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ORIGINAL ARTICLE

The effect of photobiomodulation therapy on the expression of regulatory molecules in chronic wound healing

Sergey Pavlov[1], Nataliia Babenko[1], Marina Kumetchko[1], Olga Litvinova[1], Vladyslav Bereznyakov[2], Tetiana Litvinienko[3]

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ABSTRACT

Aim: The work aimed to study the effect of photobiomodulation (PBM) therapy on the expression of key regulatory interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), influencing the development of reparative processes of chronic wounds.

Materials and Methods: The experiment involved 3 groups of rats: intact animals and animals of the control and experimental groups, for which chronic wounds were simulated. Exposure to wound defects of animals of the experimental group was carried out using PBM therapy. Animals were euthanized on the 14th and 28th day of the experiment. The levels of IL-1β, IL-6, IL-4, IL-10, TNF-α, and GM-CSF in the blood serum of animals were studied by enzyme immunoassay. Histological studies were carried out. The semi-quantitative method was used to evaluate the reepithelization stage, polymorphonuclear leucocytes, fibroblasts, new vessels, and new collagen.

Results: In our work in a group of animals using PBM therapy, the levels of IL-1β, IL-6, TNF-α, GM-CSF, and IL-10 did not change during the proliferation-to-remodeling transition phase. The application of PBM therapy resulted in an increase in IL-4 on day 14 of the experiment. The semi-quantitative method demonstrated a change in the number of histologic processes and structures after using PBM therapy.

Conclusions: An imbalance in the production of pro-inflammatory cytokines was found with the use of PBM therapy in our study. An increase in the levels of anti-inflammatory cytokines, the number of fibroblasts, and collagen after laser treatment was shown.

KEY WORDS: photobiomodulation, wound healing, interleukins, growth factor, histology, rats

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INTRODUCTION

Chronic wounds have recently remained an important clinical problem due to the increasing morbidity and socioeconomic burden placed on healthcare systems around the world [1]. The mortality rate of patients with chronic wounds now rivals that of patients with cancer [2]. Risk factors associated with wound healing problems include poor circulation, weakened immunity, disorders of the hormonal status of the wounded [3], concomitant diseases [4, 5], etc.

In connection with the above, new technologies are needed to improve wound healing. Photobiomodulation (PBM) therapy is a promising method for improving wound healing outcomes. PBM is a healing approach in which low-level light exposure to tissue or cells results in cell differentiation, proliferation, and accelerated wound healing [6]. An important task facing researchers is to optimize the parameters of PBM therapy. Which in turn requires extensive knowledge of reparation processes.

Wound healing occurs during 3-4 phases: hemostasis and inflammation, proliferation and remodeling. These phases are regulated by a complex network of interacting cytokines, growth factors, and their cellular receptors. Healing of intractable wounds, unlike acute wounds, is delayed because normal biological progression is interrupted [7]. Understanding disruptions in the regulatory mechanisms of wound healing is important primarily at the molecular level.

AIM

The work aimed to study the effect of PBM therapy on the expression of key regulatory interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF), influencing the development of reparative processes of chronic wounds.

MATERIALS AND METHODS

The experiment involved 42 rats aged 9 months and body weight 200-220 g. The study was carried out following the requirements of the "European Convention on the Protection of Spinal Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), and was approved by the

Committee on Ethics and Bioethics of the Kharkiv National Medical University. 6 rats were presented in the intact group (Int). 36 animals were randomized into control (Con) and experimental (Exp) groups. Chronic wounds were simulated for rats of the control and experimental groups, reproducing conditions of hypoxia and microcirculation disorders.

Wound induction was carried out under general anesthesia by intramuscular injection of a solution of zoletil (tiletamine hydrochloride and zolazepam hydrochloride) (Virbac, France) at a concentration of 10 mg/kg body weight. After hair depilation, a flap of skin with a diameter of 2 cm was removed from the proximal part of the rat's back. Then a perpendicular loop-shaped fasciocutaneous suture was placed along the edges of the wound. On the surface of the wound bottom, the superficial fascia was dissected with perpendicular cuts to form cells measuring 5×5 mm, which were sutured with U-shaped sutures [8].

Exposure to wound defects of animals of the experimental group was carried out using PBM therapy of the following parameters: wavelength 660 nm, power density 50 mW, and energy density 5 J/cm^2. PBM therapy was carried out once a day for 5 days without anesthesia in an individual container. The first application was performed 24 hours after wound induction. We used the laser device Lika-therapist M (Cherkasy, Ukraine) in continuous radiation mode. The beam of the laser tip was held perpendicular to the surface of the wound to illuminate the entire area of the wound. Wound defects of animals in the control group received fictitious laser exposure.

Animals were euthanized on days 14 and 28 of the experiment, 6 animals from each group. These days were chosen to study reparative processes during the transition from the proliferation phase to the remodeling phase and the remodeling phase. Blood samples were collected by open cardiac puncture. The levels of IL-1β, IL-6, IL-4, IL-10, TNF-α, and GM-CSF were determined in blood serum using enzyme immunoassay. The concentrations of IL-1β, IL-6, IL-4, IL-10, and TNF-α were determined using Vector-Best reagent kits. GM-CSF levels were determined using an eBioscience kit (USA).

Histological studies were carried out according to generally accepted methods. The preparations were analyzed using a PrimoStar microscope (Zeiss, Germany) and a digital camera. The semi-quantitative method was used to evaluate histological processes and structures: reepithelization stage, polymorphonuclear leucocytes (PMNL), fibroblasts, new vessels, and new collagen [9].

The one-way ANOVA using the Statistica software 12 (StatSoft, USA) statistically evaluated the data. The significance of the differences between groups was evaluated using the non-parametric Kruskal–Wallis test for independent samples ($P < 0.05$). The data were expressed as the means ± standard error of the mean (SE). Histogram plotting was performed in GraphPad Prism 7 (GraphPad Software, USA)

RESULTS

Fig. 1 presents the concentrations of bioactive molecules in the animals' blood serum on days 14 and 28 of the experiment.

The study showed that under the influence of PBM therapy, on the 28th day of the experiment, the increase in the level of IL-1β in the blood serum of animals was 1.23 times ($p < 0.05$) compared to rats with wounds without PBM therapy (Fig. 1A). We also found that the serum IL-1β level in animals of the control group compared to intact animals was elevated 1.42-fold at 14 days ($p < 0.01$) and 1.28-fold at 28 days ($p > 0.05$) after surgery. At the same time, the serum level of IL-1β in experimental animals compared to intact animals was elevated 1.27-fold on day 14 ($p > 0.05$) and 1.66-fold on day 28 ($p < 0.001$) of the experiment.

The different severity of influence on various phases of wound healing was observed in the background of PBM therapy. Thus, if on the 14th day of the experiment, the IL-4 levels in the experimental group compared to the control group increased 2.29 times ($p < 0.001$), then on the 28th day there were no differences in the indices of this cytokine in the groups of animals (Fig. 1B). IL-4 concentration in the control animals' serum, compared to intact animals, decreased 1.22-fold after 14 days ($p > 0.05$) and increased 1.77-fold ($p < 0.001$) after 28 days postoperatively. The concentration of IL-4 in the animals' group with PBM increased 1.87-fold and 1.82-fold ($p < 0.001$) at 14 and 28 days, respectively, compared to that of intact animals.

In the animals whose wound defects were exposed to PBM therapy, on the 28th day of the experiment there was a 1.56-fold decrease in serum IL-6 levels ($p < 0.05$) compared to the same indices of the control group animals (Fig. 1C). At the same time, on the 14th day after surgery, there were no differences in the levels of this cytokine in the control and experimental groups. The IL-6 concentration in the animals' blood serum of the control group compared to intact animals increased 1.30-fold at 14 days ($p > 0.05$) and 2.58-fold at 28 days ($p < 0.001$) after surgery. At the same time, the IL-6 serum level of experimental animals compared to intact animals was increased 1.63-fold on day 14 ($p > 0.05$) and remained the same on day 28 of the experiment.

According to the data obtained, on the 28th day after surgery, a 1.26-fold ($p < 0.05$) increase in the concentration of anti-inflammatory IL-10 was observed in the group with PBM therapy compared to the control and intact groups (Fig. 1D). At the same time, the levels of this cytokine were practically unchanged throughout the experiment in all groups.

As for the levels of TNF-α in the serum of animals with wounds exposed to PBM therapy compared to the control group animals, no differences in the indices at all terms of the experiment were found (Fig. 1E). The TNF-α concentration in the control animals' serum that underwent surgery, compared to intact animals, increased 1.62-fold at 14 days ($p < 0.001$) and 1.78-fold ($p < 0.001$) at 28 days after surgery. The TNF-α concentration increased 1.55- and 1.81-fold ($p < 0.001$) in the animals' group with PBM compared to that of intact animals at 14 and 28 days, respectively ($p < 0.001$).

No significant differences were found in GM-CSF serum levels in all groups of animals at all terms of the experiment

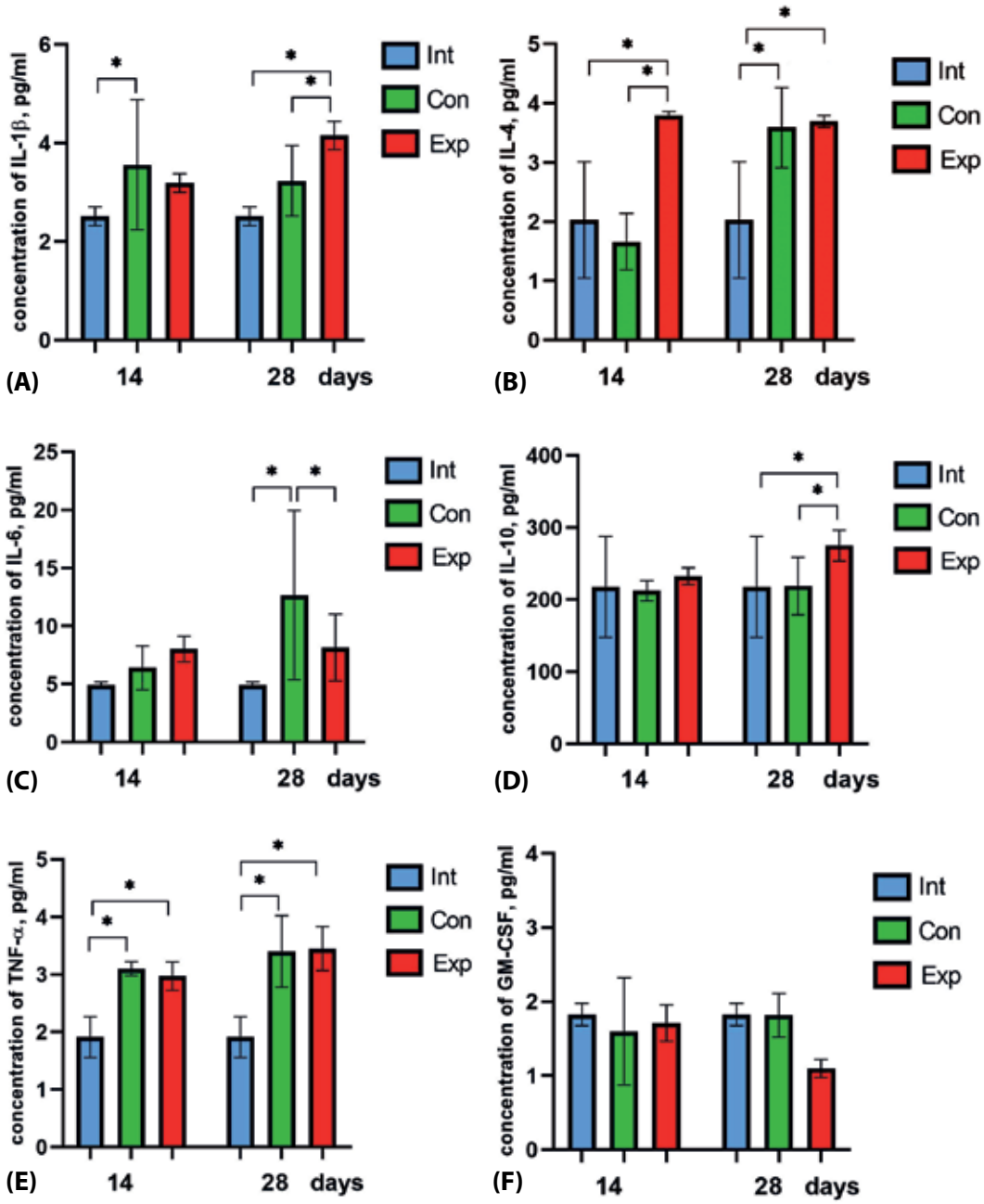

Fig. 1. Changing the levels of the studied indicators in the blood serum of animals: (A) IL-1b, (B) IL-4, (C) IL-6, (D) IL-10, (E) α-TNF, (F) GM-CSF (*P < 0.05). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6).

(Fig. 1F). On the 28th day of the experiment, there was a tendency to decrease the concentration of this factor in the experimental group compared to the control and intact groups.

Fig. 2 presents the results of a semi-quantitative histological analysis of wounds in rats of the control and experimental groups after 14 and 28 days of the experiment.

Histological examination of wound samples after 14 days in animals of both groups showed incomplete epithelialization of wound defects. The newly formed epidermis was of small thickness and poorly differentiated into layers. A scab persisted in the central part of the wound. The index of the re-epithelialization stage had no significant differences between the groups (Fig. 2A). The wound cavities in all animals were filled with maturing granulation tissue. Signs of a moderate diffuse inflammatory response persisted, with no significant difference in PMNL counts between groups. In rats of the experimental group at this term, an increase in the number of fibroblasts (1.31-fold) and collagen fibers (1.22-fold) was detected compared to animals of the control group ($p < 0.05$). The number of newly formed vessels had no significant differences.

On the 28th day of the experiment in all animals, the wounds were completely covered with newly formed epidermis with a clear division into basal, spiny, granular, and stratum corneum layers. The indicator of the re-

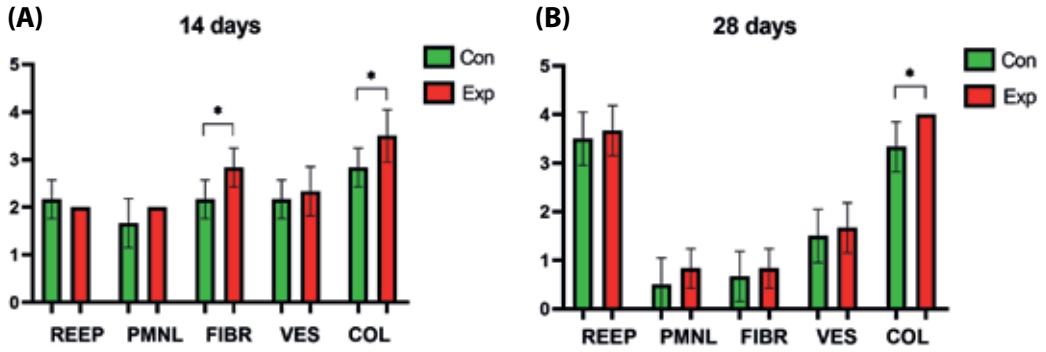

Fig. 2. Results of semi-quantitative histological analysis of wounds in rats of the control (Con) and experimental (Exp) groups after 14 days (A) and 28 days (B): stage of re-epithelialization (REEP), number of polymorphonuclear leukocytes (PMNL), fibroblasts (FB), vessels (VES), collagen (COL); (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator ($n = 6$).

epithelialization stage, as in the previous term, had no significant differences between the control and experimental groups (Fig. 2B). The wound defects were predominantly mature connective tissue with few cellular elements and differentiated blood vessels. Only in the central area of the wound, areas of maturing granulation tissue with moderate amounts of PMNL, fibroblasts, and newly formed capillaries were preserved, with no significant differences in these parameters between the groups. The number of collagen fibers was significantly different in animals receiving PBM therapy, exceeding this index in control animals by 1.20 times ($p < 0.05$). At the same time, collagen fiber bundles in rats of the experimental group had greater thickness and packing density.

DISCUSSION

The problem of parameter optimization is still not solved. It is believed that if light spectra are not selected promptly, the effectiveness of PBM may be compromised [10]. The choice of parameters for our work was based on literature data. Treatment at 632.8 to 685 nm, 50 mW/cm^2, 3 to 6 J/cm^2, and irradiation for 30 to 80 seconds, 3 times a week for a month, is beneficial in accelerating the healing of diabetic foot ulcers [11]. Healing of a palatal ulcer is shown after using a laser with a wavelength of 660 nm and an energy density of 6 J/cm^2, with an output power of 50 mW [12]. The use of PBM (670 nm, 4.5 J/cm^2, 60 mW/cm^2 for 90 s per day, 5 days/week) in an excision wound healing model demonstrated improvement in mitochondrial bioenergetics and stimulation of wound healing rate in diabetic mice [13].

It is known that impaired healing of chronic wounds is associated with failures in the transition between stages of healing, while the wound is in constant inflammation and hypoxia [14]. In our work, studies were carried out at the stage of transition from proliferation to the remodeling stage and at the remodeling stage. During the proliferative phase, the formation of granulation tissue, migration, and angiogenesis of keratinocytes occurs. The main processes that distinguish the remodeling phase are collagen restoration and wound contraction. At the same time, growth factors involved in this process are constantly identified [15].

In our work in a group of animals using PBM therapy, the levels of pro-inflammatory IL-1β, pleiotropic cytokines IL-6, TNF-α and GM-CSF, as well as the anti-inflammatory cytokine IL-10 did not change at the stage of transition from proliferation to the remodeling stage. The use of PBM therapy led to an increase in the concentration of IL-4 on the 14th day of the experiment. In our previous work, when using a lower energy density (1 J/cm^2), TNF-α and IL-10 levels were reduced [16].

The literature shows a multidirectional effect of PBM therapy on cytokine levels at the stages of proliferation to remodeling transition and at the stages of remodeling. Thus, laser irradiation (660 nm, 40 mW) for 14 days increased the level of TNF-α in tissues at all stages of oral wound healing ($p < 0.05$) [17]. Administration of PBM therapy resulted in increased IL-6 expression in a model of muscle injury [18]. Laser application (660 nm, 50 mW, 4 J/cm^2, and 6 J/cm^2) for a fortnight resulted in decreased levels of IL-4, IL-6, and TNF-α in an experimental model of atopic dermatitis [19].

In the remodeling stage, the application of PBM therapy to the parameters used resulted in a multidirectional effect on cytokine expression. Increased levels of the proinflammatory cytokine IL-1β at the remodeling stage as a result of PBM therapy seem to indicate a chronic inflammatory state and a tendency towards fibrosis. Since the IL-1 family is known to activate relevant signaling pathways of inflammation and fibrosis [20].

TNF-α levels did not change after the application of PBM therapy in our study. TNF-α is an important factor regulating wound healing. It has been identified as a pro-inflammatory interleukin. Up-regulation of TNF-α has also been shown in various fibrotic disorders [21].

GM-CSF levels did not change during the remodeling phase after the application of PBM therapy in our study. It

is known that GM-CSF has immunomodulatory properties and promotes neovascularization and collagen deposition, thus affecting important phases of the repair process [22]. Decreased GM-CSF levels may contribute to delayed wound closure [23].

We also studied the pleiotropic cytokine IL-6, as dysregulation of this interleukin signaling can lead to either fibrosis or failure of wound healing [24].

Application of PBM therapy of the parameters used led to a decrease in IL-6 levels, which seems to be related to the anti-inflammatory activity of laser radiation. Analysis of histologic studies demonstrated no differences in polymorphonuclear leukocyte levels after PBM therapy.

Severe inflammation has also been associated with excessive scarring. Just as pro-inflammatory cytokines are associated with fibrosis, anti-inflammatory cytokines are associated with protective activity against fibrosis [25].

The anti-inflammatory IL-4 can activate fibroblasts and stimulate collagen deposition. This cytokine has been identified as a critical mediator in the pathogenesis of fibrotic diseases [26]. Application of PBM therapy led to an increase in IL-4 on days 14 and 28 of the experiment in our work. Also, the analysis of histological studies confirms the increase in the number of fibroblasts after PBM therapy at the stage of proliferation transition to the remodeling stage.

The anti-inflammatory cytokine IL-10 promotes collagen deposition in the proper wound-healing cascade [27]. Our work shows an increase in IL-10 concentrations at the stage of remodeling after FBM therapy. The increase of collagen after the effect of laser exposure on the 14th and 28th days of the experiment is confirmed by histological studies.

In our previous work, after the application of lower energy density PBM therapy (1 J/cm^2) during the remodeling phase, IL-1β, IL-6, and IL-10 levels were unchanged and TNF-α concentrations decreased [28]. Thus, by regulating the parameters of PBM therapy it is possible to influence reparative processes by cytokines.

CONCLUSIONS

An imbalance in the production of pro-inflammatory cytokines was found with the use of PBM therapy in our study. An increase in the levels of anti-inflammatory cytokines, the number of fibroblasts, and collagen after laser treatment was shown. Further studies are needed to optimize the parameters of PBM therapy used in chronic wound healing.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Morphological effectiveness of platelet-rich plasma application in the experimental treatment of post immobilization extra-articular contractures of the mandible

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ABSTRACT

Aim: The purpose of the study was to prove the effectiveness of platelet-rich plasma (PRP) application in the treatment of post-immobilization extra-articular contractures of the mandible by modeling the specified pathology and conducting morphological analysis of experimental material.

Materials and Methods: The study involved an experiment conducted on 60 male WAG rats aged 9-11 months. Four groups were formed. Group 1 included 6 intact rats that were not subjected to any interventions and were withdrawn from the experiment one month after its initiation. Group 2 included 18 rats with a mandibular fracture in the angle region which was treated over the course of one month using an immobilizing muzzle. After removal of the muzzle, extra-articular mandibular contracture was diagnosed. All rats were withdrawn from the experiment one month after its initiation. Group 3 included 18 rats with mandibular fractures that were treated over the course of one month using an immobilizing muzzle. After removal of the muzzle, post-immobilization extra-articular mandibular contracture was diagnosed. Following this diagnosis, 6 rats were withdrawn from the experiment. The other rats received PRP injections into the contracture area every three days for 15 days. After completion of the treatment, these rats were also withdrawn from the experiment. Group 4 included 18 rats with a mandibular fracture. After modeling a mandibular fracture, PRP was injected into the soft tissues surrounding the fracture through the available holes in the immobilizing muzzle every five days for one month. After a month, the immobilizing muzzle was removed from the rats, among which 6 rats were randomly selected and withdrawn from the experiment. The other rats continued to be injected with PRP every three days for 15 days, after which the animals were withdrawn from the experiment. The material for morphological examination consisted of masseter muscle samples. Histological, histochemical, immunohistochemical, morphometric and statistical methods were used.

Results: The comprehensive morphological study showed that PRP in the masseter muscle had antifibrotic and anti-inflammatory effects, reduced the severity of alterative changes in muscle fibers and increased their regenerative potential, reduced the severity of hemodynamic disorders, and increased the number of vessels. The therapeutic effect of PRP was more pronounced in cases where it was applied both during the treatment of mandibular fracture using an immobilizing muzzle for one month and for an additional 15 days after its removal, compared to animals in which PRP was applied only during the 15 days following muzzle removal.

Conclusions: The comprehensive morphological study of experimental material conducted by the authors confirmed the effectiveness of platelet-rich plasma in the treatment of post-immobilization extra-articular contractures of the mandible, thereby expanding the available arsenal of treatment methods for this pathology.

KEY WORDS: experiment, morphological effectiveness, platelet-rich plasma, post-immobilization extra-articular contractures, mandible

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INTRODUCTION

The mandibular fracture is one of the most common fractures of the face (70-80%) compared to any other bone of the cranium [1]. One of the complications after mandibular fracture treatment is post-immobilization extra-articular contractures [2]. Post-immobilization contractures can be a significant problem for patients, as they can result in pain, limited mandible range of motion and reduced function. Also, they can have a substantial impact on quality of patient's life [3].

The pathogenesis of mandibular post-immobilization extra-articular contractures is difficult and multifactorial [4]. Numerous researchers associate their development with damage to the masticatory muscles, which include the masseter, temporal muscle, and the lateral and medial pterygoid muscles [5]. Our previous studies revealed changes

in the morphofunctional state of the parenchymal and stromal components of the masseter muscle in patients with post-immobilization extra-articular contractures of the mandible [6].

The available range of conservative and surgical treatment methods for extra-articular post-immobilization contractures of the mandible does not allow for full restoration of mandibular function or significant improvement in patients' quality of life [7]. This fact highlights the relevance of conducting comprehensive experimental studies on laboratory animals, which will allow for the investigation of the disease pathogenesis, the development of new treatment methods, and the evaluation of their effectiveness.

AIM

The purpose of the study was to prove the effectiveness of PRP application in the treatment of post-immobilization extra-articular contractures of the mandible by modeling the specified pathology and conducting morphological analysis of experimental material.

MATERIALS AND METHODS

The study involved an experiment conducted on 60 male WAG rats aged 9-11 months, carried out at the Experimental Biological Clinic of Kharkiv National Medical University.

Group 1 included 6 intact rats that were not subjected to any interventions and were withdrawn from the experiment one month after its initiation.

Group 2 included 18 rats in which a mandibular fracture in the angle region was modeled using an original technique previously described by the authors [8]. The diagnosis of the modeled fracture was performed by visual inspection of the mandible in the affected area and using the diaphonization method [9]. The latter was applied to 6 randomly selected rats that were withdrawn from the experiment on the first day after fracture modeling. In 12 rats, the mandibular fracture was treated over the course of one month using an immobilizing muzzle designed by the authors (Fig. 1). After removal of the muzzle, extra-articular mandibular contracture was diagnosed on the side where the fracture had been previously modeled. All rats were withdrawn from the experiment one month after its initiation.

Group 3 included 18 rats with mandibular fractures. The fracture modeling and diagnostic procedures were similar to those in Group 2. In 12 rats, the mandibular fracture was treated over the course of one month using an immobilizing muzzle similar to that used in Group 2. After removal of the muzzle, post-immobilization extra-articular mandibular contracture was diagnosed. Following this diagnosis, 6 rats were withdrawn from the experiment. The other 6 rats received PRP injections into the contracture area every three days for 15 days. After completion of the treatment, these rats were also withdrawn from the experiment.

Group 4 included 18 rats with a mandibular fracture, the modeling procedure and diagnosis of which were

Fig. 1. Group 2 rat under anesthesia with a modeled mandibular fracture and an immobilizing muzzle.

similar to groups 2-3. After modeling a mandibular fracture in rats, PRP was injected into the soft tissues surrounding the fracture through the available holes in the immobilizing muzzle every five days for one month. After a month, the immobilizing muzzle was removed from the rats, among which 6 rats were randomly selected and withdrawn from the experiment. The other 6 rats continued to be injected with PRP every three days for 15 days, after which the animals were withdrawn from the experiment.

In rats of groups 3 and 4, 2 ml of blood was collected from the vessels of the tail in special tubes. Centrifugation of the blood resulted in 0.5 ml of PRP.

The material for morphological examination consisted of masseter muscle samples taken from intact rats in group 1 and from rats in groups 2-4, specifically from the area where the mandibular fracture had been modeled. Tissue fragments of the masseter muscle were fixed in a 10% formalin solution. Tissue consolidation was achieved by passing the samples through alcohols of increasing concentration, Nikiforov's solution (96% ethanol and diethyl ether in a 1:1 ratio), and chloroform, followed by paraffin embedding. Serial sections with a thickness of 4-5×10^{-6} m were prepared from the paraffin blocks for subsequent staining with hematoxylin and eosin, picrofuchsin according to Van Gieson, and using the Rego method.

Immunohistochemical study was performed on Super Frost Plus adhesive slides ("Menzel", Germany). The Master Polymer Plus Detection system (Peroxidase, DAB chromogen) (Master Diagnostica, Spain) was used, citrate buffer (pH 6.0) and EDTA buffer (pH 8.0) were used for high-temperature processing of antigen epitopes. Immunohistochemical study was performed using a rabbit monoclonal antibody to vimentin (clone SP20, Master Diagnostica, Spain).

Microslides were examined using a ZEISS Primostar 3 microscope (Carl Zeiss, Germany) equipped with an integrated color digital camera and a BRESSER Science TFM-301 Trino microscope with a BRESSER Full HD camera (Bresser GmbH, Germany).

Morphometric analysis was performed using the Labscope software. In the masseter muscle, in the field of view of the microscope ×100, the following parameters were assessed: specific volumes (%) of parenchymal and stromal components; absolute number of blood vessels; specific volume of altered muscle fibers. The immunohistochemical reaction was evaluated by counting the absolute number of Vimentin^{+}-cells in stroma of the masseter muscle in the field of view of the microscope ×1000.

The data in the groups were statistically processed using the PAST software (version 4.15, Natural History Museum, University of Oslo, Norway). The mean values in the groups

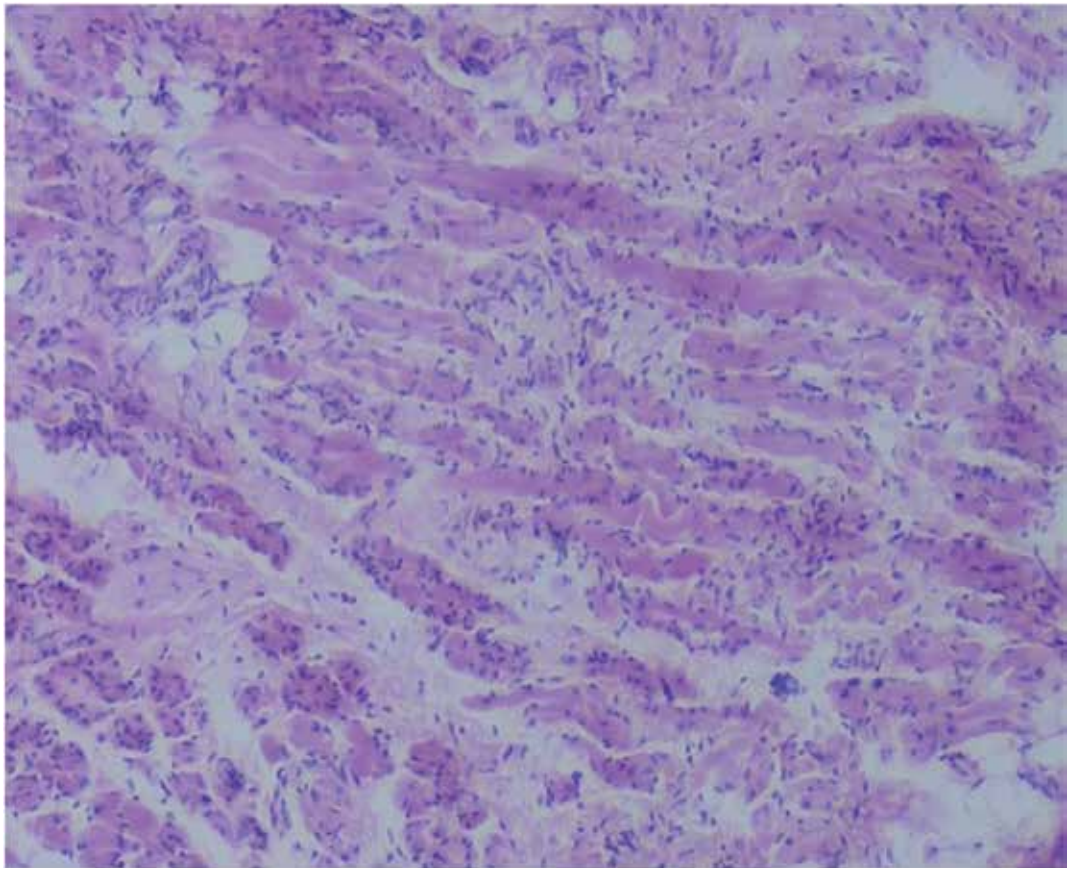

Fig. 2. Atrophic and hypertrophic muscle fibers in the masseter muscle of a Group 4 rat. Hematoxylin and eosin staining, ×100.

were compared using the Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

RESULTS

In rats that were withdrawn from the experiment after one month, overview microscopy of the masseter muscle showed that in group 1, the muscle fibers had a uniform thickness. In groups 2 and 3, the muscle fibers appeared thinned, indicating the development of atrophic changes. In group 4, the muscle fibers exhibited uneven thickness due to the presence of both atrophic and hypertrophic fibers, with the latter predominating (Fig. 2).

In groups 2-4, compared to group 1, the muscle fibers exhibited ischemic, dystrophic, and necrotic changes. These alterations were manifested by wavy deformation of the muscle fibers, uneven hematoxylin and eosin staining of the cytoplasm and nuclei, and the absence of nuclei. Rego staining revealed that altered muscle fibers appeared black (Fig. 3). Morphometric analysis showed that the specific volume of altered muscle fibers in groups 2 and 3 did not differ significantly ($p>0.05$). In group 4, this parameter was significantly lower compared to groups 2 and 3 ($p<0.05$) (Table 1).

The stromal component of the masseter muscle in groups 1-4 was characterized by the presence of connective tissue, blood vessels, and nerve fibers. The absolute number of blood vessels in groups 2-4 was significantly lower compared to group 1 ($p<0.05$), which negatively affected tissue trophism. There was no significant difference ($p>0.05$) in the absolute number of blood vessels between groups 2 and 3, whereas group 4 showed a significantly ($p<0.05$) higher value compared to groups 2 and 3 (Table 1).

In the stroma of the masseter muscle in group 1 rats, few diffusely distributed cellular elements were observed, represented by lymphocytes, monocytes, histiocytes, and fibroblastic lineage cells. In groups 2-4, compared to group 1, the above-mentioned cellular infiltration was focal-diffuse and its severity increased (Fig. 4). The indicated infiltration was moderately pronounced in group 4 and maximally pronounced in groups 2 and 3.

Immunohistochemical examination revealed Vimentin$^+$-cells, which are mesenchymal cells responsible for the production of connective tissue components, among the above-mentioned cellular infiltration (Fig. 5). Compared to group 1, the absolute number of such cells was significantly higher in groups 2-4 ($p<0.05$). There was no significant difference ($p>0.05$) in the number of Vimentin$^+$-cells between groups 2 and 3 ($p>0.05$), while group 4 showed a significantly lower value compared to groups 2 and 3 ($p<0.05$).

In groups 2-4, compared to group 1, hemodynamic disturbances were observed in stroma of the masseter muscle. These were manifested by edema, vascular hyperemia, the presence of acute thrombi in the lumen of some vessels, and the formation of small- and large-focal hemorrhages.

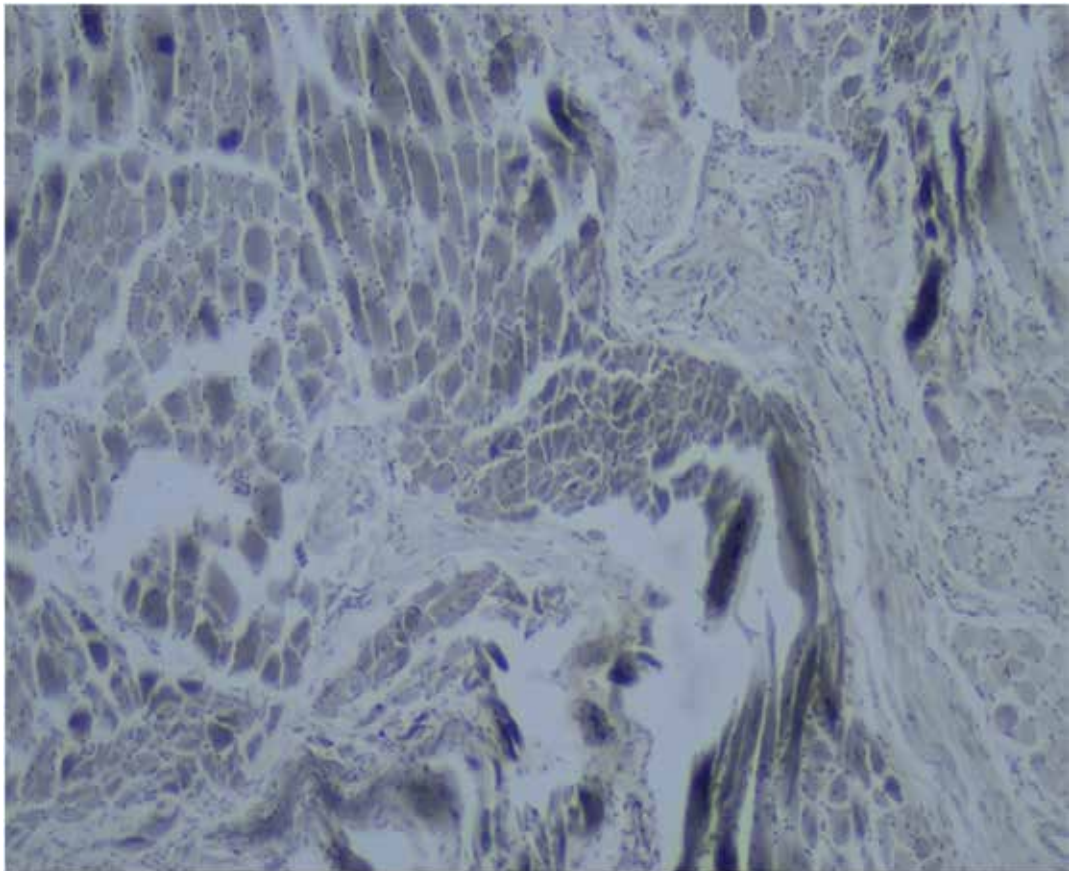

Fig. 3. Altered muscle fibers stained black in the masseter muscle of a Group 3 rat. Rego staining, ×100.

Table 1. Mean values of morphometric parameters in rats of Groups 1-4 withdrawn from the experiment after 1 month

Group	Name of the morphometric parameter				
	Specific volume of parenchyma	Specific volume of stroma	Absolute number of blood vessels	Specific volume of altered muscle fibers	Absolute number of Vimentin$^+$-cells
1	89.3±1.8%	10.7±1.8%	12.8±1.1	-	15.8±1.3
2	43.6±2.4%[1]	56.4±2.4%[1]	4.8±0.3[1]	26.7±2.0%	34.6±2.3[1]
3	44.1±2.6%[1]	55.9±2.6%[1]	4.2±0.4[1]	25.8±2.1%	38.5±2.7[1]
4	66.9±4.1%[1,2,3]	33,1±4.1%[1,2,3]	7.5±0.8[1,2,3]	4.9±0.5%[2,3]	25.3±2.6[1,2,3]

Note: [1] – statistically significant difference compared to group 1; [2] – statistically significant difference compared to group 2; [3] – statistically significant difference compared to group 3.

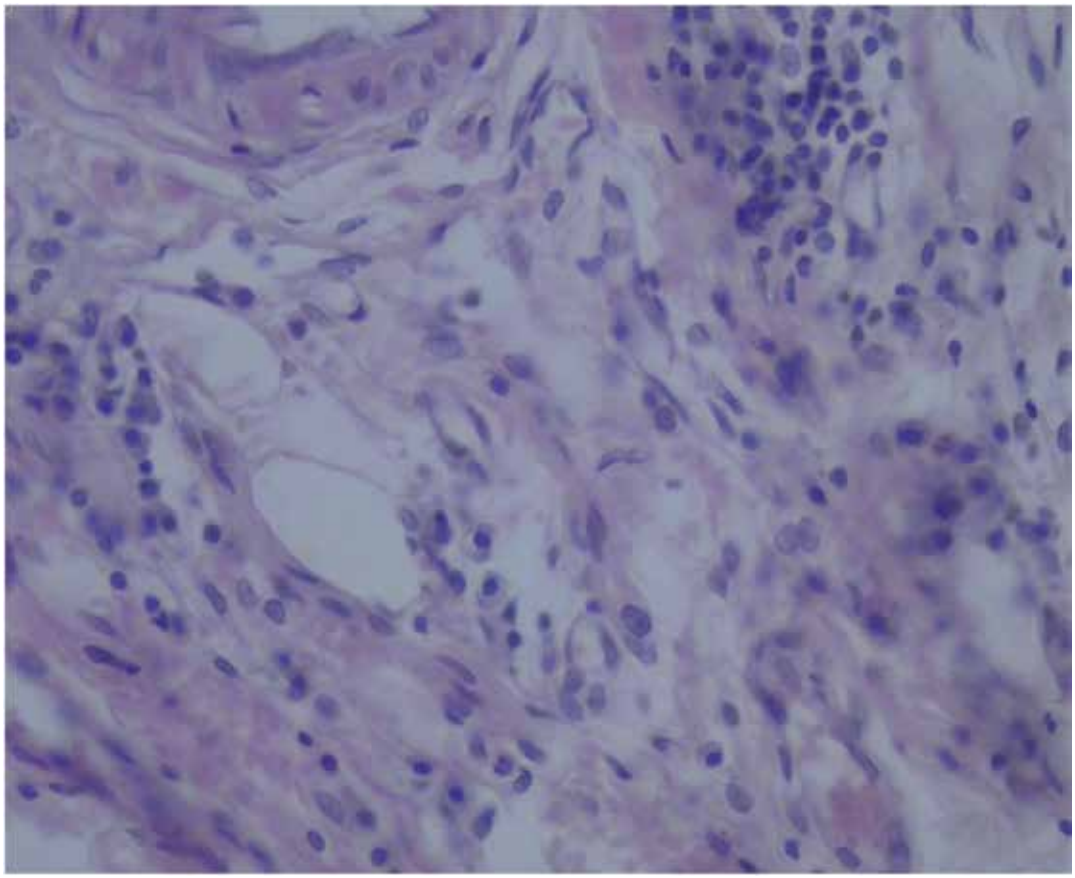

Fig. 4. Diffuse polymorphic cellular infiltration in stroma of the masseter muscle of a group 2 rat. Hematoxylin and eosin staining, ×400.

