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Randomised clinical trial in umbilical sepsis: A comparative analysis of postoperative outcomes of umbilectomy and umbilicus-retaining procedure

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Abstract. The objective of this study was to analyse the postoperative outcomes between umbilectomy and the umbilicus-retaining procedure in patients presenting with umbilical sepsis. Patients presenting to the surgical clinic with umbilical sepsis were randomised into two groups of 25 patients each based on inclusion and exclusion criteria. Postoperative factors such as pain score, wound infection, recurrence, hospital stay, and cosmetic satisfaction were analysed during follow-up. The parameters observed were statistically analysed using the chi-square test. A p-value of < 0.05 was considered statistically significant. The mean age at surgery in both groups was similar (48 vs. 49, $p = 0.9191$). Based on the pain score after surgery, there is a significant difference ($p = 0.0183$) between the two groups, with higher pain reported in patients undergoing the umbilicus-retaining procedure. Considering wound infection as a parameter, the statistical significance is less ($p = 0.096$). Hospital stay was observed in both groups, and there were no significant differences ($p = 0.7055$). The study population consisted of 23 men and 27 women. Cosmetic satisfaction of patients

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was assessed, and no statistical significance was found (p = 0.644) between the two groups, irrespective of gender. Recurrence of symptoms was observed more in the group where the umbilicus is retained (p = 0.0073). Considering all the parameters and their statistical significance, it is concluded that postoperative outcomes were comparable in both groups of patients. The results of this study provide insights for surgeons into the concept of retaining or removing the umbilicus and its impact on postoperative morbidity of patients presenting with umbilical sepsis

Keywords: cosmesis; pain scale; recurrence; infection; umbilicus; surgery

INTRODUCTION

The umbilicus is an important aesthetic landmark, marking the waistline, and its absence can result in poor self-image. The aesthetics of the abdomen mainly involve the umbilical scar. The ideal umbilicus should have a natural contour, prominent depth, minimal additional scars, and appropriate superior hooding. Surgical treatment is often preferable to conservative treatment options for many umbilical pathologies, as conservative treatment generally involves a longer healing time and a higher rate of recurrence. By focusing on these aesthetic principles and opting for surgical solutions when appropriate, healthcare providers can better address umbilical issues and enhance both the functional and cosmetic aspects of abdominal health.

K. Perez *et al*. [1] discussed an analysis of 408 patients who underwent umbilectomy and found that umbilectomy significantly decreased the number of patients presenting with wound infection but increased the risk of seroma formation. S.M. Aso *et al*. [2] discussed conservative non-operative management of 114 patients presenting with umbilical pilonidal sinus and found that umbilical preservation is highly possible using an umbilical injection mixture. G. Nisi *et al.* [3] discussed types of umbilical reconstruction techniques and the cosmetic significance of umbilical preservation using U-scoring. A. Sisti *et al*. [4] conducted a literature review on umbilical reconstruction techniques, noted that removal of the umbilicus often becomes inevitable, leaving surgeons in a dilemma whether to retain or remove the umbilicus. M. Gardani *et al*. [5] discuss multiple different options for umbilical reconstruction techniques, which clearly define the difference between umbilical preservation and neoumbilicoplasty. Y.H. Kim *et al*. [6] emphasised the importance of preoperative imaging of patients with umbilical sinus, which aids in the decision-making process regarding the possibility of removing the umbilicus intraoperatively. K. Painter *et al*. [7] highlighted the management of umbilical sepsis and also emphasised wound debridement in a selected group of patients. M. Chua *et al*. [8] discussed the significance of umbilical blood supply in umbilicus preservation techniques, which might produce umbilical necrosis if not done properly.

Literature evidence from many studies lacks clarity on several factors, such as: a) what are the most common postoperative issues patients face after umbilicus surgeries; b) which patients require umbilicus preservation; c) what are the cosmetic aspects of removing the umbilicus for the patient; d) what are the future approaches to treating patients with umbilical sepsis. This study aimed to address all these factors and guide surgeons involved in treating patients with umbilical sepsis. The objective of this study was to analyse the clinical characteristics and postoperative outcomes of umbilectomy versus the umbilicus-retaining procedure in umbilical sepsis.

MATERIALS AND METHODS

This is a prospective, randomised controlled study of postoperative outcomes in patients who underwent umbilectomy versus the umbilicus-retaining procedure for umbilical sepsis. The study included a total of 50 patients who underwent surgery for umbilical sepsis from January 2022 to September 2023 in the Department of General Surgery. The study population comprised 23 male and 27 female patients in total. Group A included 11 male and 14 female patients out of 25, and Group B included 12 male and 13 female patients out of 25. All patient-related demographics were collected while registering the patients for the study. Patients were randomised using a random allocation technique to either group of 25 patients, based on inclusion and exclusion criteria. The random allocation technique was performed by using pre-numbered opaque sealed envelopes sequentially numbered from 1 to 50. Patients were allocated to either Group A or Group B in the same order they were inducted into the study by opening the sealed envelopes. Group A consisted of patients where the umbilicus was removed as part of the surgical incision, and Group B consisted of patients where the umbilicus was retained.

All patients undergoing surgery for umbilical sepsis were elective as well as emergency. Conditions included umbilical sepsis, umbilical pilonidal sinus with abscess, and umbilical granuloma with abscess. Exclusion criteria included patients with uncontrolled diabetes, chronic smokers, morbid obesity, prior laparotomy, and those at extremes of age (paediatric, adolescent, geriatric).

The surgical technique used for both groups was almost similar, except the umbilicus was removed in the umbilectomy group. In Group A where the umbilicus was removed, the abdominal incision was either vertical or horizontal, enclosing the umbilicus all around. The incision was deepened, and the umbilicus along with its stalk, was disconnected from the *linea alba* and excised. Wound debridement was performed. After washing the wound with normal saline, the skin was sutured with non-absorbable sutures and covered with a sterile dressing. In Group B, where the umbilicus was retained, the incision was similar, either vertical or transverse, abutting the umbilicus. The incision was deepened, and infected tissue around the umbilicus was debrided. The wound was washed with saline, and the skin was sutured with non-absorbable sutures.

Patients' follow-up was analysed up to the 6-month postoperative period. Postoperative outcomes such as pain scale, recurrence of symptoms, wound infection, hospital stay, and cosmetic satisfaction were compared between the two groups of patients, and conclusions were drawn based on the statistical significance of these outcomes. Postoperative outcomes were assessed based on standard practices. Pain intensity was assessed using the Universal Pain Assessment Tool (UPAT) [9], which combines

a Visual Analog Scale with a Numerical Rating Scale, a standard pain index scoring method accepted and practised in surgical wards. Pain scoring was recorded on the 3rd and 7th postoperative days, and the average was taken for analysis (Table 1). To reduce the chance of confounding factors such as wound infection or use of painkillers [10], which can influence pain characterisation, two readings were taken on postoperative day 3 and day 7, and the aggregate was used for analysis. For the postoperative pain grading, the Universal Pain Assessment Tool was used to define the amount of pain experienced by patients in each group (Fig. 1).

Table 1. Universal Pain Assessment Tool (format)

Pain intensity	Scoring	Day 3	Day 7
No pain	0		
Very mild pain	1		
Discomforting pain (Hurts a little bit)	2		
Tolerable pain	3		
Distressing pain (Hurts a little more)	4		
Very distressing pain	5		
Intense pain (Hurts even more)	6		
Very intense pain	7		
Utterly horrible pain (Hurts a whole lot)	8		
Excruciating / Unbearable pain	9		
Unimaginable / Unspeakable pain (Hurts worst)	10		

Source: compiled by the authors

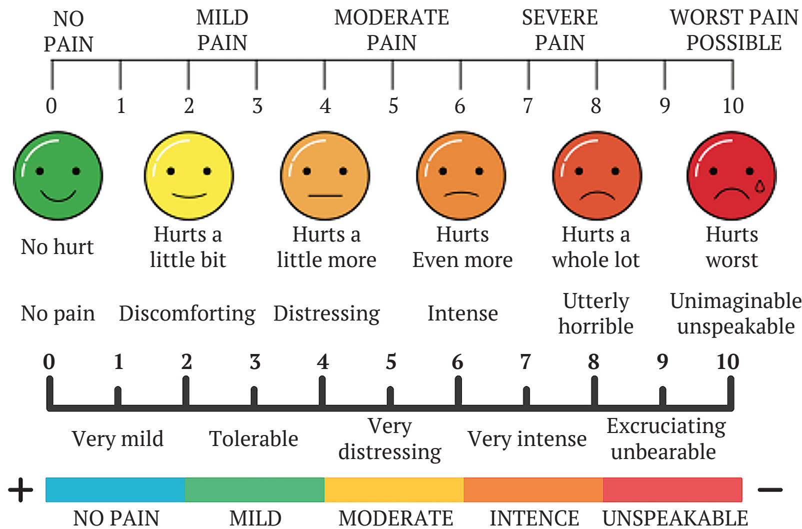

Figure 1. Postoperative pain grading

Source: [9]

Wound infection [11] was assessed by the presence of at least either discharge or slough tissue. A third-generation cephalosporin [12] was the standard antibiotic used for 3 days following surgery for all participants in both groups. Recurrence was assessed at the end of the 6-month postoperative period. Hospital stay was assessed based on the number of days patients were admitted as inpatients. The cosmetic satisfaction questionnaire was given to patients at the end of 6 months when they returned to the hospital for review, and their opinions were recorded for analysis. This questionnaire was prepared based on inputs from hospital plastic surgery and general surgery consultants and also from a previous similar study [13]. Cosmetic satisfaction was assessed by administering a questionnaire (Table 2) to all 50 patients postoperatively. It is categorised as equivocal (score 1), satisfied (score 2), and highly satisfied (score 3).

Table 2. Cosmetic satisfaction questionnaire (format)

Study Title	Randomised clinical trial in umbilical sepsis: A comparative analysis of postoperative outcomes of umbilectomy and umbilicus-retaining procedure
Patient Name	
IP number	

Continued Table 2.

How satisfied are you cosmetically regarding the wound healing post-surgery?	
Description	**✓ Please Tick**
Score 1 Equivocal	☐
Score 2 Satisfied	☐
Score 3 Highly satisfied	☐
Kindly give your feedback :	

Source: compiled by the authors

Since the same questionnaire was given to all 50 patients, standardisation of this questionnaire was maintained. The study was conducted taking into account all the ethical concerns as per the hospital's institutional ethical committee norms. Consent was obtained while registering for the study, which included analysis of collected data from patients. The statistical test used for analysis was the two-way chi-square test. A p-value of <0.05 was considered significant. The study also adhered to the ethical norms of the Declaration of Helsinki [14].

RESULTS

Statistical analysis of various factors was done, which included pain score, wound infection, recurrence of symptoms, hospital stay, cosmetic satisfaction, and conclusions were drawn. In the umbilectomy group, 15 patients (60%) experienced mild pain (score 2), 8 patients (32%) experienced uncomfortable pain (score 4), and 2 patients (8%) experienced distressing pain (score 6). In the group where the umbilicus was preserved, 10 patients (40%) experienced mild pain (score 2), 12 patients (48%) experienced uncomfortable pain (score 4), and 3 patients (12%) experienced distressing pain (score 6) (Fig. 2). The average pain score was found to be 2.96 in the umbilectomy group, and in the umbilicus-preserving group, it was 3.44. Using the chi-square test, the p-value for the pain factor was found to be 0.0183, and hence pain is considered a significant differentiating factor between the two groups. Pain levels seem higher in patients where the umbilicus is preserved.

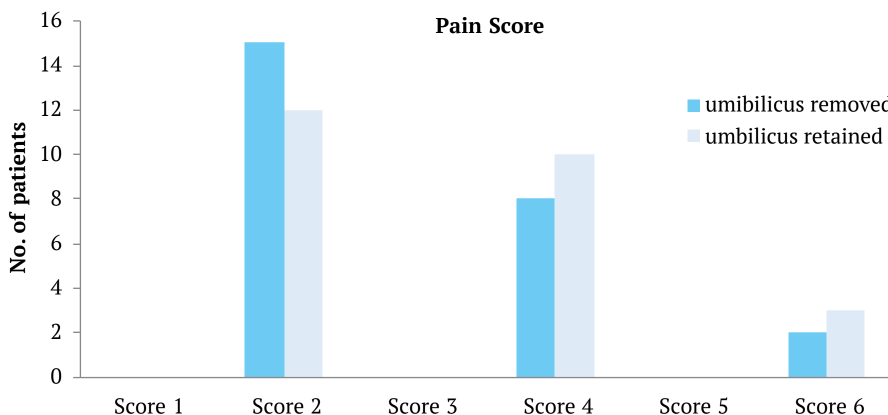

Figure 2. Pain score analysis of both groups

Source: compiled by the authors

Wound infection recorded in both groups of patients was analysed (Fig. 3). In the umbilectomy group, wound infection was noted in 5 patients (20%), and in the group where the umbilicus was preserved, wound infection was noted in 8 patients (32%) (Fig. 3), of which 2 patients developed flap necrosis of the umbilicus. All the patients were conservatively treated except for those with flap necrosis, where minimal debridement was done. The p-value for wound infection between the two groups was found to be 0.096. The statistical significance is less between the two groups, and hence, the groups are comparable.

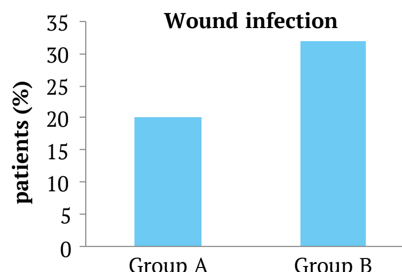

Figure 3. Wound infection in both groups

Source: compiled by the authors

Recurrence of symptoms recorded at the end of 6 months was analysed (Figure 4), and it was found that 1 out of 25 patients (4%) in the umbilicus removal group developed recurrence, whereas, in the group where the umbilicus was preserved, 4 patients (16%) out of 25 had local symptom recurrence (Fig. 4). The p-value for symptom recurrence, using the two-way chi-square test between the two groups, was 0.0073 and is considered statistically significant. Recurrence of symptoms was observed more in the groups where the umbilicus is retained.

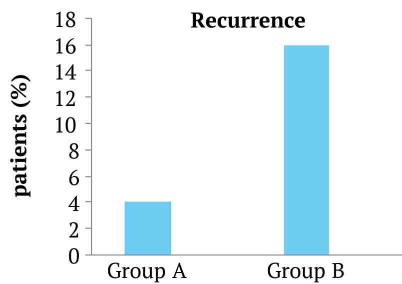

Figure 4. Recurrence in both groups
Source: compiled by the authors

Hospital stay, in terms of the number of days, recorded in both groups of patients was analysed. The average number of days patients stayed in the ward was found to be 3 days in the umbilicus removal group, whereas it was 4 days in the umbilicus preserving group. The p-value concerning hospital stay was found to be 0.7055. Although there is a small apparent advantage for the umbilicus removal group, it is not statistically significant.

15 patients were classified as satisfied, 3 patients were classified as highly satisfied, and 7 patients were classified as equivocal in the umbilicus removal group. In patients where the umbilicus is preserved, 15 patients were classified as satisfied, 4 patients were classified as highly satisfied, and 6 patients were classified as equivocal. The p-value between the two groups, while considering cosmetic satisfaction, was found as 0.644, and hence there is no statistical significance. This factor of cosmetic satisfaction must be interpreted while considering multiple other factors. In this study, we have included both male and female patients. Cosmesis concerning the umbilicus is more important from a feminine point of view. Although statistically this factor is considered non-significant in this study, there is a need for another study involving only females to achieve a proper interpretation of this factor (Table 3).

Table 3. Results with statistical analysis

S. No.	Factors analysed	Group A	Group B	p-value
1	Postoperative pain (Average score)	2.96	3.44	0.0183
2	Wound infection (%)	20%	32%	0.0960
3	Recurrence (%)	4%	16%	0.0073
4	Hospital stay (Days)	3 days	4 days	0.7055
5	Cosmetic satisfaction (Average score)	1.84	1.92	0.6440

Source: compiled by the authors

There were no reported incidences of any major complications related to anaesthesia or surgery, except for a few minor ailments which were not included in this study. Some of the factors noted included minor seroma formation and hematoma formation, which were resolved with routine care. Two patients in Group B, where the umbilicus was retained, also developed scarring that was not considered for analysis.

Postoperative outcomes such as postoperative pain, the incidence of wound infection, and recurrence were found to be comparatively higher in Group B where the umbilicus was retained. Length of hospital stay was only one day more on average in Group B. Cosmetic satisfaction was found to be better in Group B. Statistical analysis showed that postoperative pain and recurrence were higher in Group B.

DISCUSSION

The data collected in this study were compiled and analysed further. Previous studies on similar aspects were taken into account, and results from those studies were also analysed. Although many studies have been conducted on umbilical pathologies, the literature shows only a few studies that did a comparative analysis of postoperative outcomes following the removal and retention of the umbilicus.

Figure 1 shows the Universal Pain Assessment Tool, which is the standard pain scale used in this study. Figure 2 shows that only 8% of patients in Group A and 12% of patients in Group B experienced distressing pain. The majority of patients involved in the study experienced mild to moderate intensity pain (92% in Group A vs 88% in Group B), which indicates that the morbidity of the participants involved in this study is low ($p = 0.0183$). I. Hortu *et al.* [15] used the Numerical Rating Scale to assess the efficacy of postoperative analgesia at the umbilical port site. Numerical Rating Scale values were significantly lower in the study group where local anaesthetic was injected into the surgical wound (median of 2 vs 4; $p < 0.01$). Effective postoperative analgesia encourages early patient mobilisation, which is a basis for the Enhanced Recovery After Surgery (ERAS) protocol. N. Dubey *et al.* [16] used the Visual Analog Scale to compare the pain efficacy following local injection and also classified postoperative complications according to the Clavien-Dindo classification as major, minor, or moderate. Other pain scales in use include the verbal pain scale, verbal rating scale, and generic Linkert scale. The Universal Pain Assessment Tool used in this study effectively incorporates both the Numerical Rating Scale and the Visual Analog Scale.

Figure 3 shows the percentage of patients presenting with wound infection in both groups in this study (20% in Group A vs. 32% in Group B, p = 0.0960). Wound infection depends on multiple factors, including umbilical hygiene of the patient before surgery, microbial flora status for each patient, prior use of antibiotics, diabetes or other immunodeficiency conditions, and antibiotic preference post-surgery [16]. Third-generation cephalosporins were standard antibiotics used in this study to ensure that wound infection rates remained comparable. Wound infection was comparatively high (32%) in Group B, where the umbilicus was retained and only 20% in the umbilectomy group. Since the primary pathology was umbilical sepsis, the overall incidence of wound infection was high (26%) in this study. K. Tanaka *et al.* [17] discuss the importance of selecting the appropriate antibiotic in patients with umbilical infection and also the high prevalence of multidrug resistance in patients with umbilical infection. Umbilical microflora exhibits a high degree of cephalosporin resistance (46.1%). Coagulase-negative *Staphylococcus aureus* was found to be the most frequent colonising bacteria in the umbilicus. Hence, the choice of antibiotic has been found to influence the incidence of wound infection.

Figure 4 represents the comparison between Group A and Group B concerning the recurrence of symptoms. The recurrence rate was found to be 16% in Group B, which is higher than the 4% in Group A. Patients presenting with recurrence of symptoms were re-evaluated and recommended for an umbilectomy procedure, which was not a part of this study. Overall, 10% of the study population developed recurrence among those who presented with umbilical sepsis (p = 0.0073). T. Almas *et al.* [18] discussed the importance of preoperative MRI imaging in a selected group of patients to ascertain the depth of umbilical involvement. H. Huang *et al*. [19] discuss preoperative imaging, which provides an idea of the depth of involvement, the involvement of adjacent organs, and the probability of recurrence post-surgery.

Hospital stay was comparable in both groups of patients, measured as the number of days admitted as inpatients (mean days: 3 vs. 4, p = 0.7055). C.A. Steiner *et al.* [20] assessed the option of hospital-based ambulatory surgery to reduce the number of days of hospital stay, which thereby reduces the cost factors involved in treatment for patients. As hospitals become more capable of handling outpatient surgery, ambulatory surgery or outpatient surgery accounts for a majority of surgeries in recent years, showing an increase in the trend from inpatient care to hospital-based ambulatory surgery (57% in 1994 to 66% in 2014). A comparison of ambulatory surgery with inpatient surgery can provide insights into postoperative complications, length of hospital stay, and hospital cost savings.

Cosmetic satisfaction was assessed at 6 months of age based on a questionnaire-analysed score (mean score: 1.84 vs. 1.92, p = 0.6440). M.E. Miscia *et al*. [21] assessed cosmetic satisfaction in umbilical incisions and found that transumbilical incision provides better cosmetic results compared to subumbilical incisions, but with a higher incidence of wound infection in the transumbilical incision. Operating time, incidence of granuloma, and surgical site infection were also assessed [15]. J. Raakow *et al*. [22] evaluated long-term outcomes regarding cosmesis and chronic pain in umbilical surgery using the Patient and Observer Scar Assessment Scale (POSAS). Pain, itching, colour, pliability, thickness of scar, and relief are considered for cosmetic assessment in the POSAS score. Standardised cosmetic evaluations were done based on photographs of the patient's scar by independent surgeons. K.S. Yazar *et al*. [23] used an aesthetic outcome questionnaire given to patients and two independent surgeons to assess the effectiveness of the umbilicoplasty technique.

Overall, all the postoperative outcomes assessed were compiled and subjected to statistical analysis using the two-way chi-square test. Statistical significance was categorised by p-value. Based on the assessment of postoperative outcomes and their statistical significance, conclusions were drawn.

CONCLUSIONS

The primary objective of this study was to compare the postoperative patient outcomes between umbilectomy and umbilicus-retaining procedure for umbilical sepsis. This objective was reasonably achieved based on a carefully designed methodology, the surgical team involved, cooperation from patients, and proper data analysis. The evaluated postoperative outcomes, which have the potential to affect patient morbidity, were chosen based on multiple literature searches conducted prior to the start of this study. In this study, it was observed that the pain factor (p-value = 0.0183) and recurrence of symptoms (p-value = 0.0073) were comparatively higher in patients where the umbilicus was preserved. Hospital stay (p-value = 0.7055), wound infection (p-value = 0.0960) and cosmetic satisfaction (p-value = 0.6440) were comparable in both groups without any significant difference. Regarding the postoperative pain factor, in the umbilicus-retaining group, the umbilicus was fixed to the *linea alba* with Vicryl, which could have possibly caused increased pain in visual analogue scoring; thus, this statistical difference should be interpreted with caution. Regarding the recurrence of symptoms at the end of 6 months, patient umbilical hygiene post-discharge from the hospital could not be monitored between the two groups of patients, which could have impacted the statistical significance. Hence, in this study, considering the overall perspective authors have concluded that postoperative outcomes were comparable in both groups of patients.

The study is practically significant as it guides clinicians in deciding on umbilectomy in a given scenario. Retaining the umbilicus or removing the umbilicus should be judged based on the individual situation. Factors such as patient symptoms, duration of symptoms, scar, cosmesis, recurrence, emergency or elective settings, and patient preference must be carefully analysed, and a decision should be made accordingly. Future studies with additional parameters, if conducted, could provide more insights into the decision-making process of removing or retaining the umbilicus.

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CONFLICT OF INTEREST

All authors declare no conflict of interest in this article.

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Рандомізоване клінічне дослідження при пупковому сепсисі: порівняльний аналіз післяопераційних результатів омбілектомії та процедури збереження пупка

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Анотація. Метою цього дослідження було проаналізувати післяопераційні результати між умбілектомією та процедурами збереження пупка у пацієнтів з пупковим сепсисом. Пацієнти, які звернулися до хірургічної клініки з пупковим сепсисом, були випадковим чином розподілені на дві групи по 25 пацієнтів у кожній, відповідно до критеріїв включення та виключення. Післяопераційні фактори, такі як шкала болю, інфекція рани, рецидив, тривалість госпіталізації та косметичне задоволення, були проаналізовані під час подальшого спостереження. Спостережені параметри були статистично проаналізовані. Статистичний аналіз проводився за допомогою тесту хі-квадрат. Значення $p < 0,05$ вважалося статистично значущим. Середній вік на момент операції в обох групах був схожим (48 проти 49, $p = 0,9191$). На основі шкали болю після операції спостерігається суттєва різниця ($p = 0,0183$) між двома групами, де біль виявився більшим у пацієнтів, у яких пупок зберігався. Щодо інфекції рани як параметра, статистична значущість менша ($p = 0,096$). Тривалість госпіталізації в загальному числі днів була спостережена в обох групах, і суттєвих відмінностей не було ($p = 0,7055$). Популяція дослідження складалася з 23 чоловіків і 27 жінок, де косметичне задоволення пацієнтів оцінювалося і не було статистичної значущості ($p = 0,644$) між двома групами, незалежно від статі. Рецидив симптомів спостерігався більше в групі, де пупок зберігався ($p = 0,0073$). Враховуючи всі параметри та їх статистичну значущість, можна зробити висновок, що післяопераційні результати були порівнянні в обох групах пацієнтів. Результати цієї роботи надають хірургам уявлення про концепцію збереження або видалення пупка та його вплив на післяопераційну морбідність пацієнтів з пупковим сепсисом

Ключові слова: косметичний результат; шкала болю; рецидив; інфекція; пупок; хірургія

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Influence of mesenchymal stromal cells of different origins on behavioural reactions of rats with cerebral ischemia-reperfusion

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Abstract. A new direction in cell therapy for ischemic stroke has been the use of mesenchymal stromal cells, which have shown a positive impact on functional changes in the central nervous system due to their neuroprotective effects, reduction of ischemia-reperfusion-induced injury, inhibition of ischemia-reperfusion-induced apoptosis, and restoration of motor function. This study aimed to investigate the effect of mesenchymal stromal cells of different origins, their lysate, and citicoline on the functional state of the central nervous system in rats with experimental brain ischemia-reperfusion. The study considered the effect of mesenchymal stromal cells derived from human umbilical cord Wharton's jelly, human and rat adipose tissue, rat embryonic fibroblasts, as well as mesenchymal stromal cell lysate and citicoline on the emotional and behavioural responses of sexually mature Wistar rats (3-4 months) weighing 160-190 g. The behavioural responses of rats were studied using the open field test on the 7th and 14th days of the experiment; the following behavioural acts were recorded: ambulation (locomotion), climbing, rearing, and grooming. The significance of differences was determined using the non-parametric Mann-Whitney U test. It was established that after ischemia-reperfusion, animals with control pathology showed a significant decrease in the duration of episodes of ambulation in peripheral and central squares, vertical locomotor activity, and exploratory activity compared to the sham-operated group. In rats that received citicoline and transplanted human umbilical cord Wharton's jelly mesenchymal stromal cells, a significant increase in the duration of episodes of horizontal locomotor activity was observed compared to other types of stem cells and the control. Intravenous administration of rat embryonic fibroblasts increased the emotional activity of the experimental animals. The least impact on locomotor and adaptive exploratory activity in rats with ischemia-reperfusion was registered in groups of animals that received mesenchymal stromal cells from human and

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rat adipose tissue, as well as mesenchymal stromal cell lysate. The practical significance of the study lies in the search for the most effective class of stem cells with neuroprotective properties for the creation of an injectable drug for intravenous transplantation in the treatment of patients with acute ischemic stroke

Keywords: stem cells; ischemia-reperfusion; adaptive behaviour; emotional activity; open field test

INTRODUCTION

Vascular pathology, and the associated ischemic stroke, according to the 2019 report from the American Heart Association's Statistics Committee, are leading causes of neural dysfunction, characterised by high morbidity, disability, and mortality [1]. The Stroke Expert Collaboration Group [2] has highlighted the substantial socioeconomic impact of stroke on households, underscoring the immense burden this disease places on families, communities, and nations. Stem cell transplantation has emerged as a promising avenue within regenerative strategies. Stem cells possess the unique ability to differentiate into various cell types and continuously self-renew [3]. Y. Zhang *et al*. [4] have demonstrated that stem cell transplantation can effectively treat neurodegenerative diseases, including stroke. Among stem cells, mesenchymal stromal cells (MSCs) are the most commonly employed cell type in advanced therapies for a wide range of diseases, many of which involve inflammation [5]. MSCs constitute a heterogeneous population of stem cells and exhibit neuroprotective properties when used to treat ischemic stroke. These properties manifest in reduced ischemic-reperfusion injury, inhibition of ischemia-reperfusion-induced apoptosis, and restoration of motor function [6]. Numerous preclinical studies have demonstrated the ability of MSCs to mitigate tissue damage, thereby promoting functional recovery through various mechanisms, including immunomodulation, pro-angiogenic signalling, secretion of neurotrophic factors, and neuronal differentiation [4, 7]. For instance, a research group led by J. Li *et al*. [7] discovered that MSCs can facilitate cell migration, angiogenesis, immunomodulation, neuroprotection, and the restoration of neural circuits. The paracrine action of MSCs can also provide neurotrophic effects and enhance functional recovery. In a few clinical studies, notably the STARTING-2 study conducted by a research team led by J.W. Chung *et al.* [8], autologous modified MSCs demonstrated significant improvements in lower limb motor function in patients with chronic ischaemic stroke. Furthermore, MSCs offer several advantages over other stem cell types due to easier procurement methods, a low risk of tumorigenicity, and the absence of ethical concerns [9]. However, several questions remain regarding the nature of the cell product, the delivery route, and the optimal cell dose and administration schedule [10]. MSCs can be derived from adipose tissue, dental buds and pulp, bone marrow, liver or umbilical cord, umbilical cord blood, and placenta [11]. Previous studies [12] have shown that different types of MSCs can influence mortality and neurological deficits in rats subjected to a model of brain ischaemia-reperfusion (IR).

Numerous studies investigating the effects of various factors on the behavioural responses of rats have not addressed how ischaemia-reperfusion injury of the brain in rats, or correction using MSCs, affects adaptive exploratory behaviour. Therefore, this study aimed to investigate the effects of MSCs from different sources, MSCs lysate, and citicoline on the functional state of the central nervous system (CNS) in rats with experimental brain IR.

MATERIALS AND METHODS

The study was conducted between 2021 and 2023 at the educational and research laboratory for preclinical evaluation of new drugs and biologically active compounds "Farmadar" of the National Pirogov Memorial Medical University, Vinnytsya (NPMMU) (technical competence certificate No. 031/18 valid until 31.10.2023), using sexually mature (3-4 months old) Wistar rats weighing 160-190 g, bred in the vivarium of NPMMU. Animals were kept in standard vivarium conditions with natural lighting and had free access to water and food. MSCs and MSCs lysate were obtained from the Institute of Molecular Biology and Genetics (IMBG) of the NAS of Ukraine. The transfer of cells was carried out based on the Agreement on Scientific Cooperation between IMBG NASU and NPMMU dated 22.09.2017. Cell viability was assessed by staining the cell sample for live-dead with trypan blue while counting the number of cells in the Goryaev chamber, with viability ranging from 95-98%. Cell quality was checked on a flow cytometer for the presence of minimal MSC markers – CD34, CD73, CD90, CD105. Occasionally, the ability of cells to differentiate into adipocytes and chondrocytes, which is characteristic of MSCs, was checked. Cell morphology was constantly monitored by assessing the shape (spindle-shaped).

Rats were used as experimental animals because the angioarchitecture and morphology of the rat cerebral cortex are similar to those of humans. An experimental model of IR was created by bilaterally ligating the internal carotid arteries (ICAs) for 20 minutes under propofol anaesthesia (Propofol-novo, LLC Novofarm-Biosyntez, Ukraine, 60 mg/kg). The chosen model reflects the clinical picture of cerebral infarction and is optimal for experimental studies of potential neuroprotective substances [13]. The distribution of animals into experimental groups is presented in Figure 1.

The test substances were administered intravenously into the femoral vein immediately after IR, as early transplantation of MSCs has been shown to promote better neurological recovery, reduce infarct volume, and require a smaller number (1×10^6) of donor cells to achieve a positive effect [14]. Adaptive behaviour and emotional reactivity of the animals were assessed using the open field test twice: on the 7th and 14th days after the experimental model of brain IR. Observations of animals in the open field were carried out using a standard setup – a rectangular chamber (100×100 cm) with transparent walls 40 cm high. The floor was divided into 25 (20×20 cm) equal squares [15]. Recording was performed using the EthoWatcher: A TOOL FOR BEHAVIORAL AND VIDEO-TRACKING ANALYSIS IN LABORATORY ANIMALS software-computer complex for studying the behavioural reactions of animals, for two days

at the same time of day under the same conditions in the laboratory, which was located in the vivarium of NPMMU. The exposure time for each animal in the open field was 3 minutes. The special room had a stable temperature (18-22°C), relative humidity (40-60%) and illumination of 250 lux. The first and second testing of the experimental animals was carried out to study the effect of treatment on the behavioural reactions of animals with brain IR. In the individual behaviour of rats, the following behavioural acts were recorded: ambulation (locomotion) – gradual movement of the animal in a horizontal plane (crossing central and peripheral squares); according to indicators of vertical locomotor activity – climbing (wall vertical stance on hind legs) and rearing (free vertical stance on hind legs) and emotional activity – grooming (number of washes and number of boluses during defecation).

Group 1 (n = 10): sham-operated animals + intravenous injection of 0.9% sodium chloride solution, 2 mL/kg

Group 2 (n = 40): ischemia-reperfusion + intravenous injection of 0.9% sodium chloride solution, 2 mL/kg

Group 3 (n = 20): ischemia-reperfusion + intravenous transplantation of human umbilical cord Wharton's jelly MSCs, 10^6 cells/animal

Group 4 (n = 20): ischemia-reperfusion + intravenous transplantation of rat embryonic fibroblasts, 10^6 cells/animal

Group 5 (n = 25): ischemia-reperfusion + intravenous transplantation of human adipose tissue MSCs, 10^6 cells/animal

Group 6 (n = 25): ischemia-reperfusion + intravenous transplantation of rat adipose tissue MSCs, 10^6 cells/animal

Group 7 (n = 25): ischemia-reperfusion + intravenous injection of cell lysate from human umbilical cord Wharton's jelly MSCs, 0.2 mL/animal

Group 8 (n = 25): ischemia-reperfusion + intravenous injection of citicoline ("Neuroxon", Corporation "Arteium", Ukraine), 250 mg/kg

Figure 1. Distribution of animals in the experiment

Source: compiled by the authors

Statistical analysis of the obtained data was performed using Microsoft Excel 2010 and Statistica 6.1 software. The significance of differences was assessed using the unpaired nonparametric Mann-Whitney U test. Differences between the measured parameters were considered statistically significant at $p < 0.05$. All manipulations with experimental animals were carried out following the International Rules and Standards of the Directive of the Council of the European Communities 86/609/EEC and the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [16] and the Law of Ukraine dated 21.11.2006 No. 3447-IV "On the Protection of Animals from Cruelty" [17]. The research protocol was approved by the Bioethics Committee of NPMMU (protocol No. 2 dated January 31, 2024).

RESULTS

The open field test allows for the examination of rat behaviour by assessing levels of emotional and behavioural reactivity, the dynamics of individual behavioural elements, and locomotor stereotypy (Table 1).

