case, the first clinical manifestations appeared approximately eight days after the traumatic injury. This incubation period falls within the commonly reported range of 3–21 days described in the literature [1,5]. Shorter incubation periods are generally associated with more severe disease because of the shorter distance between the site of toxin production and the central nervous system [1]. Early manifestations in our patient included progressive trismus and dysphagia, which represent typical initial symptoms of tetanus.

However, these symptoms are nonspecific and may mimic several other clinical conditions. The differential diagnosis of trismus includes temporomandibular joint disorders, odontogenic infections, peritonsillar abscess, dystonia, and other neurological disorders affecting the brainstem [6,7]. Because of these diagnostic challenges, the disease may initially be overlooked, particularly in regions where tetanus has become rare due to effective vaccination programs.

The differential diagnosis of trismus after craniofacial trauma is shown in Table 2.

One of the most important clinical features of cephalic tetanus is its high risk of progression to generalized disease. Previous studies indicate that approximately 60–70%

Table 2. Differential diagnosis of trismus after craniofacial trauma

Trismus after facial trauma	
Infectious causes	Tetanus (including cephalic tetanus)
	Peritonsillar abscess
	Odontogenic infections
Musculoskeletal causes	Temporomandibular joint dislocation
	Temporomandibular joint trauma
	Masticatory muscle spasm
Neurological causes	Dystonia
	Brainstem lesions
	Drug-induced muscle rigidity
Oncological causes	Tumors of the oropharynx or jaw
Inflammatory causes	Osteomyelitis of the mandible
	Severe dental infection
Clinical "red flags" suggesting tetanus:	progressive trismus
	dysphagia
	neck rigidity
	generalized muscle spasms
	absence of vaccination history

Source: compiled by the authors of this study

Table 3. Clinical characteristics of cephalic and generalized tetanus

Feature	Cephalic tetanus	Generalized tetanus
Frequency	Rare (<3% of cases)	Most common form (>80% of cases)
Typical trigger	Craniofacial trauma, ear infection	Contaminated wounds anywhere on the body
Initial symptoms	Trismus, cranial nerve palsy, facial weakness	Trismus, neck stiffness, generalized muscle rigidity
Cranial nerve involvement	Common (especially facial nerve)	Rare
Muscle spasms	Usually localized initially	Generalized spasms and opisthotonus
Risk of progression	May progress to generalized tetanus in ~66% cases	Already generalized
Severity	Variable	Often severe with autonomic dysfunction
Need for intensive care	Sometimes required	Frequently required
Mortality risk	Moderate	Higher mortality risk

Source: compiled by the authors of this study

of patients with cephalic tetanus subsequently develop generalized tetanus within several days after the onset of neurological symptoms [4,8]. Generalized tetanus is characterized by diffuse muscle rigidity, painful spasms, and involvement of respiratory muscles, which may result in respiratory failure and severe autonomic instability [1,2].

The clinical characteristics of cephalic and generalized tetanus are summarized in Table 3.

The clinical course observed in our patient followed a similar pattern. Initial localized symptoms gradually progressed to generalized disease requiring intensive care management. The need for ICU admission highlights the potentially life-threatening nature of this condition even in patients who initially present with moderate clinical severity.

Another important aspect of this case is the delayed presentation to medical care. The patient sought hospital treatment approximately ten days after the traumatic injury. Delayed recognition and treatment are well-known factors associated with more severe disease and an increased risk of complications [3,10]. Early wound management and timely administration of tetanus prophylaxis remain essential components of prevention.

Treatment of tetanus is primarily aimed at neutralizing circulating toxin, controlling muscle spasms, and providing adequate supportive care. Administration of human tetanus immunoglobulin remains the cornerstone of therapy, as it neutralizes unbound toxin and limits further progression of the disease [2,11].