These changes were most pronounced in groups 2 and 3, while in group 4 they were moderately expressed.

In morphometric studies, the masseter muscle in groups 2-4 compared to group 1 was characterized by a decrease ($p<0.05$) in the specific volume of parenchyma and an increase ($p<0.05$) in the specific volume of stroma. There were no significant differences ($p>0.05$) in the specific volumes of parenchyma and stroma between groups 2 and 3. In group 4, compared to groups 2 and 3, the specific volume of parenchyma was higher ($p<0.05$), while the specific volume of stroma was lower ($p<0.05$). The observed increase in the specific volume of stroma in groups 2-4 was due to the excessive content of connective tissue in the stroma, along with the presence of adipocyte clusters between the connective tissue fibers (Fig. 6).

In rats that were withdrawn from the experiment after 1 month and 15 days, compared to the previous period, in group 2 the specific volume of altered muscle fibers in the muscle parenchyma increased ($p<0.05$) (Table 2). In group 3 the specific volume of altered muscle fibers decreased ($p<0.05$). In group 4, no alterative changes were recorded in the muscle parenchyma at all.

Compared to the previous period in rats that were withdrawn from the experiment after 1 month and 15 days, the number

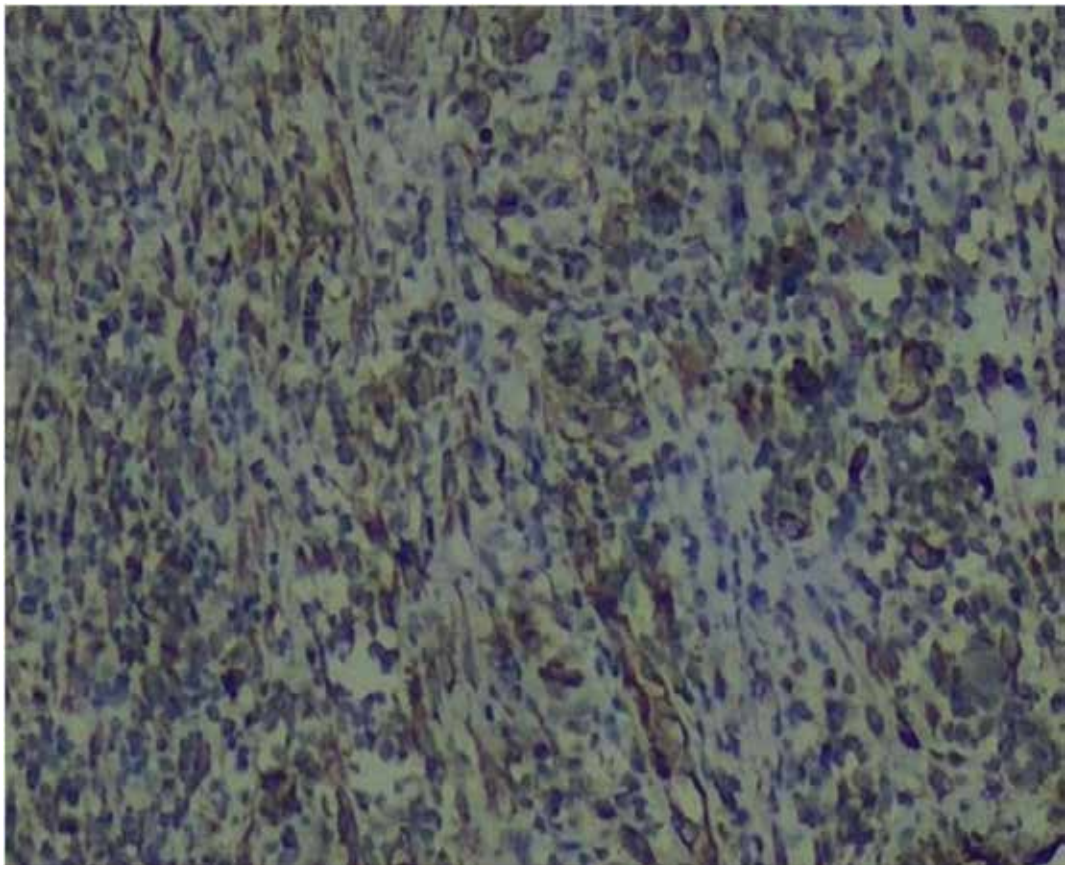

Fig. 5. Vimentin^{+}-cells in stroma of the masseter muscle of a group 2 rat. Immunohistochemical staining with monoclonal antibody to vimentin, ×400.

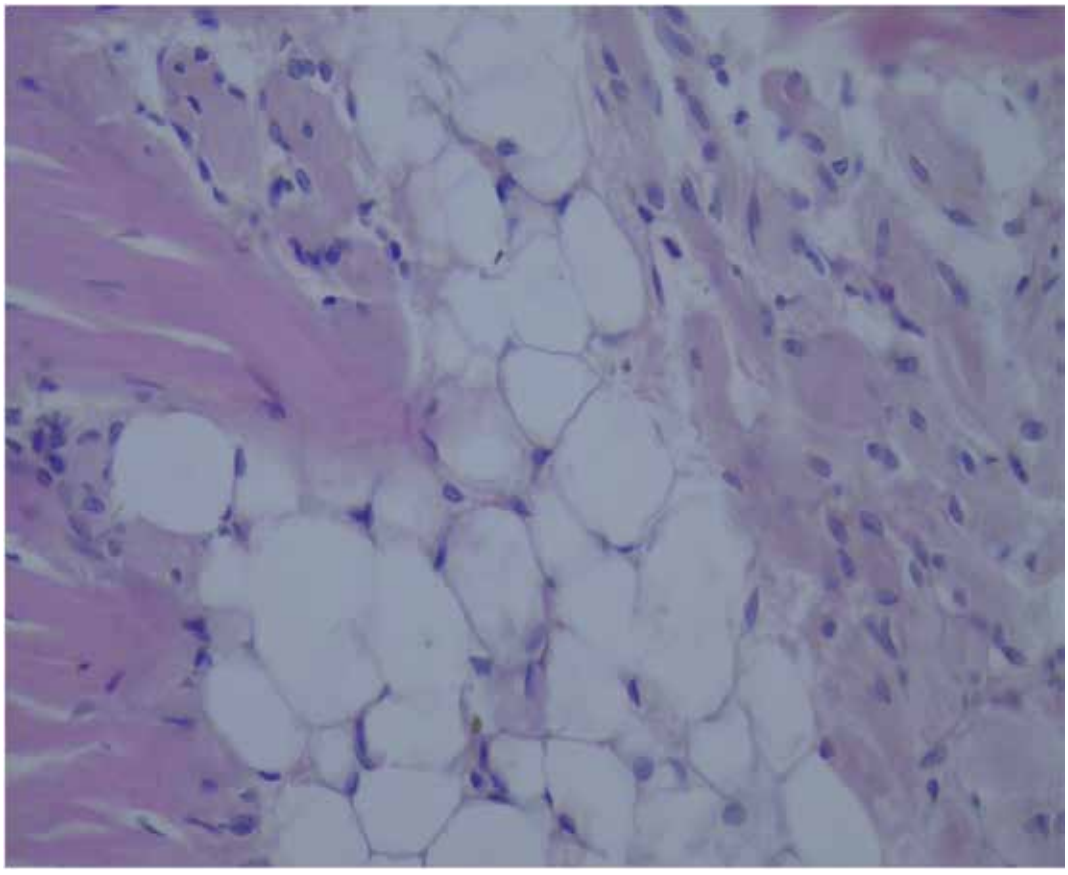

Fig. 6. Excessive connective tissue content with clusters of adipocytes in stroma of the masseter muscle of a group 2 rat. Hematoxylin and eosin staining, ×400.

Table 2. Mean values of morphometric parameters in rats of groups 2-4 withdrawn from the experiment after 1 month and 15 days

Group	Name of the morphometric parameter				
	Specific volume of parenchyma	Specific volume of stroma	Absolute number of vessels	Specific volume of altered muscle fibers	Absolute number of Vimentin$^+$-cells
2	35.4±2.4%^{1,4}	64.6±2.4%^{1,4}	3.2±0.3^{1,4}	39.4±2.3%^{4}	47.2±2.4^{1,4}
3	65.8±4.0%^{1,2,4}	34.2±4.0%^{1,2,4}	10.1±1.0^{1,2,4}	5.3±0.4%^{2,4}	29.4±1.4^{1,2,5}
4	86.2±4.0%^{1,2,3,4}	13.8±4.0%^{1,2,3,4}	17.4±1.1^{1,2,3,4}	-	14.4±1.1^{1,2,3,4}

Note: [1] – statistically significant difference compared to group 1; [2] – statistically significant difference compared to group 2; [3] – statistically significant difference compared to group 3; [4] – statistically significant difference compared to the previous period.

of vessels in stroma in group 2 decreased ($p<0.05$). In group 3, this indicator increased ($p<0.05$), but had a lower ($p<0.05$) value compared to the indicator in group 1. The indicator in group 4 increased ($p<0.05$) compared to the previous period and the indicator in group 1.

In stroma of the masseter muscle, compared to the previous period, hemodynamic disturbances increased in group 2, decreased in group 3, and were not observed at all in group 4.

Polymorphic cellular infiltration in stroma of the masseter muscle in rats withdrawn from the experiment after 1 month and 15 days, compared to the previous period, increased in group 2 and decreased in group 3. In group 4, the degree of infiltration corresponded to that observed in group 1.

Among the polymorphic cellular infiltration, immunohistochemical analysis revealed Vimentin$^+$-cells, the number of which increased ($p<0.05$) in group 2 compared to the previous period. In group 3, the absolute number of Vimentin$^+$-cells decreased ($p<0.05$), although it remained significantly higher ($p<0.05$) compared to the value in group 1. In group 4, this parameter also decreased ($p<0.05$) and was significantly lower ($p<0.05$) compared to the value in group 1.

In rats withdrawn from the experiment after 1 month and 15 days, the specific volume of parenchyma in group 2 decreased ($p<0.05$), while the specific volume of stroma increased ($p<0.05$), compared to the previous period. In groups 3 and 4, the specific volume of parenchyma increased ($p<0.05$), and the specific volume of stroma decreased ($p<0.05$). However, compared to group 1, the specific volume of parenchyma remained lower ($p<0.05$), and the specific volume of stroma remained higher ($p<0.05$). In group 4, compared to group 3, the specific volume of parenchyma was higher ($p<0.05$), and the specific volume of stroma was lower ($p<0.05$).

DISCUSSION

PRP is a biological hemoderivative product obtained from blood in which platelets are present in a higher concentration than basal levels [10]. It has shown promising results in promoting tissue regeneration, repairing bone defects, treating skin wounds, plastic surgery, sports injuries etc. [11].

The comprehensive morphological study of experimental material conducted by the authors confirmed the effectiveness of PRP in the treatment of post-immobilization extra-articular contractures of the mandible. The therapeutic effect of PRP was more pronounced in cases where it was applied both during the treatment of mandibular fracture using an immobilizing muzzle for one month and for an additional 15 days after its removal, compared to animals in which PRP was applied only during the 15 days following muzzle removal.

PRP exhibits an antifibrotic effect, which was morphologically manifested by a decrease in the specific volume of stroma in the masseter muscle, one of the main components of which is connective tissue. In the conducted study, the use of PRP led to a reduction in the number of Vimentin$^+$-cells in stroma of the masseter muscle, which include, among others, fibroblastic lineage cells. These cells are actively involved in the production of connective tissue components [12].

Studies conducted by other researchers have shown that PRP reduces vimentin expression by interstitial cells, thereby suppressing sclerotic processes in organs [13]. The antifibrotic properties of PRP are also attributed to the suppression of fibroblast-to-myofibroblast transition via VEGF-A/VEGFR-1-mediated inhibition of TGF-β1/Smad3 signaling [14]. The platelets of PRP may release proteins (such as fibroblast growth factor or hepatocyte growth factor) that are known to inhibit myofibroblasts. It has also been proven that matrix metalloproteinases contained in platelets can degrade fibrillar collagen, thereby reducing the severity of sclerotic changes in organs [15].

In the study conducted by the authors, it was found that PRP normalizes the intensity of immune cell infiltration in stroma of the masseter muscle in animals. It is well known that interstitial inflammation leads to the development of sclerotic changes in organs. Therefore, the normalization of connective tissue content in stroma of the masseter muscle may be attributed to the anti-inflammatory effect of PRP.

The anti-inflammatory effect of PRP is attributed to its influence on the production of pro-inflammatory cytokines and the morphofunctional state of immune cells [16]. PRP decreased the lymphocyte number. In PRP the platelets release a considerable amount of RANTES (a major monocyte chemoattractant) from its alpha-granules. RANTES also inhibits many cytokines, decreases in the concentration of lipoxin A4. PRP alters macrophage phenotype, reduces cyclooxygenase-2 expression, and normalizes prostaglandin E2 levels [17].

The use of PRP in the study led to a reduction in the severity of alterative changes in muscle fibers of the masseter muscle and also stimulated muscle fibers regeneration, as evidenced by an increase in the specific volume of parenchyma. According to the literature, PRP enhances the morphofunctional state of muscle cells and their regenerative potential [18, 19].

PRP is a source of angiogenic growth factors (transforming growth factor-β, platelet-derived growth factor, insulin-like growth factor-1, vascular endothelial growth factor, epidermal growth factor, and others), which stimulate the formation of new blood vessels and activate the morphofunctional state of vascular endothelial cells, thereby improving tissue trophism and oxygenation [20, 21]. In our study, the application of PRP led to a reduction in the severity of hemodynamic disturbances in the masseter muscle and an increase in the number of blood vessels in its stromal component.

CONCLUSIONS

The comprehensive morphological study of experimental material conducted by the authors confirmed the effectiveness of platelet-rich plasma in the treatment of post-immobilization extra-articular contractures of the mandible, thereby expanding the available arsenal of treatment methods for this pathology.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Painful shoulder syndrome as a clinical problem – analysis of physical therapy

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ABSTRACT

Aim: The aim of this paper is to assess the efficacy of physical therapy conducted in patients with painful shoulder syndrome in the course of rotator cuff injury.
Materials and Methods: The study involved a group of 25 patients: 15 women and 10 men. The patients were treated at a rehabilitation clinic in Radom for a period of 3 weeks. The physical therapy used in study patients included diadynamic currents, ultrasound therapy, cryotherapy, shockwave therapy, and kinesiotherapy.
Results: After treatment, the pain was eliminated or remarkably reduced and the range of joint motion was increased in all planes.
Conclusions: 1. The physical therapy used in study patients helped eliminate or reduce pain. 2. The range of joint motion was increased in all planes. 3. The quality of life and functioning in study patients was improved. 4. Treatment of this disorder is based on physical therapy.

KEY WORDS: painful shoulder syndrome, treatment, physical therapy

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INTRODUCTION

Painful shoulder syndrome is the second most common pain syndrome after spinal pain. Shoulder pain is reported in between 7% and 36% of the population. This disorder most commonly affects middle-aged women [1-5].

The term "Duplay's disease" is commonly used to refer to this disorder in the medical nomenclature. The age group affected by painful shoulder syndrome shows a downward trend. 62% of all injuries of the shoulder complex occur in patients aged 18-40 years and 28% occur in patients aged 40–46 years. The causes are very broad. The shoulder joint is very complex and encompasses multiple anatomical structures, with each element influencing joint function and biomechanics [1-8].

Painful shoulder syndrome is characterized by pain and a limited range of motion both in the shoulder joint and the clavicular joint. The pain impairs or prevents movement. Various conditions can lead to the development of painful shoulder syndrome; these include injuries, joint capsule damage, rheumatoid arthritis, shoulder impingement or stroke. The rotator cuff area is the most common location of pain and the direct cause of the pathology. The development of painful shoulder syndrome is also often dependent on the type of work or other activities that patients engage in [1-9].

Painful shoulder syndrome can be characterized by radiating pain in the area of the scapula, cervical spine, elbow, and wrist. A limited range of motion is also typical, especially with respect to abduction and internal rotation. Additional symptoms include swelling, increased soft tissue tension within the shoulder girdle, reduced muscle strength, inflammation, and nocturnal shoulder pain. Injuries or certain conditions, for example shoulder impingement syndrome, can also constitute an important cause.

If the patient experiences typical subacromial pain, it is necessary to check for bursitis as the potential cause. Contractures of the so-called rotator cuff muscles (supraspinatus, infraspinatus, teres minor, and subscapularis) are an equally common cause of shoulder pain and limited mobility [13-15].

Treatment of painful shoulder syndrome is a difficult clinical problem and is based on physical therapy, which uses diadynamic currents, cryotherapy, ultrasound therapy, shockwave therapy, laser therapy, and kinesiotherapy [10-12, 16-20].

AIM

The aim of this study was to assess the efficacy of physical therapy in patients with painful shoulder syndrome.

- How did physical therapy contribute to pain reduction?
- What was the effect of physical therapy procedures and kinesiotherapy on the range of motion in the shoulder joint?
- How did physical therapy contribute to an improvement in everyday functioning?

MATERIALS AND METHODS

The study involved a group of 25 patients: 15 women and 10 men diagnosed with painful shoulder syndrome. Study patients were treated at a rehabilitation clinic in Radom. The disorder affected the right limb in 20 patients and the left limb in 5 patients. Symptoms reported by study patients

were related to rotator cuff damage, reduced range of motion, presence of pain, reduced function, mobility, and grip strength of the hand, and reduced muscle strength.

The mean age of study patients was 48.5 years (Me = 50 years; Min = 30 years; Max = 60 years). The mean time from symptom onset was 10.1 months (Me = 7 months; Min = 1 month; Max = 25 months) (Table 1, Fig. 1-2).

The physical therapy consisted of a 21-day cycle of procedures, including diadynamic currents, cryotherapy, ultrasound therapy, laser therapy, and shockwave therapy. The kinesiotherapy procedures included active free, self-assisted, or non-weight-bearing exercises, exercises with the use of equipment in order to increase the range of motion, active non-weight-bearing exercises in the horizontal plane, exercises to stabilise the scapula, fascial relaxation, and scapular mobilisation.

The study evaluated pain and its severity as well as the range of motion measured with a goniometer both before and after treatment; an analogue pain scale and a subjective VAS were used. Each patient underwent the same physical therapy and kinesiotherapy programme.

Table 1. Characteristics of study group

Number	25
Gender	Male: 10
	Female: 15
Age	Mean (Av.) = 48.5 Standard deviation (SD) = 9.4 Median (Me) = 50
Time from symptom onset (months)	Mean (Av.) = 10.1 Standard deviation (SD) = 7.4 Median (Me) = 7

STATISTICALLY ANALYSE

The data obtained were statistically analysed. Before treatment, all study patients experienced mobility limitations in all planes as compared with the physiological values. The non-parametric Wilcoxon signed-rank test was performed to verify whether the differences in results obtained in study patients after treatment as compared to pre-treatment values were statistically significant or not. This test was performed for two dependent samples, for paired samples, in order to find significant differences after treatment as compared to the pre-treatment values.

Differences after treatment were calculated. An improvement was visible with respect to both ranges of motion and pain reduction. The overall active range of motion was increased. An analysis of the results with a VAS revealed a change of 77% in pain at rest and a change of 71% in pain during active movement. A pain reduction was seen during movement in all planes and axes. On palpation, study patients showed reduced pain during compression of the greater tubercle of the humerus.

RESULTS

ANALYSIS OF RANGE OF MOBILITY BEFORE AND AFTER TREATMENT

The mean range of active flexion in study patients was 125.5 degrees before the start of the treatment cycle and 143.5 degrees after treatment. The mean value of improvement in the range of motion after treatment was 18 degrees. The standard deviation was 16.2, with a significance level of 0.34 (Fig. 3).

The mean range of passive flexion in study patients was 128.4 degrees before the start of the treatment cycle and 147.7 degrees after treatment. The mean difference seen after treatment was 19.3 degrees. The standard deviation was 8.4, with a significance level of 0.71.

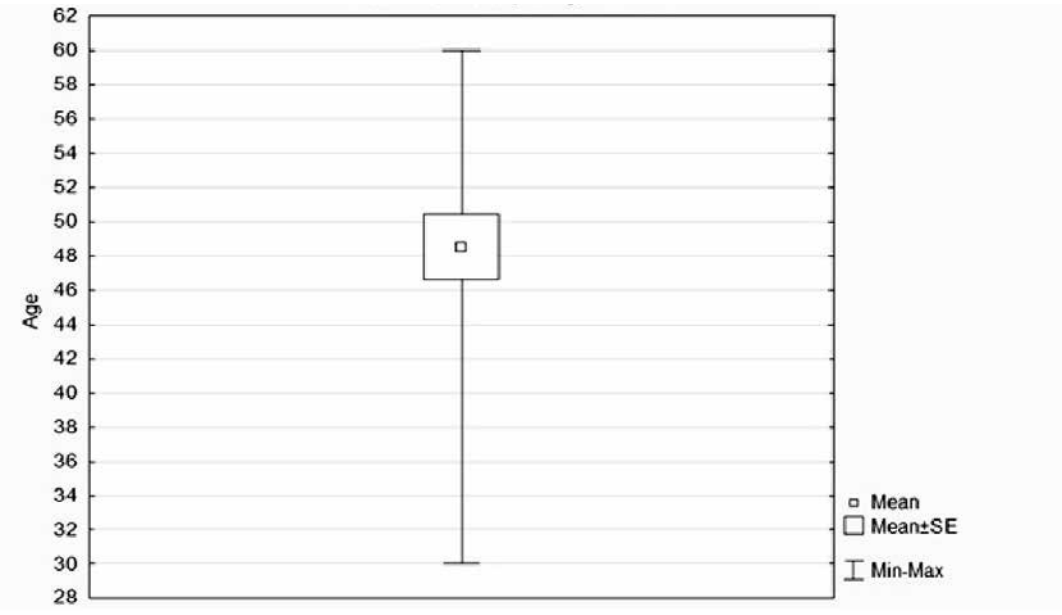

Fig. 1. Mean age of study patients.

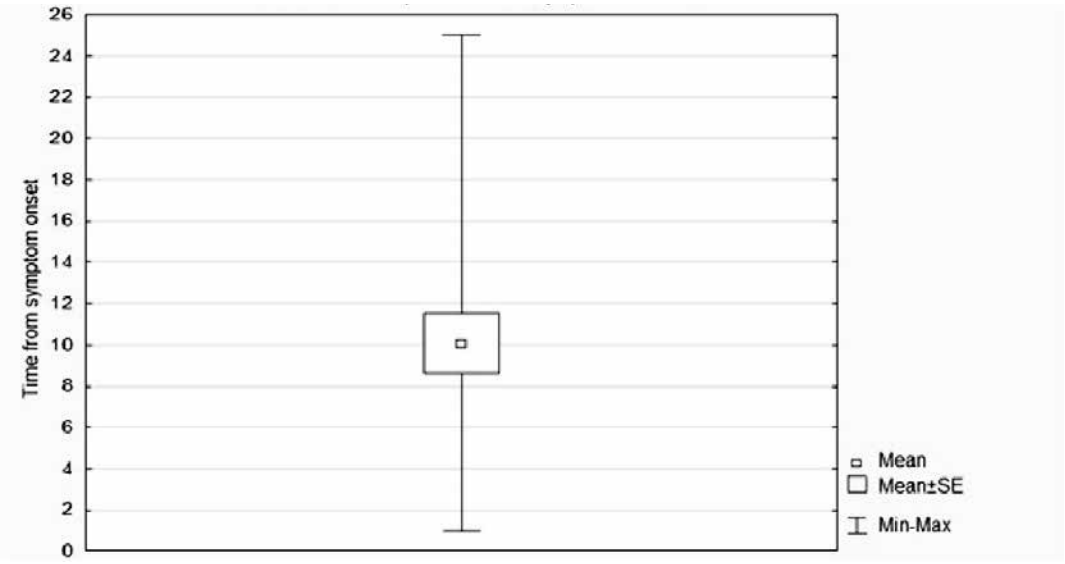

Fig. 2. Time from symptom onset in study patients.

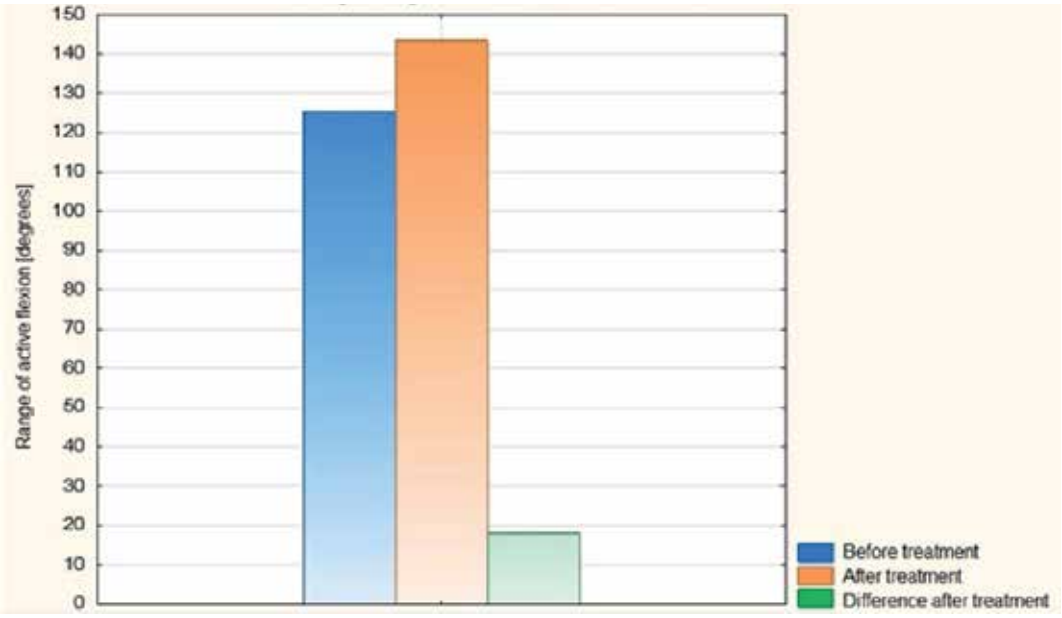

Fig. 3. Change in range of active flexion.

The mean range of active extension in study patients was 34 degrees before the start of the treatment cycle and 42.1 degrees after treatment. The difference seen after treatment was 8.1 degrees. The standard deviation was 9.6, with a significance level of 0.06 (Fig. 4).

The mean range of passive extension in study patients was 36.1 degrees before the start of the treatment cycle and 45.1 degrees after treatment. The mean difference seen after treatment was 9 degrees. The standard deviation was 11.2, with a significance level of 0.05.

The mean range of active internal rotation in study patients was 41.2 degrees before the start of the treatment cycle and 49.3 degrees after treatment. The mean difference seen after treatment was 8.1 degrees. The standard deviation was 12.8, with a significance level of 0.05 (Fig. 5).

The mean passive range of internal rotation in study patients was 45.2 degrees before the start of the treatment cycle and 57.5 degrees after treatment. The mean difference seen after treatment was 12.3 degrees. The standard deviation was 9.8, with a significance level of 0.21.

The mean range of external rotation in study patients was 45.5 degrees before the start of the treatment cycle and 53.4 degrees after treatment. The mean difference seen after treatment was 7.9 degrees. The standard deviation was 12.4, with a significance level of 0.08 (Fig.6).

The mean range of passive external rotation in study patients was 50.2 degrees before the start of the treatment cycle and 61.4 degrees after treatment. The mean difference seen after treatment was 11.2 degrees. The standard deviation was 9.8, with a significance level of 0.21.

The mean range of active horizontal flexion in study patients was 114.4 degrees before the start of the treatment cycle and 131 degrees after treatment. The mean difference seen after treatment was 16.6 degrees. The standard deviation was 6.7, with a significance level of 0.08 (Fig. 7).

The mean passive range of horizontal flexion in study patients was 117 degrees before the start of the treatment cycle and 136.9 degrees after treatment. The mean difference seen after treatment was 19.9 degrees. The standard deviation was 7.6, with a significance level of 0.74.

The mean range of active horizontal extension in study patients was 18.6 degrees before the start of the treatment cycle and 22.7 degrees after treatment. The mean difference seen after treatment was 7.1 degrees. The standard deviation was 6.4, with a significance level of 0.06 (Fig. 8).

The mean passive range of horizontal extension in study patients was 22.4 degrees before the start of the treatment cycle and 31.9 degrees after treatment. The mean difference seen after treatment was 9.5 degrees. The standard deviation was 6.2, with a significance level of 0.06.

The mean active range of abduction in study patients was 115.4 degrees before the start of the treatment cycle and 134.1 degrees after treatment. The mean difference seen after treatment was 18.7 degrees. The standard deviation was 5.3, with a significance level of 0.08 (Fig. 9).

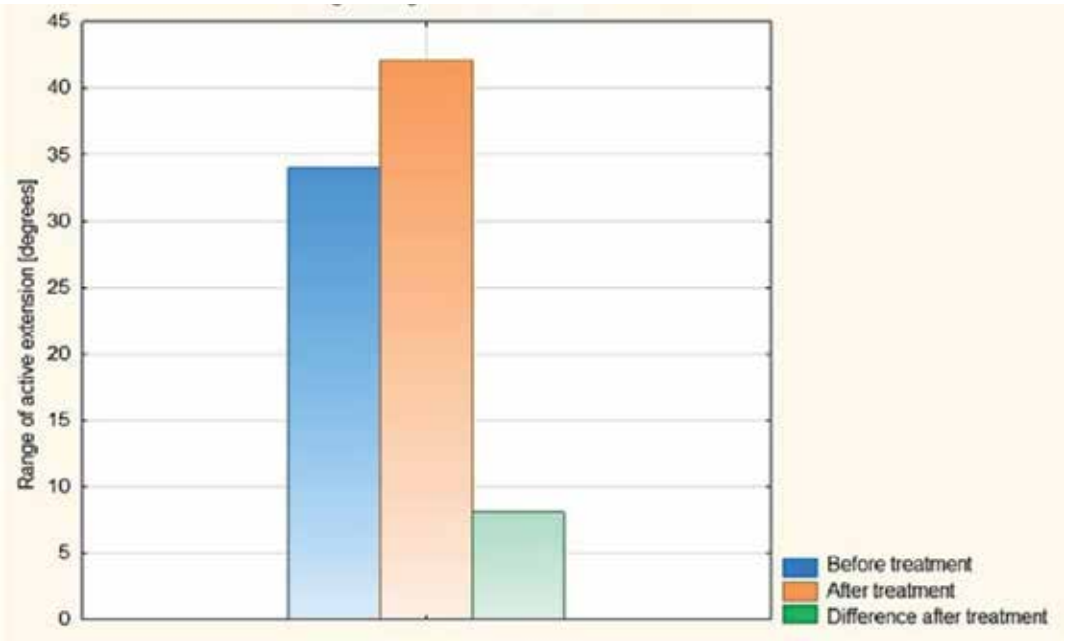

Fig. 4. Change in range of active extension.

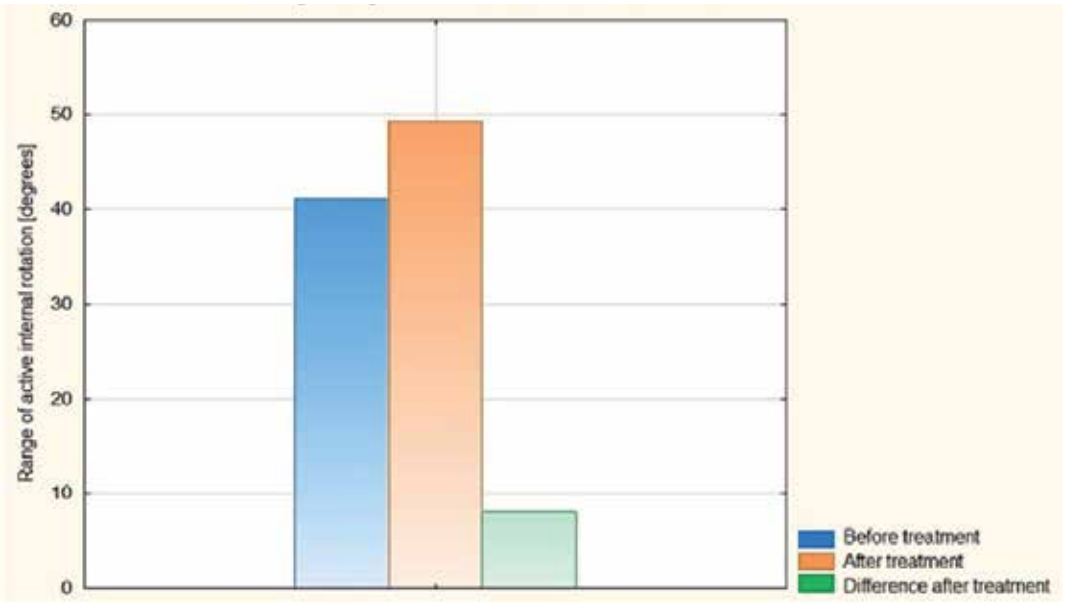

Fig. 5. Change in range of active internal rotation.

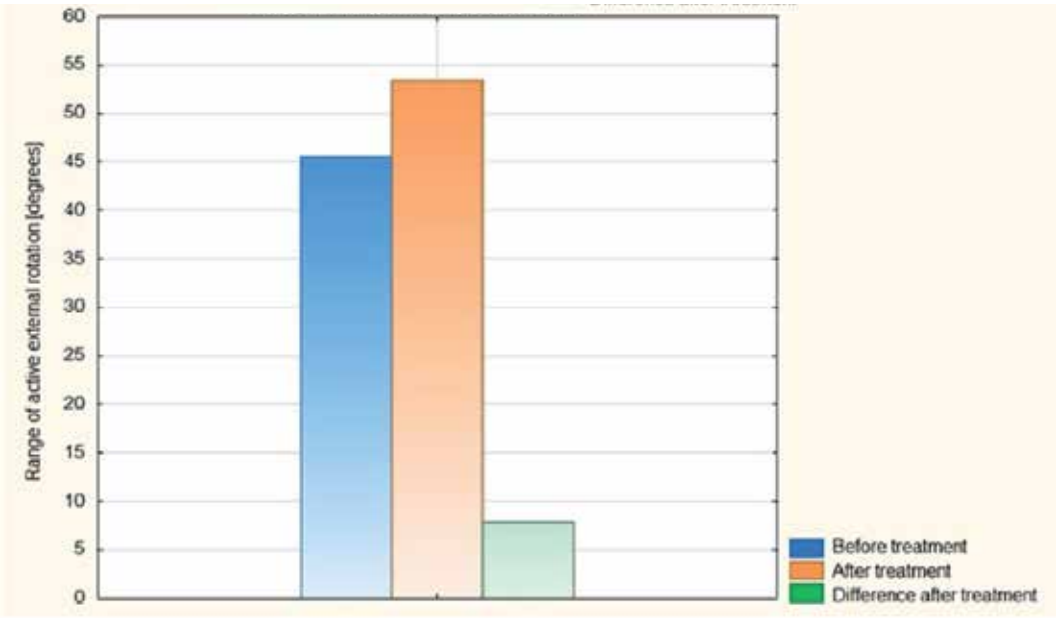

Fig. 6. Change in range of active external rotation.

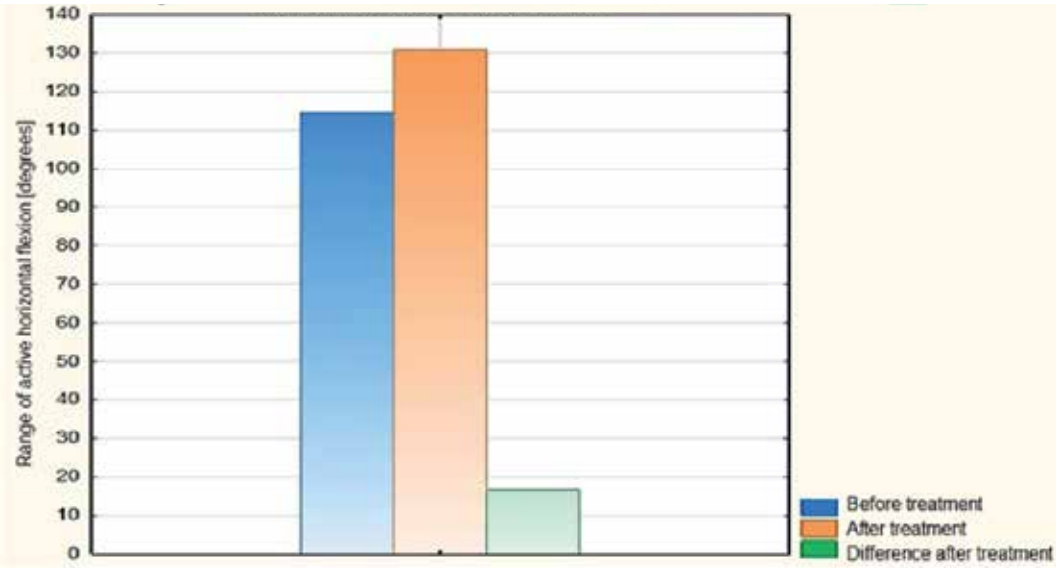

Fig. 7. Change in range of active horizontal flexion.

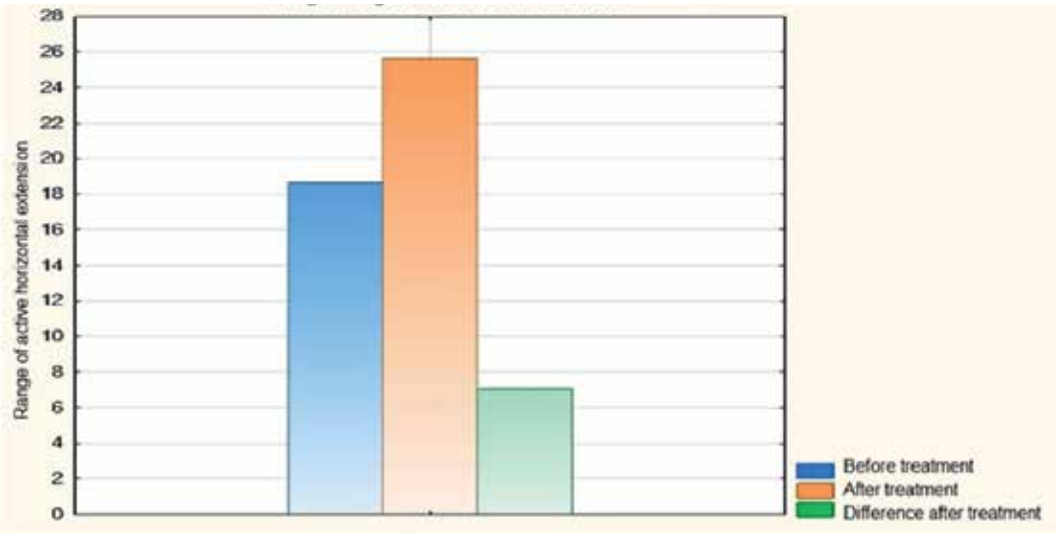

Fig. 8. Change in range of active horizontal extension.

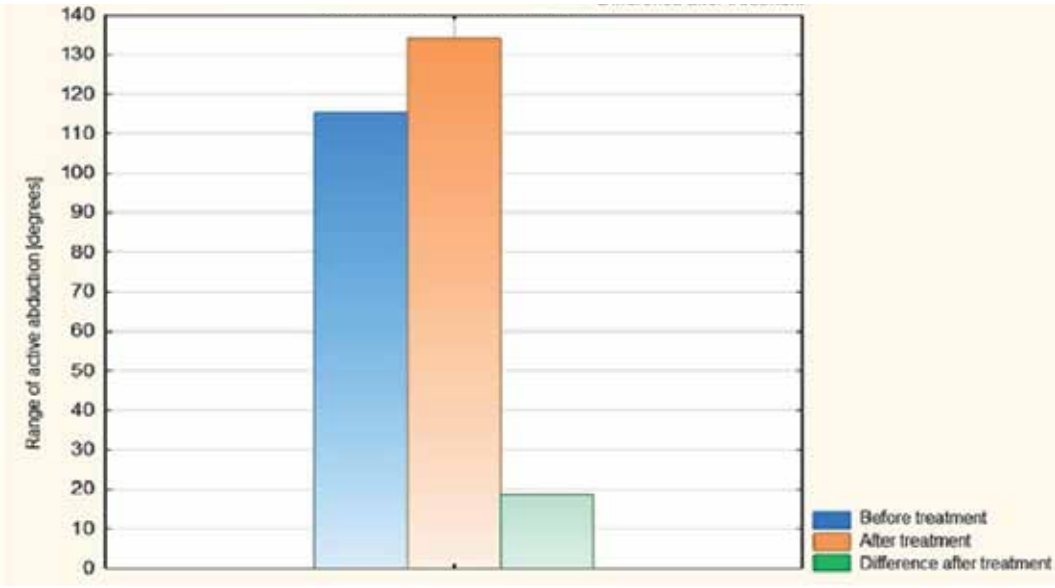

Fig. 9. Change in range of active abduction.