Table 1. Comparison of behavioural response indicators in rats with a model of brain ischemia-reperfusion and amid a correction (M ± m)

Sham-operated						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days
Ambulation (peripheral)	2.26 ± 1.03	0.30 ± 0.18	18.00 ± 1.22	12.43 ± 1.68	115.27 ± 3.28	101.20 ± 9.48
Ambulation (centre)	5.05 ± 3.28	21.11 ± 19.17	3.14 ± 0.60	1.43 ± 0.52	11.97 ± 1.81	3.05 ± 1.61
Climbing	13.89 ± 3.07	34.07 ± 12.56	11.43 ± 0.88	5.86 ± 1.83	23.47 ± 2.87	14.82 ± 4.67
Rearing	33.41 ± 22.77	0 ± 0	0.86 ± 0.50	0 ± 0	1.52 ± 0.85	0 ± 0
Grooming	96.90 ± 14.06	41.02 ± 10.57	2.43 ± 0.88	3.29 ± 0.81	24.02 ± 4.55	40.26 ± 7.46
Ischaemia-reperfusion (IR)						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.16 ± 0.17	0.65 ± 0.71	16.00 ± 3.01	12.83 ± 2.14	74.05 ± 11.38*	91.66 ± 13.64
Ambulation (centre)	12.03 ± 6.49	3.35 ± 2.20	1.83 ± 0.59	1.50 ± 0.68	5.23 ± 1.91*	5.65 ± 2.26
Climbing	16.14 ± 8.12	20.41 ± 12.40	9.83 ± 2.76	5.50 ± 1.46	20.50 ± 4.29	12.11 ± 3.19
Rearing	5.02 ± 5.50	14.42 ± 15.79	0.17 ± 0.18	0.50 ± 0.55	0.28 ± 0.30	0.90 ± 0.99
Grooming	61.13 ± 12.59	61.51 ± 18.8	1.67 ± 0.23	2.17 ± 0.34	13.91 ± 4.36	16.36 ± 6.18*
IR + human Wharton's jelly MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.29 ± 0.94	5.72 ± 5.48	14.57 ± 2.18	12.57 ± 2.89	114.13 ± 12.58#	99.57 ± 21.15

Table 1. Continued

	Latent period		Number of episodes		Duration of episodes	
IR + human Wharton's jelly MSCs						
Ambulation (centre)	16.03 ± 10.58	5.16 ± 2.76	2.57 ± 0.88	2.00 ± 0.53	8.63 ± 3.10	6.98 ± 3.64
Climbing	10.99 ± 3.23	16.94 ± 4.76	7.14 ± 1.55	6.14 ± 1.79	11.02 ± 3.70*	12.52 ± 3.63
Rearing	16.36 ± 11.41	0 ± 0	0.43 ± 0.32	0 ± 0	0.43 ± 0.36	0 ± 0
Grooming	62.27 ± 22.98	40.09 ± 9.58	2.86 ± 1.01	3.00 ± 0.71	14.74 ± 4.70	19.18 ± 4.01*
IR + rat embryonic fibroblasts						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.50 ± 1.07	0.55 ± 0.44	14.50 ± 1.12	15.67 ± 3.17	87.10 ± 13.45*	76.94 ± 10.03
Ambulation (centre)	6.00 ± 6.04	3.24 ± 3.10	2.17 ± 0.34	1.83 ± 1.00	7.57 ± 2.49	4.13 ± 2.42
Climbing	8.83 ± 1.29	15.57 ± 7.12	7.50 ± 0.97	8.17 ± 2.67	13.98 ± 3.64	17.81 ± 6.64
Rearing	15.51 ± 17.00	30.16 ± 26.27	0.17 ± 0.18	0.50 ± 0.37	0.28 ± 0.30	0.55 ± 0.43
Grooming	35.84 ± 8.34*	13.41 ± 4.64#	4.17 ± 0.52#	4.33 ± 0.67#	39.12 ± 11.83#	25.66 ± 5.78
IR + human adipose MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.55 ± 0.39	3.05 ± 1.80	13.50 ± 2.03	6.83 ± 1.15*#	105.34 ± 14.84	53.99 ± 12.44*
Ambulation (centre)	12.12 ± 8.53	0 ± 0	1.83 ± 0.77	0.50 ± 0.24	4.95 ± 2.23	3.05 ± 1.80
Climbing	34.23 ± 23.13	0.39 ± 0.40*	6.67 ± 2.03	1.00 ± 0.75*	14.89 ± 4.28	2.24 ± 1.66*#
Rearing	8.70 ± 9.53#	0 ± 0	0.33 ± 0.37	0 ± 0	0.54 ± 0.59	0 ± 0
Grooming	52.65 ± 17.27	43.62 ± 31.31	2.83 ± 0.87	0.83 ± 0.44*	21.84 ± 8.32	4.22 ± 2.32*#
IR + rat adipose MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.01 ± 0.01	0.01 ± 0.01	14.50 ± 3.30	7.33 ± 1.08*	69.48 ± 14.09*$	63.47±10.50*
Ambulation (centre)	7.27 ± 5.68	0 ± 0	1.00 ± 0.40	0 ± 0	2.99 ± 1.57*	0 ± 0
Climbing	6.21 ± 3.12	13.73 ± 6.31	5.50 ± 2.80	3.00 ± 0.69	11.63 ± 6.65	7.92 ± 2.66
Rearing	11.84 ± 8.21	0 ± 0	0.67 ± 0.46	0 ± 0	1.17 ± 0.82	0 ± 0
Grooming	54.41 ± 23.71	25.10 ± 9.43	4.33 ± 0.88#	2.00 ± 0.69	29.14 ± 13.40	31.30 ± 18.22
IR + cell lysate from human Wharton's jelly MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.55 ± 1.70	0.52 ± 0.36	16.83 ± 4.19	9.83 ± 3.27	75.48 ± 13.34*$	69.36 ± 6.36*
Ambulation (centre)	8.38 ± 7.92	3.17 ± 3.48	1.67 ± 0.73	0.50 ± 0.24	6.06 ± 2.12	0.78 ± 0.38
Climbing	20.73 ± 14.87	8.79 ± 7.17	9.00 ± 3.49	3.33 ± 2.44	20.95 ± 7.94	6.50 ± 4.23
Rearing	27.63 ± 19.87	0 ± 0	0.50 ± 0.37	0 ± 0	0.74 ± 0.57	0 ± 0
Grooming	48.03 ± 17.59*	53.74 ± 29.40	3.00 ± 1.33	1.83 ± 0.96	16.12 ± 8.83	15.25 ± 8.06*
IR + Citicoline						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	3.10 ± 1.21	0 ± 0	14.17 ± 2.07	11.33 ± 2.11	125.90 ± 10.91#	79.66 ± 16.34
Ambulation (centre)	3.03 ± 3.32	11.36 ± 10.25	2.50 ± 0.68	0.83 ± 0.44	9.68 ± 2.34	1.42 ± 0.70
Climbing	13.95 ± 2.02	6.03 ± 2.55*	8.33 ± 2.01	5.83 ± 1.40	14.52 ± 4.24	10.91 ± 2.48
Rearing	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Grooming	47.40 ± 7.58*	43.19 ± 24.74	3.00 ± 0.75	2.33 ± 0.78	21.97 ± 7.34	19.28 ± 7.67

Notes: * – $p < 0.05$ compared to the sham-operated rats index; # – $p < 0.05$ compared to the control pathology index; $ – $p < 0.05$ compared to the citicoline group index
Source: compiled by the authors

The analysis of rat behaviour in the open field test, based on the "number of crossings" parameter, characterises the overall level of activation. As shown in Table 1, on the 7th day after IR, in animals with control pathology, the duration of ambulation episodes in peripheral and central squares decreased on average by 35.8% and 56.3% respectively ($p < 0.05$), compared to the sham-operated group. Therapeutic correction of IR injury using embryonic fibroblasts and MSCs from rat adipose tissue, as well as MSCs lysate, did not have a positive effect on the horizontal motor activity of the experimental animals, as observed in a significant decrease in the duration of episodes of hor izontal locomotor activity on average by 24.4%, 39.7%, and 34.5%, respectively, compared to the sham-operated group. The use of citicoline, as well as the transplantation of human umbilical cord Wharton's jelly MSCs during the study period, proved to be the most effective methods of treating rats with IR brain injury when studying behavioural reactions in the open field, as manifested in an increase in the duration of episodes of horizontal locomotor activity on

average by 70.0% and 54.1% ($p < 0.05$), respectively, compared to the control.

On the 14th day of the experiment, there was a trend towards a decrease in horizontal motor activity in the experimental groups of rats during open field testing compared to the results obtained on the 7th day of observation (Table 1). A decrease in horizontal locomotor activity with crossings of peripheral squares was also observed in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate, on average by 46.6%, 37.3%, and 31.5% respectively ($p < 0.05$), compared to the sham-operated group.

Vertical motor activity indicates the exploratory behaviour of the animals. A greater number of stances suggests a lower level of anxiety. Therefore, in the studied periods of the experiment, in groups of rats with control pathology and with its correction, compared to the sham-operated group, a tendency towards a decrease in vertical locomotor activity and exploratory behaviour was observed. In rats that received MSCs from human adipose tissue in the context of IR, on the 14th day of the experiment (Table 1), wall rearing was significantly less frequent compared to sham-operated animals and those with control pathology.

Emotional activity in the experimental animal groups on the 14th day of observation (Table 1) led to a decrease in the duration of grooming episodes by an average of 2-3 times compared to the sham-operated group. At the same time, a significant increase in the number of grooming episodes was observed on the 7th and 14th days of the experiment in the group of animals that received rat embryonic fibroblasts as a correction, on average 2.5 and 2 times, respectively, as well as on the 7th day of observation in the group of rats that were transplanted with MSCs from rat adipose tissue in the context of IR, on average 2.6 times, relative to the control pathology indicator. Acts of defecation and urination during the study in the open field conditions were absent in animals of all groups.

The 20-minute model of IR ICAs induced functional disturbances in the CNS of rats, manifested by a significant decrease in the number of vertical stances, squares crossed, and entries into the centre, indicating a suppression of locomotor and exploratory activity. There was also a tendency towards a decrease in emotional activity, and reduced feelings of anxiety and fear under stressful conditions, as represented by the open field test. The therapeutic use of citicoline and human umbilical cord Wharton's jelly MSCs in rats with IR brain injury led to a significant increase in the duration of episodes of spontaneous horizontal motor activity compared to the group of animals with control pathology. The use of rat embryonic fibroblasts as a therapeutic correction contributed to an increase in the emotional activity of the experimental animals with ischemic-reperfusion brain injury.

DISCUSSION

There is no consensus in the literature regarding the motivations that determine rat behaviour in the open field. Most authors believe that the factors determining this behaviour are exploratory motivation and so-called emotional reactivity [18]. The latter, in turn, is associated with such brain structures as the limbic system and hippocampus. For example, a study by Q. Lei *et al.* [19] using a mouse model with permanent middle cerebral artery occlusion found that transplantation of MSCs derived from the bone marrow of knockout ($SRC3^{-/-}$) mice had a minimal impact on reducing cognitive disorders, motor impairments, and anxiety, as assessed by the Morris water maze test and open field test. However, in another study conducted by M.K. Tobin *et al.* [20], it was shown that intravenous administration via the retro-orbital sinus of MSCs contributed to functional recovery in experimental animals with a 90-minute occlusion of the right middle cerebral artery according to the open field test.

Ischemia leads to progressive cerebral injury. Clinically, treating ischemic stroke remains challenging. Therefore, an increasing number of researchers are focusing on finding effective methods to reduce ischemic reperfusion injury in cerebral ischemia. MSC transplantation may be effective in slowing or stopping this process [21, 22]. Recent studies have shown that MSC transplantation therapy has a positive impact on the course of cerebral ischemia [23, 24]. In a study by J. He *et al.* [23], it was found that olfactory mucosa MSCs during cerebral ischemia-reperfusion attenuate apoptosis and oxidative stress in models of ischemic stroke, reduce infarct volume, and improve neurological deficits in rats. Many preclinical studies, as well as clinical trials, have demonstrated the efficacy of MSC therapy in preclinical stroke models and the safety of MSC treatment in clinical trials [14, 20, 25]. Researchers L. Zhou *et al*. [24] determined that MSC therapy is safe and effective for acute, subacute, and chronic ischemic stroke. In the acute phase of ischemic stroke in rats, MSC therapy enhanced neuronal plasticity and functional recovery by protecting mitochondria, suppressing neuronal pyroptosis and apoptosis, and reducing microglia activation in the penumbra. In the subacute phase of ischemic stroke, human umbilical cord MSC therapy effectively improved behavioural deficits, reduced infarct volume, and glial scar formation, and promoted angiogenesis in the ischemic penumbra. In a chronic stroke model in rats, transplantation of human umbilical cord MSCs maintained blood-brain barrier integrity, improved behavioural responses in animals, and promoted neurogenesis and angiogenesis.

In the study of Y. Chen *et al.* [14], it was noted that sensory and motor functions were significantly improved following the therapeutic application of human MSCs in rats that had undergone ischemic stroke. Additionally, a significant improvement in the recovery of behavioural responses and a reduction in infarct volume was observed in the group of animals that received an intravenous injection of $1x10^6$human MSCs per animal.

However, the extent of recovery following MSC treatment is not fully understood. Most clinical trials using stem cell therapy have applied this treatment to patients with subacute stroke [25-27]. Other experiments have focused on patients with chronic stroke [28, 29]. For instance, research by K.R. Nalamolu *et al.* [26] established that treatment with MSCs derived from human umbilical cord blood mitigates post-stroke brain damage and significantly improves neurological recovery in both male and female rats with induced stroke. In previous preclinical studies, scientists have demonstrated better neurological recovery after stroke in rats that received exosomes isolated from MSCs, as manifested by a reduction in infarct size and ipsilateral hemisphere swelling, preservation of neurological function, and facilitated recovery of rats [27]. Based on the

results obtained, the authors concluded that treatment with exosomes secreted from MSCs, under appropriate experimental conditions, attenuates post-stroke brain damage and improves neurological deficits. Y. Ogawa *et al.* [28, 29] found in their studies that intravenous transplantation of bone marrow mononuclear cells was insufficient for the treatment of chronic stroke, however, cell therapy-assisted training was effective.

The results of the few clinical trials have been inconsistent, particularly the intravenous transplantation of allogeneic adipose-derived MSCs (AMASCIS) for acute ischemic stroke (AMASCIS). E. de Celis-Ruiz *et al.* [30] conducted a randomised, double-blind, placebo-controlled, single-centre, pilot clinical trial that included elderly patients with moderate to severe acute stroke, as measured by the National Institutes of Health Stroke Scale (NIHSS) of 8-20 points. A two-week course of MSC treatment did not show significant differences in outcomes after 24 months of follow-up (median NIHSS scores were not significantly lower compared to the placebo group). For this reason, the authors of this study decided to determine whether MSCs of different origins and MSCs lysate could be beneficial for treating acute ischemic stroke. To do this, rats were subjected to 20 minutes of cerebral ischemia-reperfusion and intravenously transplanted with the studied MSCs immediately after reperfusion; the behavioural adaptation of the rats was assessed using the open field test.

J. Zheng *et al.* [31] aimed to investigate whether mesenchymal stem cells (MSCs) could improve their survival and alleviate cerebral ischemic injury in an ischemic microenvironment. The researchers used ischemic brain tissue to culture MSCs and evaluated the functional changes in rats after the administration of pre-treated MSCs with brain tissue following stroke. It was established that transplantation of MSCs promoted proliferation and the release of growth factors, enhanced neurogenesis, reduced behavioural changes, decreased infarct size, and suppressed apoptosis, representing an effective strategy for the treatment of cerebral ischemic injury.

J.-R. Chen e*t al.* [32] found that MSC transplantation significantly improved behavioural deficits in rats associated with induced ischemia-reperfusion. Behavioural improvements in the performance of elevated body swing test and forelimb stride length were achieved as early as 7 days after MSC implantation. The authors observed a sustained improvement in the elevated body swing test and stride length during locomotion, but only a slight improvement at one time point for the adhesive sensory stimulus-induced test. According to the authors, this suggests that MSC transplantation primarily improves motor functional recovery.

In the study by M.K. Tobin *et al.* [20], a significant improvement in functional recovery of the CNS was demonstrated in experimental animals with ischemic stroke that received treatment using interferon-γ-activated mesenchymal stem cells (aMSCγ). In animals that received aMSCγ, a significant reduction in infarct size and inhibition of microglial activation was observed. In another study, conducted by Y.S. Fu *et al.* [33], it was shown that MSCs derived from human umbilical cord Wharton's jelly, when transplanted into rats with middle cerebral artery occlusion, had therapeutic benefits for chronic ischemic stroke. The open field test enabled for the detection of changes in adaptive behaviour and emotional reactivity in animals, as well as disorders in locomotor stereotypy resulting from ischaemic reperfusion injury to the brain in rats. It also assessed the corrective effects of transplanting MSCs of various origins, MSCs lysate, and the reference drug citicoline. Thus, the analysed data are consistent with those of other researchers; however, the results of this study demonstrated an increase in the duration of episodes of spontaneous horizontal motor activity when using citicoline and MSCs from human umbilical cord Wharton's jelly. Intravenous transplantation of rat embryonic fibroblasts promoted increased emotional activity in experimental animals with IR brain injury. The least impact on locomotor and exploratory activity in rats with IR was recorded in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate.

CONCLUSIONS

The results of the study revealed a positive therapeutic effect of MSCs of various origins, MSCs lysate, and the reference drug citicoline on the functional state of the CNS in rats with experimental brain IR. An experimental study of the effects of 20 minutes of brain ischemia-reperfusion on the behaviour of rats in the open field test revealed a significant decrease in the duration of episodes of ambulation of peripheral and central squares by an average of 35.8% and 56.3%, respectively, on day 7, and a tendency towards a decrease in vertical locomotor, exploratory and emotional activity, a decrease in feelings of anxiety and fear, compared to the sham-operated group of rats. The use of citicoline and transplantation of MSCs from human umbilical cord Wharton's jelly proved to be the most effective treatment methods, as evidenced by an increase in the duration of episodes of horizontal locomotor activity by 70.0% and 54.1% ($p < 0.05$), respectively, compared to the group of animals with control pathology. The least impact on locomotor and exploratory activity in rats with ischemia-reperfusion was recorded in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate: a significant decrease in horizontal locomotor activity with the crossing of peripheral squares was observed on day 14 of the experiment, which averaged 46.6%, 37.3%, 31.5%, respectively, compared to the sham-operated group of rats. The use of rat embryonic fibroblasts as a therapeutic correction promoted an increase in the emotional activity of experimental animals with cerebral ischemia-reperfusion injury with an increase in the number of grooming episodes by 2.5 times; when MSCs from rat adipose tissue were administered – by 2.6 times, relative to the control pathology indicator ($p < 0.05$).

The data obtained from this research will be used to explore new avenues for treating brain ischemic reperfusion injury. These results will provide experimental evidence to justify clinical trials of an injectable drug based on the most effective class of MSCs for a new application, namely as a neuroprotectant in patients with ischemic stroke.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Вплив мезенхімальних стромальних клітин різного походження на поведінкові реакції щурів із церебральною ішемією-реперфузією

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Анотація. Новим напрямком клітинної терапії при ішемічному інсульті стало використання мезенхімальних стромальних клітин, що виявило позитивну динаміку на функціональні зміни в центральній нервовій системі, завдяки їхньому нейропротекторному ефекту, зменшенню ішемічно-реперфузійно-індукованого пошкодження, інгібуванню ішемічно-реперфузійно-індукованого апоптозу та відновленню рухової функції. Мета роботи полягала у вивченні впливу мезенхімальних стромальних клітин різного походження, їх лізату та цитиколіну на функціональний стан центральної нервової системи щурів з експериментальною ішемією-реперфузією головного мозку. Розглянуто вплив мезенхімальних стромальних клітин, отриманих із пуповинної тканини людини, жирової тканини людини та щура, ембріональних фібробластів щура, а також лізату мезенхімальних стромальних клітин та цитиколіну на стан емоційно-поведінкових реакцій статевозрілих щурів (3-4 міс) лінії Вістар із масою тіла 160-190 г. Поведінкові реакції щурів досліджували за тестом «відкрите поле» на 7-у та 14-у добу експерименту; реєстрували такі поведінкові акти як амбуляція (локомоція), кламбінг, рерінг і грумінг. Достовірність відмінностей визначали з використанням непараметричного U критерію Манна-Уітні. Встановлено, що після ішемії-реперфузії у тварин з контрольною патологією, тривалість епізодів амбуляції периферійних та центральних квадратів, вертикальної локомоторної активності та дослідницької активності достовірно знижувались, порівняно з групою псевдооперованих щурів. У щурів, яким вводили цитиколін і трансплантували мезенхімальні стромальні клітини Вартонових драглів пуповини людини, виявлено достовірне збільшення тривалості епізодів горизонтальної локомоторної активності, порівняно з іншими типами стовбурових клітин та контролем. Внутрішньовенне введення ембріональних фібробластів щура підвищувало емоційну активність піддослідних тварин. Найменший вплив на локомоторну та орієнтувально-дослідницьку активності у щурів з ішемією-реперфузією було зареєстровано в групах тварин, що отримали мезенхімальні стромальні клітини із жирової тканини людини та щура, а також лізат мезенхімальних стромальних клітин. Практична цінність дослідження полягає в пошуку найбільш ефективного за нейропротекторними властивостями класу стовбурових клітин з метою створення на його основі ін'єкційного препарату для внутрішньовенної трансплантації при лікуванні хворих із гострим ішемічним інсультом

Ключові слова: стовбурові клітини; ішемія-реперфузія; адаптивна поведінка; емоційна активність; тест «відкрите поле»

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Assessment of physical (somatic) health of young men in the construction of health improving strength training

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Abstract. The systematic review aimed to determine the effect of strength training on physiological and morphological adaptive capacities in healthy young men. A search was conducted for randomised clinical trials containing information on the effect of resistance exercise on physical and functional changes in young men aged 18-45 years. As a result, 13 publications that met the search criteria were found, of which 9 studies were selected after excluding inappropriate ones. Most of the studies were assessed as good-quality research with a score of 7-9 on the PEDro scale. Long-term strength training has been shown to significantly improve strength, muscle volume and explosive abilities in trained individuals. Strength training 3 times a week can increase lean body mass and left ventricular mass index, with a decrease in body fat. Light training stimulates hypertrophy of the gastrocnemius muscle more than heavy training, which has a greater impact on the middle and lateral heads of the gastrocnemius muscle. The sequence of exercises does not affect the increase in maximum strength, but the effect on pectoral muscle hypertrophy may be better when performing multi-joint exercises after isolated exercises. The appearance of microRNAs does not show specificity in the early acute state of training, with changes in expression observed 8 hours after training. The duration of weightlifting training has a positive effect on anthropometric and physiological parameters, but not on biochemical parameters

Keywords: resistance exercise; systematic review; functional adaptation; muscle hypertrophy; sports medicine

INTRODUCTION

Lack of physical activity can have significant negative health consequences. Systematic exercise can improve health by contributing to the prevention of cardiovascular, musculoskeletal, and metabolic disorders [1]. Strength training (ST) is becoming increasingly popular among young men as a means of improving physical fitness and increasing muscle mass and strength [2]. However, many young men lack adequate information about safe and effective training methods. To properly prescribe and monitor ST, clinicians need to know the health effects of exercise to design optimal exercise programmes and reduce the risks of injury and complications. There are many studies describing the general effects of ST, but there is a lack of systematic reviews that evaluate specific changes in functional and physical status in men. This study will provide a comprehensive assessment of the impact of ST on somatic health, considering physiological and biochemical changes that occur in the body.

Muscle strength is defined as the ability to produce force against resistance [3]. The stress-recovery-adaptation model assumes that training stress causes the body to recover and adapt to the load, which is manifested in increased performance. This cycle of stress, recovery, and adaptation is the basis of an effective ST programme [2]. The regeneration of damaged tissues occurs through the transfer of daughter nuclei from satellite cells after they have multiplied and fused. Over time, bones also increase their mineral density to withstand increasing loads [4].

Resistance training helps to increase muscle strength and mass, improve balance, bone mineral density, walking speed, and the ability to climb stairs [5]. They reduce systolic blood pressure (SBP), back pain, and local and total fat mass, and increase intestinal transit time, which can reduce the risk of colon cancer in healthy men [6]. The mechanism of prevention of metabolic syndrome and type 2 diabetes mellitus is associated with a decrease in

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visceral fat and an increase in lean body mass, as well as the ability to insulin-independent glucose uptake, which is activated by muscle contraction [7]. ST is an effective method of counteracting sarcopenia, which is characterised by a decrease in muscle mass and strength. Scientific studies show that ST significantly improves grip strength and upper limb function in patients with sarcopenia [8]. Individually tailored and periodised ST programmes can slow the progression or development of sarcopenia by increasing muscle strength [9].

The effectiveness of different modes of training loads for the development of physical qualities in students was studied by I. Skibytskyi *et al.* [10] in an experiment during gymnastics classes. As determined, the greatest increase in muscle mass was observed at 80% of the maximum weight. For strength development, 90% of the maximum weight was the most effective, while 60-70% of the maximum weight was the best for strength endurance. The recommended training regimes include 3 sets with a 3-minute rest for strength development, 3 sets of 12 repetitions for muscle growth, and 3 sets with a 60-second rest for strength endurance.

The study of the relationship between the risk of low muscle mass and the frequency and duration of ST involved 126,339 people from Korea. The results of the study by J.H. Park *et al.* [11] showed that regular ST (3-4 days a week) reduces the risk of low muscle mass by 22% and performing more than 5 days a week – by 27%. The duration of training also matters training for 1-2 years reduces the risk by 19%, and for more than 2 years – by 41%. The greatest effect is achieved with training for more than 2 years.

M.H. Stone *et al.* [12] and H. Momma *et al.* [13] studied aspects of ST and their impact on health. It was found that STs confirm the existence of a strength-endurance continuum (S-EC), with two main aspects: high-load, low-repetition exercises (power stimulus) and high-volume, low-load exercises (HIEE stimulus). Dynamic matching of training principles improves the transfer of results to real-world performance. Studies have also shown that muscle-strengthening activity is associated with a 10-17% reduction in the risk of all-cause mortality, cancer, cardiovascular disease, metabolic disorders and lung cancer, while no association was found with the risk of colorectal, renal, bladder and pancreatic cancer.

The effect of a single high-intensity ST on memory was studied by T. Hashimoto *et al.* [14]. The training group demonstrated improved memory two days after the strength training session, particularly in cued recall and free recall. Free recall was associated with increased connectivity in the left posterior hippocampus. This suggests that short, intense ST can have a positive effect on memory and neural plasticity without requiring repeated training.

The study of the long-term impact of strength training on the physical health of young people, addressing individual differences, psychological aspects and optimal recovery methods, remains relevant. The study presents a systematic review of studies of the effect of ST on functional and physical changes in men aged 18-45 years.

MATERIALS AND METHODS

This systematic review was conducted following the PRISMA 2020 guidelines for systematic reviews and meta-analyses [15]. Relevant scientific publications were selected by searching PubMed, Google Scholar and Wiley Online Library databases. A combination of keywords in English was used, and a time filter was set to select publications published between 2019 and 2024. To ensure the accuracy and completeness of the search, logical operators AND/OR were used. The following combination of search queries was used for Google Scholar: ("young men" AND "strength training" AND "health" AND "research") OR ("healthy men" AND "physiological changes" AND "waist circumference" AND "volume"). The search phrases used in PubMed and Wiley Online Library were "young men" AND "strength training" AND "health" AND "research". The process of literature selection was carried out following the PRISMA flowchart, which allows us to systematise and visualise the stages of publication selection.

Study inclusion criteria were developed based on the PICOS model (population, intervention, comparison, outcomes, study design). The study included publications that analysed functional and physiological changes in healthy men aged 18-45 years under the influence of strength training of varying intensity. Studies where the population consisted of both men and women were included only if separate indicators for men were available. The intervention consisted of ST of different intensities: low, moderate and high. It also included a combination of strength exercises with other types of training. The somatic assessment of participants' health was carried out before and after the training period, and a comparison was made between groups with different intensities of exercise, and exercises for different muscle groups.

The results were assessed by comprehensive indicators of physical characteristics, anthropometry, muscle function and biochemical parameters related to strength training. Exclusion criteria included the presence of acute or chronic diseases of the cardiovascular, musculoskeletal or respiratory systems, and metabolic disorders that could affect exercise performance. Individuals with bad habits and those who used androgenic and anabolic steroids during the study were also excluded.

Only randomised controlled trials were included in the review. The risk of bias was assessed using the PEDro scale. Data from the selected studies were organised and entered a Microsoft Excel spreadsheet. The collected data elements included the author's name and year of publication, number of participants, their level of physical fitness at the beginning of the study, specifics of the STs performed, including duration, frequency and intensity, and combination with other training exercises. Methods for assessing physical activity, somatic condition, and genetic and biochemical parameters were also carefully documented. The data also included baseline measures of participants' condition at the beginning of the study or before the intervention, as well as the dynamic changes that occurred as a result of regular resistance exercise. A systematic search for relevant articles identified 9 publications. The search process is shown in Figure 1.

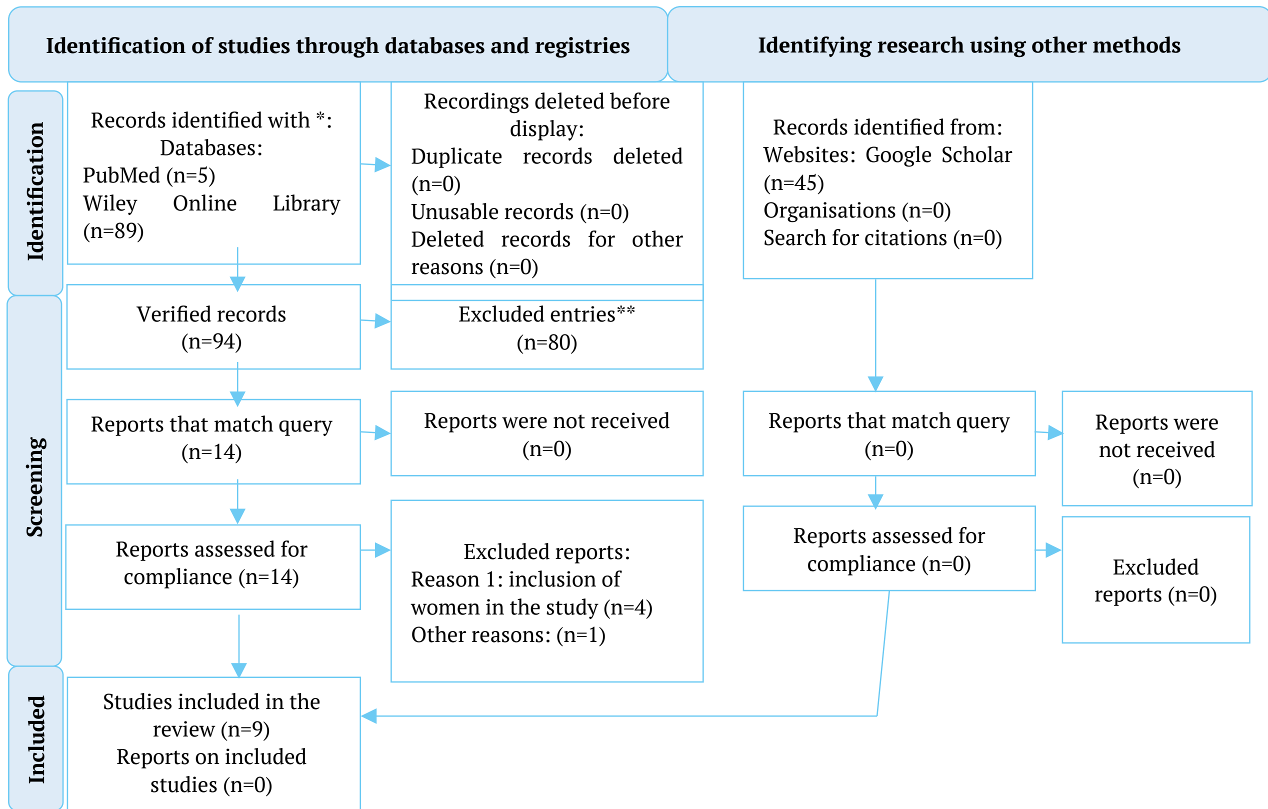

Figure 1. PRISMA flowchart for selecting literature that meets the inclusion criteria
Source: compiled by the author

RESULTS AND DISCUSSION

ST is based on progressive overload: a gradual increase in the load, which stimulates physiological adaptation. At the initial stage, this can be achieved by simply increasing the weight while keeping other training parameters unchanged [2]. Most studies scored between 7 and 9 on the PEDro scale (Table 1), which indicates a high quality of methodology, but there are limitations to objectivity due to the lack of blinding of participants and researchers. The main results of the included studies are presented in Table 2. The studies evaluated the effect of ST on the physiological parameters of men of different ages and fitness levels. Participants underwent various ST programmes that included exercises for the upper and lower extremities with varying intensity and volume. Muscle strength, endurance, muscle hypertrophy, changes in cardiac and vascular function, as well as cellular respiration and levels of inflammatory biomarkers were assessed. The pre-and post-intervention scores were compared to determine physiological changes, such as increased muscle mass, strength, improved cardiac function and endurance.