Table 4. Reported cases of cephalic tetanus in the literature

Author, year	Patient age / sex	Triggering event	Initial symptoms	Progression to generalized tetanus	Outcome
Fabris F, 2023 [19]	54 / M	Facial wound	Trismus, facial nerve palsy	Yes	Recovery
Adeel M, 2012 [20]	12 / F	Otitis media	Trismus, dysphagia	No	Recovery
Adeleye AO, 2012 [21]	young/M	Head trauma	Trismus, neck stiffness	Yes	Pneumonia, recovery
Reda Hamdi, 2023 [17]	43 / M	Idiopathic facial palsy	Cephalic form	Yes	Recovery
Abdirahin Mohamed Abdulkadir, 2026 [10]	17 / M	Head injury	Trismus	Yes	Recovery
Present case (2025)	67 / M	Facial trauma (bicycle accident)	Trismus, dysphagia	Yes	Pneumonia, recovery

Source: compiled by the authors based on [10, 17, 19-21]

An additional aspect of this case that deserves consideration is the initial dose of tetanus immunoglobulin administered at hospital admission. The patient received 500 IU of tetanus immunoglobulin on the third day after the onset of clinical symptoms. Although current international recommendations generally suggest higher doses for treatment of tetanus, several clinical reports indicate that the optimal dose of human tetanus immunoglobulin remains a matter of discussion, and effective neutralization of circulating toxin may occur even with lower doses when administered early in the disease course [11,12].

In the present case, the initial clinical presentation was assessed as moderately severe, and the diagnosis of tetanus was not yet fully established at admission. After rapid clinical deterioration and progression toward generalized tetanus, an additional 2000 IU of tetanus immunoglobulin was administered in the intensive care unit. This stepwise therapeutic approach reflects the evolving clinical picture and highlights the importance of continuous reassessment of patients with suspected tetanus. Similar treatment adjustments during disease progression have also been reported in previous case reports of cephalic tetanus progressing to generalized disease [8,9].

Additional treatment measures include antimicrobial therapy to eradicate *Clostridium tetani*, sedation to control muscle spasms, and intensive supportive care, including respiratory support when necessary. In the present case, administration of tetanus immunoglobulin together with sedation and oxygen therapy contributed to gradual clinical improvement.

Respiratory complications represent one of the most frequent causes of morbidity in severe tetanus. Pneumonia may develop as a result of impaired respiratory mechanics, prolonged immobilization, and difficulties with airway clearance [8,11]. In our patient, pneumonia developed during hospitalization but responded well to antimicrobial therapy and supportive care.

Despite the severity of the disease, the patient demonstrated gradual clinical improvement following comprehensive intensive therapy, including sedation, respiratory support, antimicrobial treatment, and administration of tetanus immunoglobulin. Advances in modern intensive care have significantly improved survival rates among tetanus patients, particularly in specialized centers where early supportive therapy can reduce mortality [2,13,14-17].

Several previously reported cases of cephalic tetanus have demonstrated similar clinical progression characterized by initial cranial nerve involvement followed by generalized muscle rigidity. A comparison with selected cases from the literature is presented in Table 4. In most reports, craniofacial trauma or ear infection represented the primary trigger of infection, and approximately two-thirds of patients progressed to generalized tetanus. The clinical course observed in our patient is consistent with these findings, including the development of respiratory complications during hospitalization.

Finally, this case highlights an important public health issue. Although the global incidence of tetanus has declined considerably due to widespread vaccination programs, sporadic cases continue to occur, particularly among elderly individuals who may not have received booster immunizations for many years [3,15]. Waning immunity in older adults remains a recognized risk factor in many countries, emphasizing the importance of maintaining adequate vaccination coverage throughout adulthood [12,16,18].

Overall, this case illustrates the typical progression of cephalic tetanus to generalized disease and emphasizes the importance of considering tetanus in the differential

diagnosis of trismus and dysphagia following craniofacial trauma. Early diagnosis, prompt administration of tetanus immunoglobulin, and adequate intensive care management remain critical for improving patient outcomes.

CONCLUSIONS

1. In elderly patients, generalized tetanus may present with bulbar and respiratory manifestations (trismus, dysphagia, difficulty breathing) without typical generalized seizures, which complicates early diagnosis.
2. Increased dyspnea in the horizontal position is an important clinical marker of functional upper airway obstruction due to spastic damage to the oropharyngeal muscles and requires immediate airway patency.
3. Early clinical diagnosis of tetanus and timely use of antitoxin, control of muscle spasms, and respiratory support are key factors in a favorable course of the disease, especially in older patients.
4. The absence or insufficient level of immunization in adults and elderly patients remains a significant risk factor for the development of tetanus, which emphasizes the need for revaccination throughout life.
5. Early diagnosis and timely administration of antitoxin are the greatest predictors of survival in patients with tetanus.

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CONFLICT OF INTEREST
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