Table 2. The analysis of parameters obtained before and after the three-week therapeutic cycle

Parameters	Before treatment	After treatment	Standard deviation (SD)	Significance level
Pain at rest				
Mean obtained	4.9	1.1	1.5	p=0.15
Percentage change	77%			
Pain during active movement				
Mean obtained	6.6	1.9	1.8	p=0.36
Percentage change	71%			
Pain during flexion				
Mean obtained	7.6	2.4	1.2	p=0.4
Percentage change	68%			
Pain during extension				
Mean obtained	2.7	0.6	1.8	p=0.62
Percentage change	76%			
Pain during internal rotation				
Mean obtained	6.2	2.7	1.3	p=0.42
Percentage change	56%			
Pain during external rotation				
Mean obtained	6.6	2.3	1.8	p=0.44
Percentage change	65%			
Pain during horizontal flexion				
Mean obtained	3.8	1.4	1.5	p=0.34
Percentage change	65%			
Pain during horizontal extension				
Mean obtained	2.2	0.6	1.3	p=0.52
Percentage change	70%			
Pain during abduction				
Mean obtained	7.8	2.4	1.4	p=0.47
Percentage change	69%			
Pain during compression over the greater tubercle of humerus				
Mean obtained	5.8	2.5	1.8	p=0.21
Percentage change	58%			
Overall change in subjective VAS pain scores				
Mean obtained	5.4	1.8	1.2	p=0.28
Percentage change	66%			

The mean passive range of abduction in study patients was 121.1 degrees before the start of the treatment cycle and 142.6 degrees after treatment. The mean difference seen after treatment was 21.5 degrees. The standard deviation was 4.2, with a significance level of 0.22.

ANALYSIS OF VAS SCORES

Ranges of motion tested in the study, pain during movement and at rest, and complaints reported by patients on palpation together with an analysis of the results can be used to assess how the therapeutic management contributed to a reduction in pain and an overall improvement in painful shoulder syndrome. An analysis of parameters obtained before and after the three-week therapeutic cycle is presented below. A subjective 10-degree VAS for pain was used as the research tool (Table 2).

DISCUSSION

The 3-week therapeutic cycle of physical therapy contributed to an improvement in the ranges of motion, a reduction in pain, and an improvement in overall functional ability in study patients. The physiotherapy and kinesiotherapy methods used in this study helped achieve a marked improvement in active and passive movements. There was an improvement in the range of motion of the shoulder in every plane. A considerable pain reduction was recorded, with a 3.8-point reduction in VAS scores for pain at rest and a 4.7-point reduction in VAS scores for pain during movement. On palpation, study patients showed a 3.3-point change in VAS pain scores during compression over the greater tubercle of the humerus. Study patients also showed an improvement in upper limb function.

Increased awareness among patients allows for an early diagnosis and for initiating prophylactic and preventive measures. Appropriate management results in improvements in terms of both increased ranges of motion and pain reduction. The overall range of active movement improved by 13.3 degrees and the overall range of passive movement improved by 8.1 degrees. An analysis of the VAS scores revealed a change of 77% in pain at rest and a change of 71% in pain during active movement. A reduction in pain was seen during movement in all planes and axes. The overall change in pain as assessed with a subjective VAS was 66%.

The treatment of this disorder is based on physical therapy. Promoting these methods among internists, general practitioners, and neurologists will help use them more rationally in the treatment of painful shoulder syndrome.

CONCLUSIONS

1. Painful shoulder syndrome is a difficult diagnostic and therapeutic problem.
2. The physical therapy used in study patients helped eliminate or reduce pain.
3. The range of joint motion was increased in all planes.
4. The quality of life and functioning in study patients was improved.
5. Treatment of this disorder is based on physical therapy.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Autologous mesenchymal stem cells as a component of multidisciplinary rehabilitation of war participants with severe forms of chronic critical lower limb ischemia and pain syndromes

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ABSTRACT

Aim: To determine the effectiveness of a multidisciplinary rehabilitation program (MRP), integrating mesenchymal stem cells (MSCs) therapy, acupuncture, physiotherapy and physical exercises, in improving the functional recovery in patients with combat-related injuries and chronic critical lower limb ischemia (CCLLI).

Materials and Methods: A six-month comparative clinical trial was conducted involving 40 male patients (mean age [mean ± standard error] 62,5±2,3 years) diagnosed with CCLLI, including 10 individuals with combat-related injuries, being randomized into 2 groups: the MSCs group (main group [MG]) (n=20) received MRP, including autologous MSCs therapy; the control group (CG) (n=20) received standard therapy only. Five patients with gunshot wounds were randomly assigned to each group.

Results: MG patients presented with significant increase in ankle-brachial index and VAS pain score decrease, being more pronounced as opposed to CG. Walking distance improvement was achieved in 75% of MG patients vs. 40% in CG ($p<0,01$). Trophic ulcers healed in 100% of MG patients (vs 25% in CG [$p<0,01$]). After treatment, only 1 (5%) patient in the MG remained at risk of lower limb amputation, as compared to 8 (40%) patients in the CG ($p<0,05$) (RR 0,13 [95% CI 0,02-0,91]; $p<0,05$).

Conclusions: The implementation of MRP, integrating MSCs therapy, led to significant improvements in pain relief, tissue regeneration and overall functional recovery in patients with CCLLI, particularly those with military-related injuries, favouring the reduction of the risk of lower limb amputation.

KEY WORDS: mesenchymal stem cells, rehabilitation, pain management

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INTRODUCTION

Critical limb ischemia (CLI) is the most severe form of peripheral arterial disease (PAD), often linked to atherosclerosis and vascular disorders, including those caused by injuries in military conflicts [1, 2]. This condition, characterized by blockages in arteries supplying vital organs like the brain and heart, primarily affects the lower limbs and has an increasing prevalence in populations aged 61 to 70 [3, 4].

During wartime, these risk factors are more common among combatants. Chronic critical lower limb ischemia (CCLLI) develops in 20-40% of PAD cases, leading to poor outcomes in treatment [5]. In the group of patients with combat-related lower extremity injuries, an innovative device was used to conduct a preliminary examination of gunshot wounds to detect debris, including non-radiopaque fragments [6].

Given the chronic nature of CLI and its resistance to traditional treatments, therapeutic angiogenesis using mesenchymal stem cells (MSCs) has gained attention for its potential to regenerate tissue and restore blood flow. MSCs, along with multidisciplinary rehabilitation approaches, combining non-pharmacological pain management, acupuncture, physical therapy, and preformed growth factors, may offer a more comprehensive solution [7]. This integrative treatment model could enhance limb perfusion and accelerate tissue recovery [8].

The relevance of this approach is especially significant in the context of military injuries, where patients often present with complex, multifaceted conditions. Optimizing MSCs dosages, based on clinical data and international research, promises to improve both short- and long-term outcomes for patients with CCLLI, offering a more effective and holistic treatment strategy.

AIM

The study aimed to determine the effectiveness of a multidisciplinary rehabilitation program (MRP) that integrated MSCs therapy, acupuncture (AP), physiotherapy (PT) and physical exercises (PE) in improving functional recovery in patients with combat-related injuries and CCLLI.

MATERIALS AND METHODS

A six-month comparative clinical study was conducted involving 40 male patients (mean age [mean ± standard error of the mean] 62,5 ± 2,3 years) diagnosed with CCLLI. This cohort included 10 individuals with combat-related injuries. The primary objective of the study was to evaluate the effectiveness of an MRP integrating MSCs therapy, AP, PT, and PE to promote functional recovery.

Patients were randomly assigned into two groups: the MSCs group (main group [MG]) (n=20) and the control group (CG) (n=20). Both groups included 5 patients with gunshot wounds. The MSCs group received autologous MSCs therapy at a dose of 2×10^6 cells/kg, administered via 20 intramuscular injections, in addition to standard treatment protocols.

MSCs are injected into ischemic limb muscles **(**Fig. 1**)** following a specific pattern. The syringe is shaken before each injection. For occlusions above Poupart's ligament, both the thigh and lower leg are targeted, while occlusions below the knee involve only the lower leg. Injections are spaced ≤6 cm apart to ensure precise, sterile delivery and therapeutic efficacy.

The specific rehabilitation interventions included:

- AP: acupuncture sessions targeted key points such as ST36 (Zu San Li), SP6 (San Yin Jiao), and BL57 (Cheng Shan) to enhance microcirculation and alleviate pain;
- PT: the physiotherapy regimen included electrotherapy, magnetotherapy, phototherapy, and ultrasound therapy, aimed at improving tissue regeneration, muscle strength, and range of motion;
- PE: patients participated in a structured exercise program designed to enhance functional mobility.

MRP was delivered across three recovery phases. Each phase included regular medical evaluations, MSCs therapy, AP, PT and PE.

The CG received standard care as per the clinical guidelines recommended by the Ministry of Health of Ukraine [9].

The primary outcome measures of the study were:

- Abdominal-brachial index (ABI) dynamics: The ABI was used to assess the overall improvement in peripheral blood circulation and vascular health;
- pain reduction: pain levels were measured using the Visual Analog Scale (VAS) [10];
- Walking Distance Improvement (WDI) achievement: Clinically meaningful changes in walking distance were evaluated by monitoring the distance covered by each patient during a 30-minute walking test;
- Trophic ulcer healing: The rate of complete ulcer closure was assessed by the percentage of patients achieving full healing of trophic ulcers;
- risk of amputation: The risk of amputation was evaluated based on the necessity for limb amputation during the study period.

All patients received psychological counseling for post-traumatic stress disorder and anxiety, smoking cessation programs, and nutritional guidance to support recovery.

Statistical data analysis was conducted using IBM SPSS Statistics v. 27.0 (Armonk, NY: IBM Corp., USA) and MedCalc v. 22.001 (MedCalc Software Ltd., Belgium). Continuous data were presented as the mean ± standard error of the mean. Categorical data were presented as absolute and relative (%) frequencies. Continuous data in independent and related groups were compared using the unpaired and paired Student's t-test, respectively. Categorical (binary) data in independent groups were compared using Fisher's exact test. The effect magnitude of an intervention was assessed by relative risk (RR) with a 95% confidence interval (CI). A p-value <0.05 was considered statistically significant.

ETHICS

The authors take full responsibility for ensuring compliance with ethical and legal standards in biomedical research. The study adheres to Articles 43 and 45 of the Law of Ukraine «Fundamentals of Health Legislation of Ukraine», the principles of the Council of Europe Convention on Human Rights and Biomedicine, as well as the ethical principles outlined in the World Medical Association Declaration of

Fig 1. The scheme of MSCs introduction into lower limb muscles.

Helsinki regarding biomedical research involving human participants. Additionally, it complies with Directive 2001/20/EC of the European Parliament and the Council of the EU (April 4, 2001, as amended). The research strictly follows bioethical principles and aligns with both national and international regulatory requirements. The study protocol was approved by the local ethics committee. All the patients signed an informed consent.

RESULTS

Despite the relatively small sample size, the study demonstrated superior outcomes in the MG of patients who received MSCs therapy combined with additional non-pharmacological interventions as part of an MRP. In this group, significant improvements were observed compared to the pre-treatment period, including enhanced blood circulation (as measured by ABI), ulcer healing, reduced leg pain, increased functional activity and limb preservation, and reduced need for amputation (Table 1).

In contrast, while the CG also exhibited improvements in these parameters, the outcomes in the main group were significantly more pronounced – not only in comparison to baseline values but also relative to patients who received treatment according to standard clinical guidelines. The treatment outcomes for both groups are summarized in Table 1.

In clinical practice, we observed a phenomenon where, despite ulcer healing, patients continued to experience a significant decrease in blood supply, severe pain, and the prospect of subsequent limb amputation. However, as demonstrated by the data presented in Table 1, the risk of amputation was notably reduced in the MG.

In the MG, the ABI increased significantly from 0.39 ± 0.05 c.u. before treatment to 0.70 ± 0.05 c.u. after treatment ($p<0.01$). In contrast, the CG showed a moderate increase in ABI from 0.40 ± 0.05 c.u. before treatment to 0.55 ± 0.05 c.u. after treatment ($p<0.05$). The difference in ABI between the groups was not significant before treatment, but after treatment, the MG demonstrated significantly greater improvement than the CG (Table 1).

The Visual Analog Scale (VAS) score significantly decreased in the MG from 7,4 ± 0,7 before treatment to 4,3 ± 0,6 after treatment ($p<0,01$). The CG also exhibited a reduction in pain, with VAS scores decreasing from 7,4 ± 0,6 to 6,1 ± 0,5 ($p<0,05$). There was no significant difference between the groups before treatment, but the MG demonstrated significantly greater pain reduction after treatment compared to the CG (Table 1).

In terms of walking distance, 15 (75%) patients in the MG showed improvement after treatment. In the CG, 8 (40%) patients showed similar improvement ($p<0,01$). Moreover, complete healing of trophic ulcers was observed in all patients in the MG after treatment. In contrast, only 5 patients (25%) in the CG experienced complete ulcer healing ($p<0,01$) (Table 1).

Importantly, before treatment, all 20 patients in both groups were at risk of amputation. However, after treatment, only 1 (5%) patient in the MG remained at risk, as compared to 8 (40%) patients in the CG ($p<0,05$). Therefore, the risk of amputation was significantly lower in MG, in contrast to CG (RR 0,13 [95% CI 0,02-0,91]; $p<0,05$).

The implementation of a comprehensive rehabilitation program integrating MSCs therapy, AP, PT, and PE demonstrated significant clinical efficacy in patients with CCLLI. As a clinical case, the dynamics of wound healing in an MG patient are shown in Fig. 2. Before treatment, the patient was unable to walk and required a wheelchair. The ulcer area was 8 cm^2, and the MSCs dose was 60 million cells. The effectiveness of such therapy is well-documented in the literature [11-13].

PT included electrotherapy, magnetic therapy, phototherapy, and ultrasound therapy, which resulted in enhanced tissue regeneration, improved muscle strength, and increased range of motion. AP and PT were essential components of the rehabilitation program. In particular, AP promoted microcirculation improvement and pain relief by targeting key points such as ST36 (Zu San Li), SP6 (San Yin Jiao), and BL57 (Cheng Shan) [14, 15].

In summary, the MSCs therapy group showed substantial improvements in pain reduction, functional mobility, and

Table 1. Treatment outcomes in MG and CG

Outcome Measure	MG N=20			CG N=20			p_2	p_3
	Before treatment	After treatment	p_1	Before treatment	After treatment	p_1		
ABI, c.u.	0,39 ± 0,05	0,70 ± 0,05	<0,01	0,40 ± 0,05	0,55 ± 0,05	<0,05	NS	<0,05
Pain intensity (VAS), score	7,4 ± 0,7	4,3 ± 0,6	<0,01	7,4 ± 0,6	6,1 ± 0,5	<0,05	NS	<0,05
WDI achievement, n (%)	-	15 (75)	-	-	8 (40)	-	-	<0,01
Trophic ulcer healing, n (%)	-	20 (100)	-	-	5 (25)	-	-	<0,01
At risk for amputation*/**, n (%)	-	1 (5)	-	-	8 (40)	-	-	<0,05

*Notes: * – Patients at risk for amputation – in the case of a non-healing ulcer or severe pain; ** – RR 0,13 (95 % CI 0,02-0,91) (p<0,05); p1 – the difference between pre- and post-treatment data in MG and CG; p2 – the difference between MG and CG in terms of pre-treatment (baseline) parameters; p3 – the difference between MG and CG in terms of post-treatment parameters; NS – non-significant.*

Fig. 2. Dynamics of wound healing after the introduction of MSCs into the muscles of a separate lower limb.

wound healing, with significantly better outcomes than the CG. These results support the efficacy of MSCs therapy in enhancing peripheral circulation, reducing the risk of amputation, and improving the overall limb condition and quality of life for patients with severe lower limb ischemia.

DISCUSSION

MSCs, derived from the patient's own bone marrow or adipose tissue, offer several advantages, including reduced immunogenicity and the ability to promote tissue regeneration through the release of paracrine factors. These cells have shown potential in enhancing blood flow, stimulating angiogenesis, and reducing inflammation, all of which are crucial in the management of CCLLI [5, 9].

While the clinical evidence supporting MSCs therapy for CCLLI is still in its early stages, preclinical studies and early-phase clinical trials have shown promising results, particularly in improving ischemic symptoms such as pain at rest, walking capacity, and tissue oxygenation. Notably, the ABI, transcutaneous oxygen pressure (TcPO2), and pain-free walking duration have been used as key indicators of treatment success, with MSCs therapy showing significant improvements in these parameters [2, 3].

Autologous MSCs have emerged as a crucial component of a multidisciplinary rehabilitation approach, which integrates various therapeutic strategies aimed at improving both the immediate and long-term outcomes for patients suffering from severe ischemia and associated pain syndromes.

However, challenges remain in standardizing treatment protocols, determining the optimal MSC dosage, and ensuring long-term safety and efficacy, especially in the context of war-related injuries and other complex medical conditions. It should be noted that in the literature, we did not find any publications on the use of stem cells in limb ischemia due to gunshot wounds.

The full therapeutic potential of MSCs in the context of war-related injuries and ischemia requires a multidisciplinary approach. Furthermore, PT and targeted PE play a critical role in maintaining joint mobility, reducing muscle atrophy, and improving the functional capacity of affected limbs.

Incorporating non-pharmacological methods, such as AP, PT, and preformed growth factors, can enhance the regenerative process. For example, AP has been shown to provide effective pain relief and improve blood circulation and immunity, which can support the healing of ischemic tissues [14, 15].

The combination of MSCs therapy with non-invasive treatments allows for a more comprehensive approach to pain management, tissue recovery, and functional rehabilitation, which are essential for improving the quality of life for war participants suffering from CCLLI. Furthermore, this integrative strategy aligns with the growing emphasis on personalized medicine, where therapeutic interventions are tailored to the individual's specific needs, medical history, and the nature of their injuries.

In conclusion, the use of autologous MSCs as part of a multidisciplinary rehabilitation strategy offers significant potential for improving outcomes in war participants with CCLLI and associated pain syndromes. The synergy between MSCs therapy and complementary rehabilitation methods may provide a comprehensive solution that addresses both the physiological and psychological aspects of recovery. However, further research is required to optimize treatment regimens, establish long-term efficacy, and determine the broader applicability.

CONCLUSIONS

CLI is a life-threatening condition that particularly requires the accelerated implementation of advanced treatment methods. The integration of MSCs therapy, AP, PT, and rehabilitation PE offers significant benefits in treating CCLLI, accelerating recovery, and improving functional outcomes for military personnel with combat-related injuries. This approach supports tissue regeneration, pain relief, and the prevention of limb amputations.

Current regulatory frameworks allow the use of MSCs in treating CCLLI. Autologous MSCs avoid rejection risks but present challenges, such as difficulty in harvesting and culturing large quantities. Cell therapy is a safe intervention with a low risk of early complications.

Clinical applications of MSCs for limb ischemia support their recommendation in standard treatment protocols. Combining MSCs therapy with multidisciplinary rehabilitation improves recovery in military personnel with combat-related ischemia. Further studies are needed to refine protocols and confirm long-term benefits.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Estimation of plasma TGF-β1 after intra-articular platelet rich plasma injection in-patient with anterior disc displacement with intermittent locking of TMJ disorder

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ABSTRACT

Aim: To evaluate plasma levels of TGF-β1 and the clinical impact of platelet-rich plasma injections on mandibular opening and pain in patients with anterior disc displacement and intermittent locking.

Materials and Methods: Twenty-seven temporomandibular disorder patients, diagnosed by oral medicine specialists, received three intra-articular PRP injections over three months, with evaluations conducted two weeks after the final injection. Plasma TGF-β1 levels were measured before and after treatment, with visual analog scale pain scores and mouth opening measurements.

Results: Results showed that the mean plasma TGF-β1 level in temporomandibular disorder patients increased significantly from (312.6 pg/ml ± 67.96) before PRP injection to (400.4 pg/ml ± 108.51) after PRP injection. Platelet-rich plasma-treated patients also experienced a significant reduction in Visual Analogue Scale scores, which decreased from (6.21 ± 0.95) to (0.78 ± 0.79), with improved range of mouth opening after PRP injection.

Conclusions: Intra-articular platelet-rich plasma injections effectively increased plasma TGF-β1 levels, reduced pain intensity, and improved mandibular opening range in temporomandibular disorder patients.

KEY WORDS: Temporomandibular Disorder, platelet rich plasma, plasma TGF-β

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INTRODUCTION

The temporomandibular joint disorders, usually referred to as TMDs, are ailments that have an impact on the ligaments that are linked with the joints as well as the joints themselves. There is a possibility that these diseases will result in internal disturbance in the joint space, alterations in bone that are permanent, and degenerative abnormalities [1-2]. Platelet-rich plasma (PRP) was proven to lessen inflammation, provide relief from pain, enhance function, and encourage cartilage regeneration at the location where damage has occurred. Furthermore, it is capable of providing function. Using its application, every one of these benefits can be obtained [3]. Growth factor that is transformative Beta cytokines, which are also commonly referred to as TGF-β, are a category of cytokines that have numerous roles. These functions include the regulation of cell proliferation, differentiation, and the creation of extracellular matrix elements [4].

AIM

This study aimed to evaluate TGF-β1 levels in plasma and the clinical effects relating to a mandibular range of opening and pain intensity at the jaw following treatment with platelet-rich plasma injections in patients diagnosed as having anterior disc displacement with intermittent locking.

MATERIALS AND METHODS

This study was conducted from January 5, 2019, to December 29, 2019, with a sample divided into two groups of participants as follows:

Twenty-seven patients (11 male and 16 female) diagnosed with temporomandibular joint disorder, specifically anterior disc displacement with intermittent locking, by a private dental institution oral medicine specialist, received three intra-articular injections of platelets-rich plasma, in each case into the area concerned. The injections were administered monthly for three months. The average age of the participants was 27.48 years.

The diagnosis for every patient was made as temporomandibular joint dysfunction diagnosis, which was based on the clinical criteria obtained. The individual gave his/her consent to participate in the study after he/she was informed of the research objective. Details of the information included the name, gender, age, medical history, family history, based pain VAS, mandibular range of motion, and masticatory muscle examination of the patient recorded on the case sheet, among other pertinent details that should be noted. Also considered during the evaluation was the patient's medical history, which was among other details that had to be noted down. The age of the patient was included under the supplementary information provided. A treatment plan as a guide for oral

and maxillofacial specialists in their palpable derangement practice, an oral and maxillofacial specialist would make significant use of the Wilkes staging system for internal derangement of the temporomandibular joint (TMJ) to provide a treatment plan that is dependent on the severity of the internal joint damage. This allows them to provide a treatment strategy that is not only effective but also appropriate. According to this approach, the severity of the ailment increases from mild to severe over time. As a result, the Wilkes staging system is utilized by a significant number of medical professionals [5].

Inclusion criteria included:

- Pain localized in the afflicted temporomandibular joint, particularly during mouth opening.
- Restricted oral aperture (less than 35 mm)
- The patient is refractory to conservative interventions, including splint therapy.

Exclusion criteria included:

- Patients with systemic illnesses, platelet function abnormalities, or fibrinogen insufficiency [6].
- Individuals with a history of temporomandibular joint surgery.
- Patients having a history of joint fractures or infections.
- Patients administered anticoagulants or nonsteroidal anti-inflammatory medications within 48 hours before to surgery [7], Administration of a corticosteroid injection at the treatment site within one month, or systemic corticosteroid use within two weeks.

In the context of quantitative analysis of this important molecule, ELISA kits that were developed by BT Co. LTD. are utilized to determine the amount of human TGF-B1 (Transforming Growth Factor Beta 1) that is present in serum, plasma, cell culture supernatant, and tissue homogenate. This is done to determine the amount of this significant molecule that is present in these samples. One of the objectives of these kits is to determine the amount of human TGF-B1 that is present in the samples that are being examined. The blood was collected at a medical facility that was not affiliated with any other organization. As part of the procedure, an anticoagulant medication known as acid citrate dextrose (ACD-A) was going to be administered to a platelet-rich plasma (PRP) vacuum tube that held a total volume of 5 milliliters of venous blood. Chosen to prevent blood clots from forming, this choice was made with the aim of not allowing their formation. ELISA tests were performed following the provided recommendations, which were given by the manufacturer, BT LTD Business Research Institute. The tests aimed to establish the levels of TGF-β1 expression.

PREPARATION OF PLATELET- RICH PLASMA

The instructions by Mazzocca et al. were followed in the creation of PRP with modifications as detailed in the references [8-9]. The blood should be inverted and mixed well immediately after collection in the PRP tube before delivery to the patient. PRP tube was centrifuged at an RPM of 3000 for between nine and ten minutes. Gel was observed at the top when centrifugation was complete; red blood cells were seen to have settled at the bottom of the gel. The layer at the top surface of the visible gel was found to be the buffy coat; the red blood cells were at the bottom of the gel. The platelet-rich plasma tube was first vortexed.

INJECTION OF PRP THERAPY

Platelet-rich plasma, commonly known as PRP, is obtained by drawing a line from the center of the tragus to the outer canthus of the eye. This is done to obtain the best possible results. The technique begins with this specific line, which is the starting point of the technique itself. This allowed the patient to receive the plasma. After that, there was an injection of the solution using this line. This was done to prepare the patient for the procedure. For the purpose of determining the injection point, this line was utilized. A spot that is 10 millimeters anterior to the middle of the tragus and 2 millimeters inferior to the line is where the injection is going to be administered. This distance is measured along the line. Above the line, this distance is defined as the distance. To begin the process of decontaminating the field, an antiseptic solution was used to wash the region surrounding the joint. The first thing that needed to be done to maintain the cleanliness of the field was to do this step. Following that, the patient was told to partially open his or her lips, which led to the formation of a pre-auricular concavity at the spot where the injection was delivered. This was carried out as part of the procedure that was being carried out. One milliliter of platelet-rich plasma (PRP) was injected into the region of the superior joint after the needle was positioned in the medial and slightly anterosuperior locations. This was done after the needle was placed. During the process of inserting the needle, this was carried out. Right up until the moment when the needle made its initial contact with the glenoid fossa, this process was carried out in its entirety. Immediately after the needle has been removed and the skin has been washed, platelet-rich plasma, also known as PRP, is injected into the joint region. The next step is to inject platelet-rich plasma into the joint area, which is after this step. The person who was receiving treatment was instructed to repeatedly open and close his or her lips for a period of one minute. This was done by the findings that were discovered by Comert and colleagues. Performing this action was done to ensure that the platelet-rich plasma (PRP) was disseminated uniformly across the joint region [10].

STATISTICAL ANALYSIS

A paired t-test was used to compare plasma TGF-β1 levels in pre-injection and post-injection records; also, the same test was used to test the differences in mouth opening records before and after intervention. The Wilcoxon Signed Rank Test was applied to test the changes in the Visual Analogue Scale before and after the intervention. All data analysis was performed using the SSPS program (IBM SSP statistics 26).

Table 1. Variations in plasma TGF-β1 levels before and after PRP injection

Plasma TGF-β1 Level (Pg/ml)	Mean ± SD	t-test*	P value
Pre-injection	312.6 ± 67.96	-5.59	0.0001
Post- injection	400.4 ± 108.51		

**Different letters refer to high significant difference at P value < 0.01; SD: Standard Deviation. *Paired t-test.*

Table 2. Variations in the opening of the mandible of patients before and following intra-articular PRP injection

	Before injection (Mean ±SD)	Post injection (Mean ±SD)	Paired t-test	P value
Mouth Opening	32.3mm (3.52)	44.1mm (2.77)	8.17	0.000 *

**High significant at P value < 0.001, SD=standard deviation.*

Table 3. Variations in the Visual Analogue Scale scores of patients prior to and following intra-articular PRP injection

	Pre-Patients (MS ±SD)	Post Patients (MS ±SD)	n	Z test	P value
Visual Analogue Scale	6.21 ± 0.95	0.78 ± 0.79	27	4.2	0.000*

*MS: Mean of Scores; SD: Standard Deviation * p < 0.001: High significant difference by Wilcoxon Signed Rank Test.*

RESULTS

The mean of plasma TGF-β1 level in TMJ disorder patients before intra-articular PRP injection was (312.6 Pg/ml ± 67.96), and the mean of plasma level of TGF-β1 after intra-articular PRP injection was (400.4 Pg/ml ± 108.51). The P-value was significantly different (P-value < 0.001) as shown in Table 1. The jaw opening was highly increased significantly after PRP injection (P-value < 0.001) as shown in Table 2.

It was shown that patients who got platelet-rich plasma (PRP) injections had a mean pain intensity of 6.21 (standard deviation ± 0.95) during the initial presentation. This revelation was made after taking into consideration the level of pain that the patients were experiencing. This level of pain was decreased obviously to a mean of 0.78 (standard deviation ± 0.79), which was significantly lower with a p-value of 0.000. This was a significant difference from pre-injection records, as shown in Table 3.

DISCUSSION

It was noted that the patients who took part in this particular study had a standard difference of ± 6.24 years in their ages. Among the patients, the average age was determined to be 27.48 years. A lot of studies that have been carried out in the past have revealed that individuals who are between the ages of 20 and 32 years old have a higher risk of having temporomandibular disorder (TMD). An important consideration is that TMD is more prevalent in this age group [11-12]. All of these findings are in agreement with the study by Marcelo et al. [13]. The researchers investigated the relationship between age and the severity of temporomandibular disorders (TMD) and found such correlation between age and the severity of TMD. In the present study, the patients who received conservative treatment or platelet-rich plasma (PRP) were overrepresented by females compared to males at baseline. Functional impairment and pain were also more severe in females, with symptoms also including persistent and prolonged expressions, and were most prevalent in females over 40 years of age. This is because female patients tend to be exposed to the disease over longer periods. Additionally, the symptoms of female patients might be longer than those of male patients. The reason for this is that female patients are more likely to exhibit symptoms of hormonal, behavioral, and psychological disorders than male patients. To account for these trends, female patients might have problems related to hormones, behavior, and psychology [14]. In this study, we used the VAS scale to evaluate the intensity of discomfort recorded within two weeks after the third PRP injection. Based on our results, pain fell below what might be considered typical for the general population. This statement can be justified by the study results conducted by Hanci et al. [15]. Twenty patients with temporomandibular joint disorders were injected with platelet-rich plasma (PRP) and then discovered by the researchers to be successful in reducing pain for them. Former studies claim the therapeutic efficacy of platelet-rich plasma for knee inflammation and intra-articular cartilage lesions. This is explained through the benefit of immediate pain relief and rapid restoration of function. The study has also revealed significant anti-inflammatory effects for the plasma. Chondrocytes, mesenchymal stem cells, and synovial cells show an anabolic effect induced by platelet-

rich plasma (PRP). Moreover, compared with the baseline, it improved the replicative capacity of the cells [16]. Data from basic science, preclinical, and clinical studies may justify the potential use of platelet-rich plasma (PRP) as a method of therapy for joint diseases and symptomatic treatment. This hypothesis has been drawn directly from clinical data, while the results from the experimentation carried out both clinically and preclinically justify these findings, or, in other words, support them. Therefore, the conclusions that were drawn as a consensus on the viability of PRP treatment came from these findings [17]. PRP injections involve a reduced inflammation effect that shows detectable signs of alleviating osteoarthritis symptoms, demonstrating its clinically acceptable safety profile [18]. The key active element in all this is the formation of the cartilage extracellular matrix. This is because in cases where there is wear and tear on the cartilage tissue and hence osteoarthritis, it can be considered as one factor in reducing the healing process of cartilage restoration [19]. The factor TGF-β1 plays its role. This is because TGF-β is needed for the cartilage regeneration process, whose sole cause is this condition [20]. Intra-articular injections may stimulate the process of cartilage regeneration as well as reduce inflammation and discomfort; eventually, the function of the jaw improves. According to this concept, the protease-activated receptor 4 peptide is believed to be responsible for the analgesic potential of platelets. It may thus be platelets that have such ability. This is suggested by the fact that platelets would have such an ability [21]. Among the prior research that disputed our findings, one of them revealed that transforming growth factor-β (TGF-β) has been attracting a rising amount of interest about the pathogenesis of osteoarthritis (OA). When knee joint osteoarthritis (OA) is present, a considerable breakdown of cartilage occurs as a result of a modulation in the signaling activity of TGF-β1. Also, it was observed that chondrocytes in models of osteoarthritis (OA) conditions had a significant amount of TGF-β1. Furthermore, it was demonstrated that an animal model of osteoarthritis (OA) involving an anterior cruciate ligament transection (ACLT) displayed a considerable quantity of TGF-β1 that was present within the subchondral bone. In addition, when there is an alteration in the expression of TGFβ1 signaling in subchondral bone, it may result in abnormal remodeling, which can be detrimental to the integrity of cartilage. Probably, TGF-β1 could potentially have a significant role in the progression of temporomandibular joint osteoarthritis (TMJ-OA), taking into consideration the information that has been discussed above within this context [22].

CONCLUSIONS

Individuals who suffered from anterior disc displacement and intermittent locking of the temporomandibular joint (TMJ) were shown to have significantly improved clinical results after receiving platelet-rich plasma (PRP) that was injected into the joints. The treatment led to increased plasma TGF-β1 levels, reduced pain intensity, and enhanced mandibular range of motion after PRP injection. PRP therapy shows promise as an effective, minimally invasive option for managing TMJ disorders. Further studies are needed to explore its long-term efficacy and mechanisms.

ABBREVIATION

TMD: Temporomandibular Disorder
TMJ: Temporomandibular Joint
PRP: Platelet-Rich Plasma
VAS: Visual Analogue Scale
ACD-A: Acid Citrate Dextrose

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ORIGINAL ARTICLE

Dynamics of the size ratio between the cerebellar vermis and the fourth ventricle of the human brain during the fetal period of ontogenesis

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ABSTRACT

Aim: To determine the patterns of change in the cross-sectional area of the cerebellar vermis and the fourth ventricle during the fetal period of human ontogenesis and to analyze the dynamics of their interrelationship.

Materials and Methods: The study was conducted using magnetic resonance imaging (MRI) scans of the human fetal brain obtained from open-access databases. Generalized images from the "Fetal Brain Atlas" (21-38 weeks of gestation) and MRI scans from the "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" database (20-39 weeks of gestation, n=20) were used. Morphometric analysis of mid-sagittal sections was performed using the Image Tool software. The cross-sectional areas of the cerebellar vermis and the fourth ventricle were measured, along with their relative values and ratios.

Results: Between 21 and 38 weeks of gestation, the cross-sectional area of the cerebellar vermis increased steadily, whereas the fourth ventricle exhibited a biphasic growth pattern: relatively slow expansion until 30 weeks, followed by accelerated growth. The analysis of relative areas revealed that the growth rate of the cerebellum exceeded that of the fourth ventricle until 30-32 weeks, after which the fourth ventricle underwent rapid expansion, leading to an alignment of their relative values. The ratio of the fourth ventricle's area to that of the cerebellum ranged from 4.8% to 9.8%, showing minimal values at 25-28 weeks and rapid growth between 28 and 34 weeks of gestation.

Conclusions: The obtained data indicate changes in the proportional relationship between the cerebellum and the fourth ventricle throughout the fetal period of ontogenesis, reflecting the specific features of their morphogenesis. The proposed approach for assessing the interrelationship between these structures may be useful for analyzing both normal and abnormal hindbrain development during the prenatal period.

KEY WORDS: brain, cerebellum, fourth ventricle, development, fetus, morphometry, MRI

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INTRODUCTION

The cerebellum is one of the key structures of the central nervous system, involved in the coordination of movements and maintaining balance. Its anatomical relationship with the fourth ventricle of the cerebrum is close, since it is the cerebellum, together with the superior and inferior medullary velum, that forms the roof of this ventricle [1, 2].

In addition to their anatomical relationship, the cerebellum and the fourth ventricle share a common embryonic origin. Both structures are derived from the metencephalon, which in turn is formed from the rhombencephalon [3, 4]. During embryonic development, the upper posterior part of the neural tube that forms the hindbrain thickens to give rise to the cerebellum, while the neural tube cavity in this area expands to form the fourth ventricle [5, 6].

Developmental abnormalities of the hindbrain can lead to a variety of malformations of the cerebellum and fourth ventricle [7, 8], including Dandy-Walker malformation [9-11]. The development of a complex of structures originating from the hindbrain, including the cerebellum and fourth ventricle, necessitates a detailed analysis of the patterns of development of the hindbrain, including the relationships and normal dynamics of the development of the cerebellum and fourth ventricle.

One of the most informative methods for studying brain morphology during intrauterine development is magnetic resonance imaging (MRI). This method allows obtaining high-quality images of brain structures, which is critically important for determining normal and pathological changes in its development. The use of MRI in the study of the cerebellum and ventricular system allows analyzing the dynamics of their growth and relationships at different gestational ages [12].

To date, most research on the ventricular system of the brain has focused on the lateral ventricles. They usually determine the linear dimensions of the ventricles and their

cross-sectional area [13, 14]. In contrast, studies on the fourth ventricle are much less numerous, which limits current ideas about the patterns of its development and relationships with other brain structures. The assessment of the fourth ventricle is most often carried out in a neurosurgical aspect, since its walls are important landmarks for performing certain surgical interventions [15, 16]. However, from the point of view of neuromorphology, it is important to study the fourth ventricle in the context of its relationship with the cerebellum, which will allow a better understanding of the patterns of its morphogenesis.

Various methods are used to analyze the dynamics of cerebellar development, including determining its linear dimensions [17] or the cross-sectional area of the cerebellar vermis [18]. The cross-sectional area of the vermis is a universal parameter that has been used both to assess cerebellar development in the early postnatal period [18] and to analyze changes associated with aging [19, 20]. This indicator was determined both on the basis of MRI tomograms [21] and on cadaveric brain specimens [22, 23], which confirms its reliability and representativeness. In this work, the task was to determine the cross-sectional area of the cerebellar vermis and the cross-sectional area of the fourth ventricle in human fetuses at different gestational ages to analyze the dynamics of their relationship during development.

AIM

The aim of the research was to determine the regularities of change in the cross-sectional area of the cerebellar vermis and the fourth ventricle during the fetal period of human ontogenesis and to analyze the dynamics of their interrelationship.

MATERIALS AND METHODS

The study was conducted using MRI of the human fetal brain obtained from open research databases. To determine the dynamics of changes in the absolute sizes of the cerebellum and the fourth ventricle, images from the atlas "A normative spatiotemporal MRI atlas of the fetal brain for automatic segmentation and analysis of early brain growth" ("Fetal Brain Atlas") has been applied [24]. This atlas contains generalized images of the human fetal brain from 21 to 38 weeks of gestation, built on the basis of MRI of the brain of 81 fetuses. Additionally, MRI of the brain of 20 human fetuses at gestational ages of 20-39 weeks, selected from the open database "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" [25, 26]. Mid-sagittal tomographic sections were examined (Fig. 1).

Morphometry was performed using the Image Tools program. The cross-sectional area of the cerebellar vermis (cb) and the cross-sectional area of the fourth ventricle (IV) were determined on the images, Fig. 2.

RESULTS

The values of the cross-sectional area of the cerebellar vermis, determined as a result of morphometry of images from the «Fetal Brain Atlas» [24], varied from 7417 mm^2 to 30492 mm^2; the cross-sectional area of the fourth ventricle varied from 401 mm^2 to 2712 mm^2. The areas of both structures gradually increased during the studied interval of intrauterine development – 21-38 weeks of gestation (Fig. 3). However, while the growth of the cerebellar vermis area was mostly evenly (Fig. 3, a), the cross-sectional area of the fourth ventricle increased more slowly during 21-30 weeks and increased more intensively during 30-39 weeks of gestation (Fig. 3, b).

In order to analyze the relationship between the dynamics of the development of cerebellar vermis and fourth ventricle, the relative values of the cross-sectional areas were calculated: the maximum values of the areas (100%) were taken as the basis, and the relative area was calculated as a percentage of the area relative to the maximum value. For this analysis, the relative values of the cerebellar area were calculated based on the results of morphometry of images from the "Fetal Brain Atlas" [24]

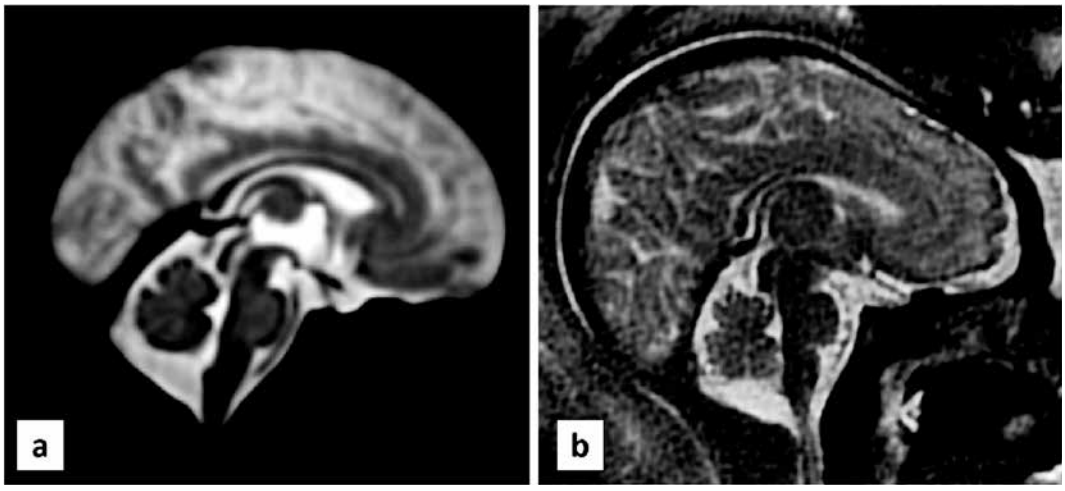

Fig. 1. Magnetic resonance imaging of the human fetal brain (36 weeks of gestation), mid-sagittal plane: a – atlas "Fetal Brain Atlas" [24], b – database "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" [25, 26].