Table 1. Evaluation of the quality of studies of the effect of ST on the body of young men using the PEDro scale

Author's name and year	A. Grandperrin *et al.* [16]	B.J. Schoenfeld *et al.* [17]	T.G. Balshaw *et al.* [18]	B.J. Schoenfeld *et al.* [19]	E.A. Dawson *et al.* [20]	E.I. Lähteenmäki *et al.* [21]	L. Brandão *et al.* [22]	G.D. Telles *et al.* [23]	P. Deku *et al.* [24]
The selection criteria were defined as follows	1	1	1	1	1	1	1	1	1
Participants were randomly assigned to groups	1	1	1	1	1	1	1	1	0
The distribution was hidden	1	1	0	0	0	0	1	0	0

Table 1. Continued

Author's name and year	A. Grandperrin *et al.* [16]	B.J. Schoenfeld *et al.* [17]	T.G. Balshaw *et al.* [18]	B.J. Schoenfeld *et al.* [19]	E.A. Dawson *et al.* [20]	E.I. Lähteenmäki *et al.* [21]	L. Brandão *et al.* [22]	G.D. Telles *et al.* [23]	P. Deku *et al.* [24]
The groups were similar at baseline in terms of the most important prognostic indicators	1	1	1	1	1	1	1	1	1
All participants were anonymised	0	0	0	0	0	0	0	0	0
All therapists who conducted therapy were anonymised	0	0	0	0	0	0	0	0	0
All evaluators who measured at least one key outcome were anonymised	0	0	0	0	0	0	0	0	0
More than 85% of the participants achieved one outcome	1	1	1	1	1	1	1	1	1
All participants received treatment or control condition as defined	1	1	1	1	1	1	1	1	1
Results of intergroup statistical comparisons are reported for at least one key outcome	1	1	1	1	1	1	1	1	1
The study provides both point estimates and measures of variability for at least one key outcome	1	1	1	1	1	1	1	1	1
ST	8	8	7	7	9	7	8	7	6

Notes: ST – total score 0-10 points
Source: compiled by the author based on [25]

Table 2. Data from studies of power loads on the physiological characteristics of young men

Author, date	Sample	Impact/intervention	Assessment/ surveillance/ inspection	Metrics before the intervention	Physiological changes associated with ST (after the intervention)
A. Grandperrin *et al.* [16]	17 men aged 18-40 years. Exercise no more than 1 hour/per 3 years	CT 3 times/week for 16 weeks. 70% of 1-RM (leg press, squats, leg extension and flexion), trunk (butterfly, bench press, incline bench press, vertical pull-ups and horizontal rowing), arms and shoulders (triceps curl, rope pull, military press, barbell biceps curl, side/front raises, dumbbell biceps curl). 4 sets of 10 repetitions with a 90-second rest between sets	of the 16-item Global Physical Activity Questionnaire version 2, adapted (GPAQ-2) to collect data on sedentary behaviour and physical activity in three areas: commuting, work activities and leisure activities during a typical week. Anthropometry, blood pressure, and date. Cardiac output, body composition, function and morphology	1-RM bench press (kg) 67.9 ± 19.1; 1-RM squat (kg) 82.5 ± 20.9; fat-free body weight (kg) 36.9 ± 3.9; body fat mass (%) 23.0 ± 6.1; cardiac morphology and function: LVMi (gm^{-2}) 89.1 ± 9.6; wave E (cms^{-1})71.7 ± 16.8; wave A, (cms^{-1}) 35.2 ± 6.9; E′ mean (cms^{-1}) 10.49 ± 1.81; EF (%) 62.2 ± 4.4; GLS, % – 19.56 ± 1.76; LAVI ($ml.m^{-2}$) 23.9 ± 3.9; reservoir function LP(%) 30.5 ± 7	1-RM bench press (kg) 87.9 ± 17.5; 1-RM squat (kg) 118.7 ± 16.4; fat-free body weight (kg) 37.6 ± 3.9; body fat mass (%) 22.6 ± 5.7; cardiac morphology and function: LVMi (gm^{-2}) 109.2 ± 12.9; wave E (cms^{-1}) 77.6 ± 15.5; wave A (cms^{-1}) 29.5 ± 6.1; E′ mean, (cms^{-1}) 10.60 ± 1.37; EF (%) 63.9 ± 2.9; GLS (%) – 18.65 ± 1.39; LAVI ($ml.m^{-2}$) 25.3 ± 4.2; LV reservoir function (%) 30.8 ± 9.1
B.J. Schoenfeld *et al.* [17]	34 men aged 18-35 years, experience of ST (3/ week≥1 year. 3 groups: low volume of CT (1SET) (n = 11), moderate volume (3SET) (n = 12), high volume (5SET) (n = 11)	8 weeks, 3 r/week, (1SET) 1 set per exercise per training session, (3SET) – 3 sets per exercise per training session, (5SET) – five sets in the upper and lower extremities. 7 exercises per session for all muscle groups: bench press, military bench press, wide-grip lateral stretches, seated cable row, barbell back squat, simulator leg press, and unilateral leg extension in the simulator. 8-12 repetitions performed to the point of instantaneous concentric failure, 90 seconds of rest, and 120 seconds between exercises.	Anthropometry, muscle mass thickness (ultrasound). The endurance of the upper body muscles was assessed by bench pressing at 50% of 1-RM until the moment of maximum failure. Muscle hypertrophy was examined using B-mode ultrasound for elbow flexors and extensors, as well as for the middle and lateral thigh	Squats (1RM) (kg): 1SET: 104.5 ± 14.2, 3SET: 114.9 ± 26.1, 5SET: 106.6 ± 24.0. Bench press 1-RM (kg): 1SET: 94.1 ± 16.1, 3SET: 100.2 ± 20.6, 5SET: 91.1 ± 20.9. Bench press endurance (repetitions): 1SET: 21.3 ± 5.0. 3SET: 2.1 ± 5.6. 5SET: 23.6 ± 7.4. Muscle thickness (mm): biceps 1SET: 39.7 ± 4.7, 3SET: 42.2 ± 4.0, 5SET: 41.7 ± 4.6. Triceps (mm): 1SET: 47.4 ± 4.6, 3SET: 47.7 ± 6.1, 5SET: 47.2 ± 6.8. Rectus femoris muscle (mm): 1SET: 54.2 ± 5.3, 3SET: 52.2 ± 5.0, 5SET: 54.9 ± 5.4. Lateral broad muscle bone (mm): 1SET: 57.9 ± 6.8, 3SET: 56.4 ± 5.6, 5SET: 57.9 ± 6.4	Squats (1RM): 1SET: 123.4 ± 12.9, 3SET: 126.6 ± 25.0, 5SET: 122.2 ± 19.0. Bench press 1-RM: 1SET: 102.6 ± 15.5, .3SET: 108.6 ± 20.6. 5SET: 100.7 ± 22.3. Bench press endurance: 1SET: 23.0 ± 4.2, 3SET: 24.9 ± 5.2, 5 SET: 25.3 ± 8.0. Biceps muscle thickness: 1SET: 40.7 ± 4.7.3SET: 43.6 ± 4.1. 5SET: 44.6 ± 4.7. triceps: 1SET: 48.2 ± 4.7, 3SET: 49.4 ± 6.2, 5SET: 50.2 ± 6.6. rectus femoris muscle after: 1SET: 55.3 ± 5.8, 3SET: 54.6 ± 5.8, 5SET: 57.3 ± 5.8. The lateral broad muscle bone after: 1SET: 59.0 ± 6.7, 3SET: 58.8 ± 5.7, 5SET: 62.6 ± 5.8

Table 2. Continued

Author, date	Sample	Impact/intervention	Assessment/ surveillance/ inspection	Metrics before the intervention	Physiological changes associated with ST (after the intervention)
T.G. Belshaw *et al.* [18]	n = 63.2 groups: UNTs (n = 49) had not engaged in lower body ST for >18 months in total. Physical activity level according to MOFA: 2326 ± 1337 IU min/week. LT-MST group (n = 14): performed systematic heavy quadriceps ST for ⩾3 years. Physical activity level: 5568 ± 1457 IU min/week	Long-term maximal ST (LT-MST) several knee extensions exercises 3 times a week (squats, lunges, step up and leg press). Participants underwent an introductory session, which included unilateral isometric voluntary maximal and explosive contractions, as well as evoked contractions on an isometric dynamometer for knee extension/ flexion. Neuromuscular measurement sessions with the dominant leg. LT-MST group performed knee extension exercises 3 times per week	CSA of the quadriceps ($QACSA_{MAX}$); quadriceps by MRI). Maximal voluntary torque (MVT), Electromyography (EMG) from quadriceps (QEMG), hamstring muscles (HEMG)	For UNT: MVT knee extension (nm): 245 ± 45; QEMG (cm^2): 90 ± 12	For LT-MST MVT, knee extension: 407 ± 63; QEMG: 138 ± 14; LT-MST showed significantly higher maximal strength and CSA values of +66% and +54%, respectively. The absolute explosive power was also higher in LT-MST (+41% to +64%). The relative explosive power was lower in LT-MST (by 11% to 16%). LT-MST showed slower contractile properties, which did not depend on differences in the activation of the neuromuscular system
B.J. Schoenfeld *et al.* [19]	26 untrained men. Average metrics Height: 175.7 cm; weight: 77.3 kg; adipose tissue: 20.5%; age: 22.5 years	LT (20-30 repetitions) and WT (6-10) for the calf muscles. Sitting and standing shin raises 2/ week 8 weeks, 4 sets, 90s rest between sets, 3 min between exercises	Muscle thickness (ultrasound) and muscle strength (dynamometer)	WT: soleus (mm) 18.8 ± 4.4; medial calf muscle (mm) 18.3 ± 3.2; lateral calf muscle (mm) 15.9 ± 2.6; isometric plantar flexion (N·m) 154 ± 48. LT: soleus (mm) 18.2 ± 4.3; medial calf muscle 17.7 ± 3.0; lateral calf muscle 15.6 ± 2.8; isometric plantar flexion 153 ± 47	VT: soleus 20.1 ± 4.6; medial calf muscle 19.7 ± 3.1; lateral calf muscle 17.9 ± 2.5; isometric plantar flexion 170 ± 41. LT: soleus 19.7 ± 4.6; medial calf muscle; lateral calf muscle 17.9 ± 3.2; isometric plantar flexion 168 ± 41. Hypertrophy of the psoas muscle: LT: 10 ± 10% more; WT: 7 ± 8% more. Hypertrophy of the calf muscles: LT: 15 ± 30% more; VT: 20 ± 25% more

Table 2. Continued

Author, date	Sample	Impact/intervention	Assessment/ surveillance/ inspection	Metrics before the intervention	Physiological changes associated with ST (after the intervention)
E.A. Dawson *et al.* [20]	35 healthy young men	Two programmes of 4 weeks: ST and endurance training (END). ST 3 times a week on a leg extension machine. 4 sets of 10 repetitions of 80% of 1-RM for each leg, 2 min break between sets. END was performed on a cycle ergometer and 30 minutes of cycling with a maximum heart rate of 70% in the first 3 sessions. In sessions 4-6, 5 intervals of 1 min at 90% of the maximum heart rate, followed by 5 min at 70% of the maximum heart rate. Sessions 7-9 30 minutes cycling at 80% of the maximum heart rate. Sessions 10 and 11 included 5 intervals of 1 min each with an intensity of 90% of the maximum heart rate and 5 min with a load of 80% of the maximum heart rate	Anthropometric measurements, CPET testing, brachial artery vascular function assessment (ultrasound), peak VO_2, RER, genotype PCR, power output for END	For CT: 1-RM (kg) 56 ± 14; total load (kg) 5,874 ± 1,456; peak VO_2 (ml·min^{-1kg-1}) 47.5 ± 11.0 For END: Output power (W) 113 ± 23; maximum CPET: VO_2 peak, (ml·min^{-1} kg^{-1}) 46.5 ± 9.4	For CT: 1-RM (kg) 67 ± 13; total load (kg) 7,208 ± 1,563; peak VO_2, (ml·min^{-1} kg^{-1}) 46.4 ± 104. For END: Output power (W) 123 ± 25; maximum CPET: Peak VO_2, (ml·min^{-1} kg^{-1}) 49.6 ± 10.4 Total load increased by 23% (7,208 ± 1,563 kg), a 1-RM increase from week 1 to 4. 1-RM, one maximum repetition: 67 ± 13. Peak VO_2 ml·min -1 kg -1: 46.4 ± 10.4. Output power (END) 123 ± 25. ik VO_2, ml·min-1·kg-1: 49.6 ± 10.4
E.I. Lähteenmäki *et al.* [21]	12 trained healthy men	Isokinetic bench press on the Smith isokinetic machine with 5 sets of 10 repetitions of maximum load with a rest of 2 minutes. Subjects performed (1) concentric (C) only, (2) eccentric (E) only, or (3) combined eccentric-concentric (E+C) contraction exercises in random order on the Smith isokinetic machine for 3 to 5 s with 15 s rest between trials	The levels of leukocytes, interleukin 6*(IL-6)*, C-reactive protein (CRP), creatine kinase (CK), venous blood lactate and maximal voluntary isometric strength were measured at the same time points. Cellular respiration of intact VSMCs was measured using a high-resolution respirometer	Maximum isometric force (N) = 1.084 ± 4.1; PBMC cellular respiration: Regular breathing (μmol O_2/ min/ml): 2.5 ± 0.2; Free routine activity: Before training: 1.0 ± 0.1; ET-capacity (μmol O_2/min/ ml): 3.0 ± 0.2; Lactic acid (10 mmol L-(+)-lactic acid): pH = 7.4	Maximal isometric force = 1.084 ± 4.1; 1 min after E+C: reduced to 950 ± 5.0; 24 h after E+C: restored to 1.050 ± 4.8. Regular breathing: 5 minutes after E+C: reduced to 1.8 ± 0.3; 24 h after E+C: restored to 2.4 ± 0.2; free routine activity: 5 minutes after E+C: reduced to 0.7 ± 0.1; 24 h after E+C: restored to 0.9 ± 0.1. ET-capacity: 5 minutes after E+C: reduced to 2.3 ± 0.3. 24 hours after E+C: restored to 2.9 ± 0.2.

Table 2. Continued

Author, date	Sample	Impact/intervention	Assessment/ surveillance/ inspection	Metrics before the intervention	Physiological changes associated with ST (after the intervention)
E.I. Lähteenmäki *et al.* [21]					Lactate (20 mmol L-lactate sodium): insignificant effect on cellular respiration. Lactic acid (10 mmol L-(+)-lactic acid): 5 min after exercise: pH decreases to 7.2. 24 hours after exercise: pH restored to 7.3
L. Brandão *et al.* [22]	43 young men	CT 10 weeks 2 r/week group 1: bench press with bench triceps press (MJ+SJ, n = 12); group 2: bench triceps press plus bench press (SJ+MJ, n = 10); group 3: bench triceps press (SJ, n = 11) group 4: bench press (MJ, n = 10). 8 repetitions of 50% 1RM, 2 min rest, 3 repetitions of 70% 1RM and single repetitions of heavier weights until failure	CSA of the cross-sectional area of the pectoralis major muscle (PMM) and the triceps brachii of the shoulder (MRI)	1-RM for bench press (kg) MJ: 72.3 ± 19.3; SJ: 77.6 ± 21.1; MJ+SJ: 75.2 ± 23.5; SJ+MJ: 76.6 ± 11.5; elbow extension (kg): MJ: 36.6 ± 9.5; SJ: 42.9 ± 12.6; MJ+SJ: 37.2 ± 14.0; SJ+MJ: 39.8 ± 6.6. CSA (cm^2) for VGM: MJ: 413 ± 3.7; SJ: 40.5 ± 8.9; MJ+SJ: 39.1 ± 9.4; SJ+MJ: 41.0 ± 4.2; for TM: MJ: 36.6 ± 9.5; SJ: 42.9 ± 12.6; MJ+SJ: 37.2 ± 14.0; SJ+MJ: 39.8 ± 6.6	1-RM for bench press increased by (%) MJ 27.1 ± 17.7; MJ+SJ: 23.6 ± 144; SJ+MJ: 22.3 ± 15.4; for SJ: 9.9 ± 10.9 (there was no significant difference); for triceps press, it increased by (%): MJ: 36.6 ± 9.5; SJ: 23.2 ± 14.0; MJ+SJ: 35.3 ± 26.3; SJ+MJ: 26.3 ± 17.2; for MJ 26.3 ± 17.2 (no significant difference). CSA increased by (%) for PMM: MJ: 9.1 ± 5.6; SJ: 40.5 ± 8.9; MJ+SJ+MJ: 5.6 ± 5.1; for SJ, the metric was 0.8 ± 1.9; for TM: SJ: 9.5 ± 4.8; MJ+SJ: 11.5 ± 5.1; SJ+MJ: 10.4 ± 6.1; for MJ metric was 4.8 ± 4.2
G.D. Telles *et al.* [23]	Nine untrained young men	2 sets of 10 repetitions of ST, 2 sets of leg press and leg extension HIIE – 12 sets of 1-minute sprints with a 1-minute rest. SE (HIIE after ST) separated by 1 week	Skeletal muscle biopsy before training, during training (0 h), after 4 and 8 hours, followed by RNA quantification	Expression of *miR-1-3p*, *miR-133a-3p*, *miR-133b*, *miR-181a-3p*, *miR-486* 8 hours after training was higher than before exercise. Expression was lower after HIIE compared to CT and SE	

Table 2. Continued

Author, date	Sample	Impact/intervention	Assessment/ surveillance/ inspection	Metrics before the intervention	Physiological changes associated with ST (after the intervention)
P. Deku *et al.* [24]	66 weightlifters	The majority (61.3%) trained for 120 minutes per session, 46.7% trained 5 days a week, and 41.3% had been training for about 1-5 years	BREWTQ questionnaire, anthropometry, physiological (SBP, DBP, heart rate), biochemical studies (total cholesterol, triglycerides, total protein, creatinine, urea, LDL, HDL)	A positive association was found between DBP and arm circumference (r = +0.331, P = 0.022). Differences in the mean values of CC (P = 0.013) and AC (P = 0.010). Maximal oxygen consumption was positively correlated with HDL, total cholesterol, total protein and globulin levels, with the strongest correlation with cholesterol levels. BP was positively correlated with BMI, hip circumference, CC, creatinine and LDL-C levels, with the most pronounced relationship being with creatinine levels. Positive correlation between SBP and total cholesterol, CC, arm circumference, creatinine and HDL. HR had a positive correlation with all anthropometric and biochemical parameters, except for total cholesterol, LDL, cholesterol, urea, total protein and globulins, which had a negative correlation	

Notes: 1-RM – one repetition with maximum weight; DBP – diastolic blood pressure; LVMi – left ventricular mass index; EF – emission fraction; LP – left atrium; LAVI – left atrial volume index; CSA – cross-sectional area; LT – light training; WT – hard workout; UNT – untrained; LT-MST – Long-Term Maximal Strength Training; MOFA – international physical activity questionnaire; ME – metabolic equivalent; CPET – maximum cardiopulmonary exercise capacity; RER – respiratory exchange rate; peak VO2 – oxygen uptake; PCR – polymerase chain reaction; PBMC – peripheral blood mononuclear cells; VGM – pectoralis major muscle; TM – triceps brachii muscle; HIIE – high-intensity interval training; CT – cardio training; SBP – mean arterial pressure; BMI – body mass index; HDL – high-density lipoprotein; LDL – low-density lipoprotein; CC – chest circumference
Source: compiled by the author

The ST program led to improvements in muscle strength, and body composition, and contributed to morphological remodelling of the heart (enlargement of the LV, RV) in a study by A. Grandperrin *et al.* [16].

B.J. Schoenfeld *et al.* [17] demonstrated that a significant increase in muscle strength can be achieved in individuals who engage in ST with only three 13-minute sessions per week. The results of such training are similar to those achieved with significantly more time spent on medium-load training (8-12 repetitions per set). This is relevant for those with time constraints, allowing for efficient strength gains that can contribute to a greater commitment to physical activity among the population. The increase in muscle hypertrophy is dose-dependent, with greater gains being achieved with higher training volumes. To maximise muscle growth, it is recommended to spend more time training every week. However, the amount of training does not affect the endurance of the upper body muscles.

T.G. Belshaw *et al.* [18] also confirmed that prolonged maximal ST significantly improves strength, muscle volume and explosive muscle capacity in trained individuals compared to untrained individuals. B.J. Schoenfeld *et al.* [19] determined that a light training programme induced greater hypertrophy of the gastrocnemius muscle compared to a heavy training programme, while heavy training promoted greater hypertrophy of the middle and lateral heads of the gastrocnemius muscle.

ST and END have a positive effect on men's physiological parameters. E.A. Dawson *et al.* [20] determined that peak VO_2 increased significantly after endurance training, and brachial artery vascular function increased after both types of training. Both training plans led to a significant improvement in endothelium-dependent vasodilation of the brachial artery, but the overall adaptation to peak VO_2 was more significant after END. Eccentric exercises without concentric exercises have a significant impact on PBMC respiration. Combined eccentric-concentric

exercise caused the greatest muscle fatigue, reducing PBMC respiration and lactate levels, while eccentric exercise alone had the least effect. The effect of anaerobic metabolism did not change PBMC respiration in the study by E.I. Lähteenmäki *et al.* [21].

The sequence of exercises does not affect the increase in 1-RM in bench press and bench triceps extension. L. Brandão *et al.* [22] argued that performing these exercises in any sequence is effective for achieving maximum strength. There was a moderate decrease in CSA increase in the pectoralis major when an isolated triceps exercise was performed before a multi-joint exercise. It may be worth performing exercises where the pectoral muscles are the main agonist muscles first in the sequence if the goal is to maximise hypertrophy of this muscle complex. Performing a combination of exercises that vary in length-tension ratio is preferable for maximising the development of all three triceps heads.

The microRNA responses in the study by G.D. Telles *et al.* [23] were specific, in the early acute state during different types of skeletal muscle training, no specificity was observed for *miR-1-3p, miR-133a-3p, miR-133b, miR-378aa-5p*, as well as for the expression of *miR-181a-3p* and *miR-486* between ST, HIIE or CT in untrained individuals. This indicates that changes in expression occurred mainly approximately 8 hours after training. ST had a more pronounced effect on the expression of *miR-23a-3p* and *miR-206* compared to *HIIE*. This is relevant for the formation of the molecular basis of adaptive responses for each type of exercise. P. Deku *et al.* [24] determined that the duration of weightlifting training had a positive effect on anthropometric and physiological parameters, but not on biochemical parameters (glomerular filtration rate and total protein). Increasing the duration of training was associated with an increase in breast and arm volumes ($P < 0.05$). There was a significant increase in DBP with training duration ($P = 0.038$).

A systematic review by B.S. Currier *et al.* [26] included 192 studies evaluating the effects of various resistance training protocols on muscle strength and hypertrophy. The highest effects for both indicators were observed in protocols with high load and training frequency. The overall risk of bias in the studies was moderate, suggesting that the results should be interpreted with caution. The main conclusion is that high load and frequency of training provide the greatest gains in strength and hypertrophy, highlighting the importance of intensity in ST programmes.

In this review, most studies used different levels of volume and intensity in the number of sets (1, 3, 5) and repetitions (10-30). After analysing 2083 articles comparing the responses to training with different volumes to induce muscle hypertrophy, E. Baz-Valle *et al.* [27] determined that for the quadriceps and biceps brachii there were no significant differences between moderate and high-volume training, but high volume training was better at stimulating muscle mass gain in the triceps brachii. The optimal volume for muscle hypertrophy in young, trained men was 12-20 sets per week.

STs, as an acute stressor, affect the sympathetic and parasympathetic nervous systems, as well as the hypothalamic-pituitary-adrenal axis (HPA axis). During intense training or competition, the HPA axis is activated to mobilise the body's resources [28]. The hypothalamus signals the pituitary gland to produce adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands to produce cortisol. Cortisol regulates metabolism, anti-inflammatory processes, and recovery. After exercise, cortisol levels usually decrease, allowing the body to recover and adapt. Regular training can lead to HPA-axis adaptation, where the body becomes less sensitive to stress or recovers faster after exercise [29, 30]. The HPA axis is activated when endurance exercise reaches high and prolonged intensity [31].

During muscle contractions, skeletal muscles increase oxygen consumption, which leads to the formation of oxygen-dependent free radicals that can cause tissue damage [32]. Accordingly, at the cellular level, muscles adapt through the activation of endogenous antioxidant enzymes and stress proteins (HSPs), which protect against oxidative stress during subsequent exercise [33]. The maximum hypertrophic effect of muscles can be achieved by performing multiple sets, which increases *p70S6* kinase phosphorylation and muscle protein synthesis (MPS), as opposed to exercises with a single set [17].

Before starting intense physical activity, the patient must undergo a medical examination, with a complete history, examination and necessary tests. Contraindications to strength training include valvular heart disease, ventricular hypertrophy, dangerous arrhythmias, and malignant hypertension. Patients with obesity, bronchial asthma, diabetes, and haemoglobinopathies should undergo a stress test before exercise, including measurement of heart rate, blood pressure, and electrocardiogram [6].

STs may have a positive effect on traditional cardiovascular risk factors such as blood pressure, glucose, lipids, body composition, and systemic inflammation [34-36]. Evidence for these effects is mostly based on randomised controlled trials of medium duration (2-6 months); data from studies longer than 6 months are limited. Most studies used moderate to high intensity exercise programmes (40-80% of maximal effort) 2-3 times per week [37-39].

STs contribute to an increase in muscle strength and power due to neuromuscular adaptation, an increase in muscle cross-sectional area (CSA) and changes in connective tissue stiffness. The initial rapid increase in strength during exercise mastery gradually slows down with muscle development [40]. According to some studies, both high and low loads can effectively activate muscle protein synthesis and hypertrophy, and blood flow restriction at low loads can also promote hypertrophy [41]. To achieve optimal hypertrophy, a combination of mechanical stress and metabolic stress must be ensured. Achieving optimal neuromuscular adaptation or increasing CSA is possible with different variations in the intensity and volume of training programmes [42]. Individuals seeking to optimise muscle hypertrophy are advised to implement a hypertrophic-oriented ST programme, which includes 3 to 6 sets with 6 to 12 repetitions of the programme [43]. The rest interval between sets should be 60 seconds. The intensity of the effort is from 60 to 80% of one repetition of the maximum. It is also important to increase the volume of the current load to 12-28 sets [44]. The American College of Sports Medicine (ACSM) advises untrained individuals to perform 1-3 sets of each exercise, with the number of repetitions in the range of 8-12, using a load of 70-85% of the maximum

effort per repetition. For people with training experience, it is recommended to perform 3-6 sets with the number of repetitions from 1 to 12, using a weight in the range of 70-100% of 1RM. Normally, loads in the range of one 1RM to 10RM with the number of repetitions from 4 to 12 are used.

The results of studies reflect the different effects of ST on the physiological characteristics of young men. In most studies, there was an increase in *1-RM* in exercises such as bench presses and squats. Increases in muscle mass were evident in both large muscle groups (thighs, pectorals) and smaller muscle groups (biceps, triceps). This suggests that regular strength training contributes to significant improvements in muscle strength and hypertrophy. W. Kassiano *et al.* [45] demonstrated that the choice of different exercises can affect muscle hypertrophy and strength gains. Systematic variation in the training programme enhances regional hypertrophic adaptation and maximisation of dynamic strength, whereas increased or chaotic variation can negatively affect muscle mass gain. Excessive stimulus or frequent exercise rotation can interfere with muscle adaptation.

CONCLUSIONS

The systematic search identified 9 relevant publications. Most studies showed high methodological quality (7-9 points on the PEDro scale). The review showed that different strength training regimens have a positive effect on physical and physiological parameters in healthy young men. Frequent strength training significantly improves 1-RM, lean body mass, and morphological and functional parameters of the heart. A higher volume of training (5 sets) may be more effective in improving strength performance and muscle hypertrophy compared to lower volumes (1 or 3 sets). This is confirmed by the significant increase in 1-RM and muscle thickness in the high-volume groups.

The effect of ST on physiological parameters such as muscle thickness, hypertrophy and cardiovascular function was positive, with an increase in lean body mass and a decrease in fat mass. Training also improved biochemical parameters such as leukocyte activity and levels of key metabolic markers. Combination training, which includes both concentric and eccentric exercises, has shown significant potential for improving muscle performance and functional performance. High-intensity training with a variety of exercise types can provide more pronounced results than traditional approaches.

The study was limited by the small number of participants, short intervention period, and lack of blinding of participants and researchers, which may affect the objectivity of the results. Increasing the level of control over the random allocation of participants and concealing the allocation would help to ensure greater accuracy of the results.

Given the results, it is recommended that a comprehensive approach to strength training, including a high volume of exercises and different types of loads, be applied to achieve maximum physiological benefits. Further research, development and testing of individually adapted training programmes based on the physiological and metabolic characteristics of the participants and their long-term health effects is also recommended.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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Оцінка фізичного (соматичного) здоров'я чоловіків молодого віку при побудові оздоровчого тренування силової спрямованості

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Анотація. Метою цього систематичного огляду було визначення впливу силових вправ на фізіологічні та морфологічні адаптаційні можливості у здорових молодих чоловіків. Було здійснено пошук рандомізованих клінічних досліджень, що містять інформацію про вплив вправ з опором на фізичні та функціональні зміни у молодих чоловіків 18-45 років. Було знайдено 13 публікацій, що підходять критеріям пошуку, з яких відібрано 9 досліджень після виключення невідповідних. Більшість статей були оцінені, як дослідження хорошої якості з оцінкою 7-9 балів за шкалою PEDro. Було виявлено, що тривале силове тренування значно покращує силу, м'язовий об'єм та вибухові здібності у тренованих осіб. Силові тренування 3 рази на тиждень здатні збільшити безжирову масу тіла та індекс маси лівого шлуночка, зі зменшенням маси жиру. Легкі тренування стимулюють гіпертрофію камбалоподібного м'яза більше ніж важкі, які більше впливають на середню та латеральну головки литкового м'яза. Черговість вправ не впливає на підвищення максимальної сили, але вплив на гіпертрофію грудних м’язів може бути кращим при виконанні багатосуглобових вправ після ізольованих. Поява мікроРНК не показує специфічності у ранньому гострому стані тренувань, зміни в експресії спостерігаються через 8 годин після тренування. Тривалість тренувань з підняття ваги позитивно впливає на антропометричні та фізіологічні показники, але не на біохімічні

Ключові слова: вправи з опором; систематичний огляд; функціональна адаптація; гіпертрофія м'язів; спортивна медицина

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Mouthwash as a factor in controlling the formation of soft dental plaque in patients with orthodontic treatment

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Abstract. Patients with dental braces are more likely to experience rapid accumulation of dental plaque and gum inflammation, therefore, antimicrobial mouthwashes that enhance daily oral hygiene can effectively remove and control bacterial plaque, improve gum health, and are safe preventive agents without side effects. The aim of the study was to evaluate the quantitative and qualitative composition of the soft dental plaque microbiocenosis under the influence of an alcohol-free oral hygiene product in patients undergoing orthodontic treatment. The study included patients undergoing orthodontic treatment. Samples were taken from the surface teeth of the maxilla. The obtained swabs from the surface of the upper molars were examined using a bacteriological method with subsequent identification: microscopic, cultural, and biochemical. Comparison of the effectiveness of an alcohol-free mouthwash in terms of changes in the quantitative and qualitative composition of microorganisms and the condition of periodontal tissues in patients undergoing stationary orthodontic therapy carried out after three months of use. The study revealed gram-positive and gram-negative bacteria and *Candida* fungi with a subsequent insignificant decrease in gram-negative bacteria ($p = 0.999$) after using the mouthwash. The results obtained showed changes in the taxonomic composition, namely, a decrease in the number of bacterial genera from 11 to 9. In addition to the mentioned results, changes were found at the population level of the soft dental plaque microbiota in patients who used the alcohol-free mouthwash for three months, namely, a decrease in the number of *Streptococcus* spp. α $p(x \leqslant T) = 0.9958$, *Propionibacterium* spp. $p(x \leqslant T) = 0.9837$, while changes in *Streptococcus* spp. β and *Streptococcus* spp. γ were minimal. A comparison of the population level of microorganisms before and after the use of mouthwash revealed significant differences ($p < 0.05$). Determination of the gingival index demonstrated a slight improvement in the periodontal status of the examined subjects. The conducted study is of great importance for the development of new strategies for the prevention and treatment of oral cavity diseases

Keywords: microbiocenosis; microorganisms; oral cavity; gingivitis; dental brace

INTRODUCTION

The placement of orthodontic appliances on teeth often leads to an increase in bacterial colonisation on both the visible and hidden biofilm of the tooth surface. This, in turn, plays a significant role in the inflammation of gum tissues during orthodontic treatment. Given the highly complex ecosystem of the oral cavity, inhabited by a diverse range of microorganisms, any external intervention, such as fixed orthodontic appliances, can disrupt the microbial balance within the microcenosis of the oral cavity [1, 2].

S.M. Hamdoon *et al.* [3] and D.T. Mahjoub *et al.* [4] state in their research that orthodontic treatment leads to an increase in the number of cariogenic bacteria, specifically

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Streptococcus and *Lactobacillus*, as well as fungi and other periodontal pathogens associated with dental plaque. A.K. Shahi *et al.* [5] also demonstrated the effects of various types of antibacterial agents (Ozonated Olive Oil Gel, Chlorhexidine gel, and mouthwash Amflor) on *Streptococcus mutans* and *Lactobacillus* bacteria.

N. Manashchuk *et al*. [6] conducted a review of various oral hygiene products, particularly dental elixirs and mouthwashes, and classified them based on their composition and intended use. They emphasise that any such product should be used in conjunction with toothpaste and a brush, serving as a secondary step in oral care. This is because they are used to clean hard-to-reach areas of the periodontium and interdental spaces.

N. Bila *et al*. [7] noted in their research that the use of antiseptic agents leads to qualitative changes in the composition of the oral microbiome. They emphasised the importance of consulting a healthcare professional regarding the choice of mouthwash for daily oral hygiene, as an excessive amount of sodium fluoride may have adverse effects on the human body, including potential impacts on the thyroid gland, cognitive abilities, and more.

G.M. Tartaglia *et al*. [8] and M. Selvaraj *et al*. [9] demonstrated the importance of conducting research into the side effects of mouthwashes, such as local morphological changes (oral mucosa and dental-crown staining, mucosal lesions) and functional changes (taste modifications, abnormal oral sensation). Given that mouthwashes are commonly used as an adjunct to mechanical tooth brushing, they highlight the need for detailed studies into the combinations of ingredients in these liquids and their impact on reducing dental plaque and improving periodontal tissue health.

S. Ayesha *et al*. [10] highlight the importance of selecting the right mouthwash, especially for patients undergoing orthodontic treatment, as they are prone to dental plaque accumulation, gum inflammation, and mineral loss from tooth enamel. They assert that chlorhexidine and *Aloe vera* mouthwash have shown good results in controlling inflammatory processes in periodontal tissues, gingival bleeding, and plaque accumulation. However, prolonged use of chlorhexidine can have adverse side effects, making plant-based mouthwashes an effective alternative.

However, there is a lack of research investigating the *in vivo* effects of daily oral hygiene products on patients undergoing orthodontic treatment, and analysing their impact on the oral microbiome and gingival tissues. This issue requires further study and a better understanding of the relationship between the substances contained in such mouthwashes and the composition of microorganisms forming soft dental plaque. This study aimed to analyse the quantitative and qualitative composition of the microbiocenosis of soft dental plaque in patients undergoing orthodontic treatment, under the influence of an alcohol-free oral hygiene product.

MATERIALS AND METHODS

A randomised controlled trial design was developed to assess and compare the effectiveness of an alcohol-free mouthwash in reducing the quantity and quality of microorganisms in 8 patients undergoing orthodontic treatment at a private dental clinic in Ternopil (commenced in 2023 and ongoing in 2024). These changes were assessed at regular intervals. Inclusion criteria for patients in the study group were: aged 15-40 years; at the initial or intermediate stages of dentition correction; undergoing fixed orthodontic treatment with brackets on the upper teeth. Exclusion criteria for patient selection were: no signs of tooth decalcification; no known hypersensitivity to oral hygiene products; no known medical conditions or medication that could affect oral tissues. Samples were collected from the surface of the upper molars of 8 patients undergoing orthodontic treatment. Samples were collected using a swab moistened with 0.9% saline solution. Samples were transported at a temperature of +18-22°C within 1-2 hours of collection.

For culturing on nutrient agar plates, an inoculum of microorganisms was prepared at a defined concentration (using the McFarland scale (HiMedia, India)). The inoculum, diluted to 10^{-6} in a volume of 100 µl, was applied to nutrient agar plates: Endo agar (Farmaktiv, Ukraine) for the isolation of gram-negative microorganisms, blood agar (Farmaktiv, Ukraine) for the isolation of streptococci, mannitol salt agar (Farmaktiv, Ukraine) for the isolation of staphylococci, Sabouraud agar (Farmaktiv, Ukraine) for the isolation of *Candida* species, anaerobic agar (Brewer) (Farmaktiv, Ukraine) for the cultivation of anaerobic bacteria, and Blickfeldt agar (Farmaktiv, Ukraine) for lactobacilli.

Cultivation of facultative anaerobic bacteria was carried out in a thermostat (TS-20, MIS-MA, Ukraine) at a humidity of approximately 70-80% and a temperature of +37°C for 24-48 hours. For the cultivation of *Candida* species, the incubation time was extended to five days at a temperature of +22°C. To create anaerobic conditions, a GENbox with a gas pack (bioMerieux, France) was used, and the incubation time for anaerobic bacteria was 48-72 hours at a humidity of approximately 70-80% and a temperature of +37°C. For identification, the following methods were used: colony morphology, Gram staining (BioGnost, Croatia), and biochemical tests: catalase test (Biolife Italiana S.r.l., Italy), coagulase test (Biolife Italiana S.r.l., Italy), Olkenitskyi medium (Farmaktiv, Ukraine), Voges-Proskauer test (Farmaktiv, Ukraine), and indole test (Farmaktiv, Ukraine) [11, 12]. Quantitative analysis was performed by determining the colony-forming units (CFU/mL) in 1 mL of the sample and calculated using the formula [13]:

$$TCC = \frac{N \times A}{V}, \quad (1)$$

where TCC is the total colony count, lg_{10} CFU/mL, N is the number of colonies in a sector, CFU; A is the dilution factor; V is the volume applied to the plate, mL.

To assess the microbial population, a constancy index (C) was determined using the formula:

$$C = \frac{n \times 100}{N}, \quad (2)$$

where C is the constancy index, %; n is the number of samples in which the studied species was detected; N is the total number of samples analysed.

Reference values were taken as follows: dominant >50%, frequent 20-50%, infrequent 1-19%, and rare <1%. Loe and Silness's gingival index was used as a basis for assessment [14]. The gingival index scoring scale was as follows: 0 – indicates no inflammation of the gingiva;

1 – shows mild inflammation (slight changes in gingival condition); 2 – moderate inflammation (characterised by swelling and slight enlargement); 3 – severe inflammation (marked swelling of the gums). To investigate the effect of the mouthwash on the quantitative and qualitative composition of the microcenosis and gingival status, an alcohol-free oral hygiene mouthwash with a plant-based component was selected with the following composition: water, glycerin, propylene glycol, xylitol, disodium EDTA, poloxamer 407, allantoin, methylparaben, cetylpyridinium chloride, sodium fluoride, *Aloe barbadensis* leaf juice, acesulfame potassium, propylparaben, menthyl acetate, glycerin, flavour, CI 19140, C1 42051, limonene.

The initial stage of the examination involved assessing the condition of the soft and hard tissues of the oral cavity to detect any changes during the study and determine their possible association with the use of mouthwash. Patients used the mouthwash twice a day, morning and evening, after brushing their teeth, and were informed not to use any other mouthwash throughout the study. They were also required to return the empty container to monitor product usage. Samples were collected from the tooth surface before the start of use, serving as a control in the study, and again after three months of using the mouthwash. The treatment period of 3 months was chosen because gingivitis is a chronic condition, and to avoid interfering with the hygiene habits of the individuals involved in the study. Regarding the study period, these typically last 6 months with an interim assessment at 3 months to evaluate both the efficacy and safety of the chemicals for patients. However, mouthwashes are also used and prescribed for shorter periods to assess their efficacy over shorter timeframes, which is of scientific interest [15].

Statistical analysis was performed using the STATISTICA 10 and Microsoft Excel 2016 software packages. Mean values (M) with standard error of the mean (±m) were calculated. Non-parametric tests were used for datasets with distributions differing from normal: the Mann-Whitney U-test for comparing two independent samples, the Wilcoxon signed-rank test for assessing dynamic changes within groups, and the Student's t-test. The level of significance was set at $p <= 0.05$.

The study was conducted following the recommendations outlined in the "Council of Europe Convention on Human Rights and Biomedicine" [16], taking into account the ethical principles set out in the Declaration of Helsinki of the World Medical Association regarding research involving human subjects, and in compliance with Order No. 690 of the Ministry of Health of Ukraine dated 23.09.2009, as well as the requirements of the bioethics committee of I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 75 dated 1.11.2023). All patients provided informed consent to participate in the study.

RESULTS

A study was conducted on 8 clinical samples from patients undergoing orthodontic treatment. Analysis of the gender and age composition of the patients: women predominated among the patients (6 women – 75.0%), and there were significantly fewer men (2 men – 25%, patient age from 17-37 years (median 20).

An analysis of soft dental plaque on the surface of the maxillary molar of the examined subjects with dental braces was carried out before and after the use of mouthwash after 90 days of its application. The isolated microorganisms belonged to gram-positive bacteria, the number of which was 63.63% and 70%, gram-negative bacteria 27.27% and 20.0%, and representatives of the other group 9.09% – 10.0% respectively ($p = 0.999$). The results of the paired t-test showed that there was a negligible difference between the two groups and demonstrated a slight decrease in gram-negative bacteria over the group of gram-positive bacteria (Fig. 1).

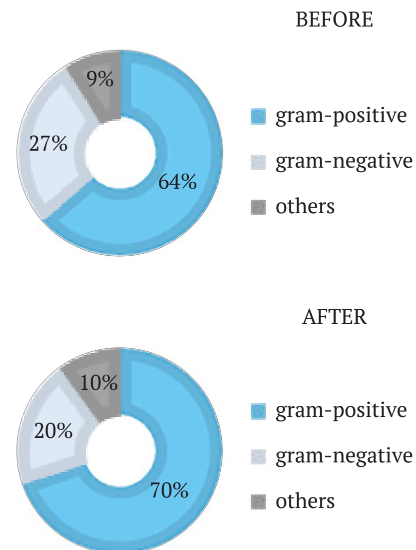

Figure 1. The proportion of microorganism groups before and after using the mouthwash in the examined subjects ($n = 8$)
Source: compiled by the authors

The taxonomic composition of the soft dental plaque microbiocenosis in the examined subjects was represented by microorganisms belonging to 11 genera of bacteria and *Candida* fungi and 9 genera and *Candida* yeasts after the use of mouthwash. Determination of the constancy index indicated the proportion of representatives of different genera in the studied samples before (group 1) and after (group 2) using the mouthwash after 3 months of application. In particular, the dominant ones include *Lactobacillus* spp., *Streptococcus* spp. β, *Streptococcus* spp. α, *Streptococcus* spp. γ, *Corynebacterium* spp., *Propionibacterium* spp., and *Candida* spp. (from 50.0-100%), which belong to both gram-negative and gram-positive bacteria, however, representatives of *Corynebacterium* spp. were not found after application. Further analysis of the obtained samples demonstrated the presence of *Staphylococcus aureus*, *Staphylococcus* spp., *Peptostreptococcus* spp., *Neisseria* spp., which belong to the frequently occurring ones (from 25.0-37.5%) with the difference between the two groups of bacteria of the genus *Neisseria* spp., which were not found in the second group. Representatives of microorganisms that are not often found were *Actinomyces* spp. and representatives of the allochthonous oral microbiota, bacteria of the genus *Escherichia*, namely *E.coli* – 12.5% respectively (Fig. 2).

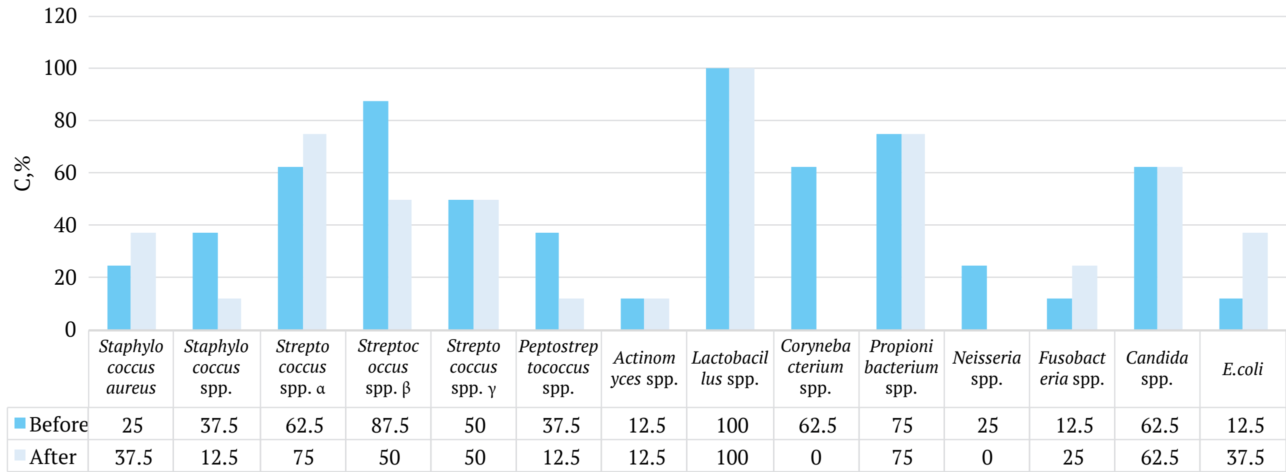

Figure 2. Constancy index in the examined patients with dental braces
Source: compiled by the authors

Study of the population level of various microorganisms in the microbiocenosis on the surface of the upper molar in patients with dental braces before and after using the mouthwash (Fig. 3).

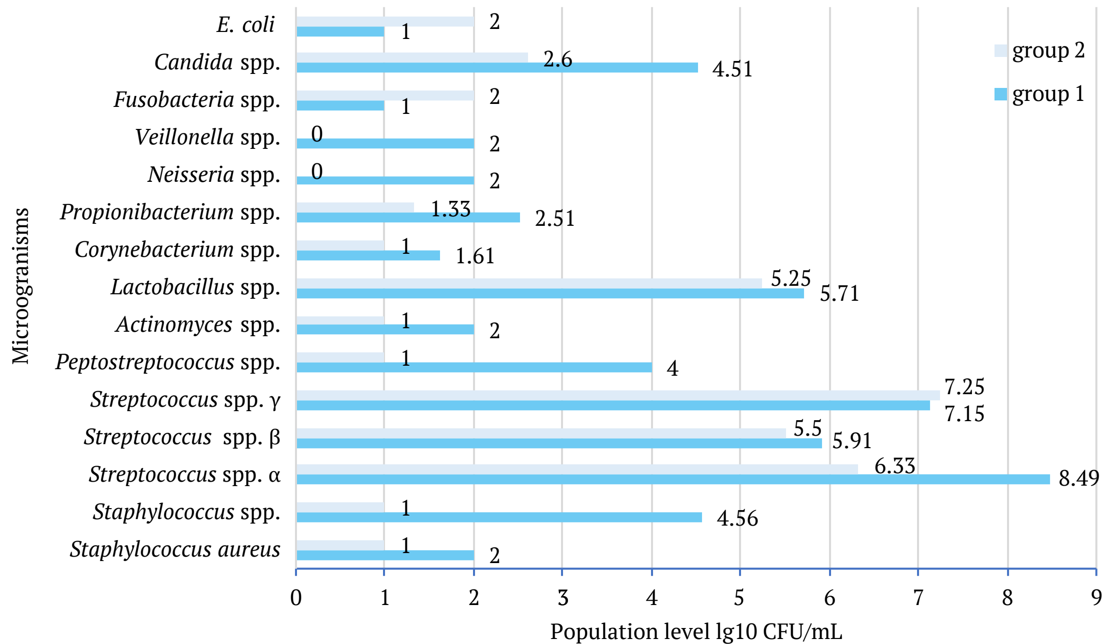

Figure 3. Population level of soft dental plaque microbiota in patients undergoing orthodontic treatment before and after using the mouthwash
Source: compiled by the authors

The composition of soft dental plaque is formed by autochthonous lactobacilli, *Streptococcus* spp. α, γ, β, which, on average, reach a significant population level and belong to the group of microorganisms with a high dominance index. The determination of differences between the population composition in patients before and after the use of mouthwash for *Streptococcus* spp. α $p(x \leqslant T) = 0.9958$, *Propionibacterium* spp. $p(x \leqslant T) = 0.9837$ is statistically significant concerning the mean values $p < 0.05$. However, when comparing the mean values for *Streptococcus* spp. β, it was found that there were no significant differences, and it was $p(x \leqslant T) = 0.6544$, so it can be considered that such differences are insignificant. For *Lactobacillus* spp. ($p(x \leqslant T) = 0.631$), *Streptococcus* spp. γ ($p(x \leqslant T) = 0.5873$) the comparison of data between group 1 and group 2 was also insufficient to be statistically significant. The determination of differences at the population level between groups 1 and 2 for the conditionally pathogenic representative of the genus *Candida* spp. by paired t-test ($p(x \leqslant T) = 0.04374$) indicates insufficient differences between the mean values in both groups.

Other conditionally pathogenic microorganisms such as staphylococci, *E. coli*, *Veillonella*, *Neisseria*, fusobacteria, and *Actinomyces* have low population-level bacterial counts. When comparing the average values of all detected

microorganisms before and after the use of mouthwash in the subjects, it was established that the differences between the groups are statistically significant $p(x \leq T) = 0.01273$, and are at the significance level $p < 0.05$. When comparing the population levels before and after the use of alcohol-free mouthwash using a paired t-test, it showed that there is a significant difference between before and after $p = 0.005$.

One of the important criteria for choosing a mouthwash is its effect on periodontal tissues. Thus, to determine this effect, the gingival index was chosen, which was determined before the use of mouthwash and after three months of its use (Fig. 4).

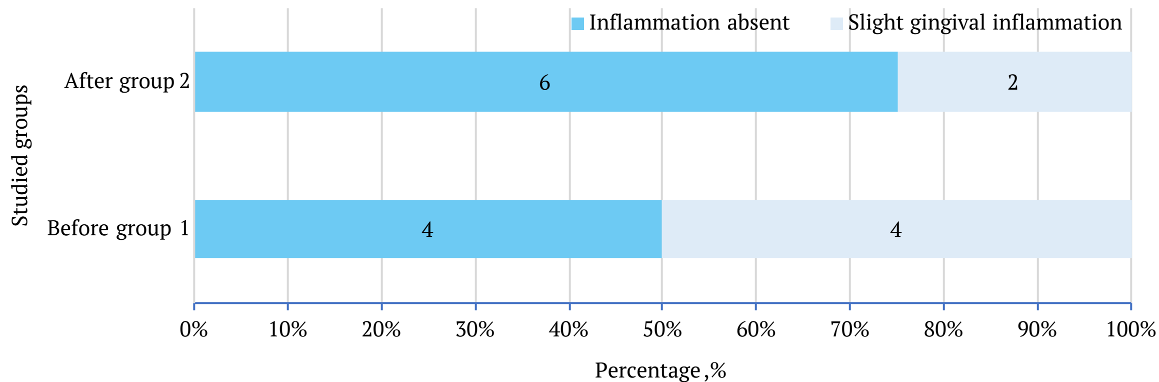

Figure 4. Comparison of periodontal tissue condition in patients before and after using the mouthwash
Source: compiled by the authors

A review of the subjects before the start of the experiment demonstrated an equal ratio of patients with mild tissue inflammation and those without it was 1:1, after use 1:3. When comparing the gingival index of the two groups based on the results of the paired t-test, a slight difference was demonstrated between before and after, $p = 0.451$, the use of alcohol-free mouthwash and was verified using the Wilcoxon signed-rank test, which confirmed that there is a small difference between before and after, $Z = -0.7$, $p = 0.484$, $r = -0.3$. As a result, the use of alcohol-free mouthwash for the oral cavity by patients undergoing orthodontic treatment did not reveal a negative impact on the mucous membrane. However, its use led to a decrease in the microbial composition at the population level and an improvement in the condition of the periodontal tissues.

DISCUSSION

The presence of orthodontic appliances in the oral cavity makes it difficult to maintain proper hygiene. To overcome this problem during the active phase of orthodontic treatment, various hygiene procedures are carried out to reduce the accumulation of bacterial plaque and reduce the risk of periodontitis in patients. The conducted study, regarding the change in the quantitative and qualitative composition of the microbiota of soft dental plaque of the maxillary molars, demonstrated insignificant changes in the taxonomic composition of the identified microorganisms, in particular, after the use of mouthwash, no representatives of the genera *Veillonella* and *Neisseria* were found, before the use of mouthwashes, the taxonomic representation was 11 genera, and after 9 genera, respectively, which was reflected in the decrease in bacteria belonging to the group of gram-negative from 27.0% to 20.0%, and an increase in gram-positive microorganisms from 64.0% to 70.0%, respectively. This is confirmed by the results of a study by a group of scientists, namely R. Bescos *et al.* [17], who indicate an increase in gram-positive bacteria (Firmicutes) and a decrease in representatives of gram-negative bacteria (Bacteroidetes), which is due to an increase in the acidity of the oral cavity pH, which is one of the factors influencing the physiological processes of microorganisms' development.

The results of the study show that the use of alcohol-free mouthwash with cetylpyridinium chloride significantly alters the oral microbiota. A comparison of the constancy index before and after three months of using the mouthwash revealed a decrease in the number of *Corynebacterium* spp. and *Neisseria* spp., *Veillonella* spp., after application. The predominance of *Lactobacillus* spp., *Streptococcus* spp., *Propionibacterium* spp., and *Candida* spp. remained high (from 50.0 to 100%). It is worth noting that in the study of Z.L.S. Brookes *et al.* [18] the results of research on the microbiome composition under the influence of chlorhexidine were demonstrated, using the genome sequencing method and indicated a decrease in the number of bacteria of the genus *Veillonella*, which led to a slight increase in fungi of the genus *Candida* spp., which is reflected in a decrease in the taxonomic diversity of microorganisms after the use of mouthwash, this is consistent with the obtained results, in particular, a decrease in the number of bacterial genera from 11 to 9, and the presence of *Candida* spp. in the study group of patients undergoing orthodontic treatment according to the constancy index refers them to the dominant group, yeasts are commensals of the oral cavity, however, they have pathogenic potential when the balance in the oral cavity is disturbed. I. Chatzigiannidou *et al.* [19] and A. Al-Kamel *et al.* [20] found that broad-spectrum antimicrobial agents do not always promote healing and can lead to further disruption of the microbiome, as confirmed by studies on the effects of chlorhexidine on oral biofilms, although chlorhexidine reduced the microbial load, however, this led to non-selective destruction of the oral microbiome and contributed to an increase in the number of taxa associated with periodontitis, namely *Fusobacterium*, which is consistent with the obtained results, regarding the increase in bacteria of the genus *Fusobacterium* at the

population level $p(x \leqslant T) = 0.01273$ ($p < 0.05$). However, the use of alcohol-free mouthwash in the subjects after three months of use revealed statistical differences ($p < 0.05$) between before and after its use, both in terms of the quantitative and qualitative composition of dental plaque.

In a study conducted by M. Rajendiran *et al.* [21], which compared the composition of different mouthwashes and their effect on plaque formation and gingivitis, it was found that the use of an oral hygiene product containing cetylpyridinium chloride demonstrated a significant reduction in gum inflammation due to disruption of dental plaque maturation. This is consistent with the results obtained during the study, the condition of the periodontal tissues improved in two patients, as confirmed by the gingival index ($p = 0.451$), and the quantitative indicators of the population level of microorganisms in the formation of soft dental plaque also decreased, as confirmed by the obtained statistical indicators.

A study conducted by A. Albert-Kiszely *et al.* [22] demonstrated that after 3 and 6 months of rinsing, there were no significant differences ($p = 0.05$) between the experimental cetylpyridinium chloride and control groups regarding overall gingivitis, gingival bleeding, and plaque accumulation when examining mean values. However, when dichotomous data were examined, there were no statistically significant differences, but they were found over time. For example, 49.0% of samples were positive for *Porphyromonas gingivalis* at baseline. At the 6-month examination, 43.9% were positive ($p = 0.24$) for *P. gingivalis*. After analysing the data on the gingival index, no statistically significant differences were found in gingival bleeding between the two study groups. However, after 3 and 6 months, a statistically significant decrease in gingival bleeding was demonstrated, but between 3 and 6 months these results were not statistically significant. Such an effect was observed in the study in some patients with bleeding gums, in whom the condition of the periodontal tissues improved, leading to a decrease in inflammation.

K. Becker *et al.* [23] suggest that the use of chemical mouthwashes as an adjunct to regular oral hygiene practices was effective in reducing plaque and gingivitis, thereby improving oral health over a 30-day experimental period. To study the effect, a 0.07% solution of cetylpyridinium chloride was used; the results showed a significant difference between pre- and post-observations at significance levels of 0.05% and 0.01% for cetylpyridinium and the control group in reducing gingival bleeding and plaque index. However, for the gingival index, such differences were not significant. This is also confirmed by studies by Z. Brookes *et al.* [24], who published a review of scientific papers on mouthwashes with different chemical compositions. They indicate that the evidence for the effectiveness of these agents on the microbiome has some limitations. In particular, while exerting a bacteriostatic and bactericidal effect on opportunistic or pathogenic microorganisms *in vitro*, acting against plaque and gingivitis *in vivo*, over-the-counter mouthwashes can cause dysbiosis of the oral microbiota. Most research on the clinical efficacy, namely the antimicrobial effect, has been conducted for antiseptic agents containing chlorhexidine. There is much less research on other such agents. In a randomised controlled trial, F.A. Adam *et al.* [25] noted that, regarding the long-term efficacy of cetylpyridinium chloride for patients without orthodontic treatment, the use of a 0.07% solution of cetylpyridinium chloride for mouth rinsing was effective in reducing plaque levels. Gingival bleeding scores did not differ after 6 months. The tested product was well-tolerated and did not cause any serious clinical adverse effects or negative impact on the microbiota. This is emphasised by studies on the effect of mouthwash on the state of the oral microbiota, as well as the condition of teeth and gums, and does not indicate a negative impact on the composition of the microbiome.

In the study by B.A. Newman *et al.* [26], it was demonstrated that the number of *Porphyromonas, Corynebacterium, Abiotrophia*, and other known periodontal pathogens did not increase in supra-gingival plaque during 21 days of experimental gingivitis induction in the presence of cetylpyridinium chloride. At the same time, a slight increase in gum inflammation and bleeding was observed compared to the beginning of the study. The authors assert that a mouthwash containing cetylpyridinium chloride, when used as the sole oral hygiene product, provides significant benefits in reducing gingival inflammation by disrupting the maturation process of dental plaque (i.e., gingivitis-associated microbiota) and balancing the diversity and composition of the oral microbiota (i.e., health-associated microbiota). The results show a decrease in the number of *Corynebacterium* spp. and a reduction in periodontal tissue inflammation after the use of alcohol-free mouthwash.

I. Chen *et al.* [27], conducting a study on changes in the microbiota during orthodontic treatment using the sequencing method, argue that there is an increase in microbial richness in such patients compared to the control. Therefore, the use of mouthwashes as an auxiliary hygiene tool is justified and is confirmed in studies by U. Hussain *et al.* [28] regarding the use of chlorhexidine, which helps to control plaque formation and gingivitis but requires careful use due to possible side effects. G.P.J. Langa *et al.* [29] and M.M.T. Oo *et al.* [30] in their scientific papers argue that the use of a mouthwash containing cetylpyridinium chloride compared to provides a reduction in plaque and gingivitis index compared to patients who do not use mouthwashes to improve oral hygiene.

To summarise the results obtained regarding the use of alcohol-free mouthwash, it can be noted that it is insufficient as the sole oral hygiene agent for reducing plaque and improving the condition of periodontal tissues. However, in a comprehensive care regimen, when used in conjunction with a toothbrush, toothpaste, and mouthwash, it can help control plaque formation and maintain oral hygiene.

CONCLUSIONS

The study demonstrated that the use of an alcohol-free oral hygiene product in patients undergoing orthodontic treatment affects the composition of the microbial cenosis in soft dental plaque. A decrease in gram-negative (from 27.27% to 20.0%) bacteria compared to gram-positive (from 63.63% to 70%) ($p = 0.999$) indicates the effectiveness of this product in reducing pathogenic microbiota. It was also found that at the population level of the microbiocenosis of the molar surface, after the application of the product, the number of representatives of *Streptococcus* spp. α $p(x \leqslant T) = 0.9958$, *Propionibacterium* spp. $p(x \leqslant T) = 0.9837$

differences were statistically significant $p<0.05$, for bacteria of the genera *Streptococcus* spp. $p(x \leqslant T) = 0.6544$, *Lactobacillus* spp. ($p(x \leqslant T) = 0.631$), *Streptococcus* spp. γ ($p(x \leqslant T) = 0.5873$) and fungi of the genus *Candida* spp. ($p(x \leqslant T) = 0.04374$) differences were insignificant, however, a trend towards their decrease was observed. A decrease in the quantitative composition of microorganisms in dental plaque leads to a decrease in the products released by them, which act as activators of gum inflammation. It has also been demonstrated that the population levels of microorganisms change significantly before and after using the product, confirming its effectiveness in reducing the number of bacteria in the oral cavity. The anti-inflammatory effect of the mouthwash on the condition of the gums of the subjects was better, due to a decrease in bleeding sites, as demonstrated by the gingival index, which was determined before and after use ($p = 0.451$). Therefore, the studied mouthwash caused insignificant changes in the quantitative and qualitative composition of dental plaque and improved the condition of the gum tissues, compared to the baseline. This study is important for creating new strategies for the prevention and treatment of oral diseases. However, it is necessary to conduct a double-blind, randomised, clinical trial to supplement the data on the impact on the oral microbiome, since even alcohol-free agents can have both positive and negative effects.

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CONFLICT OF INTEREST
None.

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Ополіскувач як фактор контролю формування м'якого зубного нальоту у пацієнтів з ортодонтичним лікуванням

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Анотація. Пацієнти з брекет-системами частіше стикаються з проблемою швидкого накопичення зубного нальоту та запалення ясен, тому антимікробні ополіскувачі для ротової порожнини, які покращують щоденний домашній догляд, можуть забезпечити ефективне видалення і контроль бактеріального нальоту, покращення стану ясен та є безпечними профілактичними засобами без побічної дії. Метою дослідження було оцінити кількісний та якісний склад мікробіоценозу м'якого зубного нальоту під впливом безалкогольного засобу для гігієни ротової порожнини у пацієнтів, що отримували ортодонтичне лікування. У дослідженні брали участь пацієнти, які проходять ортодонтичне лікування, забір матеріалу відбувався з поверхні зубів верхньої щелепи. Отримані мазки з поверхні верхніх молярів досліджували бактеріологічним методом з подальшою ідентифікацією: мікроскопічною, культуральною, біохімічною. Порівняння ефективності безалкогольного ополіскувача, щодо змін кількісного та якісного складу мікроорганізмів та стану тканин пародонту у пацієнтів, які проходять стаціонарну ортодонтичну терапію, здійснювали після трьох місяців застосування. У дослідження було виявлено грампозитивні та грамнегативні бактерії та гриби *Candida* та з подальшим незначним зменшенням грамнегативних бактерій ($p=0,999$) після застосування ополіскувача. Отримані результати показали зміни таксономічного складу, а саме, зменшення кількості родів бактерій від 11 до 9. Крім зазначених результатів було виявлено зміни на популяційному рівні мікробіоти м'якого зубного нальоту пацієнтів, які використовували безалкогольний ополіскувач протягом трьох місяців, виявлено, зменшення кількості *Streptococcus* spp. α $p(x \leqslant T) = 0,9958$, *Propionibacterium* spp. $p(x \leqslant T) = 0,9837$, щодо *Streptococcus* spp. β, з *Streptococcus* spp. γ зміни були незначними. При порівнянні популяційного рівня мікроорганізмів до та після застосування ополіскувача було виявлено достовірні відмінності ($p<0,05$). Визначення індексу гінгівіту продемонстрував незначне покращення стану пародонту у обстежуваних. Проведене дослідження має важливе значення для розробки нових стратегій профілактики та лікування захворювань порожнини рота

Ключові слова: мікробіоценоз; мікроорганізми; ротова порожнина; гінгівіт; брекет-система

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Levels of glycolytic intermediate metabolites in brain cells of rats under conditions of energy drink consumption

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Abstract. Energy drinks are popular among young people to increase performance and attention. However, their impact on metabolic processes and physiological functions has led to restrictions on their consumption in many countries. The study aimed to determine the levels of glycolytic intermediate metabolites in rat brain neurons under conditions of energy drink consumption. The study was conducted on sexually mature Wistar rats weighing 180-200 g. The animals were divided into groups, and biological material was collected for experimental purposes. Brain homogenate was prepared using a homogeniser and a cold extraction medium in a ratio of 1/9. In the obtained brain homogenate and blood serum, the concentrations of glucose, pyruvate, lactate, and the activity of the glycolytic enzyme – lactate dehydrogenase were determined. It was investigated that the consumption of an energy drink by animals led to an increase in blood glucose levels and the development of persistent hyperglycaemia. Regarding the glucose content in the brain, there was an observed increase in its utilisation by neurons. It was also established that in brain cells, the concentration of lactate (the final product of anaerobic glycolysis) and the activity of the key glycolytic enzyme, lactate dehydrogenase, increased. A decrease in the level of the glycolytic intermediate metabolites, pyruvate, was investigated in neurons. However, in blood serum, opposite changes in pyruvate levels were observed: at the beginning of the experiment, the level of pyruvate increased relative to the intact control with a tendency to normalise in the later experimental periods. By changing the level of glycolytic intermediate metabolites in the brain homogenate, it is possible to determine the course of metabolic processes and the intensity of the energy supply of brain cells

Keywords: lactate; pyruvate; brain; lactate dehydrogenase; carnitine; caffeine

INTRODUCTION

The study of the effects of energy drinks is becoming increasingly relevant today. This is because the consumption of these drinks elicits conflicting opinions: interest from young people and warnings from doctors. Energy drinks are classified as non-alcoholic, highly carbonated beverages containing carbohydrates (glucose, sucrose), alkaloids of the xanthine group (caffeine, theobromine, theophylline), extracts of guarana and ginseng, carnitine, vitamins (A, B_1, B_2, B_6, B_{12}, C, P), amino acids (taurine), and macro- and microelements: magnesium, potassium, manganese, sodium, iron, chlorine – all of these are considered stimulants for the body. Therefore, they are used to prolong the period of wakefulness, relieve fatigue, increase productivity, and enhance memorisation during exams by students, among other purposes. Most teenagers do not adhere to consumption recommendations. According to modern literature sources, the most powerful psychostimulant in the drink is caffeine, which reduces feelings of fatigue, improves cognitive function, increases heart rate, and has a diuretic effect. Under the influence of caffeine, not only mental activity

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but also physical activity increases, and the reaction-response to external influences is enhanced. The period of stimulation is followed by fatigue.

I. Klishch *et al.* [1] point out that under the influence of caffeine, such metabolic processes as glycolysis, lipolysis, general metabolism and oxygen demand accelerate, and hyperglycaemia may occur. J.P. Higgins *et al.* [2] identify another component of the drink – theobromine, but it has a less stimulating effect. It is an alkaloid of the purine series, and in its physical properties, it is transparent crystals that are bitter in taste. The therapeutic dose of theobromine is significantly higher than that of caffeine. This compound has a relaxing effect on smooth myocytes. Carnitine (a component of the drink) also enhances carbohydrate metabolism, has a positive effect on cardiovascular activity, and is sometimes used in medical practice. In her study, I. Yastremska [3] notes that L-carnitine acts as an active regulator of intermediate metabolism and energy supply processes. Therefore, the use of L-carnitine in the treatment of myocardial infarction, in addition to its pronounced energy-producing activity and antioxidant effect, had an effective impact on the endothelial function of blood vessels. Guarana and ginseng are natural stimulants with different effects, they also reduce fatigue and increase mental and physical activity. B vitamins are important cofactors in biochemical transformations but do not possess "energy-giving" properties. There are serious safety concerns about the safety of these products, especially with such a composition. In scientific publications, I.M. Nadeem *et al.* [4] mention disorders of the nervous system as a result of the consumption of energy drinks (irritability, fatigue, sleep disturbances, cramps). Therefore, studies of the effect of energy drinks on metabolic processes in the brain cells that ensure the overall functioning of the nervous system and occur with the loss of energy resources will be important. The intensity of energy metabolism is a leading factor limiting brain activity. The brain obtains energy (ATP) as a result of the aerobic breakdown of glucose (glycolysis), which ensures normal brain function: the conduction of excitation by nerve fibres, the synaptic transmission of signals, the specific activity of nerve centres, and the molecular mechanisms of integrative brain functions – memory, thinking, consciousness. Kh. Partsei [5] highlights information about the effect of energy drinks on carbohydrate metabolism in erythrocytes, in particular, an increase in glucose catabolism, as evidenced by an increase in lactate concentration and LDH activity. The authors Kh. Partsei *et al.* [6] investigated the changes in the pro- and antioxidant systems of rat erythrocytes under conditions of energy drink consumption. Their study presents the effect of energy drinks on free radical oxidation processes, which are accompanied by the activation of lipid peroxidation and protein peroxidation, which leads to a violation of the structural and functional ability of erythrocyte cell membranes, as well as an increase in the activity of superoxide dismutase. There is no data on carbohydrate metabolism in the brain. The purpose of the study was to investigate changes in carbohydrate metabolism in brain cells of experimental animals as a result of energy drink consumption.

MATERIALS AND METHODS

The experimental work was carried out at the "Centre of Bioelementology" of Ivano-Frankivsk National Medical University (accreditation certificate – CDL No. 037/19 of 13 June 2019) from October to December 2021. The study involved determining the effects of energy drinks on the metabolic processes of sexually mature male rats weighing 180-200 grams. A total of 56 animals were used in the experiment. The experimental animals were kept on a standard vivarium diet under appropriate temperature and lighting conditions (12-hour light cycle), adhering to all requirements and recommendations [7, 8]. Before the start of the experiment, all animals underwent a 10-day acclimatisation period to avoid the influence of stress factors on the measured parameters. At each stage of the experiment, the weight of the animals was recorded. The volume of the energy drink was calculated individually for each laboratory rat, based on the threshold limit value for a healthy adult human (indicated on the drink packaging) recalculated per kg of animal weight. The energy drink was administered orally. Experimental animals were divided into groups, the experimental model is presented in Table 1. All animals had free access to drinking water. The composition of the studied energy drink includes the following substances: purified water, sugar, carbon dioxide, citric acid, sodium citrate, taurine, preservatives, potassium sorbate, sodium benzoate, sugar colour, flavourings, caffeine, inositol, vitamins (niacin, B_6, B_{12}), guarana extract, and the antioxidant ascorbic acid.

Table 1. Distribution of animals in groups

Animal groups	Number of animals	Influence	Term of biomaterial collection
Group 1 (intact control)	28	rats that consumed water	1st, 10th, 20th, 30th, after discontinuation of the drink
2nd group (experimental)	7	rats that consumed the energy drink for 30 days	1 day, after discontinuation of the drink
3rd group (experimental)	7	rats that consumed the energy drink for 30 days	10 days, discontinuation of the drink
4th group (experimental)	7	rats that consumed the energy drink for 30 days	20 days, discontinuation of the drink
5th group (experimental)	7	rats that consumed the energy drink for 30 days	30 days, discontinuation of the drink

Source: compiled by the authors

Before collecting biological samples, the experimental animals were anesthetised with sodium thiopental. Since the sampling was conducted under identical conditions for all groups, the effects of the collection methods on glycolytic intermediate metabolites and the anaesthetic effect are neutralised. The brain and blood of experimental animals were collected. Brain homogenate was prepared using a homogenizer and a cold extraction medium in a 1:9 ratio. In the obtained brain homogenate and blood serum, the levels of glucose (glucose oxidase method), pyruvate (by the amount of 2,4-dinitrophenylhydrazone derivatives), lactate (by reaction with para-oxydiphenylene), and lactate dehydrogenase (LDH) activity were determined. The enzymatic activity of LDH was determined spectrophotometrically. Statistical analysis of the results was performed using the STATISTICA 8 program, taking into account the Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Glucose is the primary energy source for brain cells, so even slight fluctuations in glucose levels can disrupt metabolic transformations in brain neurons [9]. The brain uses 70% of the glucose synthesised in the liver and that obtained from food [10]. In neurons, approximately 90% of glucose is oxidised to CO_2 and H_2O in the Krebs cycle (aerobic glycolysis), 5% is spent in the process of anaerobic glycolysis with the formation of lactate, and 5% in the reactions of glycogen, sphingolipids, cerebrosides, sulfatides, glycoproteins, and neurotransmitter synthesis [11]. As a result of the conducted research, changes in glucose content were observed as early as the 1st day after 30 days of consuming the energy drink – the glucose content increased by 19.38% compared to the intact control; on the 10th day, the glucose concentration remained slightly elevated by 6.3%; on the 20th day, the glucose level was within the normal range. Such changes in glucose indicators (Fig. 1) are predictable, since according to the literature, the effect of all components of energy drinks is aimed at achieving an energy boost effect [12]. However, already on the 30th day of discontinuing the energy drink, the glucose content decreased by 11.59% compared to the control. This may lead to the exhaustion of neurons, feelings of fatigue, apathy, and other disorders of the nervous and other systems. The author Kh. Partsei [5] presented a study of an increase in glucose content in erythrocyte hemolysate under the influence of an energy drink by 1.8 times, and after discontinuation of the drink, the glucose level decreased by 1.2 times.

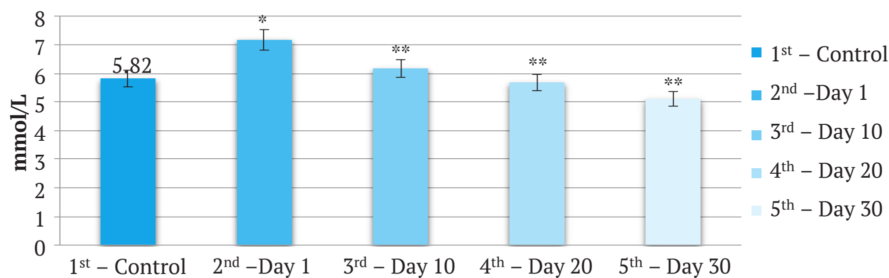

Figure 1. Changes in glucose levels in the brain homogenate in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

The level of glucose in the blood is also influenced by carnitine, a component of the drink. In the studies of H. Fathizadeh *et al.* [13], it is noted that the treatment of patients with carnitine led to a decrease in the level of glucose and insulin in fasting plasma. At the same time, this study examined the level of glucose in blood serum (Fig. 2), which made it possible to establish the development of persistent hyperglycaemia throughout the entire experimental period. In rats of the 2nd group, the glucose content in blood serum increased by 21.78% compared to intact animals and remained elevated throughout the entire study period: in the 3rd group – by 17.94%, in the 4th – by 11.27%, in the 5th – by 6.79%.

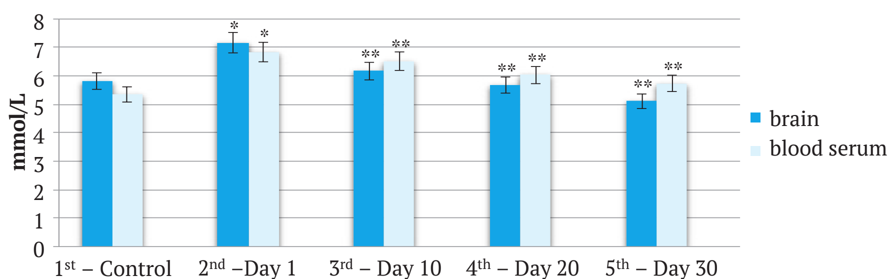

Figure 2. Comparative analysis of changes in glucose levels in brain homogenate and blood serum in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

Comparative analysis of glucose content (Fig. 2) in brain homogenate and blood serum showed an increase in the second experimental group of animals. Glucose levels in animals of the third group decreased in both studied substrates compared to the second group but remained elevated relative to the intact control (1st group). Initially, glucose levels in the brain decreased. By the 20th day of the experiment, the glucose level in the brain normalised, but in the blood serum, it remained elevated by 1.12 times. In the 5th experimental group, a decrease in the glucose level in the brain homogenate was observed, but in the serum, it exceeded the norm by 1.07 times. It is worth noting that the glucose level, after consumption of the energy drink, immediately increased in the brain and blood serum, but after discontinuation of this drink, the glucose level in the brain homogenate was lower than the intact control, while in the blood serum, it remained elevated.

To understand glucose metabolism, it is important to study the products of intermediate metabolism, in particular pyruvate and lactate. Pyruvic acid (pyruvate) is formed during glycolysis and during the conversion of lactate under the action of lactate dehydrogenase [14]. Under aerobic conditions, pyruvate is oxidised to acetyl-CoA, can enter the Krebs cycle and provide energy for the body or be used for other metabolic pathways (in particular, for the synthesis of fatty acids or cholesterol) [15]. The concentration of pyruvate in the brain (Fig. 3) on the 1st day, after discontinuation of the energy drink, was 0.231 ± 0.0012 (mmol/L), which is 10.8% lower than the intact control indicator.

On the 10th and 20th days, the level of pyruvic acid remained decreased by 12.33%, and on the 30th day – by 4.12% with a tendency to normalise (0.248 ± 0.02 mmol/L). However, in the studies of Kh. Partsei [5], the content of pyruvate in erythrocyte hemolysate steadily increased by 3.9 times compared to the intact control during the consumption of the energy drink and decreased in the subsequent periods of the study (10th, 20th, and 30th days of the experiment). R. Ostapiv [16], in his studies of the effect of taurine on energy processes in cells, notes that when a smaller dose of taurine (40 mg/kg) is administered, the glucose content decreases relative to the control group of animals, and when a larger dose is administered (100 mg/kg), the glucose level increases. Taurine is a derivative of sulphur-containing amino acids, present in energy drinks, and can increase tissue sensitivity to glucose.