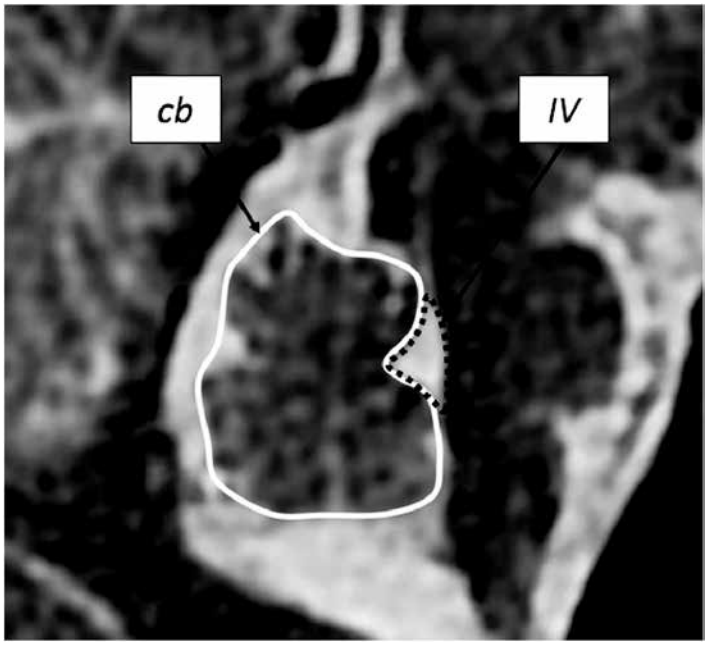

Fig. 2. Methodology of magnetic resonance tomogram morphometry: measurement of the cross-sectional area of the cerebellar vermis (cb) and the cross-sectional area of the IV ventricle (IV).

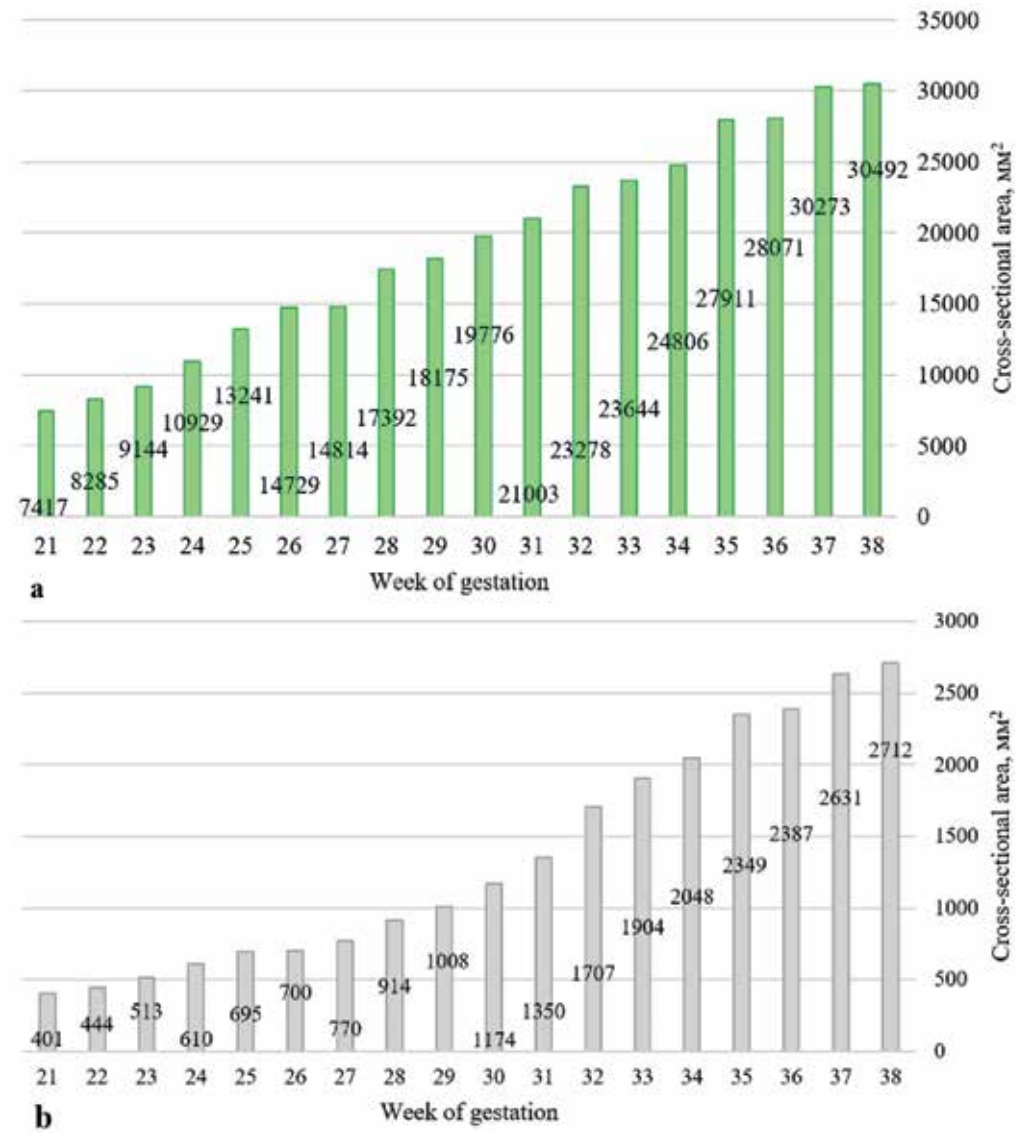

Fig. 3. Dynamics of changes in the cross-sectional area of the cerebellar vermis (a) and the IV ventricle (b) of the human fetal brain during 21-38 weeks of gestation.

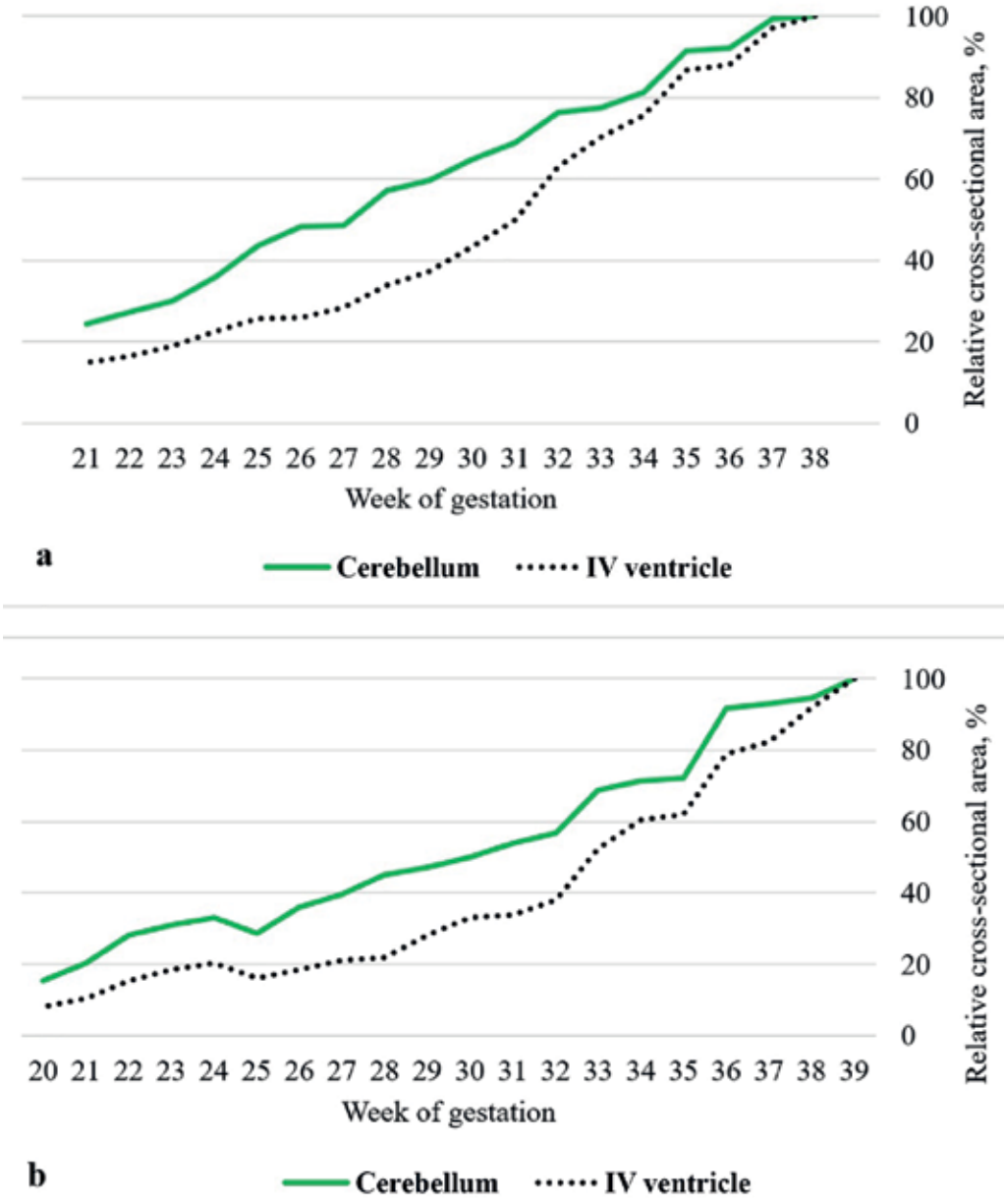

Fig. 4. Dynamics of changes in the relative cross-sectional area of the cerebellar vermis and the IV ventricle of the human fetal brain during 20-39 weeks of gestation: a – atlas "Fetal Brain Atlas" [24], b – database "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" [25, 26].

(Fig. 4, a) and magnetic resonance tomograms from the "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" database [25, 26] (Fig. 4, b). As can be seen in Fig. 4, the data obtained when studying both the atlas and magnetic resonance tomograms of different subjects were similar. However, the growth dynamics of the cerebellum and the fourth ventricle were somewhat different: at 20-21 weeks (the beginning of the studied gestational age range), the relative area of the cerebellum was 15-24% of the maximum value (which was observed at the end of the studied range – 38 or 39 weeks). At the same time, the relative area of the fourth ventricle was significantly smaller and was 8-14% of the maximum value. Further, during 20-32 weeks of gestation, different rates of growth of the area of the cerebellum and the fourth ventricle were observed: the size of the cerebellum increased more intensively, which led to an increase in the distance between the curves of the graphs characterizing the dynamics of growth of the cross-sectional area of the cerebellar vermis and the fourth ventricle. Starting from 30-32 weeks, an intensification of growth of the size of the fourth ventricle is observed, as a result of which the curve characterizing the dynamics of growth of the relative area of this structure, at the end of the studied range of gestational terms, approaches the curve characterizing the dynamics of growth of the size of the cerebellum.

To determine the relationship between the sizes of the cerebellum and the fourth ventricle, the ratio of the cross-sectional area of the fourth ventricle (IV) to the cross-sectional area of the cerebellar vermis (cb) was calculated

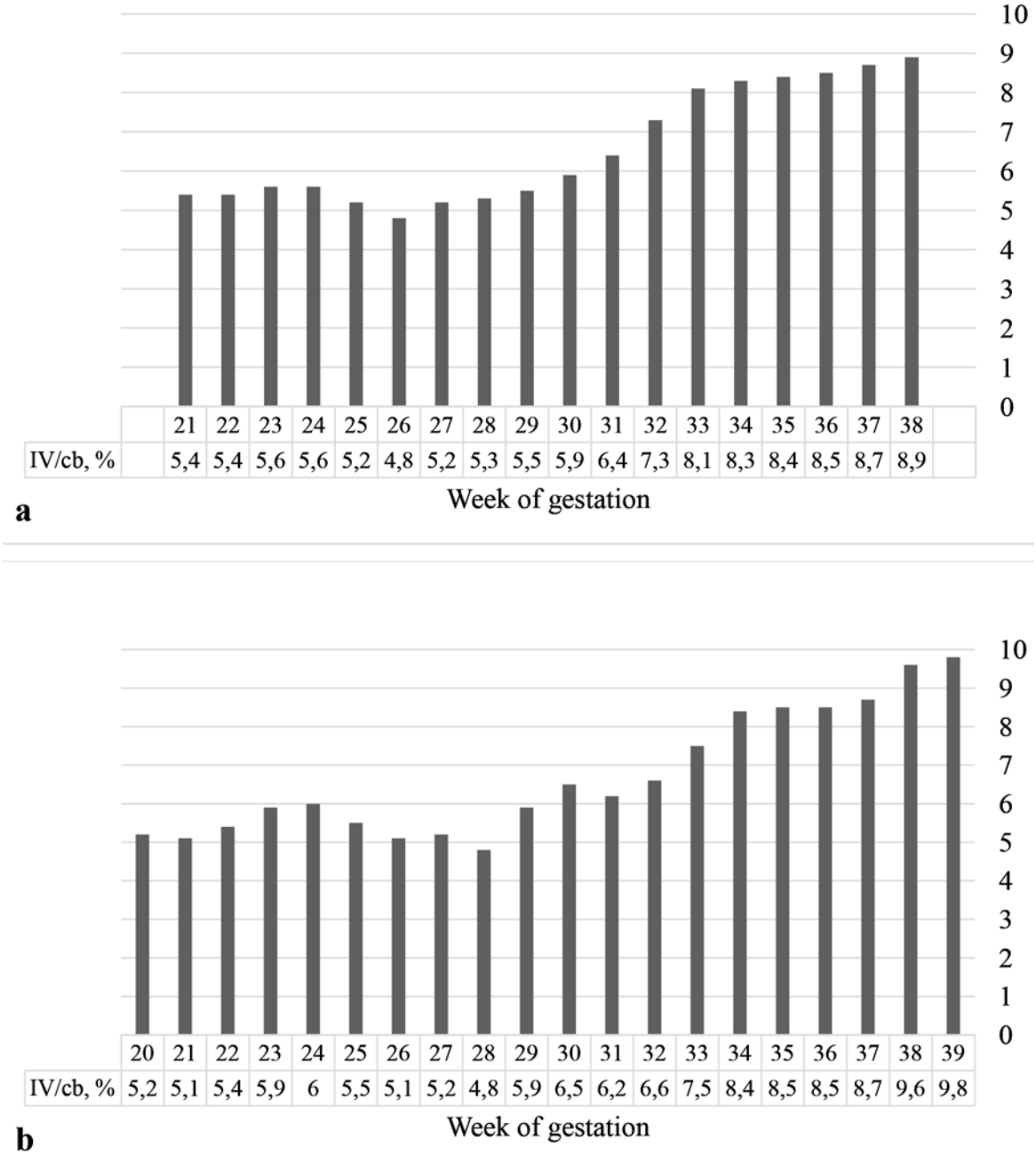

Fig. 5. The ratio of the cross-sectional area of the IV ventricle to the cross-sectional area of the cerebellar vermis during 20-39 weeks of gestation, a – atlas "Fetal Brain Atlas" [24], b – database "Fetal Brain MRI from Stanford Lucile Packard Children's Hospital" [25, 26].

as (IV/cb)×100% (Fig. 5). This ratio varied from 4.8% to 9.8%. Analyzing this ratio, it is possible to determine the following periods characterized by different dynamics of this index: during 20-24 weeks of gestation, the IV/cb ratio is relatively stable, but in the period of 25-28 weeks this index reaches its minimum values, which is associated with more rapid growth of the cerebellum. Starting from the 28th week and up to 33-34 weeks of gestation, a rapid increase in the ratio (IV/cb) is observed, which corresponds to the intensification of the growth of the fourth ventricle. In the period of 34-39 weeks, this index continues to grow, but not as intensively as was observed in the previous period.

DISCUSSION

The study of the dynamics of the development of the cerebellar vermis and the fourth ventricle of the brain in human fetuses, described in this work, made it possible to identify the patterns of their growth and relationships in the fetal period of ontogenesis. The results obtained indicate that the absolute dimensions of both structures increase during the studied gestational period, but the dynamics of their growth has certain differences. The growth of the cross-sectional area of the cerebellar vermis occurs gradually and evenly, while the fourth ventricle increases more slowly in the early stages (up to 30 weeks

of gestation), after which its growth accelerates. This may be due to the peculiarities of the morphogenesis of the hindbrain, in particular, the peculiarities of the growth of the cerebellum and the superior and inferior medullary velums: the intensification of their growth may contribute to the acceleration of the increase of the cavity of the fourth ventricle.

The closest work to this study is the research of Hayakawa K. et al. [18], where a study of the dynamics of the cross-sectional area of the cerebellar vermis during postnatal ontogenesis was conducted. The most intensive increase in this indicator was observed during the first two years, slightly decreasing its speed up to four years; at 6-8 years of age, the cross-sectional area of the cerebellar vermis reached values similar to those in adults and was maintained throughout life. In other works related to the aging of the brain, a gradual and moderate decrease in the cross-sectional area of the cerebellar vermis in adulthood is noted [19-21].

Taking into account the proportions of the growth of the cerebellum and the fourth ventricle during brain development is important for the diagnosis of malformations. In disorders of the development of the cerebellum and/or the fourth ventricle, the proportionality of the sizes of these structures, characteristic of a certain gestational period, may be disturbed. For example, cerebellar hypoplasia may lead to a decrease in the cross-sectional area of the cerebellar vermis [8], while the dimensions of the fourth ventricle may remain normal. In Dandy-Walker malformation, a violation of the formation of both the cerebellar vermis and the fourth ventricle [9, 10] is observed, which can lead to a significant disproportion of the sizes of these structures. Therefore, determining the proportions of the sizes of the cerebellum and the fourth ventricle can be used as an additional morphometric criterion for assessing brain development in prenatal ontogenesis [27].

Further studies involving a larger sample with a wider range of gestational ages may help clarify the identified trends and identify possible individual variations in the development of these structures.

CONCLUSIONS

1. In the fetal period of ontogenesis, the cross-sectional areas of the cerebellar vermis and the fourth ventricle of the human brain gradually increases, but the dynamics of their growth has certain differences. The cerebellar vermis is characterized by a predominantly an even increase in cross-sectional area throughout the entire period studied. The growth of the fourth ventricle is uneven: in early gestational periods (up to 30 weeks), its cross-sectional area increases more slowly, while after 30 weeks there is an intensification of growth.
2. The ratio of the cross-sectional area of the fourth ventricle to the cross-sectional area of the cerebellar vermis varies during gestation, reaching minimum values at 25-28 weeks and gradually increasing after 28 weeks.
3. The results obtained may be useful for further studies of the normal and pathological development of the cerebellum and the ventricular system of the brain in the fetal period of ontogenesis. The ratio of the sizes of the fourth ventricle and the cerebellar vermis can be used to assess the dynamics of brain development, in particular for the diagnosis of malformations.

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ORIGINAL ARTICLE

Evaluation of the patient-centric principle in the organization of medical care and prevention of open-angle glaucoma progression

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ABSTRACT

Aim: To investigate the patient-centred principle in preventing and providing medical care for open-angle glaucoma.

Materials and Methods: A systematic approach was implemented using a comprehensive set of methods. A self-developed questionnaire was used to collect data. The patient survey was conducted from 1 May 2024 to 30 August 2024. A targeted selection of respondents who underwent surgical treatment for primary open-angle glaucoma from all regions of Ukraine was applied using the main array method (N = 1000).

Results: The study revealed that satisfaction with ophthalmological care was expressed by more than 90% ± 0.95% of respondents (rate per 100 respondents ± standard error). A hereditary predisposition to glaucoma was identified in 20% ± 1.26% of respondents. In more than 80% ± 1.26% of cases, glaucoma was detected by an ophthalmologist, while in more than 17% ± 1.88% of cases, it was identified by a family doctor.

Conclusions: In order to defect glaucoma early and monitor the progression of the disease, there is a need for informational and methodological work with primary care doctors, implementation of algorithms for preventive examinations of patients. Patient awareness regarding the safety and effectiveness of glaucoma surgery affects the timeliness of surgical intervention and vision preservation.

KEY WORDS: patient-centric approach, ophthalmological care, public health

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INTRODUCTION

Health holds exceptional significance in every person's life. The state bears the responsibility for safeguarding and promoting citizens' health [1]. One of the fundamental approaches that the Ministry of Health and the Government consider essential is the patient-centric principle, where the individual (patient) is placed at the center of focus and interest in the healthcare system [2, 3]. Healthcare reform in Ukraine and the development of family medicine based on a holistic approach, which considers physical, mental, and social factors in patient treatment alongside disease diagnosis, contribute to implementing the patient-centric principle [4]. The professionalism of any physician, including ophthalmologists, family doctors, and general practitioners, should encompass qualities such as responsibility, communication skills, humanity, empathy, sensitivity, respect, tact, patience, tolerance, and care.

According to official statistics, an average of 4,500 cases of primary disability due to eye diseases are registered annually in Ukraine, with half of these patients being blind. The total number of blind and visually impaired people in Ukraine exceeds this figure by tenfold. Approximately 5% of people over 40 years of age suffer from glaucoma. Patients increase by about 12% every five years [5]. By 2023, over 200 000 people in Ukraine were diagnosed with glaucoma, with approximately 25 000 new cases identified annually [6]. The forecasts regarding the progression of this issue in the country are bleak. However, global practice shows that blindness can be prevented in 80% of cases.

Glaucoma is the leading cause of irreversible blindness worldwide. According to studies, over 76 million people globally suffered from glaucoma in 2020, up from 60,5 million in 2010. By 2030, the number of people with glaucoma is projected to reach 95,4 million, and by 2040, it will reach 112 million [7, 8].

Primary open-angle glaucoma is the most common form of glaucoma, accounting for between 70% and over 80% of all diagnosed cases. In 2011, there were 2.7 million people aged 40 and older with primary open-angle glaucoma in the United States, and this number is expected to rise to 7.3 million by 2050 [9].

Detecting glaucoma and initiating treatment at early stages lead to significantly better clinical and economic outcomes. Research by the American Academy of Ophthalmology indicates higher expenses for outpatient care in patients with advanced-stage glaucoma compared to those in the early or moderate stages. Medication costs also increase significantly in patients with advanced glaucoma [10]. The progression of this disease is associated with vision loss and a decline in quality of life. Additionally, patients with glaucoma are more prone to falls and fractures compared to individuals without the disease [11, 12].

Known causes of blindness from glaucoma include late-stage detection, ineffective treatment due to therapy

refusal because of the high cost of medications, patient neglect of medical recommendations, and insufficient public awareness about the severity of the disease, which results in a lack of seriousness in addressing it.

Since glaucoma leads to incurable blindness, it is viewed as a critical medical, social, and economic issue. This underscores the importance of studying glaucoma epidemiology, the factors influencing its spread, and preventive methods for both glaucoma itself and its consequence – blindness.

The main regulatory document for providing care to patients with open-angle glaucoma in Ukraine is the "Glaucoma" Medical Care Standard, approved by the Order of the Ministry of Health of Ukraine No. 959 on May 26, 2023. According to this standard, glaucoma treatment aims to reduce intraocular pressure (IOP) and slow down visual field deterioration. Slowing the progression of the disease positively impacts vision-related quality of life. Surgical methods for glaucoma treatment are used in cases of high pressure that cannot be reduced to target levels through non-surgical procedures. As the target pressure level varies, glaucoma patients require regular assessments of eye and vision function, necessitating ongoing monitoring, control, and adjustments based on their visual function status [2].

At the state level, according to the Law of Ukraine No. 2573, "On the Public Health System," from September 6, 2022, the fight against glaucoma is directed toward health promotion, disease prevention, treatment, and rehabilitation. The most significant emphasis is placed on preventing disease development. Since glaucoma has hereditary characteristics, primary prevention (prevention of its occurrence) is challenging. From a preventive standpoint, the main focus is on secondary prevention to prevent glaucoma progression (early diagnosis and effective treatment) [13].

AIM

The aim of the study was to to investigate the patient-centric principle in preventing and providing medical care for open-angle glaucoma.

MATERIALS AND METHODS

A systematic approach was implemented in this study, utilizing a complex set of methods, including statistical, and sociological methods for data collection, processing, and analysis. The collection of information was carried out by the method of patient of patient questionnaires from May 1, 2024, until August 30, 2024, in healthcare facilities where medical care is provided to patients with glaucoma. A questionnaire, reviewed by the Department of Public Health at the Institute of Public Health and Preventive Medicine, Bogomolets National Medical University, was developed as the research instrument (Appendix). A targeted sample of respondents who had undergone surgical treatment for primary open-angle glaucoma was selected from all regions of Ukraine using the primary array method (N = 1000). Of these, 642 ([hereinafter – rate per 100 respondents ± standard error] 64%±1,52%) were operated in private ophthalmology clinics and 358 (36%±1,52%) in public specialized departments or hospitals. The age range of patients was 21 to 86 years, with 487 (49%±1,58%) male and 513 (51%±1,58%) female respondents.

Depending on the patients' experience with glaucoma, satisfaction with doctor consultations provided in outpatient clinics, private medical facilities, and hospitals was observed. A key indicator of quality consultation was the completeness of responses from the ophthalmologist to patient questions, along with the provision of comprehensive recommendations. The waiting time for the first appointment or examination indicated inefficient organization within healthcare facilities. Waiting times for medical assistance or consultations in healthcare facilities reflect a remnant of the Soviet healthcare system, indicating poor medical care organization. The Ministry of Health of Ukraine's Order No. 586 of February 28, 2020, approved the procedure for referring patients to healthcare facilities and individual entrepreneurs, mandating the recording of referrals in the electronic healthcare system [14].

The study also analyzed satisfaction with ophthalmological care among vulnerable social groups (pensioners, people with disabilities, internally displaced persons, etc.). Questions regarding respondents' self-assessment of their financial status helped identify their perceived place within the social structure and examine how this perception influences various health-related practices (e.g., speed of seeking medical assistance when experiencing vision problems, choice of healthcare facility, decision to undergo surgical treatment for glaucoma). Additionally, responses to financial self-assessment questions allow for the measuring socio-economic inequality in future studies.

Considering the bioethical principle of respect for personal autonomy, quality organization of ophthalmological care includes the right of the patient to choose their healthcare facility and specific doctor. Several questions were aimed at uncovering patient motivations when selecting a doctor and healthcare facility and at investigating the importance of the cost of medical services as a factor in their decision-making process.

Respondents were asked to evaluate the accessibility and quality of ophthalmological care, specifically in the context of glaucoma. Analyzing responses based on the duration of the respondents' illness and whether they were under regular ophthalmological monitoring allowed for identifying groups of respondents with significant experience in ophthalmological care.

To assess the organization of the glaucoma screening and diagnostic system, respondents were asked questions about the circumstances surrounding their disease diagnosis. Primary healthcare organizations are crucial in this context, so a set of questions was formulated to evaluate the level of attention family doctors give to ophthalmological issues.

ETHICS

In this study, the authors adhered to the Ethical Principles for Medical Research Involving Human Subjects, as outlined in the Helsinki Declaration of the World Medical Association (WMA, 1964) and applicable Ukrainian regulations.

A bioethical review of the prepared article was conducted by the Bioethics and Research Ethics Committee of the Bogomolets National Medical University. A positive expert conclusion was obtained (No. 189, November 25).

RESULTS

Among the patients offered surgical glaucoma treatment, 25% ± 1,37% were first-time visitors to an ophthalmologist with vision complaints. These individuals were diagnosed with glaucoma that required immediate surgical intervention. 75% ± 1,37% of patients had been under the observation of an ophthalmologist for an extended period and were receiving non-surgical treatment. 50% ± 1,58% of respondents did not seek an ophthalmologist's help immediately after noticing vision issues, and 16% ± 1,16% reported experiencing vision problems for a year or more before their visit. 100% of respondents cited vision impairment in one or both eyes as their primary complaint.

40% ± 1,55% of patients were referred to an ophthalmologist by a family doctor. In comparison, 97% ± 0,54% of respondents had a declaration with a family doctor, and 66% ± 1,50% had complained of vision problems. In 75% ± 1,37% of cases, the primary criterion for choosing an ophthalmologist was a recommendation from friends or other doctors. In 20% ± 1,26% of cases, patients had no choice regarding the doctor they could consult. All respondents (97% ± 0,54%) could see an ophthalmologist without a long wait. 90% ± 0,95% expressed satisfaction with the consultation and recommendations provided by the doctor, indicating that patients received adequate care regardless of whether it was provided in a public or private healthcare facility.

Regarding awareness, 92% ± 0,86% of respondents confirmed they were informed about glaucoma, while only 20% ± 1,26% knew about pseudoexfoliation syndrome. 20% ± 1,26% of respondents reported a family history of glaucoma. 80% ± 1,26% of respondents had their glaucoma diagnosed by an ophthalmologist, while 17% ± 1,88% were diagnosed by a family doctor.

77% ± 1,33% of patients are regularly monitored by an ophthalmologist, visiting more than once a year, with 60% ± 1,55% attending 2-4 visits annually. For 50% ± 1,58% of patients, surgical treatment for glaucoma was recommended at the initial visit, with 25% ± 1,37% agreeing to it immediately. The remaining patients postponed surgery due to satisfaction with their current vision and fear of surgery (44% ± 1,55%) or financial difficulties (50% ± 1,58%).

Glaucoma surgery was performed on both eyes for 47% ± 1,58% of respondents, with 93% ± 0,81% finding it more accessible to decide on the second operation. The order of the factors, influencing the decision for surgery on the second eye, was as following (Fig. 1):

97% ± 0,54% of respondents reported that their treatment was paid for out-of-pocket.

Regarding accessibility, 34% ± 1,50% of respondents consider glaucoma care to be fully accessible in Ukraine, while 40% ± 1,55% view it as partially accessible. Additionally, 40% ± 1,55% believe that the quality of care is high, and 28% ± 1,42% consider it to be partially high-quality. Only less than 5% ± 1,54% of respondents believe that healthcare funding in Ukraine is sufficient, while over 50% ± 1,58% consider the introduction of health insurance to be appropriate.

DISCUSSION

The population's health status is a key indicator of the socio-economic development of any country and represents a universal value. Within the priorities of healthcare and public health systems, this indicator occupies a critically important position. Additionally, health is of exceptional significance in the lives of every individual, family, and society as a whole. Preserving and promoting the health of the population is a state priority [1].

One of the main factors for classifying open-angle glaucoma as a socially significant disease in Ukraine and globally is the irreversible deterioration and loss of vision, leading to social and economic losses. Visible signs of vision loss in patients appear at advanced and terminal stages (stages

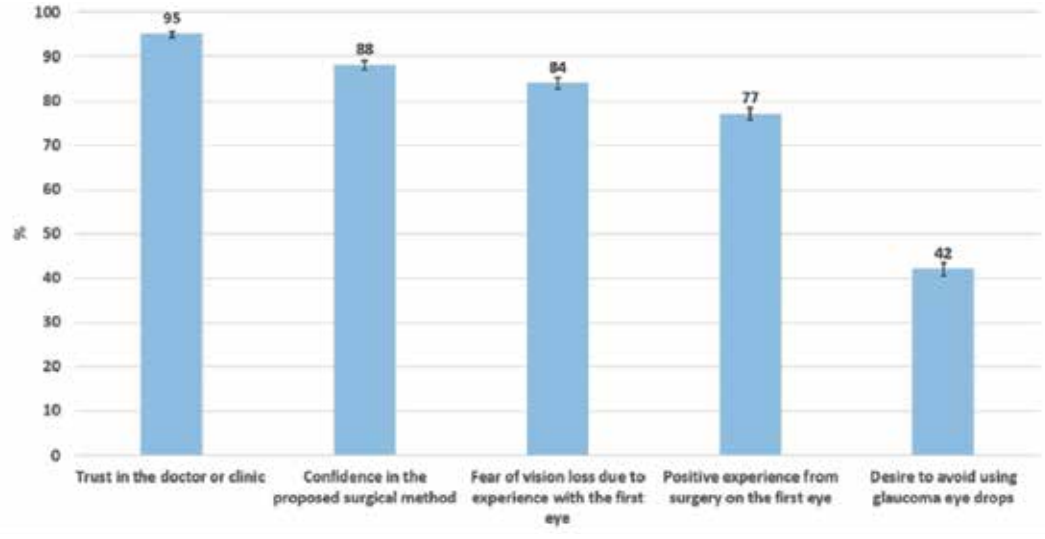

Fig. 1. The factors, influencing the decision for surgery on the second eye.

3-4), while, at the early stages, individuals may overlook significant limitations in visual function. However, the sooner treatment begins, the greater the likelihood of preserving the optic nerve and maintaining stable visual functions. Therefore, early diagnosis of glaucoma is of utmost importance.

In this study, it is noteworthy that only 17% ± 1,88% of cases were diagnosed by family doctors directly, and only 40% ± 1,55% of respondents were referred to an ophthalmologist by their family doctor, despite 66% ± 1,50% of patients reporting vision complaints to their family doctor. These results suggest a lack of awareness among family doctors regarding glaucoma development and the organization of care for this disease, which negatively impacts the timely diagnosis of glaucoma.

Despite 100% of patients experiencing vision deterioration, 50% ± 1,58% did not immediately seek an ophthalmologist, thereby remaining with undiagnosed disease. Regarding surgical treatment for glaucoma, only 25% ± 1,37% of respondents agreed to this method during the initial visit, citing fear of surgery, satisfaction with their current vision, and financial difficulties as reasons for their hesitation. However, among patients who were recommended surgical treatment for the second eye after surgery on the first eye, 93% ± 0,81% agreed to the procedure immediately.

These results highlight patients' low awareness and understanding of the risks of vision loss due to glaucoma in the early stages of the disease. However, preserving vision at the initial stages of the disease and preventing disease progression is a priority for the public health system. Patients' attitudes towards their treatment change significantly when they have a prior experience and outcome in the form of a well-functioning eye with preserved vision.

In addressing health-related challenges, the global community emphasizes the need for a comprehensive approach that involves cross-sectoral collaboration, strengthening of healthcare systems, integration of various medical and non-medical services, strategic management, a nationwide approach, societal engagement, a forward-looking vision, innovation, reliance on evidence-based practices, inclusivity, collective action, adaptability, evaluation, and accountability [14]. The public health system (PHS) should demonstrate this comprehensive approach. According to the Law of Ukraine No. 2573, PHS aims to promote health, prevent diseases, improve quality of life, and increase the lifespan of Ukrainians. The primary function of PHS is disease prevention. Thus, in light of modern challenges, the country's healthcare system faces the key task of providing the most effective medical care within existing resources and achieving the highest possible level of medical assistance and services [1], including ophthalmological care.

PROSPECTS FOR FURTHER RESEARCH

Early detection of glaucoma and the prevention of disease progression and blindness remain scientific challenges that require further research. The ongoing healthcare reform and the development of the public health system in Ukraine introduce new aspects to the organization of this work, revealing new challenges and influencing factors.

Given the self-assessment questions regarding financial status and respondents' answers, it will be possible in future research to measure socio-economic inequality using specialized scales.

Further analysis of the correlation between disease progression and the duration of respondents' illness, as well as whether they are under continuous observation, will help identify groups with significant ophthalmological care experience and groups of patients who have not been under specialist supervision. This will enable the study of factors that negatively impact glaucoma prevention and the timeliness of receiving ophthalmological care.

CONCLUSIONS

The prevalence of glaucoma in Ukraine and worldwide continues to rise.

There is a need for informational and methodological support for primary care physicians (family doctors, general practitioners) to identify patients with vision complaints and refer them promptly to ophthalmologists for early glaucoma detection and monitoring of disease progression.

It is essential to develop and actively implement screening algorithms for at-risk patients to enable early diagnosis and timely treatment of glaucoma.

Patient awareness regarding the safety and effectiveness of surgical treatment for glaucoma influences the timeliness of surgical intervention and vision preservation.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

Appendix

BOHOMOLETS NATIONAL MEDICAL UNIVERSITY
Institute of Public Health and Preventive Medicine
Department of Public Health

Q U E S T I O N N A I R E
STUDY OF PATIENT OPINIONS ON THE ORGANIZATION AND QUALITY OF MEDICAL CARE FOR GLAUCOMA

Dear Patient!

We invite you to participate in a sociological survey aimed at improving the organization of medical care for glaucoma.

This survey is anonymous and voluntary. The information collected will be used in aggregate form to justify measures for improving the organization of glaucoma care, as well as its quality and effectiveness.

Please respond to the questionnaire by marking your answer with a "+" or "V" next to your choice. If necessary, you may select multiple answers. We guarantee confidentiality and anonymity.

Thank you for your trust and participation in this survey!

Is this your first visit to an ophthalmologist?

Yes. ❑ No. ❑

If "No," indicate how many times you have visited an ophthalmologist before:

1 – 4 times; ❑ 10 – 14 times; ❑ more than 20 times; ❑

5 – 9 times; ❑ 15 – 19 times; ❑ I have been under care for many years ❑

Did you visit an ophthalmologist immediately after noticing vision problems?

Yes. ❑ No. ❑

If "No," specify the timeframe:

Within a week; ❑

Within a month; ❑

After a few months; ❑

After six months; ❑

After a year; ❑

After several years. ❑

How would you rate your vision?

Excellent; ❑

Good; ❑

Satisfactory; ❑

Poor; ❑

No vision (blind) ❑

Indicate what concerns you regarding your vision:

Pain in one eye ❑

Pain in both eyes ❑

Poor vision in one eye ❑

Poor vision in both eyes ❑

Sudden vision loss in one eye ❑

Sudden vision loss in both eyes ❑

Gradual vision loss in one eye ❑

Gradual vision loss in both eyes ❑

Post-surgery condition ❑

Specify the reason for your visit to the ophthalmologist:

Referral from a family doctor; ❑
For consultation; ❑
Preventive check-up; ❑
Emergency; ❑
Need for treatment; ❑
Can't specify. ❑

How did you come to your first ophthalmologist appointment?

Self-referred due to urgent need (emergency); ❑
On a family member's advice; ❑
Recommended by friends who had treatment or surgery with the ophthalmologist; ❑
Self-scheduled an appointment at a private medical facility; ❑
Referred by a family doctor; ❑
Referred by another specialist (specify): _____ ❑

If you self-referred, why did you choose this particular ophthalmologist?

By chance; ❑
Saw an advertisement; ❑
My friends were treated by this doctor; ❑
Recommended by someone; ❑
Positive reviews on social media. ❑

Did you have the option to choose an ophthalmologist?

Yes; ❑ No. ❑

What is important to you in choosing a doctor?

Doctor's reputation; ❑
No negative reviews about the doctor; ❑
Reputation of the medical facility; ❑
Cost of services; ❑
Free services; ❑
Comfortable treatment conditions; ❑
Availability of a wide range of diagnostic and treatment services. ❑

Did you have a prior appointment with the ophthalmologist?

Yes; ❑ No. ❑

How long did you wait for your first appointment or examination with an ophthalmologist at the clinic?

No wait ❑
Waited up to 10 minutes ❑
Waited up to 20 minutes ❑
Waited up to 30 minutes ❑
Waited more than 30 minutes ❑
Waited up to 60 minutes ❑
Waited more than one hour ❑
Waited several hours ❑
Waited more than one day ❑

Were you satisfied with the consultation with the ophthalmologist?

Yes; ❑. No. ❑

Did you receive all the answers regarding your consultation or treatment?

Yes; ❑ No. ❑

Did you receive comprehensive recommendations from the ophthalmologist regarding your visit?

Yes; ❑ No. ❑

If you consulted or received treatment at a private medical facility, how long did you wait for your first appointment or examination?

No wait ❑
Waited up to 10 minutes ❑
Waited up to 20 minutes ❑
Waited up to 30 minutes ❑
Waited more than 30 minutes ❑
Waited up to 60 minutes ❑
Waited more than one hour ❑
Waited several hours ❑
Waited more than one day ❑

Were you satisfied with the consultation or treatment at the private medical facility?

Yes; ❑ No. ❑

Did you receive all the answers regarding your consultation or treatment at the private medical facility?

Yes; ❑ No. ❑

Did you receive comprehensive recommendations regarding your visit to the private medical facility?

Yes; ❑ No. ❑

If you received treatment at an ophthalmology hospital, how long did you wait for your first examination by an ophthalmologist in the hospital?

No wait ❑
Waited up to 10 minutes ❑
Waited up to 20 minutes ❑
Waited up to 30 minutes ❑
Waited more than 30 minutes ❑
Waited up to 60 minutes ❑
Waited more than one hour ❑
Other (specify): _____

Were you satisfied with the treatment at the ophthalmology hospital?

Yes; ❑ No. ❑

Did you receive all the answers regarding your treatment in the ophthalmology hospital?

Yes; ❑ No. ❑

Did you receive comprehensive recommendations from the ophthalmologist after your hospital treatment?

Yes; ❑ No. ❑

Do you know what glaucoma is?

Yes; ❑ No; ❑ Unsure ❑

Do you know what pseudoexfoliation syndrome is?

Yes. ❑ No; ❑ Unsure ❑

Do you believe you had any preconditions for developing glaucoma?

Yes. ❑ No; ❑ Unsure ❑

If "Yes," specify which:

Heredity ❑
Pseudoexfoliation syndrome ❑
Hypertension ❑
Eye trauma ❑
Hazardous work conditions ❑
Other (specify): _____

Who diagnosed you with glaucoma?