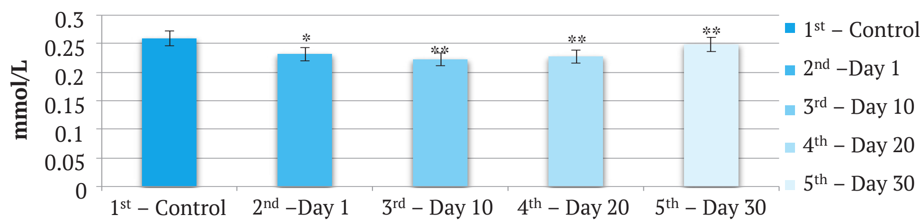

Figure 3. Changes in pyruvate levels in brain neurons in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 (control) of animals ($p < 0.01$); ** – significant compared to the data of group 2 of animals ($p < 0.01$)
Source: compiled by the authors

O. Remennyk [17] believes that an increase or decrease only in pyruvate in the blood is not a diagnostic sign; usually, the ratio of lactate to pyruvate is determined, which characterises the state of glycolytic and oxidative conversion of carbohydrates. It is well known that in cells with limited oxidative capacity or under hypoxic conditions, the tricarboxylic acid cycle is blocked, and pyruvate, under the action of lactate dehydrogenase, is converted to lactic acid, lactate. In this case, only 2 ATP molecules are formed [18] and hyperlactatemia occurs. The reasons for its appearance may be congenital diseases (type I glycogenosis), vitamin B_1 deficiency, administration of adrenaline, sodium nitroprusside into the blood, in case of alkalosis, epilepsy, the intake of too much fructose, sorbitol or xylitol [19]. An increase in the level of pyruvic acid in tissues may indicate a disruption of the balance between oxygen supply systems to tissues and the need for it. It has been established that the level of pyruvate in the blood increases as a result of decompensation for hypoxic conditions [20]. In blood serum (Fig. 4), the content of pyruvate increased on the 1st day after discontinuation of the energy drink by 29.6%, on the 10th day – by 24%, on the 20th day – remained elevated by 13.6% and with a tendency to normalise the level of this indicator on the 30th day.

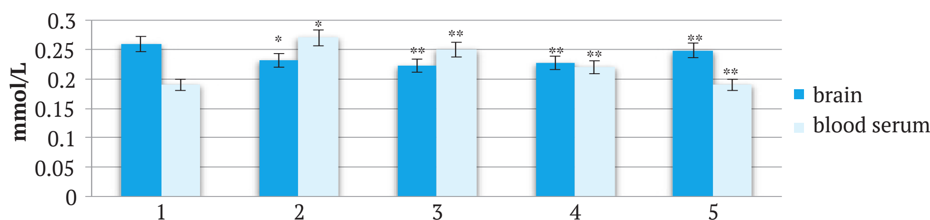

Figure 4. Comparative characteristics of changes in pyruvate levels in brain homogenate and blood serum in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

Regarding the comparative characteristics of pyruvate content in brain homogenate and blood serum, the changes are observed to be in different directions (Fig. 4). In the brain homogenate, in almost all experimental groups of animals, the level of pyruvate is lower than the intact control, only in the last period of the experiment is there a tendency towards normalisation. Regarding the pyruvate content in blood serum, an increase in this indicator was noted in the 2nd, 3rd, and 4th groups of animals and a return to normal in the 5th group relative to the intact control.

The study of lactate (Fig. 5) in brain homogenate showed an increase in this indicator in the 2nd group of animals by 20.39%, in the 3rd and 4th groups – by 13.37% and 4.98% respectively, and in the 5th group, the lactate content stabilised relative to the control group of animals. In their article, Kh. Partsei [5] also presents an increase in lactate content in erythrocyte hemolysate in the 2nd, 3rd, 4th, and 5th experimental groups by 1.9 times, 1.6 times, 1.2 times, and 1.1 times, respectively, under the influence of the energy drink.

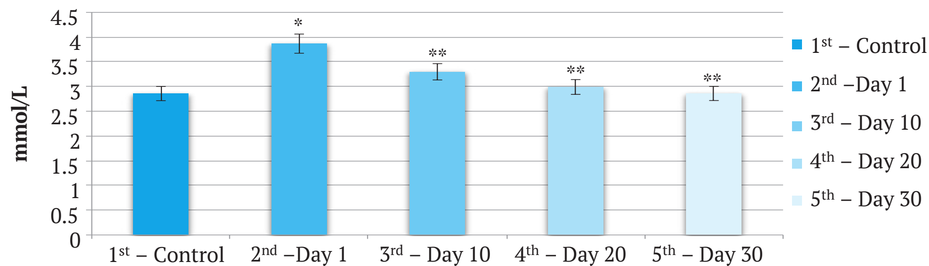

Figure 5. Changes in the level of lactate in brain neurons in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

A similar trend of increasing lactate and pyruvic acid content was observed by G. Khitry *et al.* [21] in their study of changes in carbohydrate metabolism metabolites in rats under general hypothermia. High lactate content can be observed during hypoxia, diabetes mellitus, excessive intake of sucrose and other substances, as well as with impaired lactate elimination by the liver (60%) and kidneys (30%). An increase in lactate concentration above 2 mmol/L is a diagnostic indicator of sepsis, and an increase above 4 mmol/L serves as a criterion for multiple organ dysfunction syndrome [22]. Lactate is a product of anaerobic glycolysis and is formed from pyruvate under the action of the enzyme lactate dehydrogenase with limited oxygen supply [23]. Reduces the activity of the enzyme inosine, a component of the energy drink [24]. R. Ostapiv & V. Manko [25] studied the effect of taurine on carbohydrate metabolism; the results of their research indicate that taurine regulates calcium metabolism and increases the activity of lactate dehydrogenase, therefore, it can be effective during intense muscular work.

The conducted studies of lactate dehydrogenase in brain homogenate showed a significant ($p < 0.05$) increase of 49.78% in the brains of rats in the 2nd group. As seen in Figure 6, the enzyme activity remained high (by 43% compared to the intact control) for 10 days after discontinuation of the drink, and on the 20th and 30th days, there was a tendency towards normalisation. In the study of Kh. Partsei [5], research on the activity of lactate dehydrogenase in erythrocyte hemolysate under the influence of an energy drink is presented. According to the author, the activity of lactate dehydrogenase increased by 2.6 times relative to the intact control, on the 10th, 20th, and 30th days, the enzyme activity remained elevated by 1.4 times, 1.9 times, and 2.1 times, respectively, compared to the indicators of the second group. According to the results of studies on the effect of taurine on energy metabolism, R. Ostapiv & V. Manko [25] reported an increase in lactate dehydrogenase activity in rat erythrocytes – by 59%, and in the brain – by 80% compared to the control. The authors suggest that such changes are possible with a limited oxygen supply. Prolonged administration of taurine leads to a decrease in the mass of organs, compared to the control, but the mass of the brain increases, which is explained by the positive effect of taurine on energy supply.

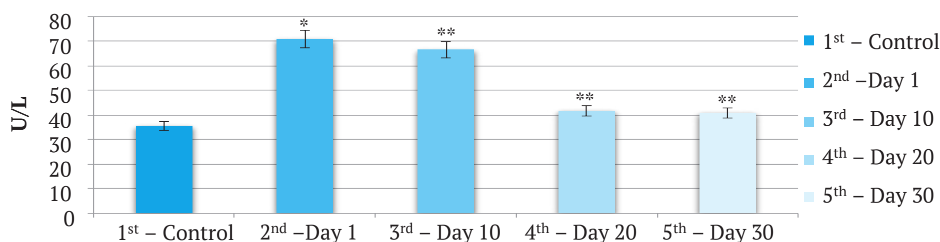

Figure 6. Changes in the activity of lactate dehydrogenase in brain cells in response to energy drink consumption (M ± m), n = 7
Notes: * – significant compared to the data of group 1 of animals ($p < 0.05$); ** – significant compared to the data of group 2 of animals ($p < 0.05$)
Source: compiled by the authors

In their research, E. Choo *et al.* [26] note that the consumption of caffeine leads to an increase in blood glucose levels. According to the results of this study, the level of glucose in blood serum and brain homogenate also increases under the conditions of consuming an energy drink. According to the article by S.M. Seifert *et al.* [27], an overdose of caffeine or energy drinks leads to loss of sleep, increased blood pressure, nausea, nervousness, muscle tone loss, cramps, changes in heart rhythm, and feelings of anxiety. Thus, the consumption of an energy drink by experimental animals leads to changes in some intermediates and enzymes of glycolysis (increased glucose and lactate content, increased lactate dehydrogenase activity, but decreased pyruvate level) in brain neurons and blood serum, which are described in the following conclusions.

CONCLUSIONS

This study aimed to investigate the impact of energy drinks on the organism of experimental animals, specifically focusing on carbohydrate metabolism parameters over 30 days of energy drink consumption and a 30-day post-experiment period. The main objective of this study was to identify changes in the content of glucose and intermediate glycolytic metabolites (pyruvate, lactate) in brain cells and serum. To assess the energy supply of neurons and the course of oxidative processes, changes in lactate dehydrogenase activity were studied. During the experiment, an increase in glucose concentration in the brain cells of experimental animals was found from 5.82 ± 0.02 mmol/L (intact control) to 7.17 ± 0.03 mmol/L on the 1st day, after 30 days of energy drink consumption. The following periods of the study were accompanied by the normalisation of this indicator to 5.11 ± 0.008 mmol/L (30th day, after discontinuation of this drink consumption). An increase in glucose content can lead to glycosylation of various structures in the brain. The research on carbohydrate metabolism metabolites in brain homogenate indicates a consistent decrease in pyruvate concentration throughout the entire experimental period, approximately by 10%. At the same time, the level of lactate, under the conditions of consuming an energy drink in rat brain cells, steadily increased from 2.85 ± 0.036 mmol/L to 3.86 ± 0.041 mmol/L. The opposite nature of changes in glycolysis intermediates – rising lactate alongside decreasing pyruvate – indicates an intensification of anaerobic glucose conversion. Confirmation of this is the increase in lactate dehydrogenase activity by 49%. Such changes are not typical for the brain and may indicate impaired functioning of the nervous system. Therefore, the perspective of further research will be to determine the activity of key enzymes of aerobic glycolysis, namely the enzymes of the tricarboxylic acid cycle (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, mitochondrial malate dehydrogenase, and Na^+/K^+ – ATPase). These enzymes will reflect the course of oxidative processes in the brain cells of experimental animals, which will help predict the effects of energy drinks on the human body to some extent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Рівень проміжних інтермедіатів гліколізу у клітинах головного мозку щурів за умов споживання енергонапою

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Анотація. Енергетичні напої популярні серед молоді як засоби для підвищення працездатності та уваги. Однак їх вплив на метаболічні процеси та фізіологічні функції призводить до обмежень їх вживання в багатьох країнах. Метою дослідження було визначення рівня проміжних інтермедіатів гліколізу у нейронах мозку щурів за умов споживання енергетичного напою. Дослідження було проведено на статевозрілих щурах лінії Вістар, масою 180-200 г. Тварин було розподілено по групах. З експериментальною метою здійснювали забір біоматеріалу. Гомогенат головного мозку готували з використанням гомогенізатора та охолодженого середовища виділення в співвідношенні 1/9. В отриманому гомогенаті мозку та сироватці крові проводили визначення концентрації: глюкози, пірувату, лактату та активності гліколітичного ензиму – лактатдегідрогенази. Було досліджено, що споживання енергетичного напою тваринами призводило до підвищення рівня глюкози в крові і виникнення стійкої гіперглікемії. Щодо вмісту глюкози в головному мозку спостерігалось посилене її використання нейронами. Також було встановлено, що у клітинах головного мозку зростала концентрація лактату (кінцевого продукту анаеробного гліколізу) та активність ключового ензиму гліколізу – лактатдегідрогенази. Було досліджено у нейронах зниження рівня проміжного інтермедіату гліколізу – пірувату. Проте, в сироватці крові досліджено протилежні зміни рівня пірувату: на початку експерименту рівень пірувату підвищувався відносно інтактного контролю з тенденцією до нормалізації в крайні дослідні періоди. За зміною рівня проміжних метаболітів гліколізу в гомогенаті мозку можна визначати перебіг метаболічних процесів та інтенсивність енергозабезпечення клітин головного мозку

Ключові слова: лактат; піруват; головний мозок; лактатдегідрогеназа; карнітин; кофеїн

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Improvement of cerebral circulation with the help of mouth guards (orthodontic appliances)

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Abstract. This study was conducted to determine the effectiveness of orthodontic appliances in improving cerebral circulation. The 24 participants of the study underwent a rheoencephalographic examination before and during the use of mouth guards. Its results analysed the changes in blood filling and blood flow in the brain vessels and evaluated the dynamics of treatment. According to the results of the study, insignificant, and slight positive treatment dynamics were detected in 58.33% of the subjects, no negative dynamics were detected, and no significant changes were recorded in 41.67% of the participants. This effect of orthodontic appliances on improving cerebral circulation is explained by the fact that by alleviating the symptoms of craniomandibular dysfunction, they affect the balance of the centre of gravity of the skull, reduce tension and spasm in the neck muscles, and protect the joints from mechanical irritation, which helps to reduce vascular tone and improves blood filling and blood flow in the masticatory muscles and jaw joint. The blood filling of the chewing muscles and jaw joint has no direct connection with the blood filling of the brain vessels, as these structures are supplied from different arteries, However, a decrease in vascular tone in the craniomandibular system and in the muscle bed of the cervical spine can affect blood pressure reduction and venous outflow in the jaw joint, which will indirectly improve cerebral circulation. The obtained results indicate that orthodontic problems can be factors of deterioration of blood filling and blood flow in the vessels of the brain, so the use of mouth guards is an effective method that should be used in the complex treatment of patients with cerebral circulation disorders

Keywords: craniomandibular dysfunction; masticatory muscles; jaw joint; blood pressure; venous outflow; carotid basins

INTRODUCTION

There are two main reasons for the importance of finding alternative approaches to improve cerebral circulation: the high prevalence of stroke, dementia, Alzheimer's disease, and other neurodegenerative diseases that affect the blood supply to the brain and the limited availability of existing treatments that are not always effective or have side

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effects. The potential advantages of using orthodontic appliances (mouth guards) to improve cerebral blood flow are the absence of side effects typical of traditional therapeutic methods and the possibility of improving cerebral circulation by reducing vascular tone due to the redistribution of masticatory pressure. To evaluate the potential of this method, it is important to conduct research to determine the biological mechanism of mouthguards' impact on improving cerebral circulation and to determine the level of their effectiveness and safety. As with any research related to the effectiveness of new therapies, there are a number of significant problems with studying the feasibility of using mouthguards to improve cerebral circulation. The first is the lack of scientific evidence on the effectiveness of their use in this area.

The few works related to this topic are mainly focused on identifying the correlation between the occlusal balance achieved with the help of certain orthodontic devices and certain indicators of brain functioning measured by instrumental methods. A study of the modification of cognitive functions caused by a functional orthodontic device was conducted by F. Pachì *et al*. [1]. Their results were based on observations of the electrical activity of the brain of 10 subjects. Cognitive functions, stress, arousal, and concentration were chosen as qualitative indicators for comparison, which, according to the results of observations, had significant differences between the three phases of the study (initial occlusion, occlusion changed by a functional orthodontic device and occlusion after removal of the device).

G. Sadvandi *et al*. [2] conducted a systematic review of the effect of experimental orthodontic tooth displacement on brain activation assessed by functional magnetic resonance imaging. The results showed that patients who used orthodontic separators experienced changes in the activation of brain areas associated with nociception, emotion, and cognitive functions.

Based on the observations of C. Kariya *et al*. [3], it was determined how the process of chewing, depending on the bite, affects cerebral blood flow. They analysed the cerebral blood flow measured by functional near-infrared spectroscopy during chewing. The results showed a correlation between jaw movement and cerebral blood flow, indicating an adverse effect of an anterior open bite on brain function. Despite the fact that all studies provide evidence of a correlation between dental occlusion and brain function, the limited size of their samples does not allow for definitive conclusions about the effectiveness of the method of improving cerebral blood flow through the impact on the bite.

Another problem with the study is the lack of understanding of the biological mechanisms by which orthodontic structures could affect cerebral circulation. P. Proff *et al*. [4] studied local vascularization during orthodontic tooth movement in a rat model. Using magnetic resonance imaging, the authors analysed the effect of orthodontic tooth movement on blood flow in the surrounding tissues and confirmed the hypothesis of increased perfusion in the areas of periodontal ligament tension during orthodontic tooth movement, but the relationship between local vascularization and cerebral blood flow was not established.

The relationship between the immune system and orthodontic tooth movement based on cellular and molecular mechanisms was analysed by Y. Gao *et al*. [5]. They investigated the mechanism of interaction of bone and soft tissue remodelling, cement resorption, orthodontic pain and relapse with immune cells and immunological active substance. And the interaction of cytokines in orthodontics was studied by F. Inchingolo *et al*. [6]. The results revealed the influence of cytokines on the cellular activity and metabolism of bone and soft tissue of the tooth through inflammatory processes and processes occurring due to tension and compression in the periodontal ligament. Although the activity of the immune system is related to the blood circulation process, the mechanisms of influence of orthodontic tooth movement on cerebral circulation have not been established in the above works.

An important problem of the study is the possibility of risks for patients using orthodontic constructions. I. Kumar *et al*. [7] emphasized the possible risk of orthodontic procedures for patients with cardiovascular diseases and blood coagulation disorders. G. Gökçe [8] identified allergic reactions and chronic fatigue syndrome among the side effects associated with orthodontic treatment. The risks that may affect cerebral circulation were not considered in these studies.

An additional problem may be created by the lack of a clear understanding of gender and age differences that may affect the results of using orthodontic appliances. When studying the impact of malocclusion on improving patients' oral health-related quality of life in adolescents, L. Schwarz *et al*. [9] found that the initial type of malocclusion has an impact on this indicator, while the gender and age of children do not correlate with it. Given that the participants of the study were adolescents, it was not possible to determine the differences in the impact of orthodontic treatment in different age groups based on its results.

Studying the relationship between the impact on oral health and personality profiles among orthodontic patients, A.A. Al. Nazeh *et al*. [10] determined that the impact of orthodontic treatment on oral health differs depending on the patient's gender. The results of the study were obtained in accordance with the methodology for assessing the profile of impact on oral health, which did not include determining the impact of orthodontic treatment on cerebral circulation or other indicators of brain function, so in the context of the tasks of this work, their use is incorrect.

The purpose of the study was to determine the effectiveness of the use of mouth guards to improve cerebral circulation. Its tasks were to conduct rheoencephalographic studies with a sample of patients, assess the dynamics of treatment and establish the biological mechanisms of the effect of orthodontic appliances on changes in cerebral circulation.

MATERIALS AND METHODS

To determine the effectiveness of using REHASPLINT mouthguards to improve cerebral circulation, rheoencephalographic studies of patients were conducted at the Kyiv Regional Mental Health Centre, a municipal non-profit enterprise of the Kyiv Regional Council, in Vorzel, from 1 to 29 March 2023. The instrumental examination was carried out using a digital rheoencephalograph, which recorded changes in the electrical resistance of the head tissues using sensors and provided the results in the form of graphs and tables. The patients were examined in a standard position. It assessed blood flow velocity and volume, vascular

tone and vascular resistance. The study group consisted of 24 men with an average age of 28.5 years. The inclusion criteria were met by participants who had cerebrovascular disorders and needed to use orthodontic appliances (mouth guards) to solve orthodontic problems of varying severity or to protect the jaw during active or vigorous activity. The exclusion criteria excluded people under the age of 18 and those with contraindications to rheoencephalographic examinations: head injuries with damage to the skull bones, intracranial haemorrhages, acute infectious brain diseases, mental disorders, neurological disorders, epilepsy, severe cardiovascular diseases and hypertension. The study was conducted in accordance with the ethical standards that must be met in research involving human subjects, as outlined in the Declaration of the Helsinki Group of the World Medical Association [11]. All participants provided informed consent to participate in the study.

In accordance with the recommendations for the use of REHASPLINT orthodontic appliances, during the month of the study, the participants wore the aligners for 30 minutes 2-3 times during the day and throughout the night. They did not eat, drink or talk while wearing the aligners.

The first rheoencephalograph measurements were taken before wearing the mouthguard, and the second during the wearing. Significant changes in cerebral circulation indices were determined by the difference in blood filling in the basin of the superior middle artery (SMA), vascular tone in the SMA, tone of the main arteries in the SMA, tone of the resistance arteries in the SMA between the two measurements. Their determination was based on the relationship between the average blood flow velocity in the frontal lobe of the brain (FMs), the average blood flow depth in the frontal lobe of the brain (FMd), the average blood flow velocity in the parieto-occipital region of the brain (CMs), average blood flow depth in the parieto-occipital region of the brain (CMd) and vascular resistance (Oa), an indicator of low-frequency blood flow fluctuations associated with vasomotor activity (α1), an indicator of medium-frequency blood flow fluctuations associated with respiration (α2), an indicator of high-frequency blood flow fluctuations associated with the pulse wave (β), the volume of blood passing through the brain vessels per unit of time (Vo), an indicator of the ratio between blood flow volume and vascular resistance (R1), blood flow velocity in the brain capillaries (MKi), blood flow volume in the brain capillaries (MKd), the ratio between the frequency and duration of blood flow fluctuations (α/T), and the biological age of the vessels (BCA). Based on the analysis of the dynamics of treatment of each of the studied patients, the effectiveness of using REHASPLINT orthodontic appliances to improve cerebral circulation was determined. The method of descriptive statistics was used to process the data.

The biological mechanisms by which orthodontic appliances can affect cerebral circulation were determined based on the results of rheoencephalography and analysis of scientific sources in the relevant field. The sources were searched for in PubMed, Web of Science, Google Scholar and Scopus databases using the following keywords: "biological mechanisms associated with the occlusion process", "relationship between occlusion and cerebral circulation", "the effect of orthodontic structures on cerebral circulation", "relationship between occlusion and cerebral blood vessels", "the relationship between dental occlusion and vascular tone", "negative effects of malocclusion", "the impact of orthodontic structures on biological processes associated with the brain", "factors affecting cerebral circulation". The inclusion criteria were papers that contained relevant information on the subject of the study (theoretical studies related to the mechanisms of orthodontic pathologies, results of cerebral circulation measurements, assessment of cognitive function, assessment of quality of life, etc.), and the exclusion criteria were papers published before 2010.

RESULTS

According to the results of the rheoencephalographic examination, all the examined patients had cerebrovascular disorders of varying severity. A descriptive analysis of the examination results before and during the use of the orthodontic appliance (mouthguard) is given in Table 1.

Table 1. Analysis of the results of rheoencephalographic examination of patients before and during the use of orthodontic structures (caps)

Patient number	Data based on measurements before using caps			Assessment of the impact of orthodontic structures on cerebral blood circulation (assessment of treatment dynamics)
	Type of cerebral circulation disorder	Localization	The factor	
1	Slight violation of venous blood flow	Mostly in the carotid pools	Hypovolaemia	No significant dynamics were found
2	Violation of blood supply	From the right sides of the VBP and carotid basins	Increase in vascular tone	A slight positive trend is observed in terms of compensation of the left sections of the VBP and carotid basins at the expense of the right sections. A slight violation of blood supply on both sides persists
3	Violation of blood supply	In carotid basins from 2 sides	Increase in vascular tone	No significant dynamics were found
4	Violation of venous outflow	In VBP from 2 sides	Increase in vascular tone in the carotid basins from 2 sides	There remains a slight violation of venous outflow compared to previous examinations, positive dynamics were noted

Table 1. Continued

Patient number	Data based on measurements before using caps			Assessment of the impact of orthodontic structures on cerebral blood circulation (assessment of treatment dynamics)
	Type of cerebral circulation disorder	Localization	The factor	
5	Decreased blood supply	In VBP and carotid basins from 2 sides	Spasm of blood vessels	There is a slight improvement in the blood supply in the VBP and carotid basins on the right side
6	Marked violation of venous outflow	In VBP from 2 sides	Hypovolaemia and increased tone of blood vessels in VBP from 2 sides	Venous stasis remains. A positive trend is observed in the reduction of vascular tone on the left side of the left ventricle
7	Violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	No dynamics detected
8	Violation of blood supply	In VBP from 2 sides	Slight increase in blood vessel tone in VBP	A slight positive trend is observed in the reduction of vascular spasm in the VBP
9	A slight decrease in blood flow	In the VBP from 2 sides (on the left side of the pool, a decrease in blood supply is observed to a greater extent)	Spasm of blood vessels	On the right side of the VBP, the blood supply has recovered, on the left – it remains at the same level, but positive dynamics are observed.
10	Violation of venous outflow	In carotid basins from 2 sides	Increase in vascular tone	There is a slight improvement in blood filling in the VBP, venous stasis in the carotid basins remains at the same level
	Violation of blood supply	In VBP, it is directly proportional from 2 sides		
11	Violation of venous outflow	In VBP and carotid basins from 2 sides. Violation of venous outflow is greatest in the carotid artery	Hypovolaemia	There is normalization of vascular tone in the carotid basins from 2 sides and improvement of venous outflow, especially in the carotid basins
12	Violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	There is a slight improvement in blood flow in the carotid basins
13	Violation of blood supply	In VBP from 2 sides	Increase in vascular tone	There is a slight improvement in blood supply, but its deficiency still persists
14	Significant violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	There is a slight improvement in blood flow in the carotid basins
	Violation of venous outflow	It is present in all pools, but is most pronounced in the carotid		
15	Violation of venous outflow	In the carotid basins on both sides (mostly observed on the left side)	Hypovolaemia	Positive dynamics were noted due to partial compensation by the right carotid basin of the violation of venous outflow in the left basin
16	Significant violation of blood supply	In carotid basins from 2 sides	Increase in vascular tone	Sjight dynamics are observed, but the increased tone of blood vessels still remains
17	Slight violation of venous outflow	In VBP and carotid basins from 2 sides	Increase in vascular tone	Indicators of blood filling are within normal limits – the volume of blood in the vessels of the brain has normalised; venous outflow has improved, but its disturbances are still observed, which are insignificant and can be considered as a variant of the norm for a specific patient
18	Violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	No significant dynamics were found
19	Slight violation of blood supply	In carotid basins from 2 sides	Hypovolaemia	No significant dynamics were found
20	Violation of venous outflow	In VBP from 2 sides	Increase in vascular tone	No significant dynamics were found

Table 1. Continued

Patient number	Data based on measurements before using caps			Assessment of the impact of orthodontic structures on cerebral blood circulation (assessment of treatment dynamics)
	Type of cerebral circulation disorder	Localization	The factor	
21	Violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	No significant dynamics were found
22	Slight violation of venous outflow	In VBP from 2 sides	Hypovolaemia	No significant dynamics were found
23	Violation of blood supply	In carotid basins from 2 sides	Increase in vascular tone	No significant dynamics were found
24	Violation of blood supply	In VBP and carotid basins from 2 sides	Increase in vascular tone	No significant dynamics were found

Notes: VBP – vertebrobasilar pool
Source: compiled by the authors

A significant number of patients (13 patients/54.17%) who underwent rheoencephalographic examination, before the use of mouth guards (REHASPLINT orthodontic constructions), had blood filling disorders, two of them had minor disorders, one – significant; in one-third of the study subjects (8 patients/33.33%) had venous outflow disorders, two of which were mild and one severe; two patients (8.33%) had both disorders; one subject had a mild venous blood flow disorder. The main factors influencing these disorders were increased vascular tone and hypovolaemia. The localization of the disorders was determined in the carotid basins and/or the VBP on one or both sides. During the use of the mouthguard, the rheoencephalogram recorded positive dynamics of various intensities associated with improved cerebral circulation in 58.33% of patients, while in 41.67% of subjects, no changes were observed. In most cases, the positive dynamics were insignificant or mild. The dynamics of cerebral circulation improvement separately by different types of disorders are shown in Figures 1 and 2.

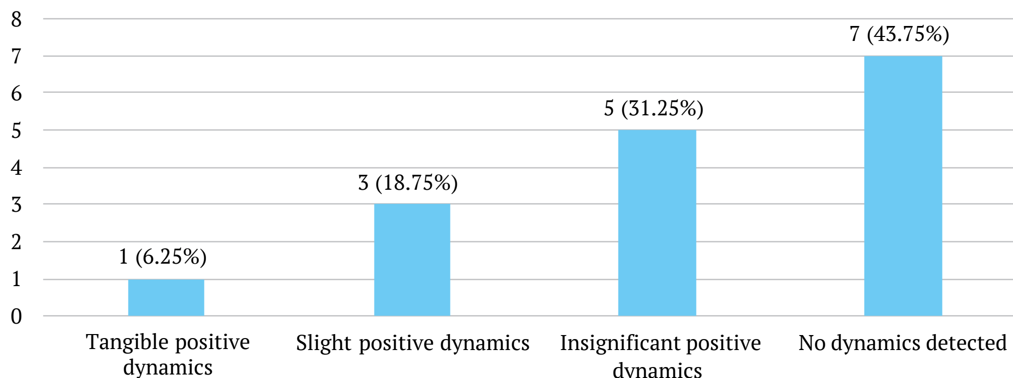

Figure 1. Dynamics of improvement of cerebral circulation in patients with blood filling disorders
Source: compiled by the authors

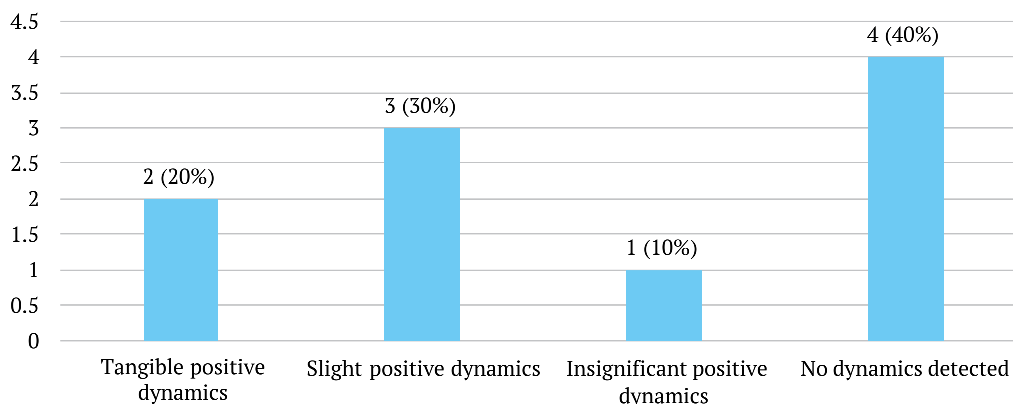

Figure 2. Dynamics of improvement of cerebral circulation in patients with venous blood flow disorders
Source: compiled by the authors

In 56.25% of patients with blood filling disorders, positive dynamics was observed due to the use of orthodontic appliances, in most of them (55.56%) it was insignificant, in one-third (33.33%) it was mild, and in 11.11% it was noticeable. The positive dynamics of the use of aligners in patients with venous outflow disorders was slightly better compared to patients with blood filling disorders (60% vs. 56.25%). Half of them had a mild improvement in outflow, one third had a significant improvement, and 16.67% had a slight improvement.

In none of the cases did the use of mouthguards as a treatment procedure lead to complete elimination of blood filling or venous outflow disorders. The main mechanisms of influence of orthodontic constructions on the positive dynamics in the restoration of normal cerebral circulation were a decrease in vascular tone, reduction of vascular spasm, compensation of blood filling or venous outflow disorders in one of the basins by another.

Analysing the results of rheoencephalographic examination, it is possible to assess the effectiveness and safety of the use of mouth guards (REHASPLINT orthodontic appliances) to improve cerebral circulation. The low level of dynamics of this treatment method indicates low efficiency. Therefore, it is inappropriate to use it for severe cases of cerebral circulation disorders. Instead, it can be effective for minor disorders of blood filling or blood flow or used as an adjunct to traditional treatments, provided that their combination is safe, which should be determined by a doctor. The use of mouth guards in combination with certain physiotherapeutic procedures, such as neck and head massage, may have potential. Given that no negative dynamics of treatment (deterioration of blood filling or blood flow during the use of mouthguards) was recorded in any of the cases, this method can be considered safe for use to improve cerebral circulation.

The development of craniomandibular dysfunction (CMD) involves biological processes associated with the dysfunction of one or more components of the craniomandibular system (CMS). Muscle dysfunction can occur under the influence of bruxism, stress or other factors that can lead to spasm and inflammation of the masticatory muscles, restriction of jaw movement, pain and discomfort, and changes in muscle tone or muscle structure. Joint dysfunctions can be manifested by inflammation of the articular disc, cartilage damage, and changes in the position of the articular head. The consequences of such disorders are pain, clicking, stiffness, restriction of movement, or dislocation of the jaw [12]. Among the neurogenic factors of CMD, the most common are trigeminal nerve dysfunction and changes in the level of neurotransmitters, in particular serotonin and dopamine, effects on nerve endings, and increased activity of the sympathetic nervous system [13].

CMD has several mechanisms of action on blood vessels. Spasms of the masticatory muscles, a common symptom of CMD, can negatively affect blood circulation. Spasmodic masticatory muscles can mechanically compress blood vessels localised in the CMS area, leading to a decrease in blood flow, and prevent venous outflow from the head and neck, causing venous congestion. At the same time, spasms of the masticatory muscles can mechanically compress the nerves, damaging nerve fibres and disrupting the transmission of signals to the blood vessels, which leads to vascular dysfunction and impairs their ability to regulate blood flow. Prolonged pain, as a result of constant spasm of the masticatory muscles, affects the sensitization of nerve endings, developing hypersensitivity to stimuli, which can lead to excessive vasoconstriction in response to minor stimuli, thus exacerbating vasoconstriction [14].

Inflammation of the joints and muscles, which is also common in CMD, can negatively affect blood vessels. It can provoke endothelial damage and the release of chemicals that constrict blood vessels, including endothelin-1 [15]. This process is accompanied by a change in vascular tone and can lead to a violation of their ability to regulate blood flow, increasing vasoconstriction. The inflammation-induced release of vasoactive substances (prostaglandins and leukotrienes), which have the ability to constrict or dilate the vessel, can lead to blood flow instability and impaired blood pressure regulation [16]. Using its own mechanisms of influence on platelets, clotting factors, fibrinogen and the fibrinological system, inflammation can lead to coagulation disorders (increased risk of blood clots) and cause circulatory disorders and damage to inflamed tissues [17, 18].

CMD can have a certain effect on blood vessels through neurogenic factors. The mechanisms of such effects include changes in the activity of the sympathetic nervous system, effects on nerve endings that regulate blood flow, and effects on the central nervous system and the noociceptive system, which is responsible for the perception and processing of pain signals [19]. In most cases, the main factors of influence are spasms or inflammation of the masticatory muscles and joints caused by bruxism, joint deformity or other CMD factors, but additional negative effects are caused by psycho-emotional factors, including stress and anxiety, and medication side effects of certain drugs, such as antidepressants, non-steroidal anti-inflammatory drugs, hormones, etc [20].

The masticatory muscles and jaw joint are supplied with blood from different arteries. The masticatory artery is the largest artery supplying the masticatory muscles, branches from the carotid artery and divides into a deep branch supplying the medial masseter, lateral masseter and temporalis muscles, and a superficial branch supplying the masseter and buccal muscles [21]. The temporalis and buccal muscles are supplied by the parotid artery, and the bicuspid and hyoid muscles by the deep neck artery. The arteries supplying the jaw joint include the superior maxillary artery, which supplies the articular disc and capsule of the jaw joint; the middle meningeal artery, which supplies the articular process of the temporal bone and the articular fossa of the zygomatic bone; and the ascending pharyngeal artery, which supplies the posterior capsule of the jaw joint [22, 23]. Depending on the individual anatomical features, the blood supply to the masticatory muscles and jaw joint may vary, but in most cases, the general blood supply to these components of the CMS corresponds to the described one.

There are several small connections (anastomoses) between the arteries of the CMS and the arteries of the brain, which allow blood to flow in small amounts from one system to the other, but they do not have a significant effect on the blood supply to the brain [24]. The arteries supplying the jaw joint and masticatory muscles do not have a direct connection to the cerebral vessels. They are part of the external carotid artery, which is one of the two main

branches of the carotid basin, a network of arteries that supplies blood to the face, neck, and scalp, and the blood supply to the brain is carried out through the internal carotid arteries, which are also part of the carotid basin, and the arteries of the vertebrobasilar system [25].

The effect of decreased blood flow in the masticatory muscles or jaw joint on cerebral circulation deterioration may be exerted by indirect mechanisms. Reduced blood flow in the masticatory artery can be a factor in increasing blood pressure, which can lead to cerebral blood flow deterioration due to reduced perfusion pressure, narrowing of the arteries and a decrease in the volume of blood circulating in the body [26, 27]. Spasm-induced mechanical compression of the vessels passing through the masticatory muscles can lead to a decrease in blood flow to the brain. Damage to nerve fibres caused by muscle spasm or inflammation can lead to disruption of nerve signals and become a factor in reducing the ability of blood vessels to regulate blood flow in the masticatory muscles and jaw joint, which can cause changes in blood pressure. When analysing the mechanisms of the impact of CMD on cerebral circulation, it should be noted that there is no direct connection between the arteries of the CMS and the brain, so impaired blood flow in the masticatory muscles and jaw joint may have a minor impact on cerebral circulation mainly through the impact on blood pressure.

Another reason for the deterioration of blood circulation in CMD is impaired venous outflow. Its main factor is mechanical compression of the veins in the jaw joint, which occurs due to displacement or curvature of the articular disc; spasm of the masticatory muscles; reduction of space in the jaw joint due to changes in the position of the articular head; swelling as a result of inflammation of the jaw joint; formation of cuspid growths caused by degenerative changes associated with arthritis; anatomical features of the structure of the venous canals that affect their width; severe impact or dislocation of the jaw [28]. Impaired venous outflow in the jaw joint area has a direct impact on the outflow of blood from the brain. The deep venous network collects blood from the muscles, bones, and skin of the face, including blood from the jaw joint, and flows into the jugular vein, which draws blood from the head and neck and then flows into the superior vena cava [29]. The submandibular plexus, located under the lower jaw, connects to the deep venous network and other plexuses that drain blood from the brain. Impaired venous outflow in the jaw joint can lead to blood stasis in these plexuses and compression of the jugular vein, which will impede blood flow from the brain and provoke an increase in intracranial pressure, nerve damage, and cerebral circulation [30].

In addition to affecting the blood vessels localised in the CMS, dental occlusion disorders lead to an imbalance in the musculoskeletal system. This occurs due to a shift in the centre of gravity of the skull and loss of support from the dental arches due to changes in the position of the temporomandibular joint. This imbalance leads to an increased load on the neck muscles to hold the head in a new position, which provokes spasms and changes in their tone and causes vertebral displacement. Such a cascade of mechanical processes triggered by a change in the position of the temporomandibular joint causes a change in the biophysics of blood vessels and nerves that are surrounded by the neck muscles. The mechanism of such changes is that under the influence of increased muscle load, the vessels located in the muscle bed of the cervical spine are compressed, resulting in a decrease in their diameter, and, accordingly, a decrease in blood flow and an increase in blood pressure. This leads to a decrease in the volume of blood filling and difficulty in the outflow of blood from the head. The use of orthodontic appliances aimed at fixing the correct position of the jaws allows balancing the centre of gravity of the skull, reducing the load on the neck muscles and eliminating vascular compression, and therefore normalizing haemodynamics – restoring the tone and diameter of blood vessels in accordance with the needs of the body and return blood pressure to the physiological norm.

Thus, by affecting masticatory muscle dysfunction and jaw joint position changes, CMD can be a factor in cerebral circulation deterioration. The use of mouthguards alleviates the symptoms of CMD by reducing muscle tension and spasms, protecting the joints and altering sensory information. Thanks to its shape, the REHASPLINT orthodontic appliance is able to correct the correct position of the lower jaw, thus reducing the load on the masticatory muscles and temporomandibular joint and reducing occlusal pressure, which helps to relax the muscles, reduce spasm and eliminate pain. As a joint protector, the mouthguard creates a cushioning effect by absorbing part of the chewing pressure, which helps protect the articular cartilage and other structures of the jaw joint from damage. At the same time, the smooth surface of the orthodontic appliance reduces friction between the teeth, eliminating the source of discomfort. An important function of the mouthguard is neuroproprioceptive stimulation – it can provide sensory information about the position of the lower jaw, helping to restore the balance of the centre of gravity of the skull, reprogramme muscle activity and reduce spasm and pain. This function is further enhanced by the mouthguard's ability to reduce nociceptive stimulation in the mouth. Having an understanding of the mechanism of the impact of aligners on cerebral circulation, it is possible to analyse the results of a rheoencephalographic study in more detail – to determine what the therapeutic effect of the orthodontic appliance is and why it turned out to be insignificant.

The therapeutic effect of using orthodontic appliances is to relax the masticatory muscles and protect the joints, reduce vascular spasm and tone, and reprogramme muscle activity through sensory information about the position of the lower jaw. These mechanisms have a direct effect on improving blood filling and venous outflow in the CMS area, and an indirect effect on improving blood circulation in the brain.

To analyse the therapeutic effect, it is important to separate cases of impaired blood filling and venous outflow, as they have different mechanisms of influence on cerebral circulation. A slight or mild therapeutic effect was found in subjects who had blood-filling disorders on one or both sides of the VBP and/or carotid basins before the procedure, due to the fact that the arteries supplying the jaw joint and masticatory muscles do not have a direct connection to the cerebral vessels. Although the external carotid artery, which includes the arteries supplying the jaw joint and masticatory muscles, and the internal carotid artery, which supplies the cerebral vessels, branch off from the common carotid artery, they do not have a direct connection to each

other. An indirect effect on the blood flow to the brain can be caused by an increase in blood pressure as a result of increased vascular tone in the masticatory muscles or jaw joint. Blood pressure has a significant impact on the blood supply to the entire body. By increasing the strength of heart contractions, it can increase blood filling, and by increasing blood flow resistance and capillary permeability, it can temporarily reduce it [31]. Hypovolaemia can also be a factor in lowering blood pressure, as fluid deficiency leads to a decrease in the volume of blood circulating in the body [32]. Impaired venous outflow in the CMS has a direct impact on the outflow of blood from the brain, as it is carried out through the only way – through the jugular vein.

Summing up the results of the rheoencephalographic study and analysis of the biological mechanisms of the effect of orthodontic appliances on cerebral circulation, it can be noted that the use of mouth guards is an appropriate and effective way to improve cerebral blood flow in cases where its disorders are caused by the effect of CMD symptoms on the vessels of the masticatory muscles and jaw joint.

DISCUSSION

Analysing the results of the study, which revealed the low effectiveness of the impact of mouthguards in improving cerebral circulation, it is worth noting that their primary task is to eliminate orthodontic pathology of moderate or mild severity. The impact of such pathology causes significant local discomfort for the patient, but does not have a significant immediate effect on the body as a whole. However, like any problem without proper attention and treatment, orthodontic dysfunctions can worsen over time and affect other body systems. In this case, by alleviating the symptoms of CMD, aligners improve the blood filling of the vessels of the masticatory muscles and jaw joint, and help to establish venous blood flow in the CMD area. Therefore, their insignificant effect, recorded at a certain moment, can be beneficial in the future, inhibiting the development of negative factors (impaired blood filling and/or venous congestion), which, when aggravated, can cause greater harm to cerebral circulation.

An observational study of the effect of the mandibular resting position on cerebral circulation and physical balance was conducted by T. Heit *et al*. [33]. The researchers' observational study involved 9 participants, seven of whom were healthy male athletes and two were women with multiple sclerosis. Cerebral blood flow was measured while the subjects clenched their teeth on both jaws using transcranial Doppler. The results of the observation showed that the physiological position of the lower jaw at rest can affect the increase in blood flow to the brain. The conclusions of scientists can be compared with the results of this study, as the use of orthodontic appliances is aimed at eliminating certain defects and bringing the jaws closer to their physiological position. Therefore, it is worth agreeing with the results of the authors and noting that for further research on the effect of orthodontic appliances on improving cerebral circulation, it would be advisable to partially use the methodology proposed by Canadian scientists, namely the use of transcranial Doppler to measure cerebral blood flow, given the accuracy and informativeness of the procedure.

The search for a link between dental occlusion and brain activity was conducted by S.S. Ulloa *et al*. [34] in their work. Scientists have found that bite changes can affect the sensorimotor cortex of the brain, so it would be fair to assume that occlusion plays a pivotal role in the development of anxiety and stress and even Alzheimer's disease or senile dementia. The authors emphasise the importance of continuing research in this area to find out how much brain functioning changes, which parts of the brain are affected by malocclusion, and what biological mechanisms contribute to this. Despite the different objectives of the study by S.S. Ulloa *et al*. [34]and the present study, both have a common aspect – confirmation of the influence of jaw position on brain function. To a certain extent, the results of the study confirm the authors' theoretical assumptions, noting that impaired venous outflow can lead to increased intracranial pressure and nerve damage, which can cause many neurological disorders, as well as anxiety and stress.

The impact of tooth loss on cognitive function was studied by P. Galindo-Moreno *et al*. [35]. After analysing data from two US national health surveys based on a total sample of 102,291 people, the researchers found that the number of teeth in the mouth directly affects the cognitive status of a person. At first glance, the work may seem to have little to do with the results of this study, but it also develops the concept of the relationship between occlusion and brain function. Given that the use of the REHA-SPLINT mouthguard can help reduce the pressure on the gums and jaw associated with tooth loss, thus reducing the risk of further tooth loss, it can be assumed that orthodontic appliances have an indirect effect on improving cognitive function.

When evaluating the effectiveness of using mouth guards as a way to improve cerebral circulation, it is important to pay attention to individual patient characteristics such as age, gender, and health status. Children have more pliable skull bones and more elastic blood vessels than adults. Therefore, it is likely that orthodontic appliances will have a greater impact on children's cerebral blood flow. It should be added that children, in most cases, do not have concomitant diseases typical of middle-aged and elderly people, which can be additional factors in orthodontic pathologies and cerebral circulation deterioration.

The difference in the effect of orthodontic structures on cerebral circulation in men and women may be explained by their anatomical differences between the masticatory muscles and the jaw joint. The temporalis muscle, masseter muscle, and medial pterygoid muscle are typically larger in men than in women, which may result in greater chewing force in men. The masticatory muscles in men may have a more pronounced shape than in women. The distribution of muscle fibres in the masticatory muscles may differ between the sexes, thus affecting the nature of their chewing movements. In men, the articular fossa of the temporal bone and the condylar fossa of the mandible are usually larger than in women. The articular fossa of the temporal bone in men is more rounded and the condylar fossa of the mandible is more conical, while in women the articular fossa is more oval and the condylar fossa is more rounded. These differences in the size and shape of the parts of the articular structure may explain the greater mobility of the male jaw. Also, the articular cartilage in men is usually thicker, and the ligaments that hold the jaw joint in place are stronger than in women, which affects the greater

resistance of male joints to certain irritations. These anatomical differences indicate that men's jaws are subjected to greater stress due to the force of chewing, which leads to greater compression of blood vessels, but at the same time they are also more resistant to technical stimuli, which reduces the risk of mechanical pressure on the arteries and veins in the occlusion area. Thus, the anatomical difference between the masticatory muscles and the jaw joint in women and men is not likely to have a significant impact on the evaluation of the effectiveness of orthodontic appliances in improving cerebral circulation. Instead, it can be affected by factors that lead to orthodontic pathologies. These include contact sports, facial injuries of varying severity, and other activities that can cause damage to the jaw vessels, which are usually more common in men. The study involved mainly young and middle-aged men, so it was not possible to analyse the effect of orthodontic appliances on cerebral circulation separately between women and men in this case. G. Kummer *et al*. [36] analysed the impact of gender on the results of orthodontic treatment based on an analysis of published studies. According to the authors, only a small proportion of published clinical trials included an assessment of the gender impact on treatment outcomes. In a quarter of those that took into account gender-specific effects, the impact of gender on the effectiveness of orthodontic treatment was reported. It is worth agreeing with the authors' conclusions about the importance of analysing the study groups taking into account not only age or physiological characteristics, but also the gender of the participants.

The effectiveness of the use of caps to improve cerebral circulation is also influenced by the health status of the subjects. Diseases of the cardiovascular system (hypertension, atherosclerosis, coronary heart disease, arrhythmias, heart failure), diabetes mellitus, hypercholesterolaemia, obesity, sleep apnoea, and inactive lifestyle, smoking and alcohol abuse are independent factors of cerebral circulation deterioration, so without a systematic approach to treatment or lifestyle changes, reducing pressure in the vessels of the lower jaw and improving their blood filling and blood flow will not significantly affect cerebral circulation.

In general, orthodontic problems have a significant impact on other body systems. Depending on their complexity, such an impact can manifest itself quickly or, on the contrary, have a cumulative effect. It is worth noting that dental occlusion disorders are often the result of a congenital defect rather than external factors. This problem was analysed by S. Kahn *et al*. [37] in their study. The main problem of the jaw epidemic, as the authors of the article called the problem of dental occlusion, they consider not genetic factors, but, despite the too limited time period for full evolutionary changes. Agreeing with the authors' conclusions, it is important to emphasise that the study of the impact of evolutionary changes on the anatomical features of the jaws is an essential component for understanding the causes and mechanisms of dental occlusion disorders and finding methods to eliminate them, in particular with the help of orthodontic appliances.

In addition to evaluating the effectiveness of mouth guards in improving cerebral circulation, this study has revealed biological mechanisms linking cerebral blood flow and blood flow to the masticatory muscles and jaw joint. Using the connection of such seemingly different structures made it possible to expand the number of methods for influencing the blood filling and blood flow of the brain vessels. Since the way to effective treatment is to take into account and eliminate all possible causes that affect the development of the pathological process, the use of mouth guards in a systemic approach to improving cerebral circulation is an effective and justified means that at the same time meets the principles of personalised medicine.

CONCLUSIONS

Rheoencephalographic studies conducted to determine the effectiveness of the use of mouth guards to improve cerebral circulation revealed a slight and mild positive trend in 58.33% of patients, in 41.67% – no significant changes were recorded, and no negative effects were recorded in any of the 24 subjects. This effect and its intensity are explained by the therapeutic effect of orthodontic appliances, which is to alleviate the symptoms of CMD. These symptoms include muscle dysfunction, which leads to spasms and inflammation of the masticatory muscles, changes in muscle tone, muscle structure, and restriction of jaw movement; joint dysfunction, which causes inflammation of the articular disc, cartilage damage, and changes in the position of the articular head. Spasms and inflammation of the masticatory muscles and jaw joint as factors of mechanical compression of blood vessels and nerves in the CMS area lead to changes in vascular tone and impaired blood flow regulation (decreased blood filling in this area and impaired venous outflow). Reduced blood filling in the vessels of the masticatory muscles and jaw joint does not have a direct impact on changes in the cerebral circulation, as these structures are supplied through various arteries of the carotid basin, but it can affect the increase in blood pressure, reducing the total volume of circulating blood and thus worsening the blood filling in the brain vessels. Disruption of venous outflow in the jaw joint has a direct impact on the same process in the brain, as it is carried out through the only way – through the jugular vein. The effect of orthodontic appliances (aligners) on improving cerebral circulation is achieved by restoring the balance of the centre of gravity of the skull, reducing muscle tension, spasms and joint protection, which leads to a decrease in vascular tone and improved blood circulation in the masticatory muscles and jaw joint.

The effectiveness of orthodontic appliances in improving cerebral blood flow and blood flow makes it possible to recommend their use in a systemic approach to the treatment of patients with cerebral circulatory disorders requiring orthodontic treatment. The limitation of the study was the insufficient gender and age representativeness of the sample. Therefore, to extend the results obtained, the main direction of further research may be to analyse the effectiveness of orthodontic appliances in improving cerebral circulation, taking into account gender and age anatomical differences in patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Покращення мозкового кровообігу за допомогою кап (ортодонтичних апаратів)

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Анотація. Дана робота була проведена з метою визначення ефективності впливу ортодонтичних апаратів на покращення кровообігу головного мозку. 24-ом учасникам дослідження було проведено реоенцефалографічне обстеження до та під час використання кап. За його результатами аналізувалися зміни кровонаповнення та крововідтоку в судинах головного мозку та оцінювалась динаміка лікування. За результатами досліджень незначна та легка позитивна динаміка лікування була виявлена в 58,33 % досліджуваних, негативної динаміки виявлено не було, в 41,67 % учасників суттєвих змін не зафіксовано. Такий ефект ортодонтичних апаратів на покращення церебрального кровообігу пояснюється тим, що, полегшуючи симптоми краніомандибулярної дисфункції, вони впливають на збалансування центру ваги черепа, зменшення напруги та спазму в м'язах шиї, а також забезпечення захисту суглобів від механічних подразнень, що сприяє зниженню тонусу судин та покращує кровонаповнення та крововідтік в жувальних м'язах та щелепному суглобі. Кровонаповнення жувальних м'язів та щелепного суглоба не має прямого зв'язку з кровонаповненням судин головного мозку, так як дані структури живляться з різних артерій, проте зниження тонусу судин в області краніомандибулярної системи та в м'язовому ложі шийного відділу хребта здатне впливати на зниження артеріального тиску та налагодження венозного відтоку в щелепному суглобі, що опосередковано впливатиме на покращення церебрального кровообігу. Отримані результати вказують на те, що ортодонтичні проблеми можуть бути чинниками погіршення кровонаповнення та крововідтоку в судинах головного мозку, тому використання кап є ефективним методом, який доцільно використовувати в комплексному лікуванні пацієнтів з порушеннями церебрального кровообігу

Ключові слова: краніомандибулярна дисфункція; жувальні м'язи; щелепний суглоб; артеріальний тиск; венозний відтік; каротидні басейни

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Recent pathogenetic aspects of hearing loss in COVID: A literature review

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Abstract. The World Health Organisation predicts that by 2050, up to 10% of the world's population will need rehabilitation to address disability-related hearing loss. The purpose of this study was to identify the main mechanisms of hearing loss associated with Severe Acute Respiratory Syndrome Coronavirus 2 infection. The study included modern English-language scientific publications, mainly those with a high citation index, through the professional platforms MEDLINE/PubMed and Index Medicus. A total of 48 sources were selected. Research papers devoted to the development of conductive or sensorineural hearing loss, which occurred directly as a result of a viral disease, or is associated with the processes that accompany it (treatment, concomitant pathology, vaccination, etc.), were analysed. It was found that the development of viral-induced hearing loss in COVID has a multifactorial nature. The heterogeneity of audiological changes is primarily conditioned by direct viral damage to auditory analyser cells that express membrane receptors of the angiotensin-converting enzyme of the second type. In addition, there is a reactivation of latent viral infection, extravasation of exudate into the middle ear cavity, blood clotting disorders, immune-mediated cell damage, local and generalised inflammatory reactions that affect both sound conduction and sound perception in one ear or both. Some cases of audiological disorders may also be of iatrogenic origin, since post-vaccination complications and ototoxic effects of medications used in the treatment of COVID-19 are not excluded, which should be considered by clinicians at all levels of healthcare to effectively manage a specific clinical scenario

Keywords: audiology; conductive hearing loss; sensorineural hearing loss; coronavirus; ototoxicity; vaccination

INTRODUCTION

The problem of hearing loss is one of the most pressing for the global medical community. More than 430 million people on the planet require rehabilitation for hearing loss, which leads to disability [1]. There are 34 million children among them. 25% of adults over the age of 60 need rehabilitation. According to forecasts of the World Health Organisation (WHO), by the middle of the 21st century, hearing loss can be diagnosed in 2.5 billion people, and at least 700 million of them will need rehabilitation. However, timely and effective treatment prevents the development of hearing loss in 60% of cases in both children and adults, especially when it is caused by exposure to noise or ototoxic drugs.

One of the causes that can cause hearing loss is viral infections, including intrauterine ones. J. Saniasiaya [2] indicates that viral infection is a recognised aetiological factor of hearing loss, which varies significantly depending on the type of causal virus: from mild to deep deafness, can be conductive or sensorineural, unilateral or bilateral, reversible and non-reversible. The prognosis is influenced

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not only by the morphological and virulent properties of the pathogen, but also by the use of specific antiviral drugs.

X. Shi *et al.* [3] describe the pathogenesis of audiological disorders in infection with cytomegalovirus, herpes simplex or herpes zoster viruses, influenza, Epstein-Barr viruses, Zika, Lassa, enteroviruses, and many others.

The first quarter of the 21st century was marked by outbreaks of coronavirus infections. In 2003, the disease with a predominant lesion of the respiratory system and a high mortality rate was called SARS-CoV – Severe Acute Respiratory Syndrome-related COronaVirus. The names of the next two epidemics reflected their geographical and temporal features. In 2012, the first cases of MERS-CoV – Middle East Respiratory Syndrome COronaVirus were recorded in the Arabian Peninsula, and COVID-19 or coronavirus disease (“COronaVIrus Disease” SARS-CoV-2) was first described in 2019 [4]. The latter, according to the statement of WHO Director-General Tedros Adhanom Gebreyesus dated May 5, 2023 [5], although it no longer has the status of an emergency, does not cease to pose a threat.

From its first days, as in the previous two, the main attention was focused on respiratory manifestations. But there are steadily increasing reports of the prevalence and diversity of sensory disorders [6] and hearing loss [7] associated with COVID-19. In particular, K.M.C. Ong *et al.* [8] noted that most individuals included in their review and had auditory and vestibular disorders, within one month of positive diagnostic tests for SARS-CoV-2 or clinical confirmation of this infection, began to experience hearing loss. In addition, sensorineural hearing loss was sudden in 23.1% of cases. According to the recommendations of the American Academy of Otolaryngology–Head and Neck Surgery, sudden sensorineural hearing loss (SSNHL) occurs within 72 hours and is a hearing loss of ⩾30 dB on at least 3 consecutive frequencies [9].

An active vaccination campaign against coronavirus has led to an increase in researchers’ interest in a possible link between it and the occurrence of otological symptoms. However, their claims are quite ambiguous, especially regarding the frequency, severity, and prognosis of hearing loss. H. Wichova *et al.* [10] reported the occurrence of tinnitus and vertigo in patients shortly after vaccination, and an increase in cases of newly diagnosed idiopathic sensorineural hearing loss doubled over a 30-day period in 2021 compared to the same period in 2019 and 2020.

In contrast, E.J. Formeister *et al*. [11], based on the analysis of the results of a survey of more than 86 million people vaccinated against SARS-CoV-2 in the United States in the winter of 2020-2021, found that the incidence of sudden SARS-CoV-2-induced sensorineural hearing loss after immunisation did not exceed those inherent in the general population.

The growing number of long-term consequences of COVID-19 [12] and not encouraging forecasts regarding the weakening of the virulent properties of the virus encourage [13] the search for modern effective methods of their prevention and treatment at all stages of medical care, from primary care physicians to specialised otolaryngological and sign language practice. And this, in turn, requires a clear understanding of the mechanisms of occurrence and development of audiological disorders. That is why the purpose of the study was to investigate the main mechanisms of hearing loss associated with SARS-CoV-2, based on world experience, by analysing available information and literature sources.

To achieve this goal, the authors analysed scientific publications mainly with a high citation index published in English in 2018-2024. The search was conducted in January 2024 using electronic database of medical and biological publications MEDLINE/PubMed. Keywords in English were used to search for potentially relevant materials: “COVID” and “Hearing Loss” (378 publications) or “SARS-CoV-2” and “Hearing Loss” (181 publications), followed by a selection of papers that met the chosen goal. A total of 48 scientific sources were selected. Later additions to the review included five papers, which were published after January 2024 and were discovered after the initial screening. In particular, the main criteria for inclusion in the systematic review and meta-analysis were: established SARS-CoV-2 infection; vaccination or treatment of this infection, first-time detected sensorineural or conductive hearing loss, and temporal correlation between the two events.

SARS-COV-2-INDUCED CONDUCTIVE HEARING LOSS

Viruses can infect and cause inflammation of the middle ear cavity. Inflammatory oedema and increased permeability of the vascular wall lead to exudate effusion into its cavity and, as a result, cause conductive hearing loss. The role of coronaviruses in the development of the inflammatory process of the middle ear has been known for more than 20 years [14]. At the beginning of the development of the coronavirus pandemic, this problem was not widely covered. It can be assumed that this is due to difficulties in establishing a diagnosis during strict epidemiological measures, a large number of asymptomatic cases of the disease, or the fact that respiratory and other manifestations of the disease came to the fore. However, there have been isolated reports and their number is growing.

Sound conduction can change as a result of the upward spread of infection from the nasopharynx, which leads to exudation into the middle ear cavity, otitis media, otalgia, and tinnitus [15]. Colonisation of the middle ear and mastoid process by SARS-CoV-2 at the beginning of the pandemic was detected during autopsy [3, 16]. Therefore, the question remained whether SARS-CoV-2 migrated to the middle ear of living patients during or possibly after primary infection, or whether it passively entered the ear post mortem [17].

In 2021, N. Raad e*t al.* [18] described clinical cases of eight patients with otitis media who were treated in two specialised clinics in Iran in April and May during the COVID-19 pandemic. All of them had no previous history of otolaryngological pathology and all of them were confirmed to be infected with coronavirus. In addition, according to the authors, the atypical time of occurrence of the problem is in favour of SARS-CoV-2-induced inflammatory process of the middle ear: in the spring and summer period, the incidence of otitis media is usually the lowest, and its peak occurs in autumn and winter. Five of the patients had clinical signs of SARS-CoV-2-related damage to other organs (cough, shortness of breath, fever, etc.), which was confirmed by instrumental studies. Two had concomitant chemosensory dysfunction. The case of a young woman who, after contact with a COVID-19 patient, experienced

only ear pain and hearing loss without any systemic manifestations of infection is interesting. Examination of the ear revealed an effusion in the middle ear on the left, fluid/air level, and severe eardrum swelling. The result of a polymerase chain reaction (PCR) smear from the oropharynx for COVID-19 was negative; however, a PCR test for COVID-19 with middle ear fluid was positive. All patients were successfully treated. A thorough analysis indicated that otitis media, which develops as a result of exudation and a decrease in pressure in the middle ear cavity, may be both one of the symptoms of COVID-19 and its complication.

S. Bhatta *et al.* [19] investigated the presence of conductive hearing impairment in 331 patients with COVID-19 at nine institutes in India and Nepal between July 01, 2020 and April 30, 2021. The criteria for inclusion in the study were not only the absence of appropriate pathology in the anamnesis, in addition, age was taken as a factor in the development of presbyacusis in older people and excessive subjectivism in the examination of children, and specific treatment for COVID-19, considering its potential ototoxicity. According to their results, only 11.2% of patients independently complained of audiological symptoms (tinnitus, hearing loss, otalgia, etc.), considering them the main ones, and 88.8% of patients noticed them only after a questionnaire and examination [19]. The most standard method for quantifying the sensitivity of an auditory analyser is tonal threshold audiometry. The degree of hearing loss is determined by the thresholds of auditory perception in the frequency range from 500 to 4,000 Hz, that is, at the so-called speech frequencies (Table 1).

Table 1. Severity of hearing loss based on audiogram metrics

Hearing Range	dB
Normal	-10 – 25
Mild	26 – 40
Moderate	41 – 55
Moderately Severe	56 – 70
Severe	70 – 90
Profound	91+

Source: [20]

According to S. Bhatta *et al.* [19], when audiometric testing with pure tone, a conductive type of hearing loss of up to 40 dB was found in 3.2% of patients with COVID-19. And the registration of tympanograms of type B and C in a number of patients indicated the presence of Eustachian tube dysfunction. The patients' hearing recovered after 3 months.

Subsequently, Y. Fan *et al.* [21] found that almost 70% of fluid samples obtained from the middle ear cavity in patients with COVID-19 were PCR-positive for SARS-CoV-2. This allowed the researchers to suggest that exudative otitis media may also be a symptom of coronavirus disease. This assumption was confirmed by the result of treatment: complete recovery was observed in 40.7%, and 51.9% of patients were diagnosed with hearing improvement, which averaged 14.5 ± 8.1 dB, with an average decrease in the air-bone interval by 13.5 ± 9.0 dB. Similar data were found for infection with the Omicron strain [22, 23], in which the incidence of otitis media increased by 15% [24]. Thus, SARS-CoV-2 can infect the middle ear cavity and cause an inflammatory response. Oedema, exudation into the tympanic cavity or mastoid cells, and auditory tube dysfunction impair sound conduction and, as a result, lead to hearing loss.

CLINICAL CHARACTERISTICS OF SARS-COV-2-INDUCED SENSORINEURAL HEARING LOSS

The relation between sensorineural hearing loss and COVID-19 was first noticed by W. Sriwijitalai & V. Wiwanitkit [7] back in spring 2020. And over time, the possibility of a negative impact of the virus on the cells of the inner ear began to attract more and more attention. V. Fancello *et al.* [25] in their study found that sensorineural hearing loss (SNHL) can occur both during and after SARS-CoV-2 infection. In addition, in 12.7% of patients, this is described as an isolated symptom; while in the majority (87.3%), hearing loss was accompanied by other additional signs. Among them, tinnitus prevailed (100%); vertigo was noted in almost half of the patients (45.5%); and facial palsy – in 5.5%. The researcher focuses on three cases when the lesion of the n. facialis had a clear connection with SNHL. Chemosensory dysfunction has also been described in 25% of patients with perceptual hearing loss: anosmia, hyposmia, ageusia, and dysgeusia. These changes were confirmed by radiological diagnostic data indicating lesions of the cochlea, cranial nerves VIII and/or VII, haemorrhagic lesions of the brain parenchyma, intra-labyrinth micro-hemorrhages, numerous cochlear fibrosis foci, etc. In 75% of cases, otoacoustic emission (OAE) results indicate possible damage to the outer hair cells. In this study, a fairly wide range of hearing loss was found: 58.7% of patients had mild to moderate hearing loss, 4.8% had moderate to severe hearing loss, and 36.5% had severe to deep hearing loss (Table 2).

Table 2. Severity of hearing loss in the patients included in the study

Hearing Range	Degrees of sensorineural hearing loss
Mild	20.6%
Mild – Moderate	6.3%
Moderate	31.7%
Moderate – Severe	4.8%
Severe	11.1%
Severe – Profound	20.6%
Profound	4.8%

Source: [25]

The positive effect of treatment (improvement of hearing from mild to almost complete recovery) was achieved in more than a third of patients. At the same time, the return of hearing acuity to the initial level (before infection with coronavirus) was observed only in 12.5% of cases [25]. A similar trend has been confirmed in other studies.

However, it is not always possible to establish a timely link between the sudden onset of hearing loss in the acute period of COVID. Most patients are hospitalised in the intensive care unit or are isolated during this period, and there is a potential risk of infection, which in combination makes impossible a performing of comprehensive audiological examination. Therefore, SNHL detection is often delayed [9], which negatively affects the urgency of prescribing adequate treatment and prognosis. According to J. Jeong *et al.* [6], in only one in ten patients, the first signs

of infection were hearing loss, tinnitus, or dizziness. The vast majority of people reported fever, cough, shortness of breath, or fatigue 21 days before the onset of audiological symptoms. In addition, after the SNHL was detected, typical general manifestations of the disease continued for another 2 weeks. Even in the latent course of the disease, in the absence of obvious clinical signs, changes in the thresholds for perception of high-frequency pure tone and OAE amplitude indicate a damaging effect of SARS-CoV-2 on hair cell function [26].

Chemosensory manifestations of coronavirus disease confirm the neuroinvasiveness of SARS-CoV-2. According to V. Fancello *et al.* [25], the site of entry of the virus into the central nervous system may be the olfactory nerve, since a quarter of patients with SARS-CoV-2-induced perceptual hearing loss reported certain changes in taste and smell, while symptoms of viral damage to the facial nerve were observed 5 times less often. Changes detected in magnetic resonance imaging (MRI) of the brain also indicated the involvement of *n. vestibulocochlearis* and *n. facialis*.

Signs of damage to these nerves, confirmed by MRI results, are described by J. Jeong, *et al.* [6]: 30% of patients showed signs of virus entry into the central nervous system; 20% had dysgeusia and anosmia. In their opinion, in these patients, the most likely spread of the virus through the cerebrospinal fluid to the cranial nerves, and subsequently to the inner ear.

Damage to the sensory systems, including the auditory system, occurs in most people infected with coronavirus. In clinical practice, tonal audiometry is mainly used to determine the presence of hearing loss. To substantiate the causal relationship between hearing loss and damage to the cells of the organ of Corti, auditory pathway or auditory cortex, the results of otoacoustic emission, registration of acoustic reflexes, auditory evoked potentials, radiological examinations, etc. are needed, timely implementation of which at the beginning of the disease is not always possible. Delayed diagnosis and, consequently, treatment may be one of the factors that slow down and complicate the full recovery of hearing in some patients. The results of diagnostic tests are often ambiguous and require careful interpretation, considering the understanding of the pathophysiology of this disease.

PATHOGENETIC ASPECTS OF SARS-COV-2-INDUCED SENSORINEURAL HEARING LOSS

Possible mechanisms for the development of virus-induced sensorineural hearing loss and other audiological or vestibular disorders can be divided into direct and indirect mechanisms. Direct viral cytotoxicity is directed at the cells of the inner ear, in particular, the spiral organ and vestibulocochlear nerve. Activation of inflammatory mechanisms, neuroinflammation, and secondary immune-mediated processes play a significant role in damage to these structures. The reactivation of latent viral infection in the inner ear is also of some importance [27]. It is not only the cells of the inner ear that are susceptible to coronavirus; this virus can affect the auditory pathway in different parts of it (Fig. 1).

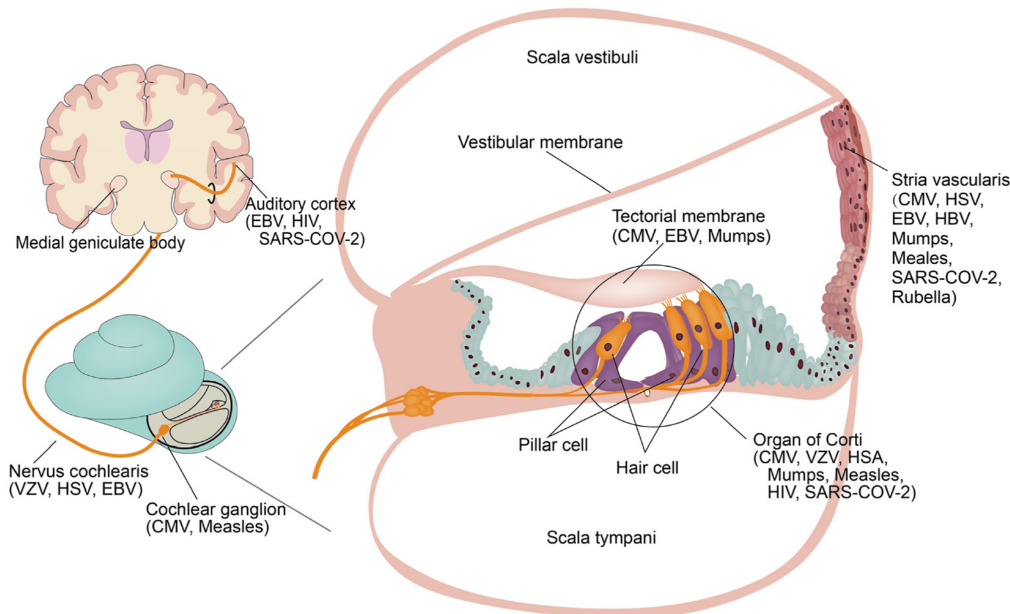

Figure 1. Schematic diagram of the infected area of the virus in the auditory pathway

Source: [3]

Nerve cell damage and the clinical manifestation of this process are observed both during the acute phase of infection and after it. Current understanding of the mechanisms of neurological symptoms remains imperfect. Most likely, the occurrence and progression of SNHL is characterised by multifactorial nature, when the key is not only the variety of additional contributing factors and mechanisms, but also the variability of their combination. These are primarily direct viral invasion/damage, hypoxia, immune-mediated damage, and blood clotting disorders [25, 28].

In the process of viral infection and further development of the disease, a significant role is played by the

unequal organ expression of specific receptors on the membranes of target cells and the selective sensitivity of viruses to them. For the coronavirus, the angiotensin-converting enzyme 2 receptor (ACE2) located on the human cell membrane is a necessary binding component for its Spike protein to the human cell [29]. W. Ni *et al.* [30] explain the lack of specific and diverse extrapulmonary symptoms in COVID-19 by the fact that these receptors are present on the cell membranes of many human organs. The SARS-CoV-2 adhesive protein binds to the host cell's ACE2 receptor. For further penetration of the virus into the cell, hydrolysis and cleavage of the ACE-2 protein by transmembrane protein serine 2 (TMPRSS2) are required [31].