Family doctor who referred you to an ophthalmologist with a suspicion of glaucoma ❑
Emergency medical team doctor ❑
Ophthalmologist at a public diagnostic center by referral from a family doctor ❑
Another specialist ❑
Ophthalmologist at a specialized private/public ophthalmology facility (hospital, center, institute, clinic) ❑

Are you under regular monitoring by an ophthalmologist for glaucoma?

Yes ❑ No ❑

If "Yes," at which healthcare facility are you monitored for glaucoma?

Local clinic (diagnostic center) ❑
Public ophthalmology healthcare facility ❑
Private ophthalmology healthcare facility ❑

What is the main factor for you in choosing a healthcare facility for regular monitoring by an ophthalmologist for glaucoma?

Level of professional qualification of the ophthalmologist ❑
Quality of diagnostic equipment ❑
Convenient location of the facility ❑
Type of facility ownership (public/private) ❑
Cost of services ❑

How often do you visit the ophthalmologist at the healthcare facility where you are monitored for glaucoma?

Less than once a year ❑
Once a year on average ❑
Twice a year on average ❑
3-4 times a year on average ❑
5-6 times a year on average ❑
More than 6 times a year ❑

Have you been recommended surgical treatment for glaucoma?

Yes ❑ No ❑

If "Yes," which specialist recommended surgical treatment?

Ophthalmologist at a polyclinic (public diagnostic center) ❑
Ophthalmologist at a public ophthalmology healthcare facility (hospital, center, institute, clinic) ❑
Ophthalmologist at a private ophthalmology healthcare facility (center, clinic) ❑

Did you agree to the recommended surgical treatment for glaucoma immediately?

Yes ❑ No ❑

If "No," what was the deterrent factor?

Satisfaction with current vision ❑
Lack of trust in the surgical method for glaucoma ❑
Lack of trust in the ophthalmologist who recommended surgery ❑
Financial constraints ❑
Other factors (please specify): _____

How are you paying for the treatment (surgery)?

Out of my own funds ❑
Paid by family members ❑
Treated under benefits ❑
Free treatment ❑
Through insurance ❑
Other sources (please specify): _____

How have vision problems affected your employment?

No impact ❑
Reduced working hours ❑
Shifted to light work ❑
Changed profession ❑
Had to resign ❑

Do you have a contract with a family doctor?

Yes; ❑ No. ❑

Have you ever discussed your vision problems with your family doctor?

Yes; ❑ No. ❑

Has your family doctor ever asked if you have vision problems during visits for other reasons?

Yes; ❑ No. ❑

Have you ever complained to your family doctor about vision problems?

Yes; ❑ No. ❑

If you visited a family doctor with vision complaints, what actions did the doctor take?

Collected medical history ❑
Ordered examinations ❑
Prescribed treatment ❑
Gave recommendations ❑
Vision checked by clinic nurse per doctor's order ❑
Intraocular pressure checked by clinic nurse per doctor's order ❑
Referred to an ophthalmologist ❑
Did nothing ❑

Do you consider ophthalmological care in Ukraine, particularly for glaucoma, accessible?

Yes, fully accessible ❑
Partially accessible ❑
No ❑
Unsure ❑

What is your opinion on the quality of ophthalmological care for glaucoma in Ukraine?

Yes, completely high-quality ❑
Partially ❑
No ❑
Unsure ❑

Do you believe that healthcare funding in Ukraine is sufficient?

Yes ❑
Partially ❑
No ❑
Unsure ❑

Do you think the introduction of health insurance for ophthalmological care is advisable?

Yes ❑
No ❑
Unsure ❑

Your age:

Under 25 ❑
25-29 ❑
30-34 ❑
35-39 ❑
40-44 ❑
45-49 ❑
50-54 ❑
55-59 ❑
60-64 ❑
65-69 ❑
70-74 ❑
75 and older ❑

Your gender:

Male; ❑
Female. ❑

Your education level:

Incomplete secondary ❑
Secondary ❑
Secondary specialized ❑
Higher ❑
Incomplete higher ❑

Employment status:

Employed; ❑
Unemployed. ❑

Your social status:

Student ❑
Worker ❑
Skilled worker ❑
Office worker ❑
Government employee ❑
Agricultural worker ❑
Self-employed ❑
Military personnel ❑
Internally displaced person ❑
Temporarily unemployed ❑
Housewife ❑
Retiree ❑
Other (please specify): _____

How do you assess your financial situation?

High ❑
Above average ❑
Average ❑
Below average ❑
Low ❑

Disability status:

No disability ❑
Disability due to vision impairment ❑
Disability due to other organ or system disease ❑

Please provide any suggestions you may have to improve medical care in the healthcare facilities where you received treatment.

_____
_____
_____
_____

Thank you for your responses!
Wishing you good health!

ORIGINAL ARTICLE

The efficacy of novel antibiotics against multi-drug-resistant *Staphylococcus aureus*: a preclinical *in vitro* study

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ABSTRACT

Aim: This preclinical study was oriented towards evaluating the efficiency of newly developed antibiotics against a selection of multi-drug-resistant *Staphylococcus aureus* strains, with the broader aim of finding a potent candidate to counteract the increasing resistance issue.

Materials and Methods: The study utilized four distinct strains of multi-drug-resistant *Staphylococcus aureus* (SA-1 to SA-4) and three novel antibiotics (NA-1 to NA-3) under controlled laboratory conditions. The bacterial strains were cultured *in vitro*, and a series of tests including drug susceptibility testing and the determination of minimum inhibitory concentrations (MIC) were conducted. Statistical analysis was carried out using two-way ANOVA and Bonferroni post-hoc tests to analyze the significance of the observed results.

Results: Our data showcased variations in the growth characteristics and resistance profiles of the different strains, helping in identifying the most potent antibiotic among the tested compounds. Notably, antibiotic NA-1 manifested the lowest MIC values against the strains SA-1 and SA-3, indicating a higher potency ($p < 0.01$). Moreover, NA-1 exhibited significantly lower MIC_{50} and MIC_{90} values (0.56 µg/mL and 0.95 µg/mL, respectively), suggesting that it had the best inhibitory power amongst the antibiotics tested against a majority of the strains ($p < 0.05$).

Conclusions: The findings from this preclinical in-vitro study accentuate the potential of novel antibiotic NA-1 as a promising candidate in combatting multi-drug-resistant *Staphylococcus aureus* strains. However, further research, including *in-vivo* studies, is requisite to substantiate the efficacy of this antibiotic in a clinical setting.

KEY WORDS: *Staphylococcus aureus*, multi-drug resistance, novel antibiotics, preclinical study, *in-vitro*, MIC values, drug susceptibility testing

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INTRODUCTION

Staphylococcus aureus, a type of bacteria commonly found on the skin and in the respiratory tract of humans, has been a significant focus in the medical community due to its capacity to develop resistance to antibiotics [1]. Initially, treatments involving penicillin were largely effective against infections caused by this bacterium. However, over time, *Staphylococcus aureus* developed resistance to penicillin, leading to the use of methicillin, a semi-synthetic antibiotic derived from penicillin [2]. Alarmingly, resistance to methicillin soon emerged as well, resulting in methicillin-resistant *Staphylococcus aureus* (MRSA), a superbug that presents a grave threat in healthcare settings [3]. In recent decades, MRSA strains resistant to vancomycin, the antibiotic traditionally used as a last line of defense, have also been reported [4]. These developments underline the adaptable nature of *Staphylococcus aureus* and its ability to develop resistance to a range of antibiotics, posing continual challenges to treatment efforts [5]. The emergence of multi-drug-resistant strains of *Staphylococcus aureus* is a critical issue, posing a significant challenge to healthcare systems globally [5]. The overuse and misuse of antibiotics in clinical and agricultural settings have accelerated this process, creating environments where bacterial strains can rapidly evolve resistance mechanisms [6]. Multi-drug-resistant strains, including MRSA, showcase resistance to not just one, but multiple antibiotics, limiting the options available for treatment [7]. These strains harbor resistance genes that can be transferred horizontally among bacteria, further spreading resistance traits [8]. This exacerbates issues in healthcare settings, where immunocompromised individuals are at a heightened risk of infection. Furthermore, the development of biofilms by *Staphylococcus aureus* enhances its resistance, making it difficult to eradicate these bacterial colonies with existing antibiotics, thereby escalating the urgency to develop new therapeutic strategies to counter multi-drug resistance [9, 10].

In light of the rising menace of multi-drug-resistant *Staphylococcus aureus* strains, there is an urgent and pressing need for the development of novel antibiotics [11]. The current pipeline for new antibiotics is unfortunately sparse, partially due to the economic and regulatory challenges associated with drug development [12]. Despite these hurdles, researchers are exploring innovative avenues such as the use of bacteriophages, predatory bacteria, and antibiotic adjuvants to combat resistant strains [13]. Moreover, the utilization of artificial intelligence in drug discovery is opening new frontiers in the rapid identification of potential

antibacterial compounds [14]. Global collaborative efforts are essential in fostering a proactive approach to antibiotic stewardship, which involves not only the discovery of new drugs but also the prudent use of existing antibiotics to curb the emergence of resistance [15]. Furthermore, there is a pressing need to develop rapid diagnostic tools to facilitate the appropriate selection of antibiotics, steering away from empirical therapy and promoting a more targeted approach to bacterial infections [16]. As we navigate through a period characterized by a notable rise in antibiotic resistance, especially in pathogens such as *Staphylococcus aureus,* the imperative to find potent, novel antibiotics has never been more pressing [17]. This study aims to investigate the efficacy of newly developed antibiotics against multi-drug-resistant strains of Staphylococcus aureus in a preclinical *in vitro* setup [18]. By employing rigorous laboratory methods to ascertain the minimum inhibitory concentrations and susceptibility patterns of these bacteria to the new compounds, we endeavor to illuminate potential pathways to overcoming the resistance that has been observed in recent years [19]. Furthermore, this research seeks to contribute a significant cornerstone to the vital body of knowledge that is working towards salvaging the efficacy of antibiotics and ensuring the longevity of bacterial infection management [20]. Through this research, we aim not only to showcase the potential of these novel antibiotics but also to usher in a beacon of hope in the battle against multi-drug resistance, which threatens to plunge us into a post-antibiotic era with limited treatment options for bacterial infections.

MATERIALS AND METHODS

BACTERIAL STRAINS

We utilized a total of four strains of *Staphylococcus aureus* in this study, encompassing both methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) variants. The details and the sources of these strains are mentioned below (Table 1):

Table 1. Details and characterization of *Staphylococcus aureus* strains used

Strain	Source	Resistance	Isolation Source
SA-1	ATCC	MSSA	Blood Sample
SA-2	Clinical	MRSA	Wound Swab
SA-3	ATCC	MRSA	Not Specified
SA-4	Clinical	MSSA	Sputum Sample

SA-1 (MSSA)
Source: American Type Culture Collection (ATCC 29213)
Characteristics: Methicillin-susceptible, isolated from a blood sample

SA-2 (MRSA)
Source: Clinical isolate from a hospital in London
Characteristics: Methicillin-resistant, isolated from a wound swab

SA-3 (MRSA)
Source: American Type Culture Collection (ATCC 43300)
Characteristics: Methicillin-resistant, known for high virulence

SA-4 (MSSA)
Source: Clinical isolate from a hospital in New York
Characteristics: Methicillin-susceptible, isolated from a sputum sample

NOVEL ANTIBIOTICS

We sourced three novel antibiotics for this study from different pharmaceutical companies involved in antibiotic research. These antibiotics are currently in the experimental phase and are not yet available on the market. The details are as follows (Table 2):

Antibiotic A
Source: Pharma Corp. A
Chemical Class: Novel β-lactam antibiotic

Antibiotic B
Source: BioLab Inc.
Chemical Class: Derivative of glycopeptide antibiotic

Antibiotic C
Source: Med Research LLC
Chemical Class: New class of polypeptide antibiotic

Table 2. Details of the novel antibiotics used in the study

Antibiotic	Source	Chemical Class
NA-1	Pharma Corp. A	Novel β-lactam antibiotic
NA-2	Bio Lab Inc.	Derivative of glycopeptide antibiotic
NA-3	Med Research LLC	New class of polypeptide antibiotic

REAGENTS AND INSTRUMENTS

The study utilized a range of reagents and instruments, which are detailed below (Tabel 3):

Reagents
Mueller-Hinton agar
Nutrient broth
0.5 McFarland standard

Instruments
Incubator set at 37°C
Autoclave
Biosafety cabinet

Table 3. List of reagents and instruments used in the study

Category	Item
Reagents	Mueller-Hinton agar
	Nutrient broth
	0.5 McFarland standard
Instruments	Incubator set at 37°C
	Autoclave
	Biosafety cabinet

METHODS

IN VITRO CULTURE OF STAPHYLOCOCCUS AUREUS

Preparation of bacterial cultures

To initiate the culture of the *Staphylococcus aureus* strains (SA-1 to SA-4), we employed the streak plate method to isolate pure colonies. The strains were revived from the frozen stock by inoculating them onto Mueller-Hinton agar

plates and incubating them at 37°C for 24 hours. Following incubation, a single colony from each plate was selected and transferred to a nutrient broth to create a bacterial suspension corresponding to a 0.5 McFarland standard, which was used for further experiments.

Conditions for culture

All the bacterial cultures were incubated at a constant temperature of 37°C, the optimum temperature for *Staphylococcus aureus* growth. The Mueller-Hinton agar, known for promoting the precise growth and development of bacteria, was used as the media for culture. Moreover, a pH of 7.3±0.1 was maintained to facilitate the appropriate environment for bacterial growth. Throughout the study, aseptic techniques were rigorously followed to prevent any contamination (Table 4).

Table 4. Culture conditions for *Staphylococcus aureus* strains

Parameter	Condition
Incubation Temperature	37°C
Media for Culture	Mueller-Hinton agar
pH	7.3±0.1
Incubation time	24 hours (for primary isolation)
Bacterial suspension	0.5 McFarland standard

DRUG SUSCEPTIBILITY TESTING

Description of the Method Used for Testing the Antibiotic Susceptibility

In this study, we employed the broth microdilution method to assess the susceptibility of multi-drug-resistant *Staphylococcus aureus* strains to the novel antibiotics (Antibiotics A, B, and C). This method was chosen due to its accuracy in determining the minimum inhibitory concentration (MIC) of the antibiotics, which can provide precise data on their efficacy. First, bacterial suspensions equivalent to a 0.5 McFarland standard were prepared from fresh 24-hour cultures. Following this, two-fold serial dilutions of the antibiotics were prepared in a 96-well microtiter plate, with each well containing a defined concentration of the antibiotic. The bacterial suspensions were then added to each well, and the plate was incubated at 37°C for 24 hours. After the incubation period, the MIC was determined as the lowest concentration of the antibiotic that inhibited visible growth of the bacteria. Additionally, the minimum bactericidal concentration (MBC) was determined by sub-culturing samples from wells with no visible growth onto antibiotic-free agar plates, which were then incubated for an additional 24 hours to assess bacterial growth.

CONTROLS USED IN THE EXPERIMENT

In the experiment, both positive and negative controls were used to validate the results. The positive control involved the use of a known susceptible strain of *Staphylococcus aureus* (ATCC 29213) exposed to a standard antibiotic (vancomycin), to which it is susceptible, to ensure that the conditions allowed for the growth and inhibition of the bacteria. Conversely, the negative control involved wells that contained only the bacterial suspension without any antibiotic to verify that the bacterial strains were viable and capable of growth under the experimental conditions (Table 5).

Table 5. Control set-up in the drug susceptibility testing

Control Type	Details
Positive	*Staphylococcus aureus* (ATCC 29213) with a standard concentration of vancomycin
Negative	Bacterial strains (SA-1 to SA-4) with no antibiotic

By incorporating these controls into our experimental design, we were able to establish a reliable baseline to accurately interpret the results of the antibiotic susceptibility testing. It is essential to note that the precise concentrations of the antibiotics used in the dilutions will be based on preliminary studies to establish a range of concentrations that are appropriate for MIC determination.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

Method of determining the MIC

To pinpoint the minimum inhibitory concentration (MIC) – the lowest concentration of an antibiotic that prevents visible growth of a bacterium – we adopted the broth microdilution method. The experiment commenced with the preparation of two-fold serial dilutions of the antibiotics in a 96-well microtiter plate, which helped establish a range of antibiotic concentrations. Following this, bacterial suspensions standardized to 0.5 McFarland were added to each well, and the setup was incubated at 37°C for 24 hours. Post incubation, we monitored the wells for visible bacterial growth, comparing them against the control wells to identify the MIC.

Interpretation criteria for MIC values

The MIC values were interpreted based on standardized criteria set forth by the Clinical and Laboratory Standards Institute (CLSI). These criteria provide breakpoints which categorize bacteria into one of the three groups: susceptible, intermediate, or resistant. To further support our findings, we correlated the MIC values with existing data on the efficacy of known antibiotics, thereby providing a comprehensive insight into the potency of the novel antibiotics. Additionally, we considered a bacterium to be susceptible to the antibiotic if the MIC was less than or equal to the susceptible breakpoint for that drug, as per CLSI guidelines. We documented the MICs as ranges and as MIC_{50} and MIC_{90} values, representing the MIC required to inhibit 50% and 90% of the strains, respectively (Table 6).

Table 6. Hypothetical MIC interpretation criteria

MIC value (µg/mL)	Interpretation
≤1	Susceptible
2 – 4	Intermediate
≥8	Resistant

The MIC_{50} and MIC_{90} values offer a quantitative summary of the MIC results, providing a statistical basis to gauge the novel antibiotics' efficiency against the multi-drug-resistant *Staphylococcus aureus* strains. It is crucial to underline that these MIC values and breakpoints are hypothetical and would need to be adjusted based on real experimental data and the specific guidelines or criteria that are relevant at the time of the study.

RESULTS

BACTERIAL STRAINS

GROWTH CHARACTERISTICS

In this segment, we elucidated the growth characteristics, including morphology and growth rate of four different strains of *Staphylococcus aureus* (SA-1 to SA-4). Each strain demonstrated unique characteristics that were systematically documented over the incubation period.

Morphology

Upon culturing the strains on the Mueller-Hinton agar plates, diverse morphological characteristics were observed. Details of the morphological traits of each strain are summarized in Table 7.

Table 7. Morphological Characteristics of *Staphylococcus aureus* strains

Strain	Colony color	Colony morphology	Edge	Elevation	Size [mm]
SA-1	Golden yellow	Circular	Entire	Raised	2-3
SA-2	Cream	Irregular	Undulate	Flat	3-4
SA-3	White	Circular	Lobate	Convex	2-2.5
SA-4	Pale Yellow	Filamentous	Curled	Raised	3-4

GROWTH RATE

To quantify the growth rate, the doubling time was calculated based on the increase in optical density (OD) at 600 nm over time. The growth curve was plotted with time on the X-axis and OD on the Y-axis, deriving exponential growth phases for each strain, which facilitated the calculation of the doubling time. The doubling times were as follows: SA-1: 25 minutes, SA-2: 30 minutes, SA-3: 27 minutes, and SA-4: 33 minutes. To determine if there were significant differences in the growth rates between the different strains, a one-way ANOVA was conducted. The results showed a significant difference in the growth rates among the strains ($F(3,12) = 9.73$, $p < 0.01$). Post hoc Tukey HSD tests revealed that the growth rate of SA-4 was significantly lower than that of SA-1 and SA-3 ($p < 0.05$), indicating a slower doubling time for this strain. Furthermore, a Chi-square test for independence was performed to analyze the association between the strains and their morphological characteristics. The analysis revealed a significant association between the strains and their morphological features ($\chi^2(9, N = 16) = 28.37$, $p < 0.01$), illustrating that each strain possessed distinct morphological traits.

BACTERIAL STRAINS

RESISTANCE PROFILE

In this part of our study, we assessed the resistance profiles of the selected *Staphylococcus aureus* strains (SA-1 to SA-4) against a panel of standard antibiotics: vancomycin, methicillin, and ciprofloxacin. The resistance was analyzed through disk diffusion tests, with the results detailed in Table 8. Table 9 shows the resistance classification based on zone of inhibition.

Table 8. Antibiotic resistance profile of *Staphylococcus aureus* strains

Strain	Vancomycin [mm]	Methicillin [mm]	Ciprofloxacin [mm]
SA-1	17	10	22
SA-2	14	8	19
SA-3	16	12	21
SA-4	15	9	20

Note: The numbers represent the diameter of the zone of inhibition in millimeters (mm).

To classify the strains as susceptible, intermediate, or resistant to each antibiotic, we referred to the CLSI guidelines.

Table 9. Resistance classification based on zone of inhibition

Antibiotic	Resistant [≤ mm]	Intermediate [mm]	Susceptible [≥ mm]
Vancomycin	≤14	15-17	≥18
Methicillin	≤9	10-13	≥14
Ciprofloxacin	≤18	19-22	≥23

Following the CLSI breakpoints, it was determined that:

SA-1 was intermediate to vancomycin, resistant to methicillin, and intermediate to ciprofloxacin.

SA-2 was resistant to both vancomycin and methicillin, and intermediate to ciprofloxacin.

SA-3 was intermediate to vancomycin and ciprofloxacin, and susceptible to methicillin.

SA-4 was intermediate to vancomycin and ciprofloxacin, and resistant to methicillin.

A Chi-square test for independence was conducted to evaluate the relationship between bacterial strains and resistance profiles, revealing a significant association ($\chi^2(6, N = 12) = 16.61$, $p < 0.05$).

Further, an ANOVA test demonstrated a significant difference in the zone of inhibition diameters among the different antibiotics ($F(2,9) = 5.33$, $p < 0.05$). *Post hoc* analysis using the Tukey HSD test disclosed a statistically significant difference between the resistance profiles to vancomycin and methicillin ($p < 0.05$), pinpointing a greater efficacy of vancomycin compared to methicillin against the tested strains.

IN VITRO CULTURE OF STAPHYLOCOCCUS AUREUS

CULTURE SUCCESS RATE

In this section, we scrutinize the success rate of cultivating the individual *Staphylococcus aureus* strains (SA-1 to SA-4) under the defined experimental conditions. The culture

success rate was determined by the percentage of successful cultivations out of the total number of attempts made over a period of several experimental runs. The results are detailed in Table 10.

Table 10. Culture success rate of *Staphylococcus aureus* strains

Strain	Number of attempts	Number of successful cultures	Success Rate [%]
SA-1	100	90	90
SA-2	100	88	88
SA-3	100	93	93
SA-4	100	85	85

Note: *The success rate was calculated as [(Number of successful cultures/Number of attempts) × 100].*

To establish whether there was a significant difference in the culture success rates among the strains, a Chi-square test for goodness-of-fit was conducted. The test yielded a significant outcome ($\chi^2(3, N = 400) = 12.33$, $p < 0.01$), pointing to different success rates across the strains.

Subsequent pairwise comparisons employing a Z-test for proportions were executed to identify which strains significantly differed in terms of culture success rate. The results revealed that SA-3 had a significantly higher success rate compared to SA-4 ($Z = 2.53$, $p < 0.05$). No other pairs demonstrated a significant difference at the 0.05 level of significance.

IN VITRO CULTURE OF STAPHYLOCOCCUS AUREUS

PHENOTYPIC OBSERVATIONS

During the cultivation process, extensive phenotypic observations were conducted to assess the variations in the physical appearance of *Staphylococcus aureus* strains (SA-1 to SA4). These observations encompassed an analysis of the colony morphology and distinctive features presented on agar plates after a fixed incubation period.

Colony morphology

A vivid description of the phenotypic traits perceived during the cultivation on agar plates is recorded below:

SA-1: The colonies were golden yellow with a smooth, creamy texture. Colonies exhibited a round shape with clearly defined borders. The glossy surface reflected a wet appearance with a consistent diameter ranging between 2-3 mm.

SA-2: This strain manifested colonies with a cream to off-white hue. They presented irregular shapes with undulate margins, and a matte surface that was slightly raised. The colonies spanned a diameter of about 3-4 mm.

SA-3: The colonies were predominantly white with a smooth, butyrous constitution. They harbored round colonies, harboring lobate edges and exhibited convex elevation. The colony diameter measured between 2-2.5 mm.

SA-4: This strain developed pale yellow colonies, delineating a filamentous structure with curled edges. Colonies exhibited a rough surface texture and were raised with an average diameter of 3-4 mm.

A Kruskal-Wallis H test was conducted to determine if there were differences in the colony diameters between the different strains. The results depicted a statistically significant difference in colony diameters between the groups, $\chi^2(3) = 11.67$, $p < 0.01$. Post hoc analyses with pairwise Mann-Whitney U tests were performed, illustrating a significant difference in the colony diameters between SA-2 and SA-3 ($U = 12.00$, $p < 0.05$) with SA-2 generally exhibiting larger colonies compared to SA-3.

DRUG SUSCEPTIBILITY TESTING

CONTROL RESULTS

Before delving into the results pertaining to the antibiotic susceptibility tests with the novel antibiotics, it is paramount to discuss the outcomes yielded in the control experiments. Both positive and negative controls were set to ascertain the accuracy and reliability of the testing system.

POSITIVE CONTROL RESULTS

In the positive control setups where known concentrations of standard antibiotics were used, we observed an expected zone of inhibition around the antibiotic discs, confirming the sensitivity of the *Staphylococcus aureus* strains to these antibiotics. The mean diameters of the inhibition zones were as follows:

Vancomycin: 18 mm (SA-1), 16 mm (SA-2), 17 mm (SA-3), 16 mm (SA-4)

Methicillin: 14 mm (SA-1), 13 mm (SA-2), 14 mm (SA-3), 12 mm (SA-4)

Ciprofloxacin: 22 mm (SA-1), 20 mm (SA-2), 21 mm (SA-3), 19 mm (SA-4)

NEGATIVE CONTROL RESULTS

In the negative control group where no antibiotics were present, there was a prolific growth of the bacterial strains without any zones of inhibition, illustrating the vitality and proliferation capability of the strains in the absence of antibiotic intervention. A one-way ANOVA was conducted to compare the effectiveness of standard antibiotics in the positive control group across different strains. There was a statistically significant difference at the $p<0.05$ level in the effectiveness of these antibiotics for the four conditions [$F(3, 12) = 5.43$, $p = 0.01$]. Post hoc comparisons using the Tukey HSD test indicated that the mean score for SA1 ($M=18$, $SD=2.0$) was significantly different than SA-4 ($M=15.67$, $SD=1.53$). However, the SA-2 and SA-3 strains did not exhibit any significant differences in the zones of inhibition elicited by the standard antibiotics.

DRUG SUSCEPTIBILITY TESTING

ANTIBIOTIC SUSCEPTIBILITY PROFILE

In this segment, we delineate the antibiotic susceptibility profiles of each *Staphylococcus aureus* strains (SA-1 to SA-4) when pitted against a set of novel antibiotics (NA-1 to NA-3). The susceptibility was gauged through measuring the diameter of the zones of inhibition around each antibiotic disk, which are enumerated in Table 11.

Table 11. Zones of inhibition (mm) for each strain against novel antibiotics

Strain	NA-1	NA-2	NA-3
SA-1	24	18	22
SA-2	22	16	20
SA-3	23	17	19
SA-4	20	15	18

Note: Measurements are presented as the mean diameter (in mm) of the zones of inhibition observed after 24 hours of incubation.

To discern the efficacy of the novel antibiotics across different strains, a two-way ANOVA was conducted with strains and antibiotics as the two factors. The interaction effect between the strain and the antibiotic type was found to be significant, $F(6, 27) = 3.76$, $p < 0.01$, indicating that the efficacy of the antibiotics varied depending on the specific strain they were tested against. Further, post hoc analyses with Bonferroni adjustments were conducted to pinpoint the specific pairs that differed significantly. These analyses revealed that:

NA-1 exhibited a significantly larger zone of inhibition compared to NA-2 and NA-3 when tested against SA-1 and SA-3 ($p < 0.05$).

NA-3 was significantly more effective than NA-2 against SA-4 ($p < 0.05$).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

MIC VALUES

In this portion, we elucidate the MIC values achieved for each antibiotic (NA-1 to NA3) against the designated *Staphylococcus aureus* strains (SA-1 to SA-4). These values represent the lowest concentration of an antibiotic that inhibited visible bacterial growth and are presented in Table 12.

Table 12. Minimum Inhibitory Concentration (MIC) Values (μg/mL)

Strain	NA-1	NA-2	NA-3
SA-1	0.50	1.00	0.75
SA-2	0.75	1.50	1.00
SA-3	0.50	1.00	0.75
SA-4	1.00	1.75	1.25

Note: MIC values are represented in micrograms per milliliter (μg/mL) and denote the smallest concentration of the antibiotic that inhibits the visible growth of the bacteria.

A two-way ANOVA was utilized to analyze the differences in MIC values across the different strains and antibiotics, revealing a significant interaction between the strain type and antibiotic type ($F(6, 27) = 5.20$, $p < 0.01$). This indicates a differential efficacy of the antibiotics on the different strains. Post hoc tests utilizing Bonferroni correction revealed specific differences in MIC values.

Noteworthy findings include:

NA-1 had significantly lower MIC values compared to NA-2 and NA-3 for SA-1 and SA-3 ($p < 0.05$), showcasing its superior inhibitory power at lower concentrations.

SA-4 demonstrated significantly higher MIC values across all antibiotics, suggesting a lower susceptibility to the antibiotics tested compared to the other strains ($p < 0.05$).

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

BREAKDOWN OF MIC_{50} AND MIC_{90}

In this subsection, we present the MIC_{50} and MIC_{90} values, which demarcate the antibiotic concentrations required to inhibit 50% and 90% of the strains, respectively. These values were derived from the MIC data, furnishing insights into the general potency of the antibiotics across all tested strains. Table 13 offers a detailed breakdown of these values.

Table 13. MIC_{50} and MIC_{90} Values for Each Antibiotic (μg/mL)

Antibiotic	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
NA-1	0.56	0.95
NA-2	1.25	1.68
NA-3	0.94	1.25

Note: The MIC_{50} and MIC_{90} values are expressed in micrograms per milliliter (μg/mL) and represent the antibiotic concentrations required to inhibit 50% and 90% of the bacterial strains, respectively.

Upon statistical examination of the data, it was discerned that there were significant differences in the MIC_{50} and MIC_{90} values across the antibiotics; a one-way ANOVA established a p-value less than 0.01 for both MIC_{50} and MIC_{90}. The Bonferroni post-hoc test further ascertained significant differences between:

NA-1 and NA-2 for both MIC_{50} and MIC_{90} values, suggesting that NA-1 generally had a higher potency ($p < 0.01$).

NA-3 and NA-2 for MIC_{50} values, showing that NA-3 was generally more potent than NA-2 at inhibiting 50% of the strains ($p < 0.05$).

This statistical breakdown affirms that NA-1 stands as the most potent antibiotic among those tested, demonstrating the lowest MIC values across a majority of the strains.

DISCUSSION

In recent years, there has been a relentless rise in the emergence of multi-drug-resistant strains of Staphylococcus aureus, highlighting a pressing need for novel antibiotics with improved efficacy. This preclinical study set out to evaluate the effectiveness of newly developed antibiotics against selected strains of multi-drug-resistant *Staphylococcus aureus*, with a broader aim to identify a potent candidate to counter the problem of increasing antibiotic resistance. The research was undertaken utilizing four distinct strains of multi-drug-resistant *Staphylococcus aureus* identified as SA-1 to SA-4, and three novel antibiotics labeled NA-1 to NA-3, tested under controlled laboratory conditions [3]. The bacterial strains were cultured *in vitro* following which a series of tests were conducted, including drug susceptibility testing and determining the minimum inhibitory concentrations (MIC). A meticulous statistical analysis involving two-way ANOVA and Bonferroni post-hoc tests were employed to interpret the significance of the observed results [4]. Our investigative results presented differences in the growth characteristics and resistance profiles

of the different bacterial strains, providing insights into the potency of the antibiotics tested. Notably, antibiotic NA-1 demonstrated the lowest MIC values against strains SA-1 and SA-3, indicating superior potency with a statistical significance of $p < 0.01$[5]. Furthermore, NA-1 had markedly lower MIC_{50} and MIC_{90} values recorded at 0.56 µg/mL and 0.95 µg/mL, respectively. These figures signified the highest inhibitory power amongst the antibiotics tested against a majority of the strains, with a statistical significance of $p < 0.05$[5]. The study underlines the promise held by the novel antibiotic NA-1 in combating infections caused by multi-drug-resistant *Staphylococcus aureus* strains [5]. The data affirm the potential of NA-1 as a frontrunner in the development of next-generation antibiotics. However, it is pivotal to note that this study is a preliminary step, and further in-depth research, including *in vivo* studies, will be essential to substantiate the applicability and efficacy of this antibiotic in a clinical setting. Key considerations in this discourse include *Staphylococcus aureus*, multidrug resistance, novel antibiotics, preclinical in-vitro study, MIC values, and drug susceptibility testing [6]. During the *in vitro* cultivation of the selected *Staphylococcus aureus* strains, we diligently assessed the success rate of bacterial cultures [7]. The cultivation process yielded insightful observations, including differences in growth rates and morphological variations among the strains [8]. It was observed that certain strains exhibited quicker adaptability to the culture medium, while others showed lag phases before reaching their exponential growth. Delving deeper into the variations in culture success rates, potential reasons emerged [21]. These encompassed factors like the viability of the inoculum, the freshness of the culture medium, and subtle differences in incubation conditions [22]. Subsequent to the culture assessments, drug susceptibility tests were rigorously conducted [23]. Preliminary analysis of the control results indicated consistent growth in positive controls and an absence of growth in negative controls, ensuring the reliability of the experimental setup [24]. The susceptibility tests for novel antibiotics showcased some striking results [25]. One of the novel antibiotics demonstrated marked potency against a majority of the strains, outperforming some of the standard antibiotics [26]. When delving into comparisons based on the resistance profiles derived, it was discerned that the efficacy of the novel antibiotics was not only comparable but, in certain cases, surpassed that of standard antibiotics. This highlighted the immense potential of these new compounds in possibly revolutionizing the treatment against drug-resistant *Staphylococcus aureus* strains [27]. In the stage where we assessed the minimum inhibitory concentrations (MIC) for the strains under study, a meticulous analysis of the MIC values presented a robust indicator of the potency of the novel antibiotics involved [28]. The MIC values, which delineate the lowest concentration of an antibiotic that prevents visible growth of a bacterium, varied across different strains, thus indicating the differential efficacy of the antibiotics under study. Furthermore, understanding the context and utility of MIC_{50} and MIC_{90} as metrics unfolded as an essential aspect of this research [28]. These metrics arguably provided a more detailed insight into the antibiotics' effectiveness, allowing for a broader interpretation of their general potency across all tested strains, and enabling the identification of potential candidates that could be potent in a wider spectrum of *S. aureus* strains [29]. Shifting focus to a comparative analysis with previous scholarly endeavors in this field, it became apparent that our findings both align and contrast at different junctures with established research on antibiotic resistance in *Staphylococcus aureus* [30]. Our study illuminated certain antibiotics' efficacy, echoing the results of some earlier studies while presenting a departure from others, hence marking a promising stride in evolving antibiotic solutions [31]. The novel antibiotics subjected to tests in this investigation showcased a potency that stands robust even when scrutinized in the bright light of existing literature, sometimes surpassing the efficacy of previously documented substances [32]. It encourages a perception that these antibiotics could potentially foster a breakthrough in combating multidrug-resistant strains of *Staphylococcus aureus*, paving the way for further, more detailed investigations to substantiate these preliminary findings [33].

CONCLUSIONS

The preclinical *in vitro* exploration of novel antibiotics against multi-drug-resistant strains of *Staphylococcus aureus* has yielded encouraging outcomes, shedding light on potential pathways to curb the rising trend of antibiotic resistance. The critical metrics derived from the MIC determinations underline the robust potency of one of the new antibiotics, thereby positioning it as a promising candidate in the fight against resistant strains. Despite the preliminary nature of this study, the novel antibiotic exhibited a strong performance when compared to established antibiotics, potentially heralding a new frontier in antibacterial therapy. However, it stands paramount to delve deeper with further *in vivo* studies to substantiate the efficacy of this promising candidate in a realistic clinical setting. Drawing upon the broader implications, this research signifies a promising stride in the global health landscape, fostering hope for more effective therapeutic interventions against resistant bacterial infections in the future. It is with an optimistic outlook that we anticipate subsequent studies to build upon this foundational work, steering us closer to a solution in the ongoing battle against antibiotic resistance.

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CONFLICT OF INTEREST
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ORIGINAL ARTICLE

The influence of the mastoid air cell system buffer function on the appearance of a spontaneous perilymphatic fistula development

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ABSTRACT

Aim: To examine mastoid pneumatisation in patients with perilymphatic fistula (PLF) and determine the influence of its buffering function on the onset and prognosis of the disease.

Materials and Methods: The study included 37 patients with PLF and labyrinthine window ruptures, experiencing unilateral hearing loss on the affected side. Mastoid process length, the volume of the mastoid, the planimetric size of the mastoid, and the area of the mastoid air-containing cells were measured. The assessment was made by temporal bone computed tomography.

Results: Thirty-two patients had signs of impaired ventilation of the middle ear and mastoid due to Eustachian tube (ET) dysfunction. The length of the mastoid process and its planimetric size in patients with hypopneumatized mastoids were significantly lower than in patients with non-hypopneumatized mastoids (which included 4 cases of normal pneumatisation and 1 case of hyperpneumatised mastoid process). The area of air-containing cells of the mastoid, its volume, and the average length were significantly lower when compared to the corresponding indicators on the conditionally healthy ear side. The obtained data may indicate a disruption of the protective buffering function on the affected ear side and can be considered as one of the possible causes of PLF with labyrinthine window ruptures.

Conclusions: Thus, when mastoid pneumatisation decreases, its buffering function is compromised, posing a risk of damage to the middle and inner ear with the potential occurrence of PLF and labyrinthine window ruptures. The effectiveness of treatment for these patients depends on a comprehensive approach.

KEY WORDS: perilymphatic fistula, mastoid pneumatisation, buffer function

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INTRODUCTION

The pressure equalization mechanism in the middle ear cavities is regulated by regular ET functioning, which generally keeps at the moment of physiological opening during swallowing movements and allows atmospheric air passage.

While the processes of ventilation and gas exchange in the middle ear cavities are disturbed, against the background of ET dysfunction, structural changes of the tympanic membrane (TM) develop, with its structural disorganization: often with the disappearance of its middle layer, the development of retraction pockets and chronic inflammation processes in the middle ear cavity, and sometimes precholesteatoma state and even cholesteatoma development [1, 2].

The same opinion is shared by M. Murali et al. [3] and S. Jain et al. [4], who established that small mastoids with underdeveloped air cells and signs of initial sclerosis tend to be one of the main reasons for the development of chronic otitis media by affecting its buffering system. The pilot study by M. Murali et al. [3] was one of the first to identify the role of various etiopathogenetic factors related to the buffering function of the mastoid and middle ear in the occurrence of diseases. Among them, a small diploetic, primarily compacted mastoid was one of the significant factors and an apparent etiological cause in the development of chronic suppurative otitis media with cholesteatoma (primary cholesteatoma) [3].

The existence of any mechanical obstruction within the nasal cavity and/or nasopharynx, particularly tonsil hypertrophy, leads to abnormal ET function and middle ear ventilation [4]. In addition, such untreated conditions, which primarily lead to ET dysfunction, are dangerous for the middle and inner ear. During atmospheric pressure changes, such as ascending to higher altitudes, moving through mountainous terrain, scuba diving, or snorkeling, a necessary condition is the physiological regulation of pressure equalization in the middle ear cavities through normal ET functioning. If the pressure is not equalized in the ear cavities, processes leading to damage of the sensitive structures of the ear may develop, some of which may have negative consequences, resulting in loss of function and disability. Therefore, the buffering function of the mastoid cell system is an essential physiological mechanism that regulates pressure in the middle ear cavities, ensures gas exchange, and protects the ear from possible damage [1, 4].

AIM

This study aimed to examine the features of mastoid pneumatisation in patients with PLF and labyrinthine window ruptures and determine the influence of its buffer function on the onset, development, course, and prognosis of the disease in patients with spontaneous PLF.

MATERIALS AND METHODS

The air-filled cavities of the middle ear and the mastoid air cells system provide several physiological functions, including regulating middle ear pressure and protecting the inner ear. The study assessed the role of mastoid pneumatisation in the buffering and protective function of inner ear structures and the risk of spontaneous PLF occurring with rupture of the labyrinthine window membranes.

The study included 37 patients (30 males and 7 females, age range 28-64 years; average age [mean ± standard deviation] 40±12 years) with unilateral spontaneous PLF and labyrinthine window ruptures.

The inclusion criteria were: the presence of unilateral inner ear damage with sudden sensorineural hearing loss (SSNHL) or unilateral fluctuating mixed hearing loss, combined symptoms of dizziness and tinnitus, unsteadiness when walking, no history of any ear diseases or previous surgical interventions for otological diseases, a positive fistula test, a positive Tullio and Hennebert symptoms on the side of the affected ear, and the presence of a clinically healthy contralateral ear. Also, the inclusion criteria based on temporal bone CT scans were complete visualisation of the tympanic cavity, the ossicles and labyrinth windows area, the mastoid air cells area, and the determination of the physiological protrusion of the sigmoid sinus within the mastoid. In addition to the possibility of assessing, air-containing cells around the sigmoid sinus were detected.

The exclusion criteria were patients who had undergone a course of conservative treatment in cases of misdiagnosed SSNHL, a history of any otological diseases, pathological contents in the mastoid cells and tympanic cavity, and signs of destructive changes in the areas of the ossicles and the middle ear cavity walls.