The development of audiological symptoms is also associated with the high affinity of SARS-CoV-2 for ACE2, which is widely represented in cochlear hair cells, on the membranes of middle ear cells, and Eustachian tube cells (Fig. 1) [32]. In addition, adult inner ear cells in addition to ACE2 receptors, according to J. Jeong *et al.* [6] also express the cofactors TMPRSS2 and FURIN, which are necessary for the virus to enter the cell.

Functional changes or damage, including viral damage, to the outer hair cells are confirmed by the results of otoacoustic emission (OAE), during which, in response to stimulation with a signal of a certain frequency, a sound created by the displacement of the cochlear basilar membrane from the base to the apex is recorded [33]. Internal hair cells, which numbers are three times smaller, form synapses of 90-95% of the auditory nerve fibres and transmit almost all acoustic information to the brain [34]. It would be logical to assume that they also undergo changes, but it is not yet possible to clinically determine their functional state (Fig. 2).

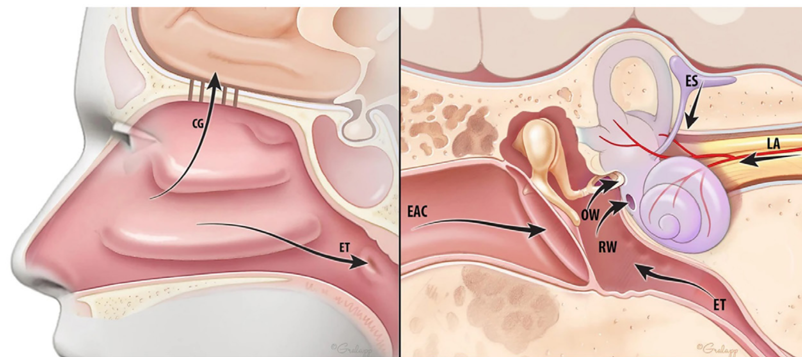

Figure 2. Potential paths for SARS-CoV-2 entry into the inner ear

Source: [6]

SARS-CoV-2 can enter the inner ear in several ways. The first pathway – through the nose and olfactory sulcus of the frontal lobe (lat. *sulcus olfactorius lobi frontalis*) to the central nervous system, as shown above in patients with chemosensory symptoms. The second pathway – through the endolymphatic sac (lat. *saccus endolymphaticus*), it is indicated by the abbreviation ES in Figure 2. Functionally, it is an immunological interface for the inner ear and regulates its fluid level. The proof of this postulate is the stated reverse development of SNHL. That is, some patients developed hearing loss due to endolymphatic hydrops due to the penetration of SARS-CoV-2 through the endolymphatic sac [6].

Third possible path – haematogenic. The virus spreads through the vascular tissue (lat. *stria vascularis*), between the epithelial cells of which a network of capillaries passes. The stria vascularis is the only structure in the spiral organ that concentrates enzymes, ensures the diffusion of ions and other substances both in and out of the endolymph. Its damage disrupts the potassium homeostasis of the cochlear endolymph and endocochlear potential. In addition, systemic inflammation increases vascular permeability, for example *arteria labyrinthi*, which in Figure 2 is indicated by the abbreviation LA. This can promote the penetration of the virus and pro-inflammatory cytokines into the inner ear. SARS-CoV-2 can damage the blood-brain barrier, and the blood-brain and blood-labyrinth barriers are structurally and functionally similar (Fig. 1, Fig. 2) [35, 36]. It can be assumed that this pathway is most likely for patients with severe COVID-19, who are characterised by uncontrolled and excessive cytokine secretion, the so-called "cytokine storm". According to the haematogenic theory described by J. Saniasiaya [2], the coronavirus attaches to haemoglobin via the β-chain, enters red blood cells, which become a kind of viral transporter, that is, spread through the blood, infecting all tissues that express ACE2.

The fourth pathway – SARS-CoV-2 penetration through the membranes of the round (abbreviation RW in Fig. 2) or oval (abbreviation OW in Fig. 2) windows. This assumption is based on data on colonisation of the mastoid process and middle ear by SARS-CoV-2 [37]. However, the likelihood of this pathway being implemented seems to be low, given the absence of signs of infection and exudation into the tympanic cavity.

A significant amount of ACE2 is found on the membranes of medulla oblongata cells, in the brain, in particular in the temporal lobe. It is in the temporal lobes of the cerebral cortex that the central (cortical) part of the auditory analyzer, the so-called auditory cortex, is located, the defeat of which also leads to perceptual hearing loss. After amplification, SARS-CoV-2 leaves the host cell and inoculates into cells of the auditory cortex that express a large number of ACE2 receptors, which, in turn, leads to hearing loss (Fig. 1) [3, 38]. Contact of the virus with the surface receptors of the temporal lobe triggers the release of inflammatory mediators, including cytokines, which are also capable of direct cell damage, that is, they deepen functional and structural changes in brain cells [2].

Among the mechanisms that lead to sudden sensorineural hearing loss, J. Saniasiaya [2] indicated not only inflammation of the auditory pathway from the cochlea to the auditory cortex, cross-reaction between inner ear cells and viral antigens, and negative effects on perilymphatic tissue. Of great importance is deoxygenation of red blood cells under the influence of coronavirus, and, as a result, constant hypoxia of the auditory cortex neurons.

Another likely hypothesis of hearing loss is reduced perfusion of the hearing organs due to ischaemia. According to R. Knight *et al.* [39], the incidence of arterial and venous thromboembolic events remains quite significant even 49 weeks after the diagnosis of COVID-19. If increased blood clotting in the veins is clinically manifested mainly by pulmonary embolism, deep vein thrombosis, usually in the legs, etc., then arterial thrombosis leads to ischemia and tissue necrosis. This hypothesis is supported by elevated levels of D-dimer in the blood and microthrombosis in SARS-CoV-2 infected individuals with audiological symptoms [40].

Figure 3 illustrates the diversity of localisation of potential thrombosis, which can lead to the development of audio-vestibular disorders due to arterial occlusion. For example, increased coagulation in the terminal capillary bed, which starts from the labyrinth artery and provides vascularisation of the inner ear, leads to cochlear SNHL [41]. Even temporary hypoxia has a stressful effect on the inner ear cells, increasing the concentration of reactive oxygen species, which can cause additional damage to hair cells. On the other hand, a blood clot in one of the vessels feeding the upper auditory pathways can lead to central hearing loss [40].

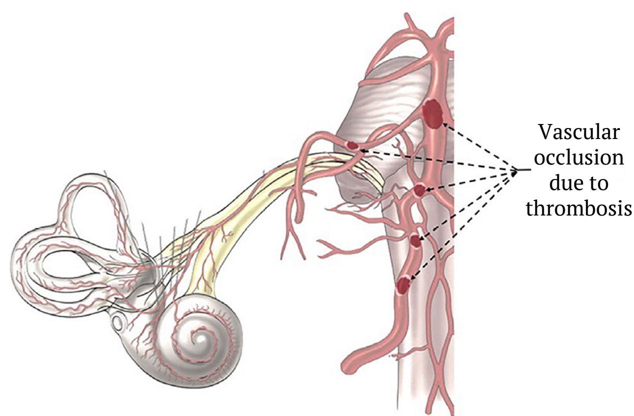

Figure 3. Indirect Virus Effect
Source: [40]

Changes in the blood clotting rate can cause both macro- and/or microthrombosis [42], followed by transient or permanent ischaemia/hypoxia in the auditory pathways, and the development of haemorrhagic complications, such as intra-labyrinth haemorrhage [43] or haemorrhagic lesions of the brain parenchyma [25], which also determines the onset, duration, and prognosis of audiological disorders. Virus-induced sensorineural hearing loss may result from direct viral damage, inflammation, and secondary effects like ischemia and thrombosis.

OTHER ETIOLOGICAL AND PATHOPHYSIOLOGICAL FACTORS OF HEARING LOSS NOT ASSOCIATED WITH THE DIRECT ACTION OF THE SARS-COV-2 VIRUS

From the first days of the fight against SARS-CoV-2, A. Ciorba *et al.* [44] focused the attention of the world medical community on the ototoxicity of certain drugs. In particular, chloroquine and hydroxychloroquine can cause sensorineural hearing loss or tinnitus, which are rarely reversible, especially if these drugs are used for weeks or months. The use of macrolide antibiotic azithromycin can also cause both reversible and irreversible sensorineural hearing loss and tinnitus, and it has often been used in combination with hydroxychloroquine to enhance the effect. And in some countries, ototoxic chloroquine and hydroxychloroquine were administered to treat SARS-CoV-2 infection at doses significantly higher than those used to treat malaria [45].

The fact that potentially ototoxic furosemide, antiviral analogues of adenosine nucleotides (remdesivir and favipiravir), and the nucleoside reverse transcriptase inhibitor lopinavir, which are associated with the occurrence of perceptual hearing loss after several weeks of administration, were used in the treatment of patients with COVID-19. The ototoxic effect of lopinavir has also been confirmed *in vitro* [25, 44]. The severity of audiological symptoms usually depends on the dose of the drug, the duration of therapy, comorbidity, and the use of other medications that may potentiate negative effects or have synergistic effects when used in combination or sequentially.

Cochlear hair cells, which have a high metabolic activity and are particularly sensitive not only to toxic, but also to hypoxic or ischemic effects, are considered the most vulnerable [44]. That is, their damage is possible both as a result of blood clotting disorders, and persistent hypoventilation and hypoxygenation in the presence of SARS-CoV-2 associated respiratory or cardiovascular pathology. In addition to significant advances in the treatment of SARS-CoV-2-related diseases, a landmark event in the fight against the virus was that some COVID-19 vaccines became commercially available in the first half of 2021. However, their use had its own risks.

F. Zoccali *et al.* [46] provide stories of a 40-year-old man and a 67-year-old woman who developed audio-vestibular disorders after the third dose of the vaccine. In man – after five days (Pfizer-BioNTech vaccine), and in woman – after seven days (Moderna vaccine). The appearance of dizziness, tinnitus, and sudden perceptual hearing loss (a unilateral increase in audiometric thresholds in a man was up to 70 dB, in a woman – up to 60 dB at each frequency), was attributed to a possible cross-immune response between the components of the vaccine and human host cells, a possible spasm of the internal auditory artery or increased blood clotting, which leads to thrombosis. Ultimately, the structures of the inner ear are very sensitive to circulatory disorders and ischaemia, which are one of the main causes of sudden idiopathic sensorineural hearing loss.

M. Canales Medina & M. Ramirez Gómez [47] also described clinical cases of newly identified postvaccinal tinnitus and acute sensorineural hearing loss. In most of the patients, these symptoms appeared a few days after the second

dose of the Astra Zeneca vaccine was administered. Hearing loss ranged from mild to severe, which was confirmed by the results of tonal audiometry. The researchers note a positive effect of the use of systemic corticosteroids to restore hearing in this cohort of patients. The researchers point to a decrease in perfusion and ischaemia of the hearing organs as a result of increased thrombosis caused by the Astra Zeneca vaccine from COVID-19 as the most likely hypothesis regarding the pathogenesis of audiological changes.

Discussing the possible link between vaccination and sudden sensorineural hearing loss, J. Jeong & H.S. Choi [48] suggested that viral antigens after vaccination, leading to the development of antibodies and the release of cytokines, can trigger autoimmune immunocomplex mediation against cells of spiral organ. Additionally, immune and inflammatory responses can lead to vasculitis and vascular ischaemia. The immunosuppressive and anti-inflammatory effects of corticosteroids, which are effectively used in the treatment of such patients, are also considered. In order to prevent post-vaccination complications, researchers [46, 48] suggest that instead of using systemic steroids, intratympanic steroids should be considered, which will suppress the systemic immune response to a much lesser extent.

Analysing the effect of vaccine immunisation on the degree of hearing loss, X.W. Liew *et al*. [20] determined the thresholds of auditory perception in pure tone audiometry. According to their results, the association between vaccination and sensorineural hearing loss (SNHL) is insignificant. In most patients, with effective treatment, hearing was restored to normal levels within a few weeks or months. According to the researchers, the incidence, prevalence, and post-vaccination occurrence of SNHL in most countries remains insignificant and is likely to remain unchanged over time. Some mechanisms of development of post-vaccination hearing loss, including delayed hearing loss, remain to be elucidated. There are assumptions about a certain effect (payload) of mRNA and an autoimmune mechanism, such as the response to the lipid nanoparticle delivery agent, and the production of immunoglobulin G in the 10-14-day period after vaccine administration, which coincides with the period of occurrence of SNHL after vaccination.

For over 20 years, the SARS-CoV-2 virus has been challenging doctors around the world: the absence of specific symptoms and the ability to prolong them, the heterogeneity of pathophysiology and multiorgan damage, and the absence of a decrease in virulence with mutation – this is not a complete list of its diversity. Hearing loss is only one of the symptoms of its harmful effects on the human body, which requires the search for new approaches in prevention, treatment, and diagnosis, since timely and effective detection of changes is an undoubted condition for significantly reducing risks. Patients with SARS-CoV-2 are completely isolated during the disease, which complicates, and sometimes makes it impossible, both otoscopic and audiometric diagnostics. This problem could be solved by developing methods for remote audiological monitoring, which is one of the challenging tasks of the future.

CONCLUSIONS

This study successfully analysed the main mechanisms by which SARS-CoV-2 infection leads to hearing loss. Despite a fairly high awareness of the aetiopathogenesis of virus-induced processes, this pandemic has become an unprecedented case in modern history and medical practice. In the pathogenesis of hearing loss caused by SARS-CoV-2, in addition to direct viral and immune-mediated damage to cells of both sound conduction and sound perception processes, a number of mediated mechanisms are distinguished. The conductive component of the disorder is mainly a consequence of an inflammatory reaction, swelling and exudation into the middle ear cavity, which are potentiated by impaired ventilation of the auditory tube.

The sensorineural component of hearing loss, in most cases, is the result of direct viral cytotoxicity directed at auditory pathway cells with high expression of angiotensin-converting enzyme 2 receptors. Damage to the hair cells of the cochlea, auditory pathway cells, and cortex is further aggravated by local and generalised inflammatory reactions, venous and arterial thrombosis, with subsequent tissue ischaemia, hypocoagulation, hypoperfusion, etc. Ototoxic medications used in the treatment of COVID patients, especially with their long-term and combined use, comorbidity and polypharmacotherapy, have an additional negative effect on auditory function. The influence of COVID vaccines as a probable etiological factor in the development of sensorineural hearing loss was investigated. Diagnosis of hearing disorders after COVID-19 includes audiometry (determination of audibility thresholds), otoacoustic emission (assessment of the function of external hair cells) and, in some cases, magnetic resonance imaging (MRI) to detect structural changes in the brain.

The results of the study are important for clinical practice. They highlight the need for early diagnosis and treatment of hearing disorders in patients who have had COVID-19. In addition, the data obtained can be used to develop new methods for the prevention and treatment of these disorders. Further research is needed to investigate the long-term effects, develop new treatments and prevention, and create effective rehabilitation systems. A multidisciplinary approach involving collaboration between otolaryngologists, neurologists, immunologists, and other professionals is essential for the successful treatment of patients with COVID-19-related hearing disorders. It is also important to consider the economic and social consequences of hearing loss, and to develop social adaptation programmes for such patients.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Сучасні патогенетичні аспекти втрати слуху при COVID: огляд літератури

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Анотація. За прогнозами Всесвітньої організації охорони здоров’я до 2050 року до 10 % населення планети потребуватимуть реабілітації для розв’язання проблеми інвалідизуючої втрати слуху. Метою цієї статті було з’ясувати основні механізми зниження слуху, пов’язаного з інфікуванням Severe Acute Respiratory Syndrome Coronavirus 2. Під час дослідження були опрацьовані сучасні англомовні наукові публікації переважно з високим індексом цитування через фахові платформи MEDLINE/PubMed та Index Medicus. Всього було обрано 48 джерел. Було проаналізовано статті присвячені розвитку кондуктивної або сенсоневральної приглухуватості, яка виникла безпосередньо внаслідок вірусного захворювання, або пов’язана з процесами, які його супроводжують (лікування, супутня патологія, вакцинація тощо). Встановлено, що розвиток вірусно-індукованої приглуховатості при COVID має мультифакторну природу. Гетерогенність аудіологічних змін насамперед обумовлена прямим вірусним пошкодженням клітин слухового аналізатора, які експресують мембранні рецептори ангіотензинперетворювального ферменту другого типу. Крім того, відбувається реактивація латентної вірусної інфекції, екстравазація ексудату в порожнину середнього вуха, порушення згортання крові, імуноопосередковане пошкодження клітин, місцева і генералізована запальна реакція, які впливають як на ланку звукопроведення, так і на звукосприйняття, як на одному вусі, так і на обох. Деякі випадки аудіологічних розладів можуть бути і ятрогенного походження, оскільки не виключені поствакцинальні ускладнення та ототоксичний вплив медикаментів, які використовуються у лікуванні COVID-19, що слід враховувати клініцистам на усіх рівнях медичної допомоги для ефективного менеджменту конкретного клінічного сценарію

Ключові слова: аудіологія; кондуктивна приглухуватість; сенсоневральна приглухуватість; коронавірус; ототоксичність; вакцинація

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Epidemiological situation of mycobacterioses in Ukraine and the worldwide at the beginning of the 21st century: A literature review

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Abstract. The lack of a unified reporting system for clinical outbreaks of mycobacterioses makes it difficult to objectively assess the epidemiological situation and identify patterns in the epidemic process, despite the growing relevance of this issue in human and veterinary medicine. The aim of this review was to study the epidemiological and aetiopathogenetic aspects of mycobacterioses in Ukraine and other countries on different continents. A comparative-geographic method and epidemiological analysis method were used in the study. As a result, it was found that in Ukraine, mycobacterioses in humans are widespread, with 94% of cases manifesting as pulmonary forms, often forming mixed infections with tuberculosis, making them difficult to diagnose. The most common aetiological factor is *M. avium complex* and disseminated mycobacteriosis usually develops in HIV-infected patients. In most of the analysed countries (Japan, South Korea, Iran, Turkey, Pakistan, Saudi Arabia, Egypt, Oman, Kuwait, China, France, Great Britain, Italy, Greece, Czech Republic, Poland, USA, Canada, Brazil, Australia and several African countries) during the period 2000-2023, there was an observed increase in the incidence of lung diseases caused by non-tuberculous mycobacteria, including an 8-fold rise in South Korea; an annual growth of 8% in the USA; and a 2.3-fold increase in Queensland (Australia) from 11.1 pcm in 2001 to 25.88 pcm in 2016. It was established that the epidemiological features of mycobacterioses are the predominant infection of patients with rapidly growing mycobacteria; an increased risk of mycobacterial infection with increasing age; detection of *M. avium complex, M. abscessus complex, M. kansasii* and *M. fortuitum* as the most common cause of mycobacterioses

Keywords: human mycobacterioses; nontuberculous mycobacteria; epidemiology of mycobacterioses; *M. avium complex*

INTRODUCTION

According to the World Health Organisation's 2022 tuberculosis report, the annual number of diagnosed cases worldwide is around 6.4 million [1]. Nontuberculous mycobacteria (NTM) are the second most common cause of mycobacterial infections. M. Lipman *et al*. [2] established that there are about 190 species and 14 subspecies, with their number continuously increasing. Data from J.E. Gross *et al*. [3] and J.W. Alffenaar *et al*. [4] show that mycobacterioses of the nontuberculosis complex are a growing problem in human medicine that is becoming increasingly relevant each year. There are many uncertainties regarding the treatment of nontuberculous mycobacterial lung diseases (NTM-LD), the peculiarities of their epidemiology have not been studied, methods of a comprehensive diagnosis of mycobacterioses and laboratory diagnosis, in particular, have not been unified, treatment protocols have not been unified, and prevention measures are extrapolated from the Guidelines for the control of other infections [5, 6].

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The nation's health is, first and foremost, healthy food free of pathogens common to humans and animals. Healthy food can be obtained from healthy animals. Currently, about a dozen species of NTM are known to be causative agents of mycobacterioses in humans and animals [7, 8]. In this context, the prevention of mycobacterioses in animals caused by NTM is an urgent problem for veterinary medicine in Ukraine [9, 10]. I. Pavlik *et al.* [8] found that every year in Ukraine, during planned allergic tests for tuberculosis in cows in 250 or more dairy farms, animals are found that give pseudo-allergic reactions to tuberculin, which indicates sensitisation of their bodies to NTM.

Research by P. Boiko *et al*. [11] indicates that pathological changes were found in the bodies of animals that reacted positively to PPD-tuberculin, and NTM were isolated, including M. *scrofulaceum* and *M. fortuitum*. This led to the conclusion that an infectious mycobacterial process was present in the cows reacting to tuberculin and that an epizootological process of mycobacteriosis caused by NTM was occurring in the herd. The isolation and identification of these pathogens are evidence of their importance as etiological factors of human mycobacteriosis. Mycobacterioses of productive, domestic and wild animals are also widespread in other countries of the world [12].

The absence of data on the diagnosis of mycobacterioses caused by NTM in official reports from government institutions responsible for epidemiological surveillance and control of infectious diseases in Ukraine, as well as in the World Health Organisation's global registry, hinders the ability to recreate a complete picture of the spatial-temporal dynamics of the epidemiological situation concerning NTM-caused mycobacterioses. Therefore, the study aimed to systematise and analyse the epidemiological results of studies of human mycobacterioses conducted by scientists from different countries, including Ukraine.

Epidemiological analysis was conducted on 49 scientific studies on the role of NTM in the infectious process of human mycobacterioses, its forms and duration, the impact of environmental factors and its manifestation in various countries worldwide from 2000 to 2023. The criteria for the scientific search were indicators that, first of all, characterise the intensity of the manifestation of the epidemiological process of mycobacterioses, the analysis of the role of various aetiopathogenetic factors, the influence of contributing and predisposing factors on the intensity of the epidemiological situation regarding human mycobacterioses for the specified period. The study used comparative-geographical and statistical research methods, as well as the method of epidemiological analysis.

ENVIRONMENTAL MYCOBACTERIA – POTENTIAL PATHOGENS OF HUMAN AND ANIMAL MYCOBACTERIOSES

Mycobacteriosis (Latin: *mycobacteriosis*) is an infectious disease of humans and animals caused by representatives of a large group of NTM, including *Mycobacterium avium, M. fortuitum, M. kansasii, M. scrofulaceum, M. xenopi* and others [13, 14]. They are also called atypical mycobacteria (ATM) or environmental mycobacteria. During 2010-2020, there was a significant increase in the prevalence of NTM and the incidence of infections caused by them [15]. Mycobacterioses has become a major public health problem worldwide. Among the numerous species of NTM, the *Mycobacterium avium* Complex (MAC) is the predominant species, which has been recognised as a ubiquitous microorganism – it is isolated from polluted water and soil [16]. However, the main causes of the increase in MAC-associated disease are not yet fully understood. This is mainly because human infection with MAC is associated with various sources of the infectious agent [17].

Identifying the causal links between the source of the infectious agent and its epidemiology is a crucial step towards developing new prevention strategies and effective control of MAC infection. This issue pertains not only to *M. avium* but also to most NTM. As research by many scientists indicates, the majority of these microorganisms are sapronoses. Only some of them, such as the *M. avium-intracellulare complex, M. kansasii, M. abscessus, M. chelonae* and some others, have formed the features of facultative, or even completed pathogens. This fully applies to M. ulcerans, which causes Buruli ulcer and is found in regions with hot tropical climates – Africa, Australia, Mexico and others [18]. This is evidence that the evolution of pathogenicity in these microorganisms continues [19].

Due to the widespread distribution of NTM in various biotic and abiotic environments, confirming their aetiopathogenetic role requires repeated isolation of these microorganisms from the source of infection. Consequently, the American Thoracic Society has developed microbiological, clinical, and radiological criteria for determining the etiological role of NTM in HIV-infected and non-HIV-infected patients [20].

Although NTM were discovered after *M. tuberculosis*, their potential ability to cause human lung disease was not recognised until the 1950s. In 1954, the Runyon classification of NTM was proposed [14], which divides mycobacteria into four main groups based on their growth rate, colony morphology, and ability to produce pigments. The most common NTM in clinical practice includes the *M. avium-intracellulare complex (MAIC-complex), M. kansasii, M. fortuitum, M. abscessus, M. chelonae, M. marinum*, and several others. These bacteria are frequently isolated from water, soil, and other environmental sources [21, 22].

Monitoring the epidemiological characteristics and aetiology of mycobacterioses in different countries of the world usually concerns NTM isolated from patients with pulmonary pathology, while skin and soft tissue infections are analysed much less frequently. However, the results of microbiological studies of clinical samples indicate that NTM were isolated from extrapulmonary tissues (maxillary and frontal sinuses, wounds and skin) no less often than from pulmonary samples [23]. These data indicate the pleiotropic pathogenic action of mycobacteria on different tissues and organs of the infected organism, which in turn suggests that NTM possess a broad spectrum of aggressive factors with genotypic control and phenotypic expression. The epidemiological process of mycobacteriosis includes the interaction of three main components: the source of infection, the mechanisms of transmission of the pathogen, and organisms susceptible to the disease. This interaction takes place under the influence of various environmental factors. At the same time, the latter factor often plays a decisive role in the manifestation of the infectious process of mycobacteriosis [24].

The sources of mycobacterial infections in humans are individuals infected with NTM. However, given the relatively young age of the problem of mycobacterioses, the first link in the epidemiological chain has been insufficiently studied. However, it has been established that NTM is present in water, biofilms, soil, and aerosols, meaning these microbes are ubiquitous. They are natural inhabitants of the human environment, especially drinking water distribution systems. Thus, it is quite likely that everyone is exposed to them daily. This is one of the reasons for the increase in the incidence of mycobacterial infections caused by environmental mycobacteria [25]. It is obvious that the incidence of mycobacterioses caused by environmental NTM will continue to grow. This growth will be partly due to increased awareness of these microbes as human disease pathogens, and partly to improved methods of detection, cultivation and identification of the pathogens themselves [26].

Mycobacterioses, according to some scientists, mainly have a tuberculosis-like clinical pattern and therefore are difficult to identify using classical diagnostic methods. They often occur in the form of mixed infections with other mycobacterioses, including tuberculosis [27]. In addition, atypical mycobacteria are characterised by a wide range of resistance and are potentially pathogenic for humans and animals. Up to 80% of cases of human mycobacterioses are caused by *M. avium complex*. Mycobacterioses are most commonly found in people infected with the human immunodeficiency virus (HIV), and a CD4+ lymphocyte count below 50/μL can lead to the development of disseminated mycobacteriosis. The causative agent of this type of mycobacterial infection is usually MAC (90% of cases) [28]. Considering that the reservoir of *M. avium* is open water bodies, NTM aerosols formed over the water surface infect people by air. Sick poultry are also a powerful source of NTM and thus play an important role in the local functioning of the epidemiological process of mycobacteriosis.

It has been established that disinfection may, in part, contribute to the resistance of *M. avium* and *M. intracellulare* in drinking water distribution systems. Thus, *M. avium* and *M. intracellulare* are many times more resistant to chlorine, chloramine, chlorine dioxide, and ozone than other waterborne microorganisms. Therefore, disinfection of drinking water leads to the predominance of mycobacteria over other water microbiota. In the absence of competitors, even slowly growing mycobacteria can proliferate in the water distribution system [29]. Studies of the spatiotemporal characteristics of the spread of mycobacterioses in Ukraine and around the world can help to identify new patterns in the development of epidemic processes and determine their defining factors for mycobacterioses.

MYCOBACTERIOSES IN HUMANS: SPATIOTEMPORAL PATTERNS OF THE EPIDEMIC PROCESS IN UKRAINE AT THE BEGINNING OF THE 21ST CENTURY

The increasing incidence of mycobacterioses caused by NTM in different regions of the world is attracting increasing attention from scientists. It has been noted that the geographical location of a country affects the species prevalence of NTM [12, 16]. Studying the epidemiological situation of mycobacterioses in Ukraine, L. Todoriko & O. Shevchenko [14] found that the incidence of this infectious disease is 2.3 per 100,000 population (2.3 pcm). It has been established that mycobacterioses in humans can manifest in various forms, including pulmonary (nodular bronchiectatic form usually occurs in older (70 years old) women; cavitary form (fibro-nodular) is typical for men 40-50 years old who smoke and abuse alcohol; hypersensitivity lung syndrome, which usually proceeds subacutely; patients are more often young people who do not smoke).

Pulmonary manifestation of the infection is the primary form and is observed in 94% of cases. In addition, disseminated mycobacterioses are often diagnosed, which develop in patients with stage IV AIDS, after kidney or heart transplantation, with prolonged use of GCs (glucocorticosteroids) and patients with leukaemia. The main causative agents are *M. avium* and *M. kansasii*. Another form of manifestation of mycobacterioses is lymph node damage, which occurs mainly in children under 5 years of age. The affected lymph nodes are typically tonsillar, preauricular, and/or submandibular on one side. The causative agent in most cases is MAC. Without treatment, the lymph node may spontaneously rupture, leading to the formation of a fistula [14].

O. Shevchenko *et al.* [27] demonstrated that in the clinic of internal infectious diseases, mycobacterioses of various types are increasingly being diagnosed, indicating a growing role of atypical mycobacteria in the development of respiratory system pathology. The clinical pattern resembles tuberculosis, they are difficult to diagnose, and often form mixed infections with tuberculosis. NTM are characterised by a wide range of resistance to disinfectants, antibacterial agents, and environmental factors and are potentially pathogenic for humans.

It has been established that risk factors for developing mycobacterioses include a history of respiratory diseases (COPD, bronchiectasis, pneumoconiosis, cystic fibrosis), previous tuberculosis (mycobacterial disease incidence increases tenfold compared to the population level), work with farm animals and poultry; increased joint stress, mitral valve prolapse, gastroesophageal reflux disease, rheumatoid arthritis, taking GCs in doses greater than 15 mg/day for more than 14 days, and immunosuppressants [14].

O. Zhurilo *et al.* [17] studied the species diversity of NTM on the territory of Ukraine during 2014-2018. Phenotypic identification of isolates was carried out using cultural and biochemical methods. The most common isolates were *M. avium-complex* – 45.5%, the number allocated *M. fortuitum* – 13.5%, less often – *M. xenopi* (7.1%), *M. kansasii* (5.8%) and *M. scrofulaceum* – 3.2%. At the same time, slowly growing mycobacteria were isolated 6 times more often than rapidly growing.

Based on the genotypic identification of mycobacterial cultures using GenoType Mycobacterium CM/AS, 11 NTM species were identified, 7 of which were assigned to slowly growing NTM groups (groups I–III according to Runyon), including *M. avium, M. intracellulare, M. gordonae, M. kansasii, M. xenopi, M. malmoence, M. scrofulaceum*, and 4 to the group of rapidly growing NTM (group IV according to Runyon), including *M. abscessus, M. chelonae, M. fortuitum, M. peregrinum*. The frequency of MAC among NTM in general was 53.24% (71/133). However, regional variations were observed, with MAC predominating among slowly growing NTMs in the Dnipropetrovsk, Zhytomyr, Chernihiv, Chernivtsi, and Cherkasy regions.

In contrast, in the Poltava and Kyiv regions, MAC was not the predominant group among slowly growing NTM. A notable feature of the Chernivtsi region was that within the MAC group, *M. intracellulare* was more common than *M. avium*, whereas, in all other regions, *M. avium* predominated within the MAC group. Among rapidly growing NTM, the predominant species in all regions of Ukraine was *M. fortuitum* (86.96% among rapidly growing NTM). Single isolates of *M. abscessus, M. chelonae* i *M. peregrinum* were identified [17].

The data show that significant attention is paid to the study of the aetiopathogenesis and epidemiological features of human mycobacterioses in Ukraine. However, the problem of mycobacterioses is multifaceted and requires a deeper study of other aspects of the infectious and epidemiological process of this infectious pathology.

EPIDEMIOLOGICAL ASPECTS OF HUMAN MYCOBACTERIOSES IN THE WORLD DURING 2000-2023

Aiming to predict the epidemiological situation and clinical manifestations of lung diseases caused by NTM, (NTM-LD) and tuberculosis, Japanese scientists conducted a multifactorial epidemiological analysis of all cases of mycobacterial isolation at Fukujuji Hospital during the period 2006-2016. Identification of mycobacterial isolates was carried out according to the microbiological criteria of the American Thoracic Society and the Infectious Disease Society of America. It was found that the most common isolates were the *M. avium* complex (87.3%), the *M. abscessus* complex (5.5%), and *M. kansasii* (3.9%) [30].

In studying the characteristics of the epidemic process and the dynamics of the intensity of the epidemic situation regarding mycobacterioses of the population of South Korea from 2001 to 2015, Korean scientists found that the incidence of NTM-LD increased nearly 8-fold during this period – from 7.0 pcm (in 2001) to 55.6 pcm (in 2015). Just like in Japan, in South Korea, the most common causative agent of NTM-LD was the *M. avium complex* (n = 1,746; 75%), *M. abscessus* (n = 519; 22%) and *M. kansasii* (n = 64; 3%) [31].

In China, lung diseases caused by NTM are being diagnosed more and more often each year. This is causing growing concern in the healthcare sector. A study of the aetiology and epidemiological characteristics of mycobacterioses in the population of South China during the period (2013-2016) showed that the most common causative agents of this infectious pathology were the *M. avium complex* (44.5%), the *M. abscessus complex* (40.5%), and much less often *M. kansasii* (10.0%) and *M. fortuitum* (2.8%). The authors believe that the epidemiological characteristics of mycobacterioses are as follows: significantly higher infection rates with rapidly growing mycobacteria compared to slowly growing ones; a sharp increase in the risk of infection with rapidly growing mycobacteria with advancing age; and pulmonary diseases caused by rapidly growing mycobacteria being more common among migrants than among the resident population [32]. The data obtained by Chinese scientists confirm the conclusions drawn by Japanese and Korean researchers and indicate that the most commonly identified causative agents of mycobacterial infections are *M. avium complex, M. abscessus complex, M. kansasii* and *M. fortuitum*.

Scientific research by a team of authors is dedicated to highlighting individual aspects of the epidemiology of mycobacterioses caused by NTM in Middle East countries [33]. They found that out of 1,084 strains isolated from primary clinical samples, 434 (40.0%) were identified in Iran, 280 (25.8%) in Turkey, 137 (12.6%) in Saudi Arabia, 116 (10.7%) in Pakistan, 47 (4.3%) in Egypt, 43 (3.9%) in Lebanon, 14 (1.2%) in Kuwait, and 13 (1.1%) in Oman. At the same time, 637 (58.7%) isolates were identified as slowly growing mycobacterial species, and 447 (41.2%) as rapidly growing; *M. fortuitum* was the most common isolate (269 out of 447; 60.1%) [34]. The data obtained give reason to assert that in Middle Eastern countries, mycobacterioses is not an uncommon phenomenon in infectious pathology, but a fully formed nosological unit that should be under constant control of infectious disease specialists.

According to scientific publications, mycobacterioses of the population caused by NTM are registered annually in almost all countries of the American continent. Thus, during 2015-2017 in the USA, the incidence of mycobacterioses caused by NTM ranged from 2.3 to 3.9 pcm. The prevalence of NTM-LD in the United States in 2019 was significantly higher than the prevalence of tuberculosis. The results of epidemiological monitoring confirm an annual increase in the incidence of NTM-LD by 8% annually [35].

Analysis of the results of microbiological studies of clinical samples from 20,617 patients with lung diseases in the population of Ontario (Canada) for the period 2001-2013 showed that in 10,936 cases, which is 53%, NTM were isolated, including *MAC* and *M. abscessus complex*, which were more often isolated from clinical samples taken from female patients [36]. This fact requires prolonged and repeated validation to be considered an objective pattern, but it merits the attention of infectious disease specialists.

Monitoring the aetiology of mycobacterioses in the USA is primarily focused on NTM isolated from patients with pulmonary pathology, while skin and soft tissue infections are analysed much less frequently. The results of microbiological studies of clinical samples from 1,033 patients in North Carolina (USA) indicate that *M. chelonae* and *M. fortuitum* (both rapidly growing species) were most often isolated from extrapulmonary tissues (maxillary and frontal sinuses, wounds and skin), while *M. avium* was isolated from pulmonary samples [37].

The incidence of pulmonary diseases caused by nontuberculous mycobacteria is also growing in South America. For example, in Brazil (Rio Grande do Sul state) in Porto Alegre, data from 100 patients with NTM-LD were analysed during the period 2003-2013. It was found that the most common NTM species isolated from patients were *M. avium complex (MAC)* – in 35% of cases; *M. kansasii* – in 17%; and *M. abscessus* – in 12% [38].