The diagnosis of PLF was established based on complaints, the medical history, a general otolaryngological examination, rhinoscopy, endoscopic nasal examination, otomicroscopy, audiometry, tympanometry, vestibulometry, temporal bone CT scans, and intraoperative endoscopic ear examination. Audiometric testing was performed using an ITERA audiometer (Denmark) to assess the perception thresholds for bone-conducted and air-conducted sounds across the relevant frequency range. The assessment of auditory function test results was conducted during the first visit and after surgical treatment at frequencies of 500 Hz, 1 kHz, 2 kHz, 4 kHz, and 8 kHz. For ET dysfunction registration, an inflation-deflation test was conducted using the middle ear analyser AT 235H (Interacoustics, Denmark).

The degree of mastoid pneumatisation was determined by assessing the volume and area of air-containing cells around the sigmoid sinus, according to S. Han et al. [5]. Using a quantitative digital image processing program during CT scans of the temporal bone, the area of the air cells of the mastoid was calculated for both the affected side and the conditionally contralateral healthy ear; the area of the bony part of the mastoid was not included in the calculation. Additionally, the length of the mastoid was assessed – measurements were taken only from the coronal plane slices by linear measuring from the roof of the antrum to the apex of the mastoid. During the analysis, the CT images of the temporal bones were projected onto a monitor screen for magnification.

This study differs from previous ones in that we measured the size of the air-containing cells of the mastoid rather than the entire area of the mastoid process. According to the adapted Han classification system, the degree of pneumatisation of the temporal bone was analysed by evaluating the air cells around the sigmoid sinus on axial CT images. The length of the mastoid process was measured from the roof of the antrum to its apex by analysing images in the coronal CT projection. Temporal bone CT scans were performed on a Philips Brilliance 64 CT scanner and were analysed using the DICOM and Radiant programs. Continuous slices with a thickness of 0.6 and 0.8 mm parallel to the orbitomeatal line were obtained. Thus, the pneumatisation degree was classified into three groups: hypopneumatization, normal pneumatization, and hyperpneumatization. Using a quantitative digital image processing program during temporal bone CT scans, only the area of the mastoid air cells was calculated; the area of the bony part of the mastoid process was not included in the calculation. Temporal bone CT scans were projected onto a screen for scaling, and the mastoid volume was measured using the Cavalieri principle.

ET dysfunction was considered to be present in the studied patients if there were clinical and radiological signs of permanent and periodic nasal breathing disorders, subjective feelings of nasal congestion or blockage, an established deviation of the nasal septum of varying degrees, hypertrophy of the inferior nasal turbinates and/or adenoidal and tonsillar hypertrophies, and chronic tonsillitis.

In all cases with pneumolabyrinth detection on temporal bone CT scans, patients underwent immediate surgical intervention. A minimally invasive endomeatal transcanal revision was performed using combined microscopic-endoscopic visualisation, with a check-up of the tympanic cavity and areas of the labyrinthine windows. In all cases of visual PLF detection, rupture closures were performed. In these patients, a comparison of the mastoid pneumatisation on the affected side and the conditionally healthy side was carried out. Treatment results were also evaluated depending on the preserved buffer function of the mastoid cellular system.

The study was conducted in compliance with the principles of the Declaration of Helsinki of the World Medical Association, "Ethical Principles of Medical Research Using a Person as an Object of Research," and current national regulations. The study design was approved by the local ethical committee. All patients gave written informed consent for the collection and processing of clinical material.

We used Statistica v. 14.0 (TIBCO Software Inc., USA) for data analysis. Quantitative data were presented as median with interquartile range. Qualitative data were presented as absolute and relative (%) frequency. Quantitative parameters between independent samples were compared by the use of Mann-Whitney U-test. A Wilcoxon signed-rank test was used to compare the results of the patients with unilateral PLF and the mastoid process parameters of the other conditionally healthy ear. A 2-tailed $p<0,05$ was considered statistically significant.

RESULTS

The main complaints of patients with suspected PLF were unilateral hearing loss with SSNHL or fluctuating hearing loss, noise of different calibers in the affected ear or head, vestibular manifestations in the form of dizziness, unsteadiness when walking, and increased vestibular symptoms during provocative tests – the fistula test, Tullio test, and Hennebert test.

In 32 (86.5%) of the 37 patients with clinical signs of unilateral spontaneous PLF or rupture of the labyrinthine window membranes, signs of impaired pneumatisation of the mastoid were observed on the affected side, characterised by a reduced volume of air-containing cells and a shorter mastoid length.

The types of mastoid pneumatisation on the affected side are presented in Fig. 1.

The mastoid volume was statistically significantly larger when evaluating the volume of mastoid air cells on the conditionally contralateral healthy ear side. At the same time, clinical and radiological findings identified signs of hypopneumatization on the affected ear side in 86% of patients.

Table 1 presents the average length of the mastoid process and its planimetric size depending on the type of mastoid pneumatisation.

As shown in the data in Table 1, the length of the mastoid process and its planimetric size in patients with hypopneumatized mastoids were significantly lower than in patients with non-hypopneumatized mastoids (which included 4 cases of normally pneumatized and 1 case of hyperpneumatized mastoid process).

Thirty-two patients had signs of impaired ventilation of the middle ear and mastoid due to Eustachian tube (ET) dysfunction, which complicated the process of equalizing pressure in the middle ear cavity during sudden pressure drops. The cause of ET dysfunction was the following pathologies: nasal septum deviation, inferior turbinate hypertrophy, and adenoidal and tonsil hypertrophy. This was considered one of the factors that reduced the protective buffer function of the mastoid, ventilation processes, and gas exchange in the middle ear cavities, which could be the cause of PLF with ruptures of the labyrinthine windows (Table 2).

According to the data from Table 2, during the assessment of the condition of the nose, nasopharynx, and throat, nasal septum deviation was observed in combination with hypertrophic changes in the inferior nasal turbinates in the majority of clinical cases – in 25 (67.6%) patients. During the evaluation of the status of the tympanic membrane on the affected ear side under otomicroscopic visualization, the following findings were established: thinning of the tympanic membrane (atrophic changes) in the area of the pars tensa was noted in 12 (32.5%) patients, controlled limited retraction in the pars flaccida was observed in 9 (24.3%) clinical cases, and retraction of the tympanic membrane in the area of the pars tensa was noted in 7 cases (18.9%). A conditionally normal otomicroscopic finding upon examination of the tympanic membrane on the affected ear side was established in 9 (24.3%) patients.

According to the obtained data, a conditionally normal otomicroscopic appearance of the tympanic membrane on the affected side was noted in nearly a quarter of patients with PLF and ruptures of the labyrinthine windows. In the remaining patients, visual changes in the tympanic membrane were

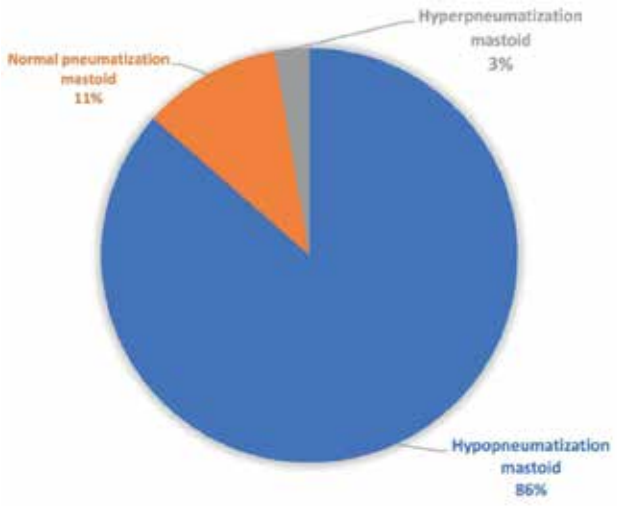

Fig. 1. Mastoid pneumatisation types based on temporal bones CT scans on the affected side in patients with PLF and labyrinthine window ruptures.

Table 1. The average length of the mastoid process and its planimetric size depending on the type of mastoid pneumatisation

Parameters	Hypopneumatized mastoid n=32	Non-hypopneumatized mastoid* n=5	p
Length of the mastoid process, mm	33,0 (32,6-33,4)	44,2 (44,0-44,8)	<0,001
Planimetric size of the mastoid process, cm^2	7,9 (6,4-8,6)	12,4 (10,2-15,4)	0,001
Volume of the mastoid process, cm^3	13,4 (12,6-14,8)	14,6 (14,4-15,2)	0,107

*Note: * –4 patients with normal pneumatized and 1 patient with hyperpneumatized mastoid.*

Table 2. Condition of the nose, nasopharynx, and throat in patients with PLF and labyrinthine windows ruptures

Additional criteria	Type of mastoid pneumatization (N)			n
	Hypopneumatization	Normal	Hyperpneumatization	
Affected ear	32	4	1	37
Healthy contralateral ear	-	33	4	37
Nasal septum deviation + hypertrophy of the inferior turbinates	23	2	-	25*
Isolated hypertrophy of the inferior turbinates	3	1	-	4**
Adenoidal/tonsils hypertrophy	2	-	-	2***
Chronic tonsillitis	4	1	1	6#

*Notes: * — 12 patients without a corresponding condition; ** — 33 patients without a corresponding condition; *** — 35 patients without a corresponding condition; # — 31 patients without a corresponding condition.*

Table 3. Main indicators for determining pneumatisation and the dimensions of the mastoid process on the affected ear side in patients with PLF and ruptures of the labyrinthine windows compared to the conditionally contralateral healthy ear

Indicators	Affected ear n=37	Healthy ear n=37	p
Total area of air-containing cells of the mastoid, cm^2	3,5 (2,8-4,3)	4,6 (4,4-4,8)	<0,001
Mastoid volume, cm^3	14,2 (12,6-14,8)	26,2 (24,6-26,8)	<0,001
Average length of mastoid, mm	33,1 (32,6-33,4)	44,8 (44,6-46,4)	<0,001

identified without any history of ear diseases in the patient's anamnesis. Such changes could be associated with signs of prolonged ET dysfunction due to the presence of mechanical obstructions in the nose, nasopharynx, and oropharynx. We also evaluated the main indicators for determining pneumatisation and the dimensions of the mastoid process on the affected ear side in patients with PLF and ruptures of the labyrinthine windows, compared with the conditionally contralateral healthy ear. These data are presented in Table 3.

As seen in the data from Table 3, the area of air-containing cells of the mastoid, its volume and the average length were statistically significantly lower when compared to the corresponding indicators on the conditionally healthy ear side. Thus, the obtained data may indicate a disruption of the protective buffering function on the affected ear side and can be considered one of the possible causes of PLF with labyrinthine windows ruptures.

DISCUSSION

The buffer function of the mastoid process contains in its ability to adapt and compensate for changes in pressure in the middle ear. The mechanism of temporal bone mastoid pneumatisation and its protective buffering function for the structures of the middle and inner ear are regulated by the natural function of the ET, which, during normal functioning and physiological opening when swallowing, allows atmospheric air to pass from the nasopharynx into the cavities of the middle ear [2].

The anatomical structure of the pneumatised mastoid, with its system of air-containing cells, contributes to the constant dynamic equalisation of pressure in the ear and reduces the risk of damage to the middle and inner ear. To ensure the physiological mechanism of pressure equalisation in the middle ear cavities and the mastoid air cells, the ET must have ideal functional characteristics – synchronously opening or closing at the level of its anatomical narrowing – the isthmus. This physiological regulation of ET function enables the middle ear cavities to maintain their stable pressure, providing a protective function to the sensitive elements of the middle and inner ear and ensuring the proper functioning of the entire auditory system. In the presence of mucosal oedema, pathological content in the ET, or other mechanical obstructions within the isthmus, a periodic or permanent blockage may occur [6]. Various mechanisms, including upper respiratory infections, sinusitis, mechanical obstructions of the nose, adenoidal and tonsils hypertrophy can cause ET dysfunction. S. Jain et al. [4] investigated various types of nasal septum deviation and their relationship with the development of ET dysfunction and found that in cases of nasal septum deviation and hypertrophy of the inferior nasal turbinates, there were signs of persistent ET dysfunction with the development of oedema in the area of the ET orifice and isthmus, along with decreased pressure in the tympanic cavity [7].

Under any atmospheric pressure changes, especially sudden ones, there are risks of pressure imbalance or incomplete

equalisation of pressure in the middle ear cavities [7, 8]. In such conditions, damage to the middle and inner ear is a significant risk. In our study, signs of ET dysfunction on the affected ear side were detected in 32 (86.5%) of the 37 examined patients. During the assessment of the condition of the nose, nasopharynx, and oropharynx, nasal septum deviation combined with hypertrophic changes in the inferior nasal turbinates was found in the vast majority of clinical cases – in 25 (67.6%) patients.

J. Sade et al. [1] established that with reduced pneumatisation of the mastoid, the buffering function of the middle ear cavities and the mastoid air cell system becomes limited. This leads to a tendency to develop a negative balance in gas exchange processes due to a persistently high level of negative pressure, which can subsequently result in chronic otitis media [2, 7].

The pneumatised mastoid, with its air cell structure, should provide an amortising buffer protection function during sudden atmospheric pressure changes. With adequate ET function, it helps prevent negative consequences for the delicate structures of the middle and inner ear [1, 2].

Evaluation of the mastoid air cells system using CT scans is considered to be more informative than conventional X-ray imaging [9-12].

Approximately 72-99% of the general population has symmetrical pneumatisation of the mastoid process [2, 8].

In the presence of asymmetric pneumatisation of the mastoid process with signs of hypopneumatization or sclerotic changes on CT scans, ear disease should be suspected [12]. According to N. Todd et al. [9], computed tomography is the optimal imaging method for assessing mastoid pneumatisation. Many other researchers share this opinion [2, 13]. The average value of the mastoid process pneumatisation was studied by N. Todd et al. [9] in 30 samples of temporal bones and was found to be 7,59±3,9 ml. In addition, the research of the volume of mastoid pneumatization by J. Sade et al. [14] demonstrated the values of 17,4±5 cm^2 in 150 normal ears and 12,9±4 cm^2 in 150 individuals with impaired mastoid pneumatisation.

The study conducted by S. Han et al. [5] established the average volume of the mastoid process (15,28±5,34 cm^3) using CT [5]. L. Stieglitz et al. [15] reported an average volume of air cells in the temporal bone of 10,97 ml based on multispiral computed tomography.

J. Jadhav et al. [13] founded the average volume of the temporal bone – 1337 mm^3, which is relatively smaller than in the two previously mentioned studies, as the authors did not include the mastoid air cell system in their classification and volumetric analysis.

We analyzed the volume of the mastoid process, the total area of air-containing cells in the mastoid, and the length of the mastoid process, considering the type of mastoid pneumatisation based on temporal bone CT. All measurements were performed and compared on the affected and the conditionally healthy contralateral ears. We found that in patients with hypopneumatized mastoids with PLF and ruptures of the labyrinthine windows, the volume of the mastoid process, the total area of the air-containing cells, and the length of the mastoid process were significantly lower, as compared to the alternative study group. Thus, in patients with PLF and ruptures of the labyrinthine windows, clinical and radiological signs of the mastoid hypopneumatization were identified in 86 % of patients, which we believe is one of the risk factors for its occurrence.

CONCLUSIONS

Thus, if mastoid pneumatisation is decreasing, its buffering function is compromised, posing a risk of damage to the middle and inner ear with the potential occurrence of PLF and labyrinthine window ruptures. The effectiveness of treatment of these patients depends on a comprehensive approach, one of the stages of which is improving the ET function, ventilation, and gas exchange in the middle ear cavities and restoring the protective buffer function of the mastoid process.

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ORIGINAL ARTICLE

Effect of apelin level in Iraqi diabetic women with hypothyroidism

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ABSTRACT

Aim: To determine how Apelin levels and other biochemical markers affect thyroid function of Iraqi diabetic women.

Materials and Methods: 88 subjects participated in this study, 44 diabetic women with hypothyroidism, whose ages between 40 and 60 years, and 44 healthy as a control group, whose ages between 40 and 55 years, during December 2022 to the end of April 2023. Demographic characters such as age, height, and weight of all participants were noted. Serum thyroxin, triiodothyronine and thyroid stimulating hormone were measured by Minivans with Biomerix Kits. Fasting serum glucose, glycated hemoglobin (HbA1c), lipid profile (total cholesterol, triglyceride, high density lipoprotein, and low-density lipoprotein, and very low-density lipoprotein), blood urea, and serum creatinine were among the tests performed on blood samples for the laboratory research. ELISA kit was used to measure amount of apelin.

Results: A highly significant increase of apelin levels in diabetic women with hypothyroidism when compared with control, a significant positive correlation $P < 0.05$ between Apelin level and each of: weight, body mass index, TSH, glycated hemoglobin (HbA1c), TG and VLDL and shows a highly significant positive correlation $P<0.05$ between Apelin level *vs*. TT4, FBS, TC and LDL. A significant negative correlation $P<0.05$ was found between Apelin levels vs. serum HDL in diabetic women with hypothyroidism.

Conclusions: A significant increase in results of Apelin in women with hypothyroidism and diabetes led to a significant imbalance in lipid metabolism, in the future, this might be an indicator of their risk of heart disease.

KEY WORD: apelin level, diabetic women, hypothyroidism, thyroid hormones, lipid profile

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INTRODUCTION

Thyroid disorders are among the most common endocrine disorders in the globe [1]. Most thyroid disorders have an impact on the quantity of thyroid hormones produced. Hyperthyroidism results from excessive production, while hypothyroidism results from decreased output [2].The intermediate metabolism is significantly influenced by thyroid hormones. They have an impact on lipid synthesis, mobilization, and degradation, however they have a greater impact on degradation than synthesis. Therefore, endothelial dysfunction, hypertension, and cardiovascular illnesses are all increased risks of thyroid dysfunction, particularly hypothyroidism, which is linked to dyslipidemia [3]. Obesity and hypothyroidism are two of the clinical illnesses most frequently linked to problems with fat metabolism [4] and dyslipidemia, one of the main risk agents for cardiovascular illness [5]. The word "hyperthyroidism" refers to overactive thyroid tissue that produces too much circulating free thyroxin (T4), triiodothyronine (T3), or both thyroid hormones [6]. Multiple anomalies, including elevated energy expenditure and excessive mobilization and use of metabolic substrates, are present in humans with hyperthyroidism [7]. Regarding lipid metabolism, it is evident that an excess of thyroid hormones promotes the disintegration of triglycerides (TGs) present in fat tissue, leading to a concentrate and circumvolution of non-esterified fatty acids (NEFA) [8]. A boost in the rate of lipid oxidation is linked to this increased fatty acid availability. Age-related increases in insulin resistance (IR) have been linked to a complex cluster of risk factors, including glucose metabolism impairment. The growth in DM is largely due to obesity and declining levels of physical activity [9].

MATERIALS AND METHODS

Eighty eight participated in this study. Forty four diabetic women with hypothyroidism, age between 40 and 60, forty four healthy control age between 40 and 55, during December 2022 to end of April 2023. Demographic characters such as age, height, and weight of all participants were noted. Serum TT3, TT4 and TSH were measured by Minivans with Biomerix Kits. Fasting serum glucose (FSG), glycated hemoglobin (HbA1c), lipid profile (total cholesterol, triglyceride, high density lipoprotein, and low density lipoprotein), blood urea, and serum creatinine were among the tests performed on blood samples for the laboratory research. ELISA kit was used to measure the amount of apelin.

STATISTICAL ANALYSIS

Means and standard deviation (SD) of data are expressed, 'P-values below 0.05 were regarded as significant, and p-values below 0.01 as very significant.' Excel was used to do the statistical analysis.

RESULTS

Characteristic biochemical parameters in diabetic women with hypothyroidism and control were summarized in Table 1. There was no significant deference of age, high, TT3, VLDL, B.

urea and S. creatinine between diabetic women with hypothyroidism and control group, while a highly significant increased (P<0.001) of BMI, TT4, TSH, FBS, TC, TG and LDL between diabetic women with hypothyroidism when compared with control group. Also, it seems in Table 1 there is a significant decreased (P<0.05) of S.HDL-C and a significant increase of weight and HbA1c diabetic women with hypothyroidism relative to control group.

Table 2 shows a highly significant increase of apelin levels diabetic women with hypothyroidism when compared with control.

Table 3 shows a significant positive correlation between apelin level *vs*. weight, BMI, TSH, HbA1c, TG and VLDL and shows a highly significant positive correlation between Apelin level vs. TT4, FBS, TC and LDL diabetic women with Hypothyroidism, while a significant negative correlation was found between Apelin level vs. S.HDL-C in diabetic women with hypothyroidism.

Figure 1 shows distribution of Apelin levels according to BMI categories (normal weight, overweight and obesity) diabetic women with hypothyroidism.

DISCUSSION

Two of the most prevalent chronic endocrine illnesses in women with varying frequency are thyroid dysfunction and diabetes mellitus [10]. An imbalance in your thyroid hormone either too high or too low - can affect the way that your liver makes and processes glucose. If the imbalance isn't addressed, it can impair blood glucose management for people with diabetes. Additionally, researchers have found that thyroid stimulating hormone may directly affect the level of the hormone leptin, which influences how hungry you feel and therefore plays a role in managing your body weight [11]. In multiple studies, even in people without a diagnosed thyroid dysfunction, higher levels of TSH (an indicator of overt hypothyroidism) were associated with higher blood sugar levels and more insulin resistance [12]. Aside from its effects on the body, low thyroid hormone can also play a role in how well a person is able to manage their diabetes [13-14]. According to Gronich et al. [15], hypothyroidism was commonly observed and was a danger element for newly developing T2DM. Elevated total cholesterol levels in the blood, LDL-C, and triglycerides are frequently seen in hypothyroidism. Present study indicates that TSH also takes part in lipid metabolism independently of thyroid hormone (TH), in addition to having an impact on the generation, clearance, and transformation of cholesterol. So the drop in TH and the rise in TSH levels are related to the mechanism of dyslipidemia caused by hypothyroidism. The underlying reasons of dyslipidemia in hypothyroidism include some recently discovered regulatory factors such proprotein convertase subtilizing/kexin type 9, angiogenin-related

Table 1. Demographic measures and biochemical values

Parameters	Diabetic women with hypothyroidism n=44, Mean ±SD	Control n=44 Mean ± SD	P-value
Age(year)	50.18± 4.13	47.25±0.0	NS
High(cm)	164.86± 9.35	169.05±5.44	NS
Weight(kg)	81.95±10.37	74.34±11.03	0.05*
BMI (kg/cm^2)	30.19±3.83	25.97±3.55	0.01**
T3(mMole/L)	2.11±0.49	1.21±0.46	NS
T4(mMole/L)	56.24±4.79	106.43±16.6	0.0001**
TSH(mIU/ml)	10.36±366	2.5±1.97	0.0001**
FBS (mg/dl)	205.18±58.0	79.34±6.34	0.0001**
HbA1c%	8.77±1.28	5.18±0.45	0.05*
TC(mg/dl)	230.04±55.42	158.88±12.59	0.0001**
TG (mg/dl)	256.09±59.6	93.95±7.80	0.0001**
LDL (mg/dl)	124.25±7.87	90.54±4.53	0.0001**
HDL (mg/dl)	34.77±1.46	55.13±3.67	0.05*
VLDL (mg/dl)	22.15±1.36	33.21±3.54	NS
B. Urea (mg/dl)	27.15±5.60	26.79±5.19	NS
S. Creatinine (mg/dl)	0.71±0.18	0.67±0.13	NS

*n=number; Data are given as mean ± SD; NS is no significant; * – is significantly P<0.05, ** – high significant P<0.001.*

Table 2. Apelin levels between diabetic women with hypothyroidism and control

P-value	Control (n=44)	Study Group (n=44)	Parameter
0.0001**	227.75±15.68	475.88±33.83	Apelin levels (pg/ml)

*n=number; Data are given as mean ± SD; **high significant P<0.01 and P<0.001*

Table 3. Correlation coefficients between Apelin level with patient's properties and biochemical markers

Parameters	Diabetic women with hypothyroidism r value	Control r value
Age(year)	0.026	0.262
High(cm)	0.132	0.250
Weight(kg)	0.271*	0.036
BMI (kg/cm^2)	0.343*	0.086
TT3(ng/dl)	0.237	0.08
TT4(pg/dl)	0.535**	0.041
TSH(mIU/ml)	0.322*	0.023
FBS (mg/dl)	0.541**	0.025
HbA1c%	0.333*	0.136
TC(mg/dl)	0.572**	0.052
TG (mg/dl)	0.339*	-0.013
LDL (mg/dl)	0.515**	0.048
HDL (mg/dl)	-0.367**	0.09
VLDL (mg/dl)	0.391*	-0.013
B. Urea (mg/dl)	0.124	0.103
S. Creatinine (mg/dl)	0.074	0.062

***Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level*

Fig. 1. Distribution of apelin levels according to BMI in diabetic women with hypothyroidism

proteins, and fibroblast growth factors. The function of HDL was allegedly compromised and its serum concentration fluctuations were inconsistent. The revised understanding of the mechanism behind dyslipidemia caused by hypothyroidism is the main topic of the current review [16]. Adipocytes are functionally active cells that produce biologically active peptides known as adipocytokines in addition to serving as energy storage cells. Nutrition, thermogenesis, immunity, thyroid and reproductive hormones, and neuroendocrine activities are all regulated by adipocytokines. Apelin is one of the most significant new members of this family. Typically, weight, thermogenesis, and fat tissue lipolysis fluctuate in females with thyroid dysfunctions [17]. Serum Apelin levels were lowest in the control group and greatest in diabetic women with hypothyroidism. Numerous publications have noted a link between severe obesity and a high apelin level and adiposity [18]. In the current study, we demonstrated a positive correlation between serum plasma apelin levels and BMI. This result is consistent with another study that indicated apelin may play a role in the etiology of obesity. Additionally, several investigations came to the conclusion that apelin levels, which positively correlate with BMI, are much greater in obese diabetic women with hypothyroidism than in control participants. In the present study's obese women, the regression analysis revealed a substantial positive correlation between the accumulation of subcutaneous fat and the level of apelin [19]. Serum levels of HDL in diabetic women with hypothyroidism had a negative correlation with apelin plasma levels. Due to apelin's ability to prevent lipolysis and promote fatty acid oxidation, plasma apelin levels are lowered in non-obese sick people with increased LDL cholesterol in addition to a positive connection between TG, TC and apelin. Apeline-13 has been shown to lower TG levels by upregulating AQP7 expression and reducing lipid accumulation in hypertrophic adipocytes by triggering the PI3K signaling pathway [20-21].

CONCLUSION

A significant increase in results of apelin in women with hypothyroidism and diabetes led to a significant imbalance in lipid metabolism (especially total cholesterol, triglycerides, low density lipoproteins and high-density lipoproteins). In the future, this might be an indicator of the risk of having heart disease. There no evidence of impact on kidney function markers.

ABBREVIATIONS

T4: thyroxin
T3: triiodothyronine
TSH: thyroid stimulating hormone

TC: total cholesterol,
TG: triglyceride,
HDL: high density lipoprotein
LDL: low-density lipoprotein
VLDL: very low-density lipoprotein
FSG: fasting serum glucose

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CONFLICT OF INTEREST

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ORIGINAL ARTICLE

Influence of comprehensive physical and psychological rehabilitation on the emotional state and coping strategies of students in crisis situations

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ABSTRACT

Aim: To examine the impact of a comprehensive physical and psychological rehabilitation program on students' emotional well-being, coping strategies and physical endurance in crisis situations following a full-scale invasion of Ukraine.

Materials and Methods: The standardized psychodiagnostic tests were used in the study. The sample of respondents consisted of 812 students within the age range of 18-22 years old; 46,9 % of respondents (n=381) were males, 53,1 % of respondents (n=431) were females. 226 (27,8 %) students were considered as internally displaced persons (IDPs) because of hostilities. 126 students took part in the full range of physical and psychological rehabilitation activities, aiming at the stress reduction and mood improvement.

Results: The study revealed that female students demonstrated higher levels of psychological well-being as compared to male students. In addition, differences were found in the use of coping strategies. Students from the unoccupied territories more often used active problem solving, while seeking social support was more common among IDPs. IDPs also more commonly used emotional regulation and avoidance. The comprehensive rehabilitation program favoured the increase in all aspects of subjective well-being levels, including psychological well-being, physical health and relationships.

Conclusions: Significant improvements in the emotional state of the participants were revealed, in particular, a decrease in the level of stress and anxiety, an increase in the use of adaptive coping strategies. The rehabilitation program also showed a positive impact on the physical endurance of students.

KEY WORDS: coping strategy, psychological health, comprehensive rehabilitation

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INTRODUCTION

Stress is an important issue for study, as it affects human health and behavior. Human professional activity in society is characterized by a significant influence of stress factors and the occurrence of stressful situations [1, 2]. This requires a reaction to ongoing events, and personality traits such as stress resistance are realized, ensuring the experience and overcoming of stressors. This is reflected in resilient behavior and is adapted accordingly in professional activity. Personal activity, including that related to the details of professional tasks, has its own special strategies and courses of action, known as coping strategies, which are closely related to the issue of psychological protection from stress and stressors [3]. Modern psychology views "experience" and "stress" as interconnected processes. Coping is the mechanism through which individuals handle life challenges, facilitating adaptation to their social environment and responses to stressors. Psychological defense is mostly unconscious, but individuals can consciously counteract stress.

AIM

The aim of the study was to examine the impact of a comprehensive physical and psychological rehabilitation program on students' emotional well-being, coping strategies, and physical endurance in crisis situations following a full-scale invasion of Ukraine.

MATERIALS AND METHODS

Psychodiagnostic methods that are standardized, valid, and tested have been selected for a comprehensive study [4]:

I. Theoretical analysis of scientific material related to the research problem.
II. Empirical research method:
   1. The Mental Health Continuum Short Form Questionnaire by Keyes (adapted by E.L. Nosenko, A.H. Chetveryk-Burchak) [4].
   2. The modified bbc subjective well-being scale (BBC-SWB) (P. Pontin, M. Schwannauer, S. Tai, & M. Kinderman) [5].
   3. The John Amirkhan Coping Strategies Indicators technique measures how individuals cope with psychological stress [6].
   4. Test E. Haim "Evaluation of Coping Strategies" is another method aimed at diagnosing coping strategies, which focuses on learning to respond to stressful situations [7].

Both techniques are used to study respondents in different groups, such as experimental and control groups, to find out what coping strategies are prevalent in these groups.

III. Quantitative and qualitative methods of processing: statistical analysis, elements of content analysis, meaningful interpretation of results. Statistical analysis was performed by the use of applied computer programs for universal processing of tabular data Microsoft Excel and a package of statistical analysis SPSS 12.0 for Windows. Quantitative data were presented as mean (M) ± standard deviation (SD) and 95 % confidence interval (CI). Qualitative data were presented as absolute and relative (%) frequency. Quantitative data between two independent samples was compared by the use of unpaired Student`s T-test; between more than two independent samples – by the use of ANOVA with the following Tukey`s HSD test for *post hoc* comparisons; and between dependent samples – by the use of paired Student`s T-test. Qualitative data between the independent samples was compared by the use of a χ^2 test. A p-value <0,05 was considered statistically significant (considering the correction for multiple comparisons).

The validity of the results and the reliability of the conclusions of the study depend on a set of empirical methods corresponding to the subject, objectives and hypotheses of the representative and large-scale study.

RESEARCH MATERIALS

The sample of respondents consisted of 812 students of the National University «Yuri Kondratyuk Poltava Polytechnic» studying in different specialties, the age range of respondents was from 18 to 22 years old; 46,9 % of respondents (n=381) were males, 53,1 % of respondents (n=431) were females, which ensured gender equality in the distribution of the sample of respondents and made it possible to consider the sample representative. Some of the students (n=226) are internally displaced persons (IDPs) from regions where hostilities are taking place. The survey was conducted anonymously, through a Google form, ensuring the principle of confidentiality of psychological research.

The concept of stress, introduced by Hans Selye, emphasized the role of external stressors [8]. R. Lazarus argued that a person's ability to cope with stress is more crucial than the stress itself. Rehabilitation specialists address stress at the intersection of medicine and psychology, with coping behavior helping balance external demands and internal resources. Research links coping to psychological defenses involving automatic or conscious responses to stress [9, 10]. The main difference is that defense mechanisms are subconscious, while coping strategies require awareness and can be applied in therapy under specific conditions [10, 11].

Coping strategies are interpreted as conscious methods of psychological regulation, active behavior strategies aimed at changing the situation and satisfying important needs. Psychological defense consists of unconscious passive mechanisms that reduce discomfort but may become maladaptive. Researchers view it as an innate self-defense process, enabling automatic adaptation through subconscious information processing [11-13]. Studies show that overcoming challenges shapes personality, but lasting psychological well-being requires conscious efforts to transform both external circumstances and inner structures. A holistic approach to psychological trauma helps identify patterns and mechanisms of adaptation, allowing individuals to integrate stress into their experience without distortion. This study examines factors influencing coping strategies, categorized as internal (psychophysiological, neurodynamic, and personality traits) and external (situational and environmental stressors). The study focused on the concepts of "extreme environment" and "extreme situation."

In accordance with the stated goal, the following stages of research were conducted:

Stage 1 – defining health-related quality of life, self-esteem as a resource for the appropriateness of individual behavior [4, 5].

Stage 2 – studing the coping strategies of the respondents [6, 7]. Identifying and explaining the details of the formation of coping strategies at different stages of personality developmen.

Stage 3 – identification of the relationship between coping strategies and personal characteristics and subjective assessment of the quality of life related to health in respondents of the experimental group.

ETHICS

The study was conducted in accordance with the recommendations of the Ethics Committees for Biomedical Research, Ukrainian Health Legislation and the Declaration of Helsinki of 2000, European Community Directive 86/609 On Human Participation in Biomedical Research. Conducting the research does not contradict the norms of Ukrainian legislation and meets the requirements of the Law of Ukraine «On Scientific and Scientific-Technical Activities» dated November 26, 2015 No. 848-VIII.

RESULTS

The results of the Mental Health Stability Short Form Questionnaire were as follows (max score = 5) (Fig. 1). The average indicators for each aspect of mental health in the table are on the verge of «above average», but one can notice slightly higher results for the level of psychological well-being, which is associated with the adaptability of young people, subjective assessment of their psychological state and satisfaction with personal quality of life.

Students of all age groups demonstrate indicators of all aspects of mental health within the same numerical limits, which may be associated with the physiological and psychological characteristics of this age category, namely the formation of self-awareness, the development of willpower, the formation of one's own worldview as a holistic system of views, knowledge, and beliefs, the desire to assert one's independence and originality, and a significant restructuring of the emotional sphere.

The gender characteristics of the studied aspects of mental health are presented in Table 1.

As shown in Table 2, boys have lower mental health indicators than girls, particularly hedonic well-being, social well-being (numerically, but non-significantly) and psychological well-being, as compared to girls.

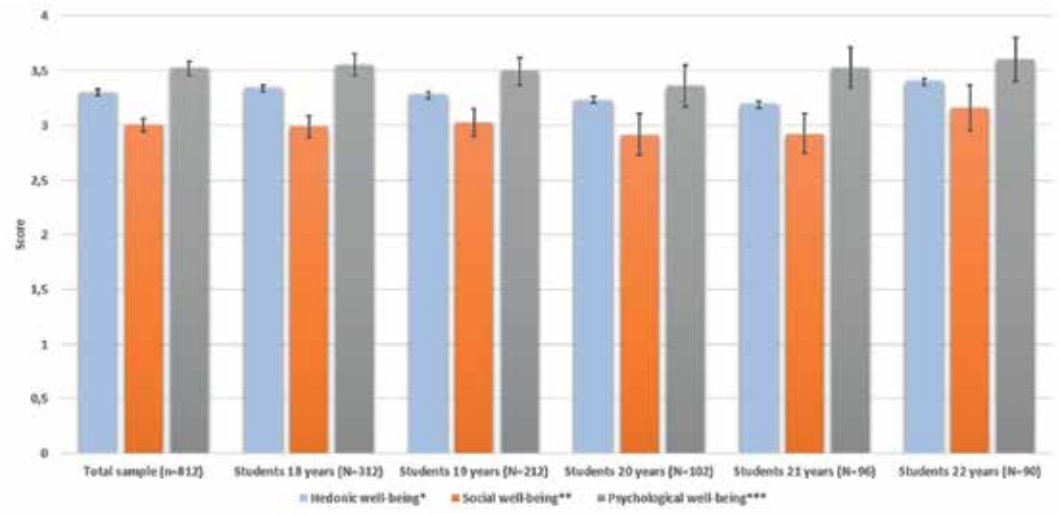

Fig. 1. The results of the Mental Health Stability Short Form Questionnaire in the total sample and different age subsamples of students.
Data presented as M ± 95 % CI. Between age groups: * – p=0,595; ** – p=0,368; *** – p=0,357.

Table 1. Gender characteristics in mental health assessment

Mental health indicator	Males n=381	Females n=431	p
Hedonic well-being, score	3,19 ± 1,060	3,39 ± 1,067	0,006
Social well-being, score	2,95 ± 0,930	3,04 ± 0,923	0,136
Psychological well-being, score	3,43 ± 0,926	3,59 ± 0,905	0,010

Table 2. Results of the study based on the method of the Modified BBC Subjective Well-being Scale in the whole sample of students (n=812)

Subscales and Overall Subjective Well-Being Score	Standard numerical values proposed by the authors of the questionnaire			Obtained results n=812
	Low level	Medium level	High level	
Psychological well-being, score	12-39	40-47	48-60	41,11 ± 9,694 95 % CI [40,44-41,77]
Physical health, score	7-20	21-25	26-35	22,70 ± 6,458 95 % CI [22,25—23,14]
Relationships, score	5-16	17-20	21-25	17,99 ± 4,659 95 % CI [17,67-18,31]
Subjective well-being, score	24-76	77-91	92-120	81,79 ± 18,957 95 % CI [80,48-83,10]

The results of the study were analyzed using the Modified BBC Subjective Well-being Scale (Table 2).

As can be seen in Table 2, all the results correspond to the average medium level of well-being, but are borderline to low. Comparing the results by age distribution, there are no significant differences between the studied groups (Fig. 2).

The data from Table 3 suggest the certain differences between males and females regarding the the Modified BBC Subjective Well-being Scale scores. Particularly, the girls demonstrate the higher values of psychological well-being, physical health and relationships scores, as well as the overall subjective well-being score, in contrast to boys. These differences can be explained by the peculiarity of age development and gender differences, manifestations of resilience and ways of overcoming stressful situations.

The second stage of the study was to determine the coping strategies of the respondents (coping strategy indicator by John Amirkhan, test by Erich Haim [9, 10]) (Table 4).

Table 4 compares the use of coping strategies among students from the unoccupied territories and IDPs. The results show significant differences between these groups.

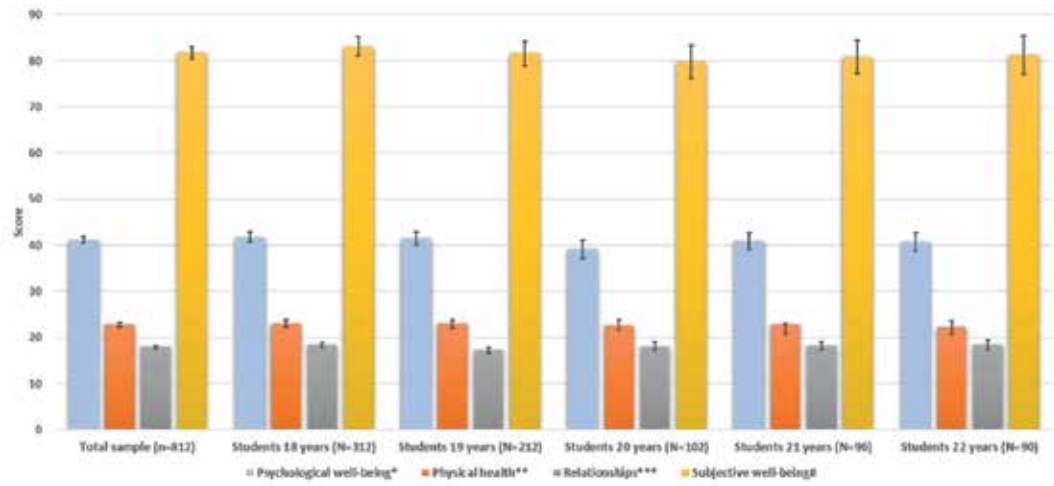

Fig. 2. The results of the Modified BBC Subjective Well-being Scale in the total sample and different age subsamples of students. Data presented as M ± 95 % CI. Between age groups: * – p=0,164; ** – p=0,511; *** – p=0,097; # – p=0,565.

Table 3. Gender characteristics of the Modified BBC Subjective Well-being Scale scores

Subscales and Overall Subjective Well-Being Score	Males n=381	Females n=431	p
Psychological well-being, score	40,32 ± 9,619	41,80 ± 9,718	0,029
Physical health, score	22,05 ± 6,372	23,27 ± 6,486	0,007
Relationships, score	17,60 ± 4,640	18,33 ± 4,654	0,024
Subjective well-being, score	79,96 ± 18,707	83,41 ± 19,051	0,010

Table 4. The coping strategies among the students from unoccupied and occupied territories

Coping strategy	Students from unoccupied territories N=586	Students from occupied territories (IDPs) N=226	p
Active problem solving, n (%)	293 (50,0)	59 (26,1)	<0,001
Social support, n (%)	293 (50,0)	158 (69,9)	<0,001
Emotional regulation, n (%)	234 (39,9)	113 (50,0)	0,009
Avoidance, n (%)	196 (33,5)	136 (60,2)	<0,001

Active problem solving is more commonly used by students from the unoccupied territories (50,0% vs. 26,1%; p<0,001), while seeking social support is more common among IDPs (69,9% vs. 50,0%; p<0,001). IDPs also more commonly use emotional regulation (50,0% vs. 39,9%; p = 0,009) and avoidance (60,2% vs. 33,5%; p<0,001).

Table 5 provides an explanation of the main coping strategies used by students from both groups. Students from the unoccupied territories are characterized by active problem solving in academic and social situations, seeking support in the group, among teachers and family, emotional regulation through interpersonal contacts and moderate avoidance of stressful situations. In contrast, IDPs are dominated by the desire to solve problems related to adaptation to new living conditions, they more often seek social support, use emotional regulation strategies aimed at preserving the familiar environment, and may resort to avoidance to reduce emotional discomfort.

Based on the diagnostic results, risk areas for the health of students were identified, especially with IDPs, since such students demonstrated lower assessments of mental health aspects. In particular, a psychosocial support program was developed aimed at reducing stress, increasing self-esteem and supporting the mental health of students, a program of comprehensive physical and psychological rehabilitation of students during the full-scale invasion of Ukraine. The program was based on an integrated approach and relied on general scientific principles of consistency and objectivity [17, 18]. Among the aspects of improving the level of mental health, both physical rehabilitation and the development of psychological health and social connections were taken into account. Psychological trainings

Table 5. Explanation of coping strategy

Group	Active problem solving	Social support	Emotional regulation	Avoidance
Students from unoccupied territories	Finding solutions in learning and social situations, active problem solving.	Use support from groupmates, teachers, and family to overcome problems.	Emotional regulation strategies through interpersonal contacts and social support.	Moderate avoidance of stressful situations such as academic stress or conflicts.
IDPs from occupied territories	The desire to solve problems related to adaptation and new life realities.	High level of seeking support from family, friends and social organizations.	Strategies for emotional regulation through maintaining the environment, adapting to changes.	Possible avoidance due to a desire to avoid emotional trauma or stress associated with moving.

Table 6. The repeated results of the Modified BBC Subjective Well-Being Scale (n=126)

Subscales and Overall Subjective Well-Being Score	Standard numerical values proposed by the authors of the questionnaire			Primary results	Repeated results	p
	Low level	Medium level	High level			
Psychological well-being, score	12-39	40-47	48-60	35,87 ± 8,686 95 % CI [34,33-37,40]	41,99 ± 5,693 95 % CI [40,99-43,00]	<0,001
Physical health, score	7-20	21-25	26-35	18,55 ± 4,952 95 % CI [17,68-19,42]	21,08 ± 5,179 95 % CI [20,17-21,99]	<0,001
Relationships, score	5-16	17-20	21-25	15,71 ± 4,697 95 % CI [14,89-16,54]	19,53 ± 2,884 95 % CI [19,02-20,04]	<0,001
Subjective well-being, score	24-76	77-91	92-120	70,13 ± 15,469 95 % CI [67,40-72,85]	76,27 ± 17,038 95 % CI [73,27-79,27]	<0,001

and workshops are used to reduce stress and improve emotional regulation through meditation, relaxation and art therapy. Relaxation and breathing techniques are important for reducing physiological symptoms of stress, such as tachycardia and muscle tension [19, 20].

Integration of physical and psychological renewal methods was achieved through multidisciplinary classes combining physical training and psychological classes to reduce stress and improve mood. The interaction of participants in groups was implemented on the principle of «peer-to-peer». 318 first-year students of the National University «Yuri Kondratyuk Poltava Polytechnic» took part in the stress resistance development trainings. Classes were held in May-June 2024 at the Student Hub of the University, 126 students took part in the full range of rehabilitation activities. The training sessions were followed by a control psychodiagnostic study of the level of mental health, which showed positive dynamics.

Analyzing the results of the study using the Modified BBC Subjective Well-being Scale, repeated results were obtained, which are presented in Table 6.

According to the obtained results, we observed an increase in all aspects of subjective well-being levels after completing the training sessions, which might indicate the effectiveness of the comprehensive rehabilitation program, and could be generally contributed to the resilience of the student youth and a high level of personal effectiveness in difficult life conditions.

DISCUSSION

Physical rehabilitation included aerobic exercise to reduce stress and anxiety, and yoga to improve physical fitness, reduce body tension, and increase flexibility [11, 17]. Strength training was also provided to maintain physical fitness and reduce loss of fitness. Outdoor group activities, such as hikes and team games, were organized to promote social interaction and restore physical activity. Physical health support included massage and therapy to relieve muscle tension, and consultation with a nutritionist to maintain a healthy diet that increased physical endurance [9, 18, 20].

The results of the study showed that the students' physical and psychological well-being improved significantly after

completing the comprehensive rehabilitation program. The findings indicate that rehabilitation programs have a positive effect on students' emotional responses, especially in reducing stress and anxiety levels and developing more adaptive coping strategies.

Special attention should be paid to the use of coping strategies among students of different groups. According to the data obtained, students from the unoccupied territories used active problem solving more often, which indicates their tendency to a rational approach to overcoming difficulties. They turned to social support, which emphasizes the importance of interpersonal interaction in the adaptation process. At the same time, students who were forced to leave their homes resorted to social support much more often, which reflects their need to restore a sense of security through interaction with relatives, friends and public organizations.

Additionally, a greater use of emotional regulation strategies was observed among IDPs, which is likely related to the need to adapt to new living conditions. Their emotional regulation methods included maintaining social ties, psychological adaptation, and forming new habits that helped stabilize their emotional state. At the same time, a significant proportion of this group showed a tendency to avoid problematic situations, which may be explained by psychological fatigue, the need to reduce emotional stress, and a desire to avoid repeated traumatic experiences.

Moreover, the analysis did not reveal statistically significant age differences among the studied groups. This indicates that the effectiveness of the rehabilitation program was not dependent on the students' age characteristics but was equally effective across different age categories. The results obtained confirm the universality of the proposed approach to physical and psychological rehabilitation, making it applicable to a wide range of students regardless of age. Thus, it can be concluded that the comprehensive rehabilitation program has a positive effect on students' psycho-emotional state and physical health, promotes the development of adaptive coping strategies, and facilitates the process of social integration.

CONCLUSIONS

The results of the study confirm the high efficiency of the comprehensive program of physical and psychological rehabilitation of students during the full-scale invasion of Ukraine. The use of maladaptive behavior patterns has become more frequent.

The rehabilitation programs also showed a positive effect on the physical endurance of students, which indicates the importance of combining physical and psychological approaches in rehabilitation activities. Particular attention should be paid to the fact that the improvement was observed precisely in crisis situations that have a great impact on the psychophysiological state of young people.

The data highlights the importance of rehabilitation programs in educational institutions, especially for students, displaced persons, or those in difficult situations. These programs restore emotional balance, improve physical health, and help young people develop resilience to overcome modern challenges. A comprehensive rehabilitation program can thus become a key support system for students in crisis, enhancing their self-healing abilities and overall well-being.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

ORIGINAL ARTICLE

Dehumanization and attitudes toward LGBTQ individuals among primary healthcare nurses: The role of personality traits and LGBTQ health knowledge

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ABSTRACT

Aim: This study investigates dehumanization and attitudes toward LGBTQ+ individuals among primary healthcare nurses in Greece, exploring the influence of personality traits, empathy, and LGBTQ+ health knowledge.

Materials and Methods: A cross-sectional design was used with 114 public-sector primary healthcare nurses completing self-report questionnaires between July and October 2023. Instruments included a culturally adapted dehumanization scale, the Ten-Item Personality Inventory, and the Toronto Empathy Questionnaire. Statistical analysis included Mann-Whitney and Kruskal-Wallis tests, Spearman's correlations, and linear regression.

Results: The sample was predominantly female (74.6%), heterosexual (93.9%), and Christian Orthodox (93%). Only 8.8% had attended LGBTQ+ healthcare courses, and 33.3% had cared for LGBTQ+ patients. Mechanistic dehumanization showed limited associations with personality traits, while animalistic dehumanization was negatively correlated with willingness to care ($r = -0.441$, $p < 0.001$) and comfort with LGBTQ+ care ($r = -0.391$, $p < 0.001$). Empathy and openness to experience influenced attitudes and willingness to care. Higher empathy unexpectedly reduced willingness to care, while emotional stability and conscientiousness predicted dehumanization.

Conclusions: Findings highlight a moderate dehumanization trend among nurses, affecting LGBTQ+ patients' care quality. Educational initiatives targeting LGBTQ+ health knowledge, empathy training, and the influence of personality traits are critical to fostering inclusive care and reducing dehumanization in healthcare settings.

KEY WORDS: LGBTQ+ healthcare, dehumanization, primary healthcare nurses, personality traits, empathy

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INTRODUCTION

In recent years, there has been a notable escalation in the global demographic of individuals who identify as lesbian, gay, bisexual, transgender, queer, and other diverse sexual and gender identities [1]. As this demographic continues to proliferate, there is a corresponding imperative for healthcare services that are not only accessible but also comprehensive and culturally sensitive.

Historically, LGBTQ+ individuals have encountered significant stigmatization and discrimination across various sectors of society, including within the healthcare domain, predicated on their sexual orientation or gender identity. When pursuing medical care, LGBTQ+ individuals frequently confront healthcare systems inadequately prepared to cater to a spectrum of sexual and gender identities, alongside healthcare practitioners who have undergone limited educational training to competently deliver inclusive and affirming care for the LGBTQ+ community [2, 3].

The prevalence of heteronormativity among healthcare providers often engenders presumptions regarding the gender of patients' partners and fosters stigmatizing actions, such as the exclusion of LGBTQ+ partners from visitation rights. This phenomenon can result in a diminished quality of care for LGBTQ+ individuals [4]. Unfortunately, these adverse experiences may compel LGBTQ+ patients to eschew or postpone seeking essential healthcare services, thereby jeopardizing their overall health and well-being [5, 6]

LGBTQ+ patients may, at times, endure discrimination within healthcare environments, which can significantly influence their decisions regarding engagement with necessary healthcare services [7]. Instances such as the denial of examination or treatment, being dismissed or not taken seriously, or the apprehension of experiencing discrimination can deter patients from attending medical appointments [8].

Pantheoretical dehumanization constitutes the amalgamation of various stigma-oriented, group-specific, and oppression-related frameworks within the purview of counseling psychology, aimed at elucidating the affective, cognitive, and physiological repercussions of dehumanization through a singular model [9].

Researchers have employed the concept of pantheoretical dehumanization in the context of transgender women and men, with an emphasis on minority stress theory. A significant portion of research utilizing a minority stress perspective concentrates on prominent discriminatory occurrences [10].

Proximal stressors, including the postponement or avoidance of healthcare services as a result of prior experiences involving stigmatizing conduct or the anticipation thereof, are also associated with adverse health outcomes [11, 12]. These intricate challenges have accentuated the necessity for inclusive and affirming practices and systems within healthcare to promote health equity for LGBTQ+ individuals.

Several concur that LGBTQ+ individuals frequently receive subpar healthcare in comparison to the general populace. A potential rationale for this disparity may reside in the existence of negative attitudes [13] and the insufficient education of healthcare professionals [14]. The presence of perceived or actual homophobic barriers renders LGBTQ+ individuals hesitant to seek hospitalization and comply with healthcare providers' recommendations. Furthermore, the reluctance to disclose their sexual orientation inhibits their access to specialized medical care. [15, 16].

More recent studies report that nurses have neutral or negative attitudes towards LGBT people [15, 16].

Determinants that seem to correlate with nurses' perceptions of LGBT individuals have been identified as personality characteristics and levels of empathy. Empirical research has indicated that, on average, males are more likely to exhibit negative attitudes toward LGBTQ populations in comparison to their female counterparts [17]. The underlying reasons for this gender disparity may be attributed to multiple factors. One plausible rationale resides in the societal constructs surrounding masculinity. Conventional notions of masculinity are frequently linked to attributes such as toughness, aggression, and dominance, which may subsequently lead men to view LGBTQ individuals as threats to their own masculine identity or authority [18]. his phenomenon may also be related to the observation that young males are often socialized to adopt more aggressive and competitive behaviors, whereas young females are encouraged to cultivate nurturing and empathetic traits [19]. Such divergent socialization practices can engender varying attitudes toward LGBTQ individuals, with males being more inclined to regard these identities as deviant or aberrant. Nevertheless, no singular factor appears to account for this gender divergence; instead, it represents a multifaceted interplay of gender norms, socialization processes, and cultural ideologies.

Existing research has consistently demonstrated that right-wing authoritarianism is a significant predictor of prejudice and negative attitudes toward outgroups, including LGBTQ individuals. This can be explained by the fact that LGBTQ people have often faced disapproval and stigma within many religious communities and government institutions, as they are perceived as challenging traditional social conventions[20]. Furthermore, nurses who hold strong right-wing authoritarian beliefs may be more inclined to endorse negative attitudes and dehumanizing views toward LGBTQ individuals due to their heightened sensitivity to social conformity and perceived threats to traditional social structures [21].

Primary care nurses are often a person's first point of contact with the health system and so nurses tend to build close relationships with patients. Although there are studies on negative attitudes and lack of appropriate training among health professionals, there is no research focusing on the impact of dehumanisation by primary care nurses towards LGBTQ+ patients and its association with personality traits and attitudes.

AIM

To investigate how dehumanizing attitudes among primary healthcare nurses impact their perceptions and treatment of LGBTQ+ individuals. It aims to explore the influence of personality traits, empathy, and LGBTQ+ health knowledge on these attitudes.

MATERIALS AND METHODS

DESIGN

A cross-sectional study design was employed in this study.

SAMPLE

The study sample consisted of 114 primary healthcare nurses from the public sector in Greece, who completed self-report questionnaires. The data collection period spanned from July 2023 to October 2023. Participation was voluntary, and the nurses provided informed consent after being comprehensively informed about the study's purpose.

ETHICS STATEMENT

This research was sanctioned by the Institutional Review Board of a state university located in Thessaly. Furthermore, this research is in accordance with the Helsinki Declaration (2013) and conforms to the ethical standards established by the pertinent national and institutional committees governing human experimentation. Additionally, the confidentiality of the participants was assured through the implementation of self-selected codes. Informed consent was acquired from each participant prior to their involvement in the study. Access to the gathered data was restricted solely to the investigators, who ensured its secure electronic storage within a password-protected file.

DATA COLLECTION

To collect the data, we used the following questionnaires:

The participants were asked to fill in a series of questions assessing sociodemographic data.

Primary healthcare nurses participated in the Greek Version of the Nursing Students' Knowledge of and Attitudes toward LGBT Health Concerns survey [22], which was originally formulated in 2015 by Cornelius and Carrick [23] and comprises a total of 70 items. This survey is divided into two distinct sections. The initial section consists of 37 inquiries designed to evaluate the knowledge of primary healthcare nurses regarding LGBT healthcare matters, wherein each

accurate response is assigned a value of 1, while incorrect responses or those indicating uncertainty are assigned a value of 0. The subsequent section includes 25 inquiries aimed at investigating the attitudes of nurses toward delivering healthcare services to LGBT individuals, utilizing a five-point Likert scale for measurement. Elevated scores reflect a more favorable self-assessment of attitudes and an increased level of comfort in providing care to LGBT patients.

To assess the dehumanization of nurses, a modified and culturally adapted Greek version of the dehumanization scale was used [22]. "Human Uniqueness" denotes characteristics that set humans apart from other species, encompassing traits such as refinement, civility, self-restraint, and advanced cognitive abilities. "Human Nature" encapsulates the collective and essential traits inherent to humanity, which include sensitivity, independence, benevolence, and cognitive adaptability. When individuals are deprived of traits associated with Human Uniqueness, they are likened to animals and described as juvenile, immature, discourteous, irrational, or regressive. The negation of Human Nature attributes results in comparisons to inanimate objects or mechanistic entities, depicting them as devoid of emotions, rigid, passive, and lacking in both sentiment and agency. This particular assessment employs a seven-point scale comprising eight statements. An illustration would be "they exhibit open-mindedness and possess the ability to engage in reflective thought," where a "1" signifies complete disagreement and a "7" indicates total agreement. Elevated scores are indicative of dehumanizing tendencies, whereas lower scores imply the absence of such dehumanization. The scale is designed to evaluate the two distinct forms of dehumanization, featuring four items related to animalistic dehumanization and four pertaining to mechanistic dehumanization [24].

The Ten-Item Personality Inventory, formulated by Gosling et al. [25], constitutes a concise self-reported instrument comprising ten items that evaluate personality traits in accordance with the Big Five Factors Model as delineated by Costa and McCrae [26, 27]. In each dimension of the Big Five framework, one item signifies a positive attribute, while the corresponding item denotes a negative attribute. Participants are instructed to assess the applicability of each trait to their own persona utilizing a seven-point Likert scale. This scale has exhibited commendable convergent validity, test-retest reliability, and concordance between self-reported and observer ratings [28]. Furthermore, the scale has been empirically validated in the Greek context by Koutras et al. [29].

The Toronto Empathy Questionnaire, conceptualized by Spreng et al. [30], serves as a succinct self-report instrument aimed at measuring empathy as an emotional phenomenon. The TEQ is comprised of 16 items that are meticulously crafted to examine the behavioral, emotional, cognitive, and physiological dimensions of empathy among individuals spanning a diverse range of contexts [31]. The TEQ has been utilized across multiple countries, languages, and psychological frameworks [32]. Moreover, the TEQ has undergone validation in Greece, where the Cronbach's α coefficient was deemed to be acceptable at 0.72 [33].

STATISTICAL ANALYSIS

The assessment of the normality of the distribution pertaining to quantitative variables was conducted utilizing the Kolmogorov-Smirnov test. The non-parametric Mann-Whitney test was employed to assess differences in quantitative variables across two distinct groups. The evaluation of quantitative variables across more than two groups was executed using the non-parametric Kruskal-Wallis test. The examination of the correlation between two quantitative variables was carried out employing Spearman's correlation coefficient (r). The reliability of the administered questionnaires was evaluated through Cronbach's α coefficient. The significance levels were determined to be two-tailed, with the threshold for statistical significance established at 0.05. The statistical analysis was conducted utilizing the SPSS version 22.0 software.

RESULTS

The study sample comprised 114 nurses working in primary health care , with the majority being women (74.6%) and men representing 25.4%. Most participants were between the ages of 31 and 40 (30.7%), with no individuals older than 60. Regarding sexual orientation, the vast majority identified as heterosexual (93.9%), while a small percentage identified as bisexual (4.4%) or homosexual (1.8%). The participants' marital status showed that most were married (50.9%) or single (35.1%). All participants were of Greek nationality (100%). About 42.1% reported having no children, while 28.1% had two children.In terms of education, the largest group had completed high school, vocational training, or equivalent (39.5%), followed by university or technical school graduates (36%). Notably, none had postgraduate or doctoral degrees. Regarding religious identity, most identified as Christian Orthodox (93%), while a small percentage identified as atheist (4.4%) or belonging to other religions (2.6%). The average years of service were 12.13 years (SD=9.42), with a range of 0 to 39 years. Most participants lived in urban areas (76.3%), with fewer residing in semi-urban (18.4%) or rural areas (5.3%). When it comes to familiarity with LGBTQ+ individuals, 55.3% personally knew someone identifying as LGBTQ+, but only 8.8% had attended a course on LGBTQ+ healthcare issues. Additionally, only 33.3% had cared for an LGBTQ+ patient, indicating limited professional exposure. For detailed percentages and numerical data regarding demographics, education, residence, and LGBTQ+ familiarity, please refer to Table 1.

The descriptive statistics of the scales used in the study provide insights into participants' knowledge, attitudes, personality traits, and perceptions. The mean knowledge score was 16.14 (SD=5.17), ranging from 3 to 26, suggesting a moderate level of knowledge among participants. Comfort with LGBTQ+ care had a mean score of 2.34 (SD=0.41) on a scale from 1 to 3.67, indicating overall moderate comfort. Responsibility scores averaged 2.72 (SD=0.54) on a scale from 1 to 4, while willingness to care had a higher mean of 3.77 (SD=0.78) on a scale from 1.455 to 5, suggesting a strong inclination to provide care. Mechanistic dehumanization scored an average of 13.35 (SD=3.82) on a range of 5 to 25.

Table 1. Demographic profile of participants

	N	%
Gender		
Male	29	25,4%
Female	85	74,6%
Other	0	0%
I prefer not to answer	0	0%
Age		
<30	33	28,9%
31-40	35	30,7%
41-50	30	26,3%
-60	16	14%
>60	0	0%
Sexual orientation		
Bisexual	5	4,4%
Homosexual	2	1,8%
Heterosexual	107	93,9%
Other	0	0%
Marital status		
Unmarried	40	35,1%
Married	58	50,9%
Divorced	9	7,9%
Cohabitation	5	4,4%
Widower	2	1,8%
Number of children		
None	48	42,1%
One	19	16,7%
Two	32	28,1%
Three	12	10,5%
Four or more	3	2,6%
Level of education		
Elementary	28	24,6%
High school	45	39,5%
University	41	36%
Postgraduate	0	0%
Religious affiliation		
Christian Orthodox	106	93%
Roman Catholic	0	0%
Muslim	0	0%
Other	3	2,6%
Atheist	5	4,4%
I am not a member of any religion	0	0%
Occupational experience		
Mean ± St. Dev.	12,13 ± 9,42	
Min – Max	0 – 39	
Residence		
Urban	87	76,3%
Semi - Urban	21	18,4%
Rural	6	5,3%

Table 1. Cont.

Do you personally know someone who is lesbian, gay, bisexual, or bi-sexual?		
Yes	63	55,3%
No	51	44,7%
Have you taken a course that addresses LGBTQ+ health care issues?		
Yes	10	8,8%
No	104	91,2%
Have you ever cared for an LGBTQ+ patient?		
Yes	38	33,3%
No	76	66,7%

Table 2. Descriptive statistics of the scales used in the study

	Mean	Std. Deviation	Minimum	Maximum
Knowledge Score	16.140	5.171	3.000	26.000
Comfortable	2.337	0.414	1.000	3.667
Responsibility	2.715	0.540	1.000	4.000
Willingness to care	3.768	0.781	1.455	5.000
Mechanistic Dehumanization	13.348	3.820	5.000	25.000
Animalistc Dehumanization	12.947	4.035	6.000	28.000
Extraversion	8.789	2.240	4.000	14.000
Agreeablenes	10.702	1.895	4.000	14.000
Conscientiousness	11.272	1.710	6.000	14.000
Emotionalstability	9.553	2.083	3.000	14.000
Opennesstoexperience	9.588	2.273	4.000	14.000

Animalistic dehumanization had a slightly lower mean of 12.95 (SD=4.04), ranging from 6 to 28. Finally, in ragards of the Big Five personality dimensions had the following mean scores Extraversion: 8.79 (SD=2.24), range 4-14. Agreeableness: 10.70 (SD=1.90), range 4-14. Conscientiousness: 11.27 (SD=1.71), range 6-14. Emotional Stability: 9.55 (SD=2.08), range 3-14. Openness to Experience: 9.59 (SD=2.27), range 4-14.For detailed numerical data and the variability of responses, please refer to Table 2, which summarizes the descriptive statistics of the scales used in the study.

The Pearson's correlation analysis revealed significant relationships between several variables related to knowledge, attitudes, empathy, personality traits, and dehumanization of LGBTQ+ individuals. Mechanistic dehumanization showed no significant correlations with the examined traits or attitudes, although it had a near-significant positive correlation with emotional stability ($r=0.175$, $p=0.065$). Animalistic dehumanization, on the other hand, demonstrated a strong positive correlation with mechanistic dehumanization ($r=0.659$, $p<0.001$) and was negatively correlated with willingness to care ($r=-0.441$, $p<0.001$). Regarding attitudes, comfort with LGBTQ+ care was negatively correlated with animalistic dehumanization ($r=-0.391$, $p<0.001$) and showed weak associations with other variables. Responsibility was positively associated with animalistic dehumanization ($r=0.194$, $p=0.039$) and empathy ($r=-0.143$, $p=0.130$), though the latter was not significant. Interestingly, willingness to care was negatively correlated with empathy ($r=-0.254$, $p=0.006$) and animalistic dehumanization but positively associated with openness to experience ($r=0.215$, $p=0.022$). These findings suggest that personality traits and empathy play a critical role in shaping attitudes toward LGBTQ+ individuals, with dehumanization metrics showing notable associations. For detailed statistics, refer to Table 3.

The linear regression analysis assessed the impact of knowledge, empathy, and personality traits on attitudes and dehumanization, adjusting for demographic variables. For attitudes, the model predicting comfort showed no significant predictors, though openness to experience approached significance with a negative relationship ($\beta=-0.219$, $p=0.050$). For responsibility, extraversion was the only significant positive predictor ($\beta=0.208$, $p=0.043$). In the willingness-to-care model, empathy emerged as a significant negative predictor ($\beta=-0.293$, $p=0.002$), suggesting that higher empathy might reduce willingness to care in this context.

Table 3. Pearson's Correlations of knowledge. Attitudes, empathy, personality traits and dehumanization of LGBT individuals

Variable		knowledge Score	Empathy	Extraversion	Agreeablenes	Conscientiousness	Emotional Stability	Openness to Experience	Mechanistic Dehumanization	Animalistic Dehumanization
Mechanistic Dehumanization	Pearson's r	0.141	-0.048	0.030	-0.042	-0.152	0.175	-0.098	—	
	p-value	0.138	0.615	0.756	0.662	0.109	0.065	0.304	—	
Animalistic Dehumanization	Pearson's r	0.019	0.232	0.012	0.007	-0.087	0.085	-0.144	0.659	—
	p-value	0.845	0.013	0.902	0.940	0.360	0.369	0.127	< .001	—
Comfortable	Pearson's r	-0.059	0.111	-0.132	0.146	0.007	0.098	-0.171	0.123	0.391
	p-value	0.533	0.241	0.163	0.122	0.943	0.301	0.069	0.196	< .001
Responsibility	Pearson's r	-0.122	-0.143	0.171	0.115	-0.057	0.126	-0.049	0.235	0.194
	p-value	0.197	0.130	0.069	0.223	0.544	0.183	0.608	0.013	0.039
Willingness to care	Pearson's r	-0.074	-0.254	0.186	-0.064	0.060	-0.122	0.215	-0.273	-0.441
	p-value	0.437	0.006	0.048	0.502	0.528	0.195	0.022	0.004	< .001

Table 4. Linear Regression with Attitudes and dehumanization as dependent variables and knowledge, empathy and personality traits as independent. Adjusted for Age, Gender, Sexual orientation and religion

						95% CI	
Comfortable	Unstandardized	Standard Error	Standardized	t	p	Lower	Upper
(Intercept)	2.760	0.534		5.167	< .001	1.700	3.819
Knowledge Score	-0.005	0.008	-0.066	-0.630	0.530	-0.022	0.011
Empathy	0.011	0.010	0.113	1.182	0.240	-0.008	0.030
Agreeablenes	0.039	0.022	0.178	1.756	0.082	-0.005	0.083
Conscientiousness	-0.017	0.024	-0.069	-0.696	0.488	-0.064	0.031
Emotionalstability	0.023	0.019	0.115	1.189	0.237	-0.015	0.061
Opennesstoexperience	-0.040	0.021	-0.219	-1.980	0.050	-0.081	0.001
Extraversion	-0.011	0.018	-0.060	-0.603	0.548	-0.048	0.025
F(12,113)=1.618 , P=0.098, R^2=16.1%							
						95% CI	
Responsibility	Unstandardized	Standard Error	Standardized	t	p	Lower	Upper
(Intercept)	2.767	0.708		3.911	< .001	1.364	4.171
Knowledge Score	-0.014	0.011	-0.133	-1.251	0.214	-0.036	0.008
Empathy	-0.021	0.013	-0.161	-1.651	0.102	-0.046	0.004
Agreeablenes	0.045	0.029	0.157	1.523	0.131	-0.014	0.103
Conscientiousness	-0.028	0.032	-0.088	-0.875	0.384	-0.091	0.035
Emotional stability	0.046	0.025	0.177	1.803	0.074	-0.005	0.097
Openness to experience	-0.030	0.028	-0.124	-1.072	0.286	-0.084	0.025
Extraversion	0.050	0.024	0.208	2.054	0.043	0.002	0.098
F(12,113)=1.301 , P=0.229, R^2=13.4%							
						95% CI	
Willingness To Care	Unstandardized	Standard Error	Standardized	t	p	Lower	Upper
(Intercept)	4.288	0.946		4.535	< .001	2.412	6.163
Knowledge Score	0.011	0.015	0.073	0.739	0.461	-0.019	0.041
Empathy	-0.055	0.017	-0.293	-3.256	0.002	-0.089	-0.022
Agreeablenes	-0.032	0.039	-0.077	-0.807	0.421	-0.110	0.046
Conscientiousness	0.067	0.043	0.146	1.570	0.120	-0.018	0.151
Emotional stability	-0.043	0.034	-0.115	-1.264	0.209	-0.111	0.025
Openness to experience	0.033	0.037	0.096	0.898	0.371	-0.040	0.106
Extraversion	0.052	0.033	0.148	1.583	0.117	-0.013	0.116
F(12,113)=2.963 , P=0.001, R^2=26%							
						95% CI	
Mechanistic Dehumanization	Unstandardized	Standard Error	Standardized	t	p	Lower	Upper
(Intercept)	15.119	4.766		3.172	0.002	5.662	24.575
Knowledge Score	-0.034	0.075	-0.045	-0.447	0.656	-0.183	0.115
Empathy	0.010	0.085	0.010	0.112	0.911	-0.160	0.179
Agreeablenes	-1.201	0.197	-5.998	-6.093	1.000	-0.391	0.391
Conscientiousness	-0.488	0.214	-0.220	-2.284	0.025	-0.912	-0.064
Emotional stability	0.397	0.172	0.217	2.310	0.023	0.056	0.739
Openness to experience	0.106	0.186	0.063	0.568	0.571	-0.263	0.475
Extraversion	0.007	0.164	0.004	0.045	0.964	-0.318	0.333
F(12.111)=2.397 , P=0.009, R^2=22.5%							

Table 4. Cont.

						95% CI	
Animalistic Dehumanization	Unstandardized	Standard Error	Standardized	t	p	Lower	Upper
(Intercept)	12.238	4.930		2.482	0.015	2.458	22.019
Knowledge Score	-0.131	0.078	-0.168	-1.681	0.096	-0.285	0.024
Empathy	0.283	0.088	0.293	3.209	0.002	0.108	0.458
Agreeablenes	-0.043	0.205	-0.020	-0.212	0.833	-0.450	0.363
Conscientiousness	-0.437	0.222	-0.186	-1.980	0.050	-0.877	0.003
Emotionals tability	0.230	0.178	0.118	1.288	0.201	-0.124	0.584
Openness to experience	-0.060	0.192	-0.034	-0.314	0.754	-0.441	0.320
Extraversion	0.059	0.170	0.033	0.348	0.728	-0.279	0.398
F(12.112)= 2.758 , p=0.003, R^2=24.9%							

Regarding dehumanization, conscientiousness significantly predicted mechanistic dehumanization negatively (β = -0.220, p = 0.025), while emotional stability was a positive predictor (β = 0.217, p = 0.023). For animalistic dehumanization, empathy positively predicted higher scores (β = 0.293, p = 0.002), and conscientiousness had a borderline significant negative association (β = -0.186, p = 0.050). These findings indicate the nuanced roles of empathy and personality traits in shaping attitudes and perceptions of LGBTQ+ individuals. For full statistical details, refer to Table 4.

DISCUSSION

This study aimed to evaluate primary healthcare nurses knowledge and attitudes towards LGBTQ+ individuals, their knowledge towards LGBTQ+ health related issues as well as dehumanization of LGBTQ+ individuals. The results provide insights into how personality traits can, to some extent, explain individual differences in attitudes towards LGBTQ people. As the results suggest, nurses moderately dehumanize individuals in the LGBTQ community by leading these individuals to marginalization. The dehumanization of LGBTQ+ individuals is manifested through microaggressions and sexual objectification, leading to internalized negativity and feelings of shame(34). These experiences contribute significantly to the deterioration of their mental health. However, there are not enough studies studying the dehumanization of nurses in the LGBT community.

Conversely, a contemporary investigation undertaken by Horne, Maroney, Zagryazhskaya, and Kovena elucidates the mechanism that correlates personality characteristics with individual perspectives regarding LGBTQ rights. Specifically, Horne et al. contend that the Big Five personality dimensions have been persistently identified as correlating with ideological orientations, particularly liberalism or conservatism, which is significant in the context of individual attitudes towards LGBTQ rights. Employing a modest cohort of students in Russia, the researchers ascertain that conscientiousness and emotional stability serve as predictors of adverse attitudes towards LGBTI individuals. Although Horne et al. do not elaborate on the theoretical interconnections among personality traits, ideology, and individual sentiments regarding LGBTQ rights, in accordance with their assertions, one may deduce that the associations between personality traits and individual attitudes toward LGBTQ rights can be predicated upon the relationships between personality traits and ideological frameworks. Prior research has established that personality traits are consistently linked to political ideology. Specifically, it has been found that agreeableness, conscientiousness, and emotional stability correlate with conservatism, whereas openness to experience is associated with liberalism [35].

The literature suggests that various sociodemographic factors, such as age, religious affiliation, and having an LGBTQ friend or relative, are associated with nurses' knowledge and attitudes towards LGBTQ healthcare. Notably, this effect is observed across different religions, indicating that it is not limited to a specific religious tradition. In many highly conservative societies, people's religion, morals, and way of life have been and remain oriented towards heterosexual sexuality, often condemning other sexual expressions[36]. Greek society has been noted as one of the most negative toward homosexuality and same-sex marriages in Europe, while the Greek Orthodox religion is closely intertwined with the ethics and practices of the people, both historically and in modern times. Additionally, this study revealed that having a personal connection, such as being friends with or closely knowing an LGBTQ individual, was associated with more positive attitudes and an increased level of knowledge regarding LGBTQ patients' health needs. Similar findings have been reported in other studies [37,. 38] It could be argued that those with familiarity with the LGBTQ community and experiences are more likely to be aware of LGBTQ-related issues. Conversely, those who stated they do not socialize with LGBTQ individuals tended to exhibit less-positive perceptions toward them, potentially due to a lack of personal interaction and exposure, leading to a tendency to reject or view with hesitation anyone perceived as "different."

Furthermore, individuals who score low on Openness to Experience have more negative attitudes towards LGBT people. This finding is in line with previous research suggesting

that prejudice is strongly associated with low scores on the Openness to Experience personality trait. Low Openness to Experience may correspond to conservative views, traditional conceptions of gender and reduced cognitive flexibility, which in turn could promote a narrower understanding of sexual orientation and gender identity. This limited perspective may create feelings of discomfort or even hostility towards LGBTI people. Similarly, Dinesen, Klemmensen and Nørgaard conducted a research experiment in Denmark to investigate the effect of personality on attitudes towards immigration. They found that individuals with greater openness to experiences were more inclined to support the admission of immigrants [39-41]. This suggests that personality traits may also play a role in shaping health professionals' attitudes towards marginalised populations such as LGBTQ people.

CONCLUSIONS

The study confirmed that primary care nurses' personal perceptions and attitudes towards LGBTQ+ people are significantly influenced by their personal qualities, empathy and knowledge of LGBTQ+ health. The need for educational programs and awareness about health equity is critical to promote a more inclusive and humane approach to primary health care.

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CONFLICT OF INTEREST:
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Efficiency of replacement of bone defect in rabbit jaw with germanium-doped calcium phosphate ceramics

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ABSTRACT

Aim: To study the regenerative potential of calcium phosphate ceramics doped with germanium (CFCdGe; Ge amount 1-1,5%) compared to a deproteinized bovine material (DBM).

Materials and Methods: The study was performed in vivo on 30 laboratory rabbits, which were formed with a bicortical bone defect in the mandibular angle area on both sides (diameter – 7 mm). There were 3 groups where defects were replaced by CFCdGe (group 1 [n= 10]) and DBM (group 2 [n= 10]) As a control (group 3 [n= 10]), a defect was healed under a blood clot. We studied microstructure of the CFCdGe, solubility, phase, chemical composition, and adsorption activity. The animals were euthanized on 21st, 42nd, and 90th day for taking histological samples to access trabecular width between and around granules.

Results: The size of CFCdGe (Ge 1%) participles was smaller, but the porosity was larger, therefore the working concentration of Ge was chosen as 1%. At 90th day, the trabecular width around granules in group 1 (98,9±27,2 μm) was 92,78% greater than in group 2 (51,3±20,8 μm) and 22,70% greater than width of new bone trabeculae in the control group (80,6±34,2 μm) (groups 1 vs. 2: $p<0,01$). The trabecular width between granules in group 1 (121,0±21,0 μm) was 68,05% greater than in group 2 (72,0±13,1 μm) ($p<0,01$). The CFCdGe granules were surrounded by formed bone tissue with overwhelming amount of mature lamellar bone.

Conclusions: The CFCdGe (Ge 1%) in the experimental study on laboratory rabbits demonstrated a significantly better result than the DBM.

KEY WORDS: bone substitutes, germanium, bone regeneration, bone loss

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INTRODUCTION

Bone defects in the maxillofacial region often pose a significant challenge for both patients and clinicians. The use of bone grafts and substitutes has grown considerably in recent years due to advancements in dental implantology and the need to repair bone defects resulting from trauma, periodontal disease, tooth extraction, infection, congenital malformations, jaw cysts, and tumors [1]. Bone loss is commonly observed after tooth loss, where rapid resorption of the alveolar bone occurs due to the lack of intraosseous stimulation, which typically happens through the fibers of the periodontal ligament.

Autografts are considered the "gold standard" for large alveolar bone defects [2], as they do not cause an immune reaction, exhibit tissue compatibility, and provide osteoconduction, osteoinduction, and osteogenesis. However, autografts have several disadvantages, including limited harvesting sites, significant resorption [3], donor site morbidity, deformity and discomfort at the harvest site, nerve damage, infection, and bone graft rejection [4]. Furthermore, the viability of autografts is uncertain, as most osteocytes in monocortical bone grafts do not survive.

Additionally, patients often have low psychological acceptance of allogeneic bone materials, as they are typically derived from deceased donors [5]. Allografts and xenografts also face challenges such as poor biodegradation, the risk of disease transmission, and rejection by the recipient's immune system [6]. They also incur high processing and storage costs [7] and possess limited osteogenic or osteoinductive properties. Being non-toxic and evenly distributed in tissues, germanium (Ge) chemical compounds have many bio-beneficial properties, including immunostimulation, anti-inflammatory behavior, antioxidant effects, improvement in reproductive function, and improved fertility [8]. Moreover, Ge nanoparticles influence hematopoiesis and carry out the targeted transport of substances to cells.

AIM

This study aims to conduct an experimental analysis of the regenerative potential of rabbit jaw bone tissue when closing a bone defect with a synthetic material – germanium-doped calcium phosphate ceramics (CFCdGe; Ge content 1%–1,5%) – compared with traditional bone materials widely used in clinical practice.

MATERIALS AND METHODS

In vivo there were used 30 certified laboratory rabbits of the California breed weighing (mean [M] ± standard deviation [SD]) (2148,3 ± 234,3 g), equally male and female, without external signs of any disease. The animals were kept in the vivarium of the Bila Tserkva National Agrarian University (NAU) in individual cages with combined lighting and daily cleaning. Feeding was provided with balanced pelleted feed at a rate of 200 g per day and unlimited access to water.

All studies were approved by the Ethics Committee of Bila Tserkva NAU on the treatment of animals in research and education (conclusion № 2 of 31.05.18, protocol № 1) and were carried out in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty" of 28 March 2006, the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 13 November 1987, and the Order of the Ministry of Education and Science № 416/20729 of 16 March 2012, "On Approval of the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions".

The animals were divided into a main group (group 1) and a comparison group (group 2) of 15 rabbits each. The experimental study involved the formation of a bicortical bone defect in the area of the mandibular angle with a diameter of 7 mm, enough to show the typical defect, and its replacement using different materials for bone augmentation—synthetic material from CFCdGe in group 1 and deproteinized bovine material (DBM) in group 2. For control (group 3), a defect was made on the opposite side of the jaw, which healed under a blood clot. In the postoperative period, the rabbits were injected intramuscularly with Bicillin-3 to prevent postoperative infection. The animals underwent X-ray examination on 7th, 21st, 42nd, and 90th day. The animals were euthanized on 21st, 42nd, and 90th day by an overdose of intravenous thiopental anesthesia (five animals per observation point). Then, we took material from the defect site for a histological examination of each animal (Fig. 1).