The data obtained indicate, on the one hand, that the dominant NTM species in human infectious pathology in Asian countries are the same as in American countries, indicating that the main pathogenicity factors of Asian NTM species differ little in virulence from American ones. On the other hand, this may be evidence that the evolution of mycobacterial pathogenicity proceeded simultaneously on all continents, with the formation of the main mechanisms of the aetiopathogenesis of mycobacterioses occurring

against the backdrop of the interaction between microorganisms and macroorganisms. In this interaction, the key factor from the microorganism's side was the development and expression of pathogenic factors, while from the microorganism's side, it was the multifaceted mechanism of specific protection against these factors.

In the European Union countries during 2011-2016, there has been an increase in the tension of the epidemiological situation regarding NTM infections, in particular, the incidence of various population groups is growing annually, both in individual countries and on the continent as a whole [39]. Thus, in Greece, the epidemiological situation regarding pulmonary NTM infections is largely unknown. However, a multifactorial epidemiological analysis and the results of bacteriological studies of clinical samples for the period 2007-2013 showed that the incidence of pulmonary NTM infections of the aetiology of the population of Greece is 18.9 pcm among inpatients and 8.8 pcm among outpatients. Identification of isolates was carried out according to microbiological criteria established by the American Thoracic Society and the Infectious Disease Society of America. At the same time, the microbial spectrum of NTM species was quite wide and amounted to 13 species, but most often *M. avium* – 13%, *M. intracellulare* – 10%, *M. gordonae* – 14%, *M. fortuitum* – 12% were identified [40].

NTM play a significant role in the aetiology of infectious lung diseases in France. Thus, among 1,582 patients with cystic fibrosis, the prevalence of NTM was between 3.7-9.6%. The most commonly identified were *M. abscessus complex* and *M. avium complex*. At the same time, isolates of the first species predominated in groups of persons aged 11-15 years, while isolates of the second species were in patients older than 25 years. Scientific observations of the prevalence of NTM-related diseases conducted at the Bordeaux University Hospital (France) for the period 2002-2013 showed that lung diseases were detected in 170 patients (54.1%), followed by skin and soft tissue infections (22.9%), disseminated cases (10.6%), lymphadenitis (7.7%), bone and joint infections (2.9%), and the remaining 1.8% were catheter-related infections. A total of 16 NTM species were identified. The most commonly isolated were *M. avium* (31.8%) and *M. intracellulare* (20%), *M. marinum* (13.5%), *M. kansasii* (10.6%), *M. xenopi* (9.4%); at the same time, rapidly growing mycobacteria accounted for 9.4%, and slowly growing mycobacteria – 5.3% [41].

Epidemiological aspects of pulmonary infections caused by NTM were studied in Poland by G. Przybylski *et al.* [42] during 2013-2022. Of the 395 patients with infectious lung pathology, 149 cases met the diagnostic criteria for NTM-LD. At the same time, *M. kansasii* (51.68%) and *M. avium* complex (46%) were most often identified.

In Italy during the period 2004-2014, 42,055 clinical samples collected from 15,000 patients with suspected mycobacterial infection were tested. At the same time, mycobacteria were isolated from 595 patients. In 448 (75.3%) patients, *M. tuberculosis complex* was isolated, and NTM was isolated from 147 (24.7%) patients. A total of 16 NTM species were identified, the most common of which was *M. avium* subsp. *hominissuis* (41.5%). This was followed by the number of identified cases: *M. intracellulare* – 14.3%, *M. gordonae* – 11.6%, *M. xenopi* – 9.5%, *M. fortuitum* – 6.8% and *M. kansasii* – 4.8% [43].

The species profile and incidence of NTM-related diseases were significantly different in the historical territories of Moravia and Silesia (Czech Republic). A team of scientists conducted an epidemiological analysis of the incidence of NTM-related mycobacterioses for the period 2012-2018 and investigated the correlation with some socio-economic and environmental factors. It was found that the most commonly isolated NTM were *M. avium-intracellulare*, *M. kansasii* and *M. xenopi*. The incidence of NTM-LD in the population was 1.10 pcm, while among men it was 1.33 pcm and 0.88 pcm among women [44].

The conclusions made by the authors suggest that geographical living conditions influence the species composition of mycobacterioses pathogens, the intensity of the epidemic process as indicated by disease incidence, and the greater susceptibility of men compared to women. This may reflect the impact of provoking (such as hypodynamia, psychogenic stress) and/or contributing (such as smoking, alcohol abuse, etc.) factors on the male organism.

Regarding the United Kingdom, there is limited analytical data on the incidence of NTM-LD. At the same time, there is no consensus on the optimal treatment regimens for this infectious pathology. A team of scientists conducted a retrospective study of NTM-LD at a London teaching hospital for the period 2000-2007. The criteria for inclusion in the retrospective analysis were as follows: only cultures of slowly growing mycobacteria were considered, the age of patients was over 18 years, they were HIV-negative, and the NTM isolates met the criteria of the American Thoracic Society. As a result, it was found that *M. kansasii* was identified in 93% of patients, *M. avium intracellulare* in 63%, *M. malmoense* in 60%, and *M. xenopi* in 25% [45].

A study of the aetiopathogenesis and the intensity of the epidemiological situation regarding NTM infections in the Netherlands from 2013 to 2019 showed that the isolation of NTM increased from 1.0% to 3.6% during this period. Single isolation of NTM in adults was 53.7% and in children – 60.0%. *M. abscessus* and *M. avium complex* were most commonly identified – 47.1% and 30.9%, respectively. The authors conclude that the increase in the number of positive cases of NTM identification is a consequence of unsatisfactory treatment outcomes and the associated decline in lung function. The researchers emphasise that NTM-LD is a serious health problem among patients with cystic fibrosis in the Netherlands [46]. Analysing the data presented in this and the previous article, it can be noted that in the aetiology of mycobacterioses, the predominant influence on the manifestation of the infectious and epidemiological process comes from contributing and predisposing factors, rather than the pathogenic properties of NTM.

The overall prevalence of NTM in pulmonary samples in Sub-Saharan Africa (Nigeria, Mali, Ghana, Zambia, South Africa, Kenya, Uganda, Tanzania, and Ethiopia) was 7.5% (7.2-7.8%). *MAC* species constituted 28.0% of all isolated NTMs. The prevalence of *MAC* complex NTM in Tanzania ranged from 15.0% to 57.8% [47]. Regional variability in the distribution of NTMs was observed. Specifically, 76.4% (2,355 isolates out of 3,084) of *MAC* complex isolates from South Africa were *M. intracellulare*, while all *MAC* complex isolates from Mali were *M. avium*. Rapidly growing mycobacteria, such as *M. fortuitum*, *M. chelonae* and *M. abscessus*, accounted for only 1.2% [48, 49].

From 2001 to 2016, there were 12,219 registered cases of NTM in patients over the age of 18 in Queensland, Australia. The most common species were *M. intracellulare* (39.1%), *M. avium* (9.8%), *M. abscessus* (8.5%), *M. fortuitum* (8.3%), *M. chelonae* (3.3%), and *M. kansasii* (2.4%). Over 15 years (2001-2016), the incidence rate increased 2.3-fold, from 11.1 pcm in 2001 to 25.88 pcm in 2016 [16].

As can be seen from the data presented, the species composition of aetiopathogenetic NTM species on the Australian continent is similar to that in most countries of the world. At the same time, there is a steady increase in the incidence of mycobacterioses in the population of Oceania, which, first of all, indicates an increasing role of NTM in the infectious pathology of the respiratory system and other body systems of humans. Summarising the above, it can be stated that the problem of mycobacterioses requires comprehensive study in each individual country and the world as a whole.

CONCLUSIONS

According to the results of the epidemiological analysis of the spatiotemporal features of the spread of mycobacterioses caused by NTM in Ukraine and on different continents of the world from 2000 to 2023, a significant increase in the incidence of these infections has been identified. In 94% of cases, mycobacterioses in Ukrainian patients manifest as pulmonary diseases, often forming mixed infections with tuberculosis. At the same time, *M. avium complex* is the isolated aetiological factor in almost 80% of cases. In most of the analysed countries, an increase in the incidence of NTM-LD was observed throughout observation, including an 8-fold increase in South Korea; an annual increase of 8% in the USA; and a 2.3-fold increase in Queensland (Australia) from 11.1 pcm in 2001 to 25.88 pcm in 2016.

Among the epidemiological characteristics of mycobacterioses, it is noteworthy that patients are predominantly infected by rapidly growing mycobacteria, the risk of infection increases with age, and there is a higher prevalence of mycobacterial lung infections among migrants compared to the local population. The most frequently isolated strains from biological samples were the *M. avium* complex (87.3%), *M. abscessus* complex (5.5%), and *M. kansasii* (3.9%). The data presented by various researchers indicate the pleiotropic of the pathogenic action of mycobacteria on tissues and organs, which, in turn, suggests a broad spectrum of aggressive factors.

Analysing the features of the infectious and epidemiological processes of mycobacterioses in different countries of the world, it can be assumed that the evolution of the pathogenicity of nontuberculous mycobacteria proceeded simultaneously on all continents, and the formation of the main mechanisms of the aetiopathogenesis of mycobacterioses was carried out against the background of the interaction of the microorganism and the macroorganism. At the same time, the determining factor from the side of the microorganism was the formation and implementation of pathogenicity factors, and from the side of the macroorganism – a multifactorial mechanism of specific protection against these factors. This, in turn, led to a special manifestation of the epidemiological process of this infection, a comprehensive study of which will reveal the details of its driving forces and will serve as the basis for the development of effective prevention measures for this infectious pathology.

The evolution of these relationships against the background of the constant or permanent influence of the stressful political-social and anthropo-ecological situation is developing towards an increase in the virulent properties of mycobacteria, which leads to a change in the way of existence of a number of mycobacteria species – from saprophytic to parasitic, thereby increasing the population of true pathogens. The study of the ecology of the most common causative agents of NTM infections, and their survival in animal products during technological processing will be the next stage of the author's scientific research, the results of which will make it possible to more effectively control the epidemiological process of mycobacterioses at the link of the mechanism, routes and factors of transmission of the causative agent of this infectious pathology of humans.

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CONFLICT OF INTEREST

None.

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Епідемічна ситуація щодо мікобактеріозів в Україні та світі на початку XXI століття: огляд літератури

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Анотація. Відсутність єдиної системи звітності про клінічні спалахи мікобактеріозів ускладнює об›єктивну оцінку епідемічної ситуації та виявлення закономірностей епідемічного процесу, незважаючи на зростаючу актуальність цієї проблеми в гуманній та ветеринарній медицині. Метою цього огляду було вивчення епідеміологічних та етіопатогенетичних аспектів мікобактеріозів як в Україні, так і в інших країнах різних континентів. В роботі використано порівняльно-географічний метод та метод епідеміологічного аналізу. Внаслідок проведеної роботи встановлено, що в Україні мікобактеріози людей мають значне поширення, у 94 % випадків перебігають у легеневій формі, часто формують мікст-інфекції з туберкульозом, а тому важко діагностуються. Найчастіше етіологічним чинником виступає *M. avium complex*, а у ВІЛ-інфікованих пацієнтів зазвичай розвивається дисемінований мікобактеріоз. У більшості аналізованих країн (Японія, Південна Корея, Іран, Туреччина, Пакистан, Саудовська Аравія, Єгипет, Оман, Кувейт, Китай, Франція, Великобританія, Італія, Греція, Чехія, Польща, США, Канада, Бразилія, Австралія та низка країн Африки) за період 2000-2023 рр. спостерігалося зростання захворюваності населення на хвороби легень, спричинені нетуберкульозними мікобактеріями, зокрема у Південній Кореї у 8 разів; у США щорічно на 8 %; у Квінсленді (Австралія) у 2,3 рази – з 11,1 pcm у 2001 році до 25,88 pcm у 2016 році. Встановлено, що епідеміологічними особливостями мікобактеріозів є переважне інфікування пацієнтів швидкоростучими мікобактеріями; зростання ризику інфікування мікобактеріями із збільшенням віку; виявлення *M. avium complex, M. abscessus complex, M. kansasii* і *M. fortuitum,* як найчастішої причини мікобактеріозів

Ключові слова: мікобактеріози людини; нетуберкульозні мікобактерії; епідеміологія мікобактеріозів; *M. avium complex*

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Hormonal and other methods of thyroid gland examination: A literature review

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Abstract. The high frequency of thyroid gland disorders is currently being established, indicating that regular assessment of its condition and hormone levels may aid in the early detection of disease development. This study aimed to analyse current scientific data on methods for diagnosing the state of the thyroid gland. A randomised systematic review of 43 scientific sources published between 2015 and 2024 was conducted. The article provides an overview of traditional and modern methods of thyroid gland examination. It has been established that modern diagnostic methods are used to determine the condition of the thyroid gland and to characterise formations. Among them, thyroid ultrasound examination is the main non-radiation diagnostic tool for establishing diseases and monitoring observation. The advantages of ultrasound examination include speed, availability, and information content of the method. In addition, an important role is played by the physical examination of the patient and laboratory tests. Currently, fine-needle aspiration biopsy is considered the gold standard for the study of thyroid nodules. Positron emission tomography combined with computed tomography is used to assess tumour response and for the diagnosis, prognosis, and staging of thyroid cancer. To determine the functional state of the thyroid gland, the level of thyroid hormones in the blood serum is established: triiodothyronine, thyroxine, thyroid-stimulating hormone, thyroid peroxidase antibody, thyroglobulin antibodies, thyroid stimulating hormone receptor antibodies, thyroglobulin, and calcitonin. Thus, various clinical, instrumental, and laboratory research methods are used to determine the state of the thyroid gland

Keywords: hormones; diagnostic methods; structure; functional state; ultrasound examination

INTRODUCTION

Over 200 million people worldwide have thyroid gland pathology [1, 2]. It has been established that in developed countries of the world, the incidence of newly diagnosed thyroid diseases has increased by 52% in women and by 17% in men [3]. According to the Ministry of Health, from 2015 to 2020, the incidence of the disease in Ukraine

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increased fivefold [3]. These changes depend on the state of the environment, iodine deficiency, a person's lifestyle, stress, nutrition, and concomitant diseases.

Thyroid gland pathologies include autoimmune thyroiditis (enlarged thyroid gland due to inflammation); hypothyroidism (hormone deficiency); hyperthyroidism (excess hormones); and thyroid gland neoplasms. For accurate diagnosis, instrumental (ultrasound examination and laboratory methods (hormone tests, tumour markers) are currently used. For a more accurate diagnosis, computed tomography (CT), magnetic resonance imaging (MRI) and biopsy of thyroid nodules are used [4].

Ultrasound is of great importance in the early detection of structural changes and thyroid cancer [4, 5]. The modern level of ultrasound technology and the improvement of the standard of living gradually increase the level of detection of thyroid nodules. This type of study can detect from 30% to 67% of thyroid nodules [4]. In most cases, due to the subjective assessment of ultrasound images by radiologists and their use of different classifications, there are difficulties in the visual differentiation of benign and malignant thyroid nodules [6, 7]. L. Yang *et al.* [4] indicate that the effectiveness of ultrasound assessment of thyroid nodules differs in different approaches to determining risk groups, which leads to the appearance of different data on the level of diagnostic specificity and sensitivity of methods. This indicates that this type of study does not allow for an accurate diagnosis of the malignancy of thyroid tumours due to the dependence on individual visualisation features, which requires the creation of detailed evaluation criteria. Currently, fine-needle aspiration biopsy allows differentiation of benign and malignant from 5% to 96% of cases. This method is effective and accurate in cases of suspected malignant neoplasms [8]. However, in about 25% of cases, the cytological result is inconclusive. In such cases, molecular tests are proposed. They help to carry out a more accurate preoperative diagnosis, which allows to reduce the number of diagnostic operations. The high cost of these molecular markers and their unavailability in all medical centres make their use in clinical practice impractical.

T.L.M. Barbosa *et al.* [8] emphasise the importance of acknowledging the strengths of various diagnostic features in current ultrasound examination programs and guidelines. Combining these features will allow the development of effective diagnostic criteria. Additionally, standardising the parameters for evaluating the thyroid gland across different hospitals will assist in multicentre validation of clinical trials and enhance diagnostic effectiveness.

A wide range of clinical, instrumental, and laboratory research methods are used to diagnose the functional and structural state of the thyroid gland. These methods allow for the detection of thyroid gland pathologies and the determination of the effectiveness (dynamics) of treatment. The aim of the study was to analyse modern scientific data on methods for diagnosing the state of the thyroid gland.

To search for publications from 2019 to 2024 in the scientometric databases PubMed, Web of Science, Scopus, and Google Scholar, the keywords "hormones", "diagnostic methods", "structure", "functional state", and "ultrasound examination" were used. A randomised systematic review of 43 scientific sources was conducted. The inclusion criteria were the study and analysis of scientific publications in Ukrainian and English. The theoretical search for publications was aimed at analysing, comparing, and summarising data from modern scientific research on effective methods for diagnosing the state of the thyroid gland. The process of selecting publications included screening and quality assessment stages, where each article was carefully analysed in terms of its scientific content and methodological aspects. This approach helps to systematically summarise the literature data and allows for a deeper consideration of the literature data and allows for a deeper consideration of the publications selected for the study.

CLINICAL METHODS OF THYROID GLAND EXAMINATION

A family history of thyroid gland pathology is a predictor of its development. The presence of malignant thyroid neoplasms in first-degree relatives increases the risk of thyroid cancer by nine times [1]. Additionally, the presence of comorbidities plays a crucial role. Often, chronic diseases and severe forms of illness lead to iron deficiency anaemia, in which thyroid nodules are more frequently detected. Common signs of thyroid gland disease include changes in body weight, mood swings, elevated body temperature, dry skin, discomfort when swallowing, changes in heart function, somnolence, and muscle pain [2].

A crucial diagnostic method is the physical examination of the thyroid gland. Palpation of the thyroid gland can be performed in various ways, including with one or both hands from the anterior or posterior approach. During the examination of the thyroid gland, the placement, shape, size, symmetry, surface condition, and degree of gland movement during swallowing are assessed. The size of the gland is crucial during the examination; if changes are detected, the nature of the enlargement needs to be determined (diffuse, nodular, or mixed); the surface condition – smooth or covered with tubercles; consistency (soft-elastic or firm); mobility; adhesions to surrounding tissues; and the patient's sensations during palpation [9].

Thyroiditis is an inflammatory process associated with the damage to the thyroid gland. It is characterised by elevated serum concentrations of thyroid antibodies, such as thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (Tg Ab). Autoimmune thyroiditis (Hashimoto's thyroiditis) is one of the most common autoimmune diseases of the thyroid gland. Researchers by O. Gąsiorowski *et al*. [10] have found that it is diagnosed five times more often in women than in men. Autoimmune thyroiditis is found in 7% of men and 27% of women, with prevalence increasing with age. Additionally, it is estimated that approximately 1% of men and 5% of women have altered thyroid gland structure. The pathological features of autoimmune thyroiditis include lymphocytic infiltration and fibrosis, which lead to stiffness of the thyroid parenchyma [10].

The prevalence of hypothyroidism depends on iodine availability, sex, and age. In 4.70% of cases, hypothyroidism is undiagnosed, while 4.11% have subclinical hypothyroidism and 0.65% have overt hypothyroidism [11, 12]. Patients with hypothyroidism typically present with a range of nonspecific symptoms that overlap with other conditions. These include low mood, feeling cold, weight gain, muscle aches/cramps, weakness, dry skin, brittle hair and nails, carpal tunnel syndrome, or dysmenorrhea. Inevitably,

many people continue to struggle with these nonspecific symptoms, possibly attributing them to other causes without discussing them with their general practitioner. This may be particularly relevant for subclinical hypothyroidism, where the severity of thyroid symptoms is likely to be less than in a person with overt clinical hypothyroidism. The diagnosis of hypothyroidism is biochemical and is established based on changes in thyroid-stimulating hormone (TSH) levels above the normal range [11]. The study by S.P. Fitzgerald *et al.* [13] found that clinical hypothyroid symptoms have correlational links with thyroid hormone levels (particularly free T4 or T3) rather than TSH. In the future, this could form the basis for recommendations and aid in better identification of patients with hypothyroidism.

Another common thyroid condition is goitre [12]. Depending on the aetiology of the disease and the presence of cancerous tissues in patients, various clinical manifestations and levels of thyroid hormones are observed. In 15% of patients with goitre, cancer is diagnosed, indicating the need for screening of patients with goitre for thyroid cancer [1].

Thyroid nodules are defined as lesions that can be differentiated from normal thyroid parenchyma through physical examination or imaging methods [1, 9]. Thyroid nodules detected by palpation are a common occurrence, affecting approximately 7% of patients, with 20% of these being cancerous [1]. The detection rate of thyroid nodules is estimated to be 2-6% on physical examination, 19-67% on ultrasound, and 8-65% in autopsy series. Thyroid lesions that are not identified during physical examination can be detected using radiological imaging methods [14]. Micronodules may increase in size over time and are more frequently found in elderly women [15]. M.A. Al-Shammari *et al*. [1] found that the presence of an enlarged thyroid gland increased the risk of thyroiditis detected by ultrasound. Meanwhile, iron deficiency anaemia, vitamin D deficiency, other autoimmune diseases, and hypertension were associated with an increased risk of thyroid nodules. Among endocrine neoplasms, thyroid cancer occupies one of the top positions and is diagnosed in 2.1% of all neoplasms worldwide (excluding carcinoma in situ and skin cancer). It should be noted that from 2000 to 2023, the incidence of thyroid cancer has been rapidly increasing [10].

Thus, the first step in the timely and high-quality diagnosis of the thyroid gland is clinical methods, which include collecting anamnestic data to determine the patient's complaints and physical examination of the thyroid gland. If necessary, further laboratory and instrumental research methods are carried out to determine the thyroid hormone spectrum, possible autoimmunity, metabolic disturbances, and assess pathomorphological changes in the thyroid gland. In addition, it is necessary to find out whether other family members (parents, brothers and sisters) had malignant neoplasms or other diseases to stratify the risk and timely carry out the prevention and diagnosis of thyroid gland diseases.

INSTRUMENTAL METHODS FOR THYROID GLAND EXAMINATION

Ultrasound is one of the main methods for visualising the thyroid gland, detecting its pathology, and monitoring dynamic changes. Moreover, this method is accessible and safe [6, 15]. When the results of ultrasound are insufficient to determine organ dysfunction, other research methods are used: MRI, CT, and PET/CT based on fluorine-18 fluorodeoxyglucose (18F-FDG). These methods are insensitive to microlesions of the thyroid gland and are more expensive and radioactive.

Currently, radioisotope studies (radionuclide scanning and scintigraphy) are necessary for patients with hyperthyroidism, in the diagnosis of various forms of goitre, and for the determination of iodine-dependent tissue following surgical intervention in thyroid cancer. Radionuclide scanning and scintigraphy are methods for obtaining gamma-topographic two-dimensional images of the gland, which determine the distribution of the radionuclide iodine-133 or 99 mTc-pertchnetate in the thyroid gland. This allows for a more accurate determination of the places where radioactive iodine or other nucleotides accumulate and are processed, as well as determining the necessity and strategy for surgical intervention. It has been established that cancer cells concentrate less radioactive iodine than healthy ones [15]. While other studies have shown the low effectiveness of these methods, it has been established that the tissues of benign formations also poorly accumulate radioactive iodine [15, 16]. The high resolution of ultrasound, its simplicity, and the absence of the need to introduce radioisotopes have been shown. This diagnostic method has high sensitivity, specificity, and diagnostic accuracy for diagnosing thyroid swellings, with values of 86.66%, 91.66%, and 90.66%, respectively [17].

Heterogeneous hypoechoic echogenicity and a lobed contour are specific grayscale features. Shear-wave elastography (SWE) is a new real-time non-invasive imaging technology for the thyroid gland that quantitatively assesses tissue stiffness. To increase the sensitivity of determining the degree of the disease and the activity of the process, B-mode ultrasound is used [18]. A direct relationship was found between SWE indicators and AT-TG levels, gland volume, between Tg Ab and TPOAb levels, and an inverse relationship between SWE and echogenicity. In addition, T. Kara *et al.* [19] established specific numerical ranges for determining thyroid gland pathology, namely, 29.45 kPa was proposed as a sensitive-specific cut-off value for determining Hashimoto's thyroiditis. However, SWE is better and more practical than other ultrasound methods for determining disease progression, as SWE allows quantitative assessment of the degree of fibrosis.

Currently, the gold standard for early diagnosis of thyroid tumours is fine-needle aspiration biopsy of nodules under ultrasound guidance with subsequent cytological examination. The results of cytological studies, together with the clinical picture and laboratory data, can help in complex and difficult cases. This method is simple, safe, requires minimal costs, and has high diagnostic accuracy [20-22]. Studies have shown that this method has 83% sensitivity and 92% specificity, and has 1 to 21% failures, which occur due to the technique and experience [23]. In another study, the authors P. Singh *et al.* [22] showed its 83.3% sensitivity and 100% specificity. Y.K. Lee *et al.* [23] established a correlation between the number of thyroid FNA and the number of thyroid cancer diagnoses.

Another method is sonography, which allows for the detection of micronodules (incidentalomas) in the thyroid gland. While sonography can provide very important

information about the nature of the thyroid lesion, it does not allow for the differentiation of benign lesions from cancer [15, 24]. To address this issue, the non-invasive diagnostic and visualisation method ^{18}F-FDG PET/CT has been proposed. PET allows for obtaining data on the quantitative parameters of the metabolic activity of tissues. 18F is a radioisotope of fluorine produced by a cyclotron, which emits positrons and has a short half-life of about 109.7 minutes [25]. This allows the labelling of numerous molecular indicators, the images of which can be obtained within a few hours after injection. FDG is a glucose analogy and is taken up by living cells through glucose transporters on the cell membrane, then incorporated into the first step of the normal glycolytic pathway. The accumulation of FDG in tissues is proportional to the amount of glucose utilisation. Increased glucose consumption is characteristic of most cancers and is partially associated with overexpression of glucose transporters and increased hexokinase activity. It has been shown that FDG PET/CT is a sensitive visualisation method for the detection, tumour volume determination, staging, and assessment of treatment response in oncology [25]. In a study by A. Akbas *et al.* [14], it was found that the frequency of malignant neoplasms with increased focal 18F-FDG uptake, accidentally detected on PET/CT, was 18.8%. It was determined that ^{18}F-FDG PET/CT uptake of the thyroid gland can be diffuse or nodular. Diffuse uptake does not require additional examination, as it is usually accompanied by benign thyroid diseases. Patients with nodular uptake with a satisfactory general condition require additional examination due to the high rates of malignancy [14].

Recent studies have allowed the identification of cancer-associated fibroblasts, which have been proposed for low-molecular-weight nuclear diagnostics. In these fibroblasts, fibroblast activation protein (FAP) is overexpressed, while it is not found in normal tissues. It has been shown that FAP is highly expressed on the membrane of cancer-associated fibroblasts in approximately 90% of epithelial tumours, which is observed in cases of tissue damage, remodelling or chronic inflammation, as well as in benign conditions [26, 27].

A new class of radiopharmaceuticals based on the FAP-specific inhibitor (FAPI) based on quinoline has been developed, which have been recognised as preclinically promising as molecular targeting imaging probes [28, 29]. FAPI PET/CT is a new diagnostic method in the imaging of oncology patients. In contrast to 18F-FDG, no diet or fasting is required before the examination, and image acquisition can potentially be started within a few minutes after the indicator application. The tumour-to-background contrast ratios were the same or even better than those of 18F-FDG. In direct comparison, 68Ga-FAPI PET/CT surpasses 18F-FDG PET/CT in sensitivity and specificity for the characterisation of primary, nodular, and metastatic lesions of various types of thyroid tumours [26, 29].

H. Liu *et al.* [30] found that diffusely increased 68Ga-FAPI uptake in the thyroid gland is mostly related to chronic lymphocytic (Hashimoto's) thyroiditis. 68Ga-FAPI uptake level correlated neither with the degree of hypothyroidism nor with the TPOAb content. Immune-related thyroiditis with immune checkpoint inhibitors may be accidentally found on 68Ga-FAPI [30]. Therefore, modern imaging methods such as ultrasound, MRI, CT, and PET/CT are used to determine the structure and size of the thyroid gland and its nodules. Additional studies of the thyroid gland are needed for more accurate diagnosis and treatment planning.

LABORATORY MARKERS FOR THYROID GLAND EXAMINATION

The thyroid gland produces the hormones triiodothyronine (T3), thyroxine (T4), and calcitonin. These hormones regulate basal metabolism, protein synthesis, and several other processes, including development [31]. Thyroid function is assessed and monitored by measuring TSH levels. Euthyroidism (normal TSH and thyroid hormone levels), overt thyroid dysfunction (abnormal TSH and thyroid hormone levels), subclinical thyroid dysfunction (abnormal TSH/normal thyroid hormone levels), and isolated hyper/hypothyroxinemia (normal TSH/abnormal thyroid hormone levels) are determined [13].

S.P. Fitzgerald *et al.* [13] believe that thyroid hormone levels are associated with clinical parameters. In their research, K. Inoue *et al*. [32] found that low levels of thyroid hormones are linked to various cardiovascular disease risk factors, including diastolic hypertension, weight gain, insulin resistance, hypercholesterolemia, and dyslipidemia. Hypothyroidism with a serum TSH level greater than 10 mIU/L is associated with a higher risk of heart failure.

Studies have shown problems that arise when interpreting laboratory data. The number of results is influenced by various physiological factors such as pregnancy, metabolic status, obesity, and the presence of comorbidities. These factors affect hormone metabolism and regulation [33]. Research by Y.C. Zhou *et al.* [34] indicates a connection between TSH and metabolic syndrome. Thyroid hormone levels influence metabolic state and energy balance. Changes in hormone levels (whether an increase or decrease) lead to insulin resistance, glucose metabolism disturbances, and alterations in lipid levels, which in turn affect the patient's metabolic parameters.

In the study conducted by A. Punda *et al.* [35], an association was found between TSH and the development of insulin resistance; a reciprocal relationship between free T3 and T4 concentrations and insulin resistance; and a reciprocal relationship between free T3 and T4 levels and cholesterol levels. Additionally, hypo- and hyperthyroidism may influence the development of atherosclerotic cardiovascular diseases, which is related to the impact of hormones on lipid metabolism and increased blood pressure [36]. TSH is associated with lipid metabolism and its seasonal fluctuations are established [37]. O. Ustinov [2] notes that an increase in hormone levels leads to weight loss, while a decrease results in weight gain.

Testing the hormonal spectrum constitutes a significant portion of the workload in laboratories worldwide. For screening, diagnosing, and monitoring thyroid diseases of various aetiologies across all age groups, different test systems for *in vitro* diagnostics and measurement devices are used. These range from classical radioimmunoassay to modern highly sensitive immunochemiluminescent methods for determining thyroid hormones and TSH levels in human serum. Identifying deviations in the hormonal spectrum allows for the assessment of thyroid gland

functional status, which complements the diagnosis of thyroid diseases. Radioimmunoassay methods for measuring thyroid hormone levels have low sensitivity. To improve sensitivity, new non-isotopic immunological analysis technologies have been developed: enzyme-linked immunosorbent assay (ELISA), chemiluminescent assays, and immunofluorescent assays. These methods are used to determine: markers of thyroid gland functional status (TSH, total and free T3, total and free T4); markers of autoimmune pathology (Tg Ab, TPOAb, thyroid stimulating hormone receptor antibodies (TSHR-Ab)); and markers of oncological pathology (thyroglobulin and calcitonin).

Many factors (population, health status, applied technology, and timing) can affect the assessment of the measured parameter. The rhythmic variations described for thyroid hormones imply that differences in sample collection timing and the duration of the study can lead to variations in the results. All these factors need to be considered during analysis. Additionally, the standardisation and effectiveness of the analytical method in terms of accuracy and specificity also impact the values obtained [33, 34]. It is evident that technological advancements will lead to changes in generations of analytical systems, meaning that over time, such analytical characteristics will significantly improve [37-39]. Therefore, laboratory marker results should be interpreted only in conjunction with the clinical picture of the disease, as well as with data from other diagnostic methods (ultrasound, CT, MRI, SWE, PET/CT).

Currently, there is progress in technological tools within medical science. Machine learning algorithms are actively being introduced into visualisation programs, which will help reduce diagnostic time and increase the diagnostic accuracy of fine-needle aspiration biopsy of the thyroid gland [40-42]. Recent research by D.D.E. Range *et al*. [43] has demonstrated the high effectiveness of machine learning in predicting malignant thyroid neoplasms. Machine learning-based diagnosis using digital imaging of biopsy samples is comparable to the effectiveness of a cytopathologist. Specifically, the machine learning algorithm for detecting malignancy achieved a sensitivity of 92.0% and a specificity of 90.5%. The area under the curve for predicting malignancy was 0.931 for cytopathologists and 0.932 for machine learning. Machine learning effectively distinguished between benign and malignant nodules in cases with indeterminate diagnoses [40]. However, machine learning-based diagnostic tools for fine-needle aspiration biopsy of the thyroid gland have not yet been implemented in practice, so further research is needed before they can be applied in clinical settings.

CONCLUSIONS

In the course of analysing the literature, the objectives of the study were achieved and contemporary scientific data on methods for diagnosing the structural and functional state of the thyroid gland were highlighted. It was determined that in the first stage of diagnosis, a thorough clinical examination of the patient and the collection of medical history are of great importance. It is essential to clearly identify the patient's complaints and their duration, the presence of other diseases, perform a physical examination (palpation) of the organ and adjust further investigations accordingly. Attention should be paid to changes in body weight, mood swings, elevated body temperature, dry skin, discomfort during swallowing, changes in heart function, somnolence, and muscle pain. In the next stage, the structural characteristics of the thyroid gland are assessed. Instrumental diagnostics are used to evaluate the structure, size, and presence of nodules or thyroid cancer. Modern imaging methods in endocrinology include ultrasound examination of the thyroid gland and its nodules, magnetic resonance imaging, computed tomography, and positron emission tomography. Additionally, determining the functional state of the thyroid gland is of significant importance. Blood laboratory tests are used to measure hormone levels (triiodothyronine, thyroxine, thyroid-stimulating hormone), thyroid-stimulating hormone receptor antibodies, thyroid peroxidase antibodies, thyroglobulin antibodies, thyroglobulin, and calcitonin. To assess the state of the thyroid gland, it is necessary to comprehensively analyse data from clinical, instrumental, and laboratory research methods. These methods and indicators are used to determine the dynamics of changes and the effectiveness of treatment. The analysis of scientific research has shown that currently there is no single method or device for accurately diagnosing changes and determining their nature. Therefore, ongoing efforts are being made to find effective markers. The implementation of artificial intelligence through machine learning algorithms for the effective diagnosis of thyroid gland pathologies is promising. It has been shown that both the structural and functional states of the thyroid gland need to be assessed. Timely detection of changes in the thyroid gland contributes to the establishment of effective treatment and prevention of thyroid diseases. The analysis conducted highlights the need for a differentiated approach to diagnosing, treating, and preventing thyroid gland diseases in patients with various pathologies. A prospective area for further research is determining the algorithm for diagnosing thyroid gland diseases using artificial intelligence.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Гормональні та інші методи дослідження щитоподібної залози: огляд літератури

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Анотація. Наразі встановлюється висока частота виявлення захворювань щитоподібної залози і це означає, що регулярне дослідження її стану та визначення гормонів може допомогти ранньому виявленню розвитку захворювання. Метою роботи було проведення аналізу сучасних наукових даних щодо методів діагностики стану щитоподібної залози. В ході роботи було проведено рандомізований систематичний огляд 43 наукових джерел, які опубліковані в період з 2015 по 2024 роки. У статті надано загальну інформацію щодо традиційних та сучасних методів дослідження щитоподібної залози. Було встановлено, що для визначення стану щитоподібної залози та характеристики утворень використовують сучасні діагностичні методи. Серед яких ультразвукове дослідження щитоподібної залози є головним безрадіаційним діагностичним інструментом при встановленні захворювань та моніторингу спостереження. До переваг ультразвукового дослідження можна віднести швидкість, доступність, інформативність методу. Крім того, важливу роль відводять фізикальному огляду пацієнта та проведенню лабораторних тестів. Наразі тонкоголкова аспіраційна біопсія вважається золотим стандартом дослідження вузлів щитоподібної залози. Позитронно-емісійна томографія разом із комп'ютерною томографією використовується для оцінки відповіді пухлини, а також для діагностики, прогнозу та визначення стадії раку щитоподібної залози. Для визначення функціонального стану щитоподібної залози в сироватці крові встановлюють рівень гормонів щитоподібної залози: трийодтиронін, тироксин, тиреотропний гормон, антитіла до тиреопероксидази, антитіла до тиреоглобуліну, антитіла до тіреотропного гормону, тиреоглобулін та кальцитонін. Отже, різноманітні клінічні, інструментальні та лабораторні методи дослідження застосовуються для визначення стану щитоподібної залози

Ключові слова: гормони; методи діагностики; структура; функціональний стан; ультразвукове дослідження

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