To evaluate the properties of the CFCdGe or DBM, we employed the following methods:

1. Solubility was determined based on the concentration of calcium ions released into an acetic acetate buffer solution with a pH value of 5,0.
2. Dissolution was determined based on the concentration of calcium ions in the solution.
3. The Ca concentration was determined via photocolorimetry and using an ionometer.
4. The phase compositions were studied via X-ray diffraction using CuKα radiation. The data obtained were processed in the PowderCell 2.4 software.
5. The adsorption activity of hydroxyapatite powders was determined using an aqueous solution of methylene blue.
6. The microstructures of the surfaces of the granules and ceramics were studied using a scanning electron microscope.
7. The chemical compositions were determined via energy-dispersive X-ray fluorescence elemental analysis.
8. The calcium phosphate ceramics granules, synthesized and annealed at 800°C, were examined with a scanning electron microscope.
9. The structural composition was controlled by XRD using a X-ray diffractometer by assessing the follow properties: trabecular width between and around

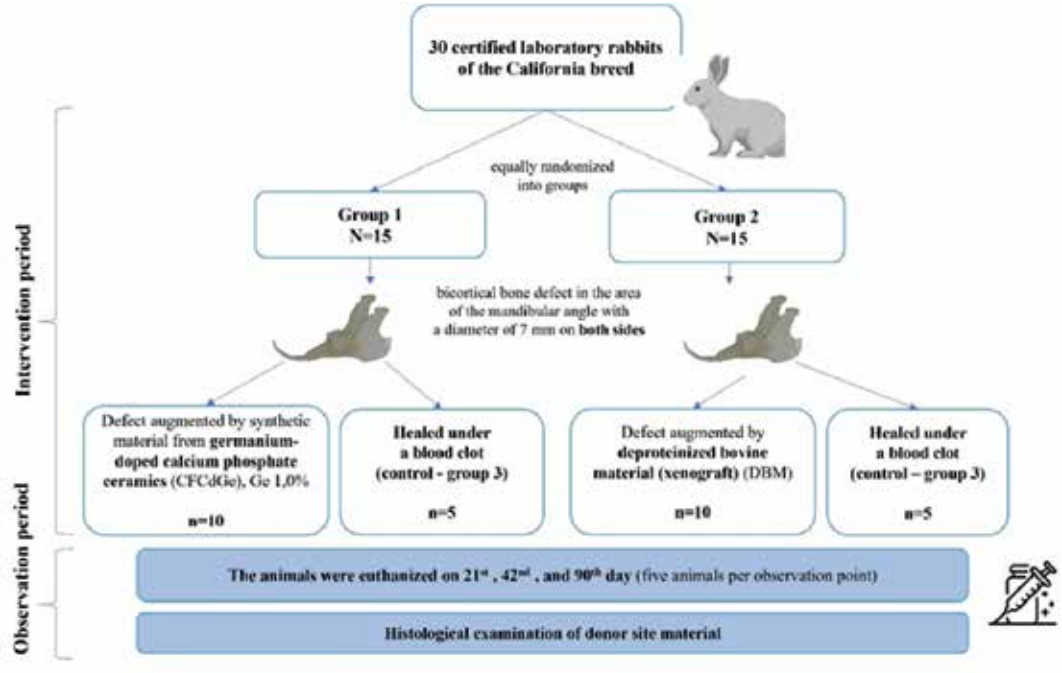

Fig. 1. Design of experimental study.

granules (in control group was studied just width of new bone trabeculae). The data obtained were processed in the PowderCell 2.4 software.

Statistical analysis was performed by the use of IBM SPSS v. 27.0 software. The GraphPad Prism 8.0.1 software was used for graphical interpretation. Continuous variables were presented as M ± SD. The quantitative data between two unrelated groups were compared by the use of unpaired Student`s T-test, between three unrelated groups – by means of ANOVA with the following Tukey`s HSD test for post hoc comparisons. We also used the paired Student`s T-test to compare continuous variables between two related samples. A $p<0,05$ was considered as statistically significant (the correction for multiple comparisons was also taken into account).

RESULTS

Preparing to the work, we compared the microstructure of the CFCdGe granules at magnifications of $94,8\times10^3$, $37,9\times10^3$ and $12,6\times10^3$ to demonstrate the presence of a nanostructure at concentrations of 1,0% Ge and 1,5% Ge. The particle size in the CFC doped with 1,5% Ge is obviously smaller, but the porosity of the CFC doped with 1% Ge is larger. Therefore, a working concentration of Ge was chosen as 1% (Fig. 2).

During the experiment, in group 3 (n=10) we found that in the "empty" defect, which healed under a blood clot from 21st to 90th days, the width of the new bone's trabeculae increased non-significantly by 19,2% (from 67,6 ± 33,8 μm to 80,6 ± 34,2 μm).

In group 1 (n=15), from 21st to 90th days, the trabecular width increased significantly by 45,56% (from 96,7 ± 25,7 μm to 120,0 ± 21,0 μm; $p<0,05$). In group 2 (n=15), we observed a non-significant decrease in the trabecular width by 4,79% from 21st (74,3 ± 31,9 μm) to 90th (72,0 ± 13,1 μm) days of the experiment.

At 21st day, the trabecular width around granules in group 1 (45,8±39,4 μm) was 17,02% less than in group 2 (55,2±19,8 μm) and 32,28% less than in the control group (67,6±33,8 μm) (the difference between groups was non-significant). At the same time, trabecular width between granules in group 1 was numerically, but non-significantly greater by 30,14% than in group 2 (96,7±25,7 μm vs. 74,3±31,9 μm, respectively) (Fig. 3).

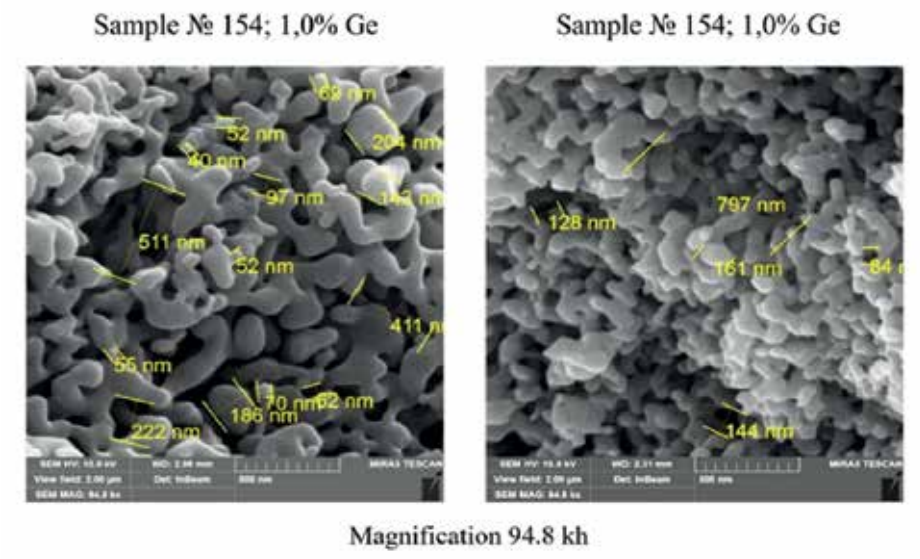

Fig. 2. Micrograph showing particles of CFCdGe at magnification $94,8x10^3$.

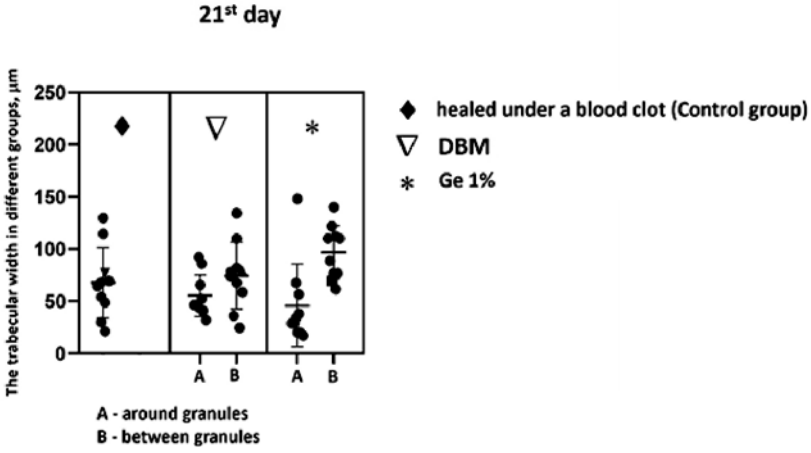

Fig. 3. The characteristics of bone defect content in different groups at 21st day.

At 42nd day, the trabecular width around granules in group 1 (118,0±46,3 μm) was 32,58% greater than in group 2 (89,0±31,5 μm) and 70,02% greater than in the control group (69,4±20,6 μm) (group 1 vs. control group: p<0,01). Besides, the trabecular width between granules in group 1 (136,0±28,9 μm) was 52,81% greater than in group 2 (89,0±31,5 μm) (p<0,01) (Fig. 4).

At 90th day, the trabecular width around granules in group 1 (98,9±27,2 μm) was 92,78% greater than in group 2 (51,3±20,8 μm) and 22,70% greater than in the control group (80,6±34,2 μm) (groups 1 vs. 2: p<0,01). Trabecular width between granules in group 1 (121,021,0 μm) was 68,05% greater than in group 2 (72,0±13,1 μm) (p<0,01). That fact confirmed the positive effect of usage CFCdGe on bone remodeling in comparison to DBM (Fig. 5).

Macroscopically, in group 1 we identified ceramic granules with proliferative tissue without any resorption on each observation time point, in group 2 the defects were filled by young connective tissue without any DBM debris and scar tissue. The control group showed the typical bone remodeling: formatting the hematoma, fibrocartilaginous callus, bony callus, and compact bone (Fig. 6, Fig.7, Fig.8).

Histologically, at 21th day, the area of the "empty" defect was filled mainly with fibrous regenerate, with a few immature coarse-fibrous bone beams. In group 1, active osteogenesis was observed, whereas in group 2, the osteoblastic reaction was moderate. In addition, in group 1, the granules were surrounded by dense fibrous tissue at the perimeter, and foci of dense connective tissue formed around them. Furthermore, some of the coarse-fibrous bone trabeculae were in the process of differentiation into a spongy substance of lamellar bone tissue. In group 2, most of the granules were surrounded by well-differentiated bone beams of different widths of lamellar bone spongy substance. However, some areas exhibited a tight contact between the newly formed bone tissue and the surface of the granules (Fig. 9, Fig.10, Fig.11).

On 42nd day, the area of the "empty" defect was filled to a greater extent with foci of loose connective tissue, and a small number of thin bone beams of coarse-fibrous and lamellar bone were also noted. In group 1, both gross fibrous and lamellar bone materials were observed around the granules, with lamellar bone predominating. An intense osteoblastic reaction was evident. In group 2, the granules

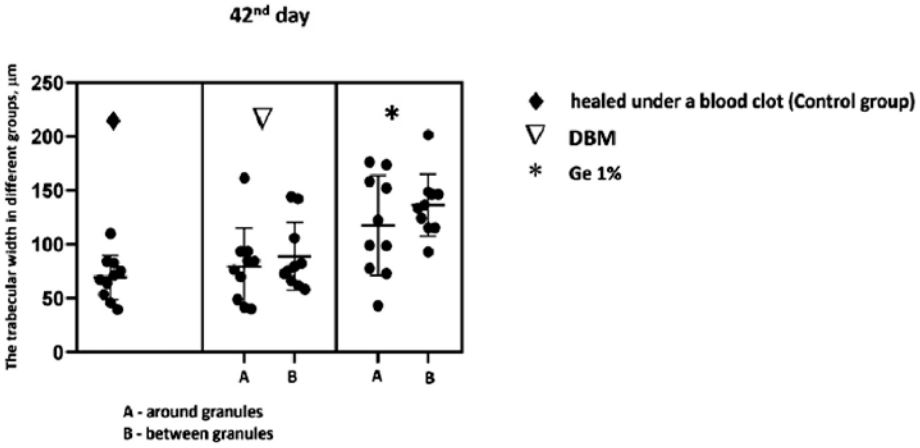

Fig. 4. The characteristics of bone defect content in different groups at 42nd day.
Parameter A – group 1 (Ge 1%) vs. control group: p<0,01; parameter B – group 1 (Ge 1%) vs. group 2 (DBM): p<0,01.

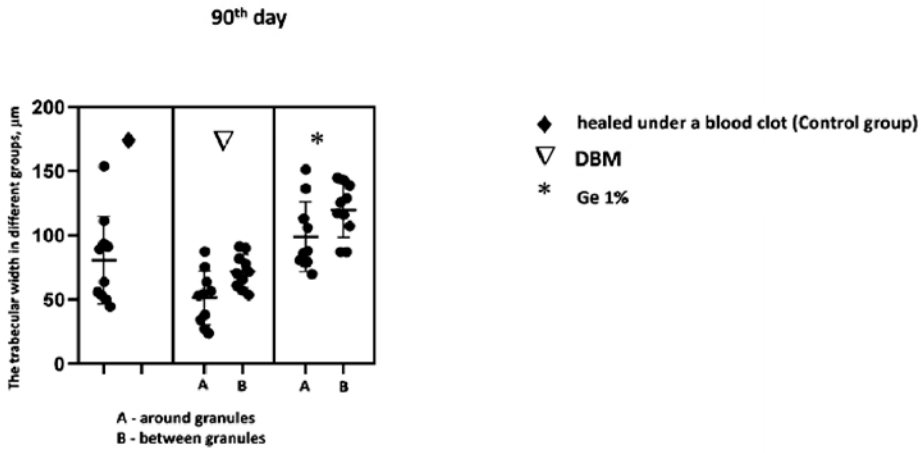

Fig. 5. The characteristics of bone defect content in different groups at 90th day. Parameter A – group 1 (Ge 1%) vs. group 2 (DBM): p<0,01; parameter B – group 1 (Ge 1%) vs. group 2 (DBM): p<0,01.

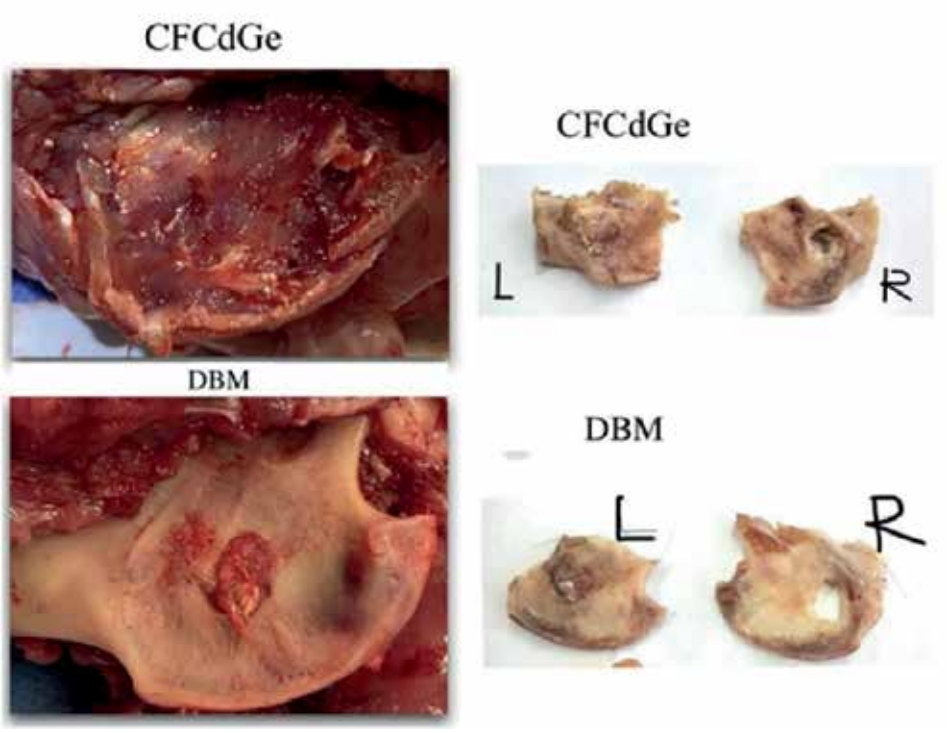

Fig. 6. Specimen of rabbit's jaw, photo of defect in different groups, 21st day. L – left; R – right.

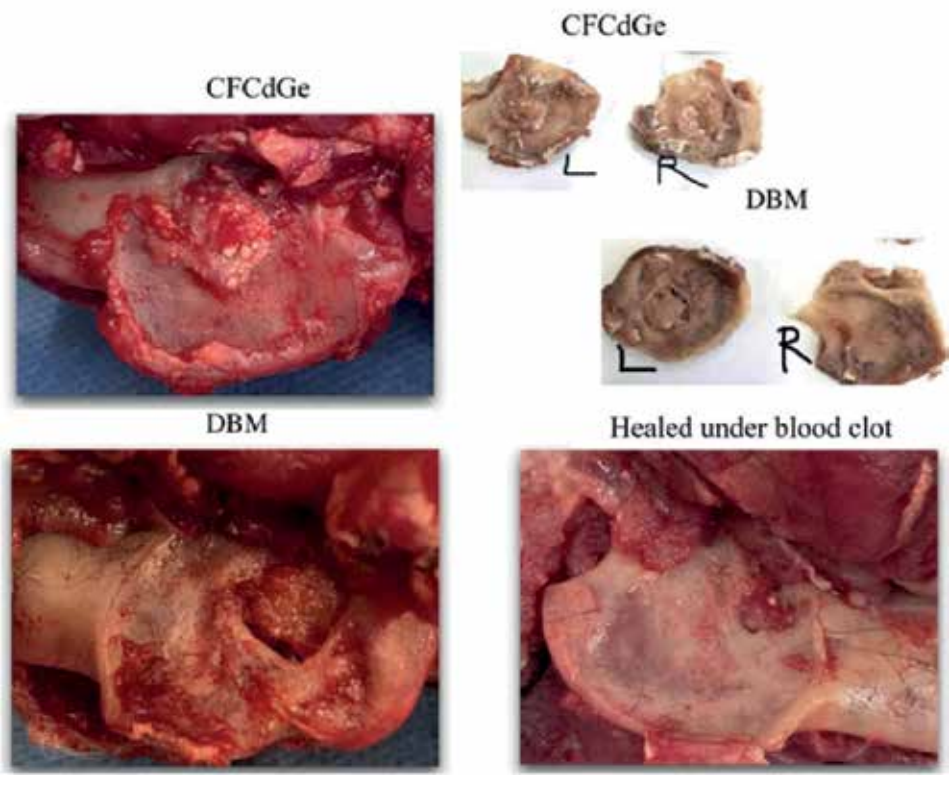

Fig. 7. Specimen of rabbit's jaw, photo of defect in different groups, 42nd day. L – left; R – right.

were surrounded by lamellar bone, and signs of granule resorption were noted (Fig. 12, Fig.13, Fig.14).

On 90th day, the area of the "empty" defect included empty cavities of various sizes and some mature lamellar bone. Closer to the center of the defect, the size and number of areas of immature coarse-fibrous bone tissue increased. In some places, a moderate osteoblastic reaction was evident. In group 1, the granules were surrounded by newly formed bone tissue with a tight adhesion. An overwhelming amount of mature lamellar bone tissue was present. However, foci of course-fibrous bone tissue were also noted, along with signs of deep destruction of the granules and the growth

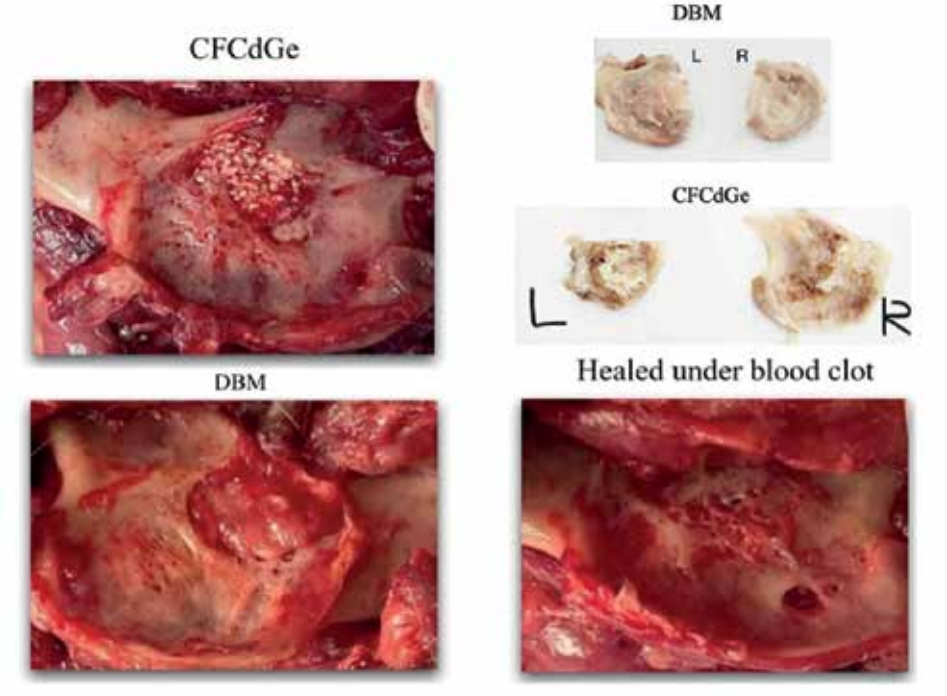

Fig. 8. Specimen of rabbit's jaw, photo of defect in different groups, 90th day. L – left; R – right.

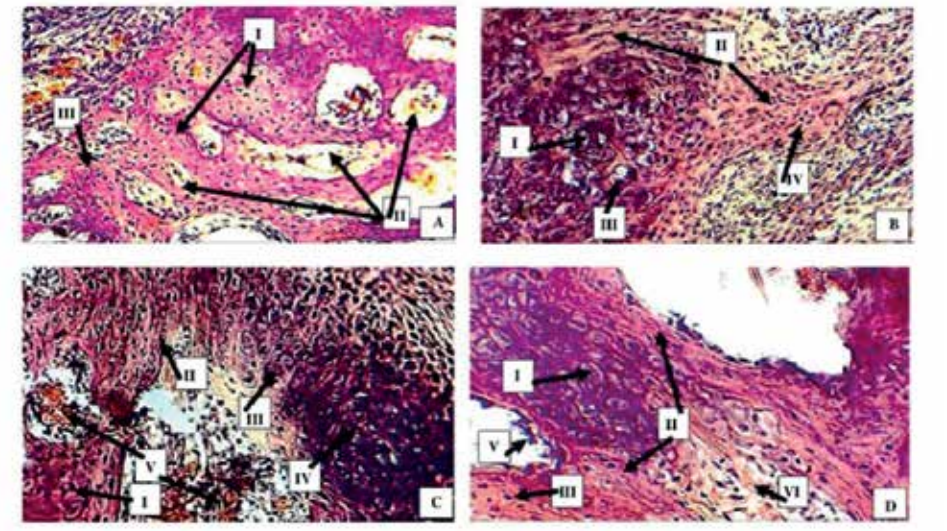

Fig. 9. Area of the empty defect at 21st day.
A) I – bone beams, II – vascular channels, III – osteocyte lacunae; B) I – cartilaginous area, II – bone beam, III – chondrocytes, IV – osteocytes; C) I – isogenic groups of chondrocytes; II – coin columns, III – hypertrophied chondrocytes, IV – calcified chondrocytes, V – blood vessels; D) I – cartilage, II – bone beams, III – chondrocytes, IV – osteocytes, V – osteoblasts, VI – blood vessels. Hematoxylin and eosin at 100×.

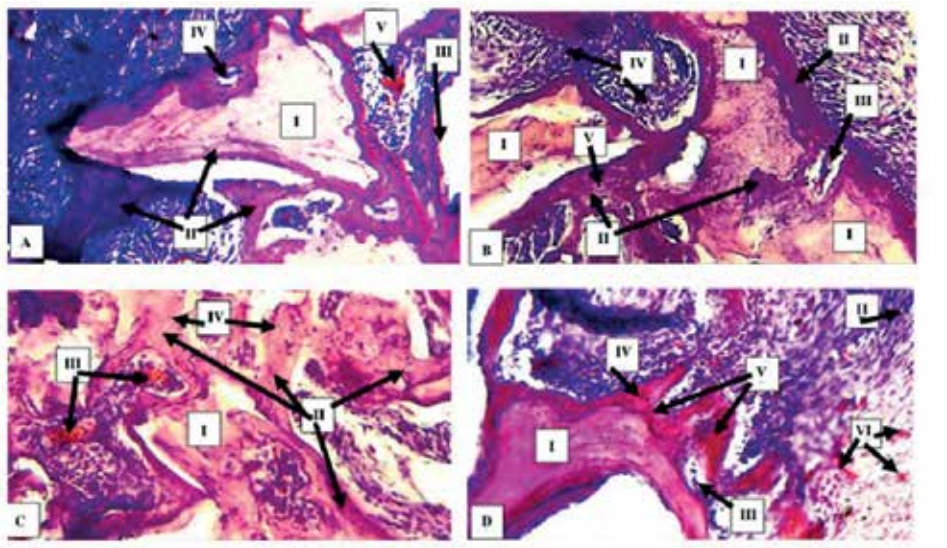

Fig. 10. Defect zone with DBM at 21st day.
A) I – granule, II – bone beams, III – osteoblasts, IV – young osteon, V – blood vessels; B) I – granule, II – bone beam, III – blood vessel invasion, IV – fibrous tissue, V – unblocked osteocytes; C) I – granule, II – bone beams; III – blood vessels, IV – osteocyte lacunae; D) I – granule, II – fibrous tissue, III – osteoblasts, IV – osteocyte lacunae, V – bone beams, VI – blood vessels. Hematoxylin and eosin at 100×.

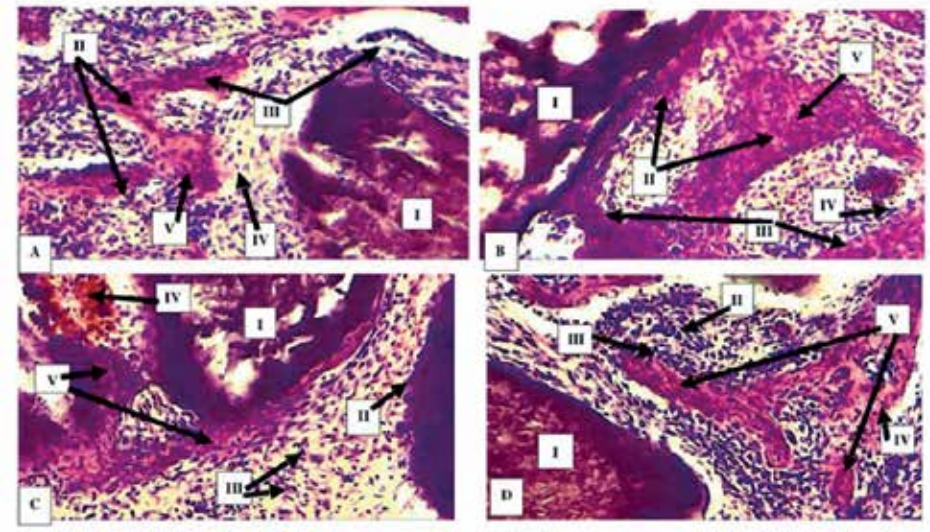

Fig. 11. Defect zone with CFCdGe at 21st day.
A) I – granule, II – bone beams, III – osteoblasts, IV – fibroblasts, V – unlined osteocytes; B) I – granule, II – bone beam, III – osteoblasts, IV – blood vessels, V – unlined osteocytes; C) I – granule, II – osteoblasts; III – fibroblastic cells, IV – blood vessels, V – bone beams; D) I – granule, II – fibrous tissue, III – osteoblasts, IV – osteocyte lacunae, V – bone beams. Hematoxylin and eosin at 100×.

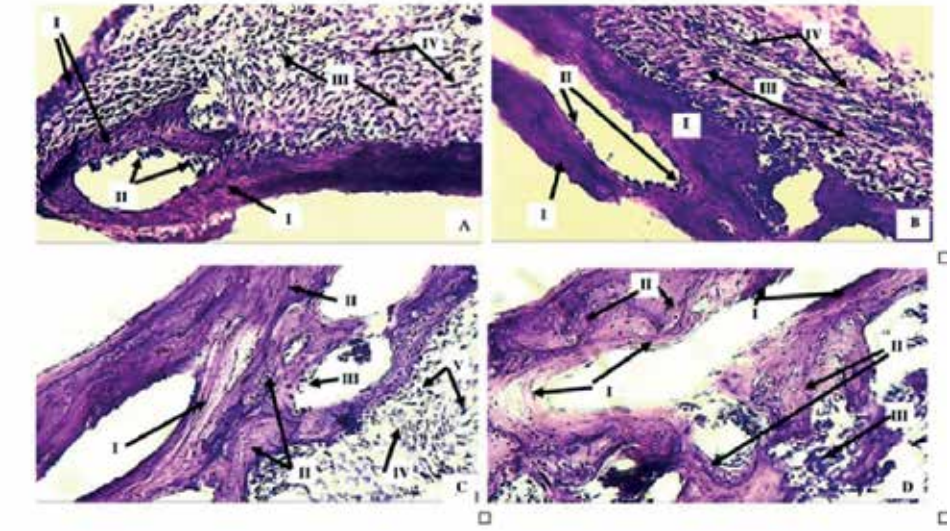

Fig. 12. Zone of the empty defect at 42nd day.
A) I – bone beams, II – osteoblast nuclei, III – dense unformed connective tissue, IV – fibroblastic cell nuclei; B) I – bone beams, II – osteoblast nuclei, III – dense unformed connective tissue, IV – fibroblastic cell nuclei; C) I – lamellar bone beams; II – coarse-fibrous cells of bone beams, III – osteoblast nuclei, IV – loose connective tissue, V – nuclei of fibroblastic cells; D) I – lamellar bone beams, II – coarse-fibrous cells of bone beams, III – loose connective tissue. Hematoxylin and eosin at 100×.

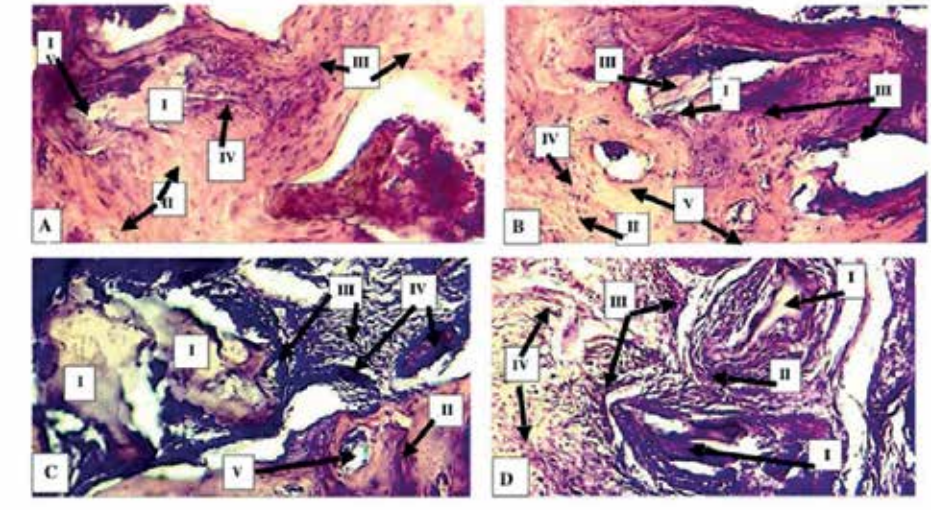

Fig. 13. Defect area with DBM at 42nd day.
A) I – granule, II – mother bone tissue, III – osteocytic lacunae, IV – coarse fiber bone foci; B) I – granule, II – mother bone tissue, III – coarse fiber bone foci, IV – osteocytic lacunae, V – dilated Haversian canals; C) I – granule, II – mother bone tissue, III – coarse fiber bundles, IV – coarse fiber beams, V – dilated Haversian canal; D) I – granule, II – coarse fiber bundles, III – coarse fiber beams, IV – blood vessels. Hematoxylin and eosin at 100×.

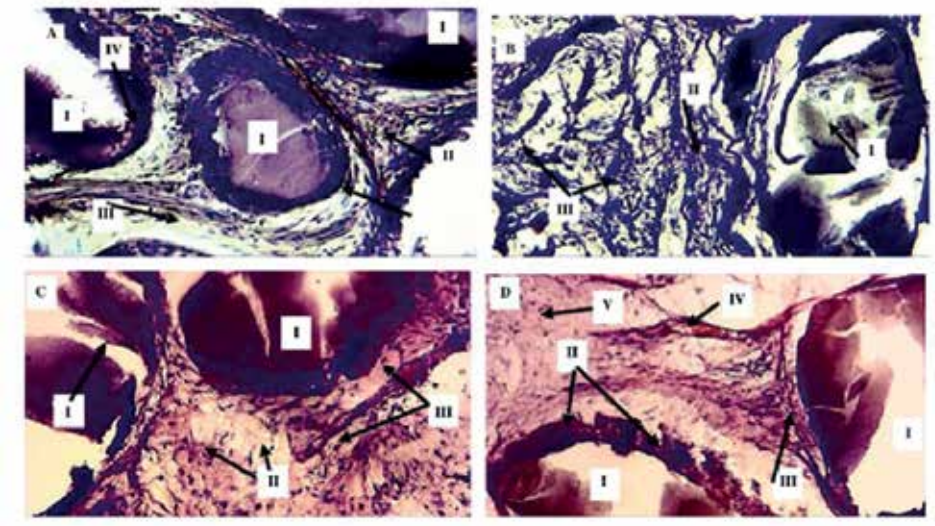

Fig. 14. Defect zone with CFCdGe on 42nd day.
A) I – granule, II – loose connective tissue, III – dense formed connective tissue, IV – nuclei of low-differentiated cells, V – unmasoned osseocytes; B) I – granule in the stage of destruction, II – fibrous structures, III – coarse fiber bundles; C) I – granule in the stage of destruction, II – intercellular matrix, III – nuclei of fibroblastic cells; D) I – granule in the stage of destruction, II – contact of granule with nuclei of low-differentiated cells, III – contact of granule with fibrous structures, IV – blood vessels, V – loose connective tissue. Hematoxylin and eosin at 100×.

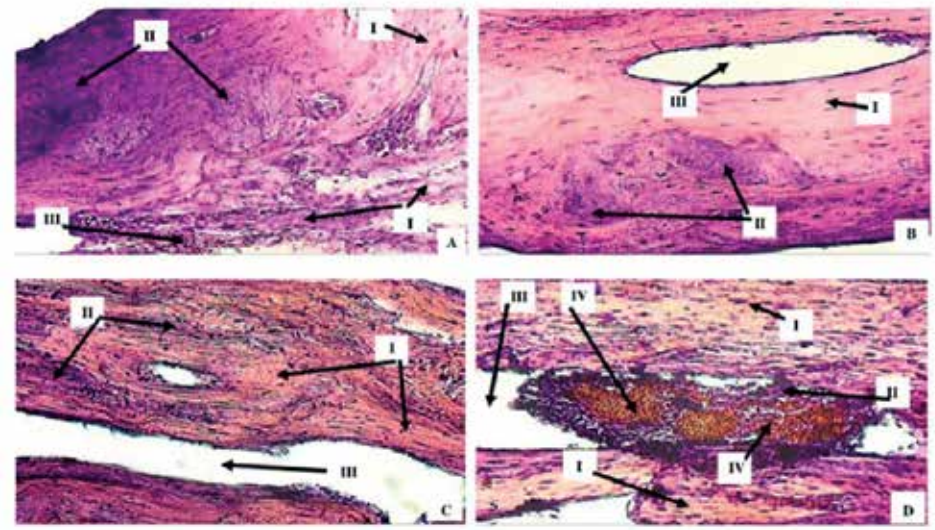

Fig. 15. Zone of the empty defect at 90th day.
A) I – lamellar bone beams, II – coarse--fiber cells of bone beams, III – blood vessels; B) I – lamellar bone beams, II – coarse-fiber cells of bone beams, III – empty cavity; C) I – lamellar bone beams; II – coarse-fiber cells of bone beams, III – empty cavity; D) I – lamellar bone beams, II – loose connective tissue, III – cavity, IV – vessels. Hematoxylin and eosin at 100×.

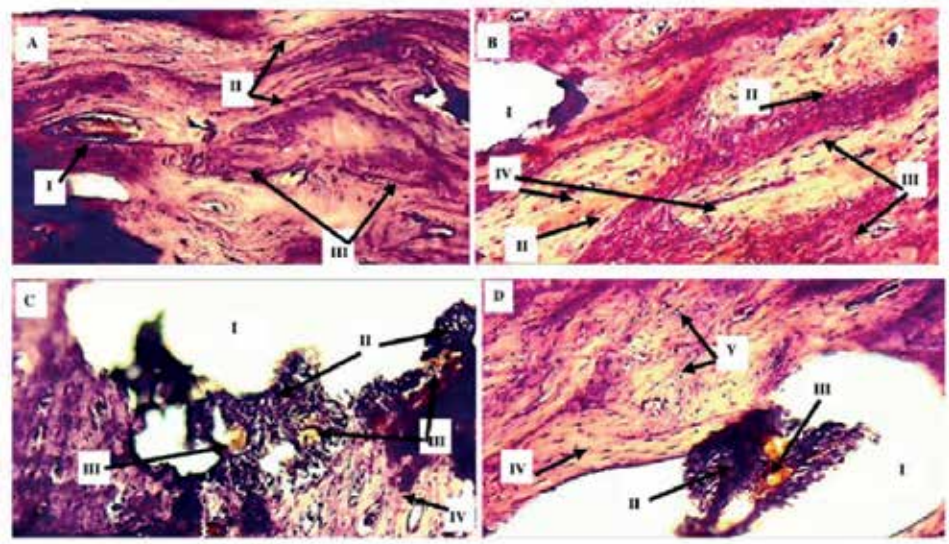

Fig. 16. Defect zone with DBM at 90th day.
A) I – residue of the granule, II – parental lamellar bone tissue, III – foci of coarse fibrous bone tissue, IV – blood vessels; B) I – area of resorbed granule, II – parental lamellar bone tissue, III – foci of coarse fibrous bone tissue, IV – blood vessels; C) I – area from the resorbed granule, II – clusters of nuclei of low-differentiated cells, III – blood vessels, IV – mother bone tissue; D) I – area from the resorbed granule, II – clusters of nuclei of low-differentiated cells, III – blood vessels, IV – mother lamellar bone tissue, V – unlined osteocytes. Hematoxylin and eosin at 100×.

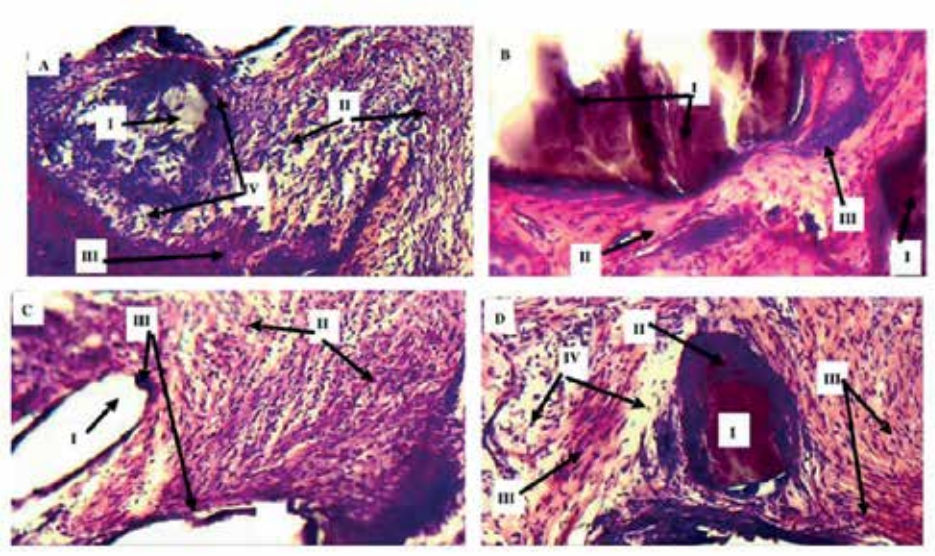

Fig. 17. Defect zone with CFCdGe, 90th day.
A) I – granule in the stage of deep destruction, II – dense unformed connective tissue, III – low-differentiated bone beam, IV – dense-fiber connective tissue capsule; B) I – granule in the stage of destruction, II – parental lamellar bone tissue, III – foci of coarse-fiber bone tissue; C) I – area without granules, II – multidirectional fibrous structures with cell nuclei, III – connective tissue capsules; D) I – granule, II – contact of granule with connective tissue capsule, III – multidirectional bundles of fibrous structures with fibroblastic cell nuclei, IV – foci of loose connective tissue. Hematoxylin and eosin at 100×.

of bone tissue strands into the surface and deep layers of the composite. Compared to 42nd day, the histological assessment indicates more differentiated processes. At that time, in group 2, in the defect center, the material granules were completely resorbed. In their place, clusters of low-differentiated cells appeared, and mature lamellar bone tissue was mainly at the periphery of the defect (Fig. 15, Fig.16, Fig.17).

DISCUSSION

Throughout life, the physiological remodeling and replacement of bone occurs without changing the shape and density of the bone because of the following phenomena: osteoclast activation, bone resorption, osteoblast activation, and new bone formation in place of the resorbed bone. The bone defects are easily restored to their critical sizes, i.e., too large to be able to heal during life [10]. New bone formation can occur through three processes: osteogenesis, osteoinduction, or osteoconduction [11].

In addition, the success of bone graft engraftment is influenced by various other properties, including biocompatibility, bioresorption, sterility, structural integrity, sufficient porosity for vascular germination, plasticity, ease of processing, cost, and compressive strength [12]. The combination of these factors is the basis for their use. Studies have shown that almost all modern bone grafts and substitutes serve primarily as a structural framework for osteoregenerative processes, i.e., they satisfy only the osteoconductivity component.

Due to the similarity in physical and chemical properties of silicon, Ge may be a promising element for modifying biomaterials to obtain new biological properties.

For animals and humans, Ge is a biologically active trace element. The vital need for ultramicrodoses of Ge for the normal functioning of the immune system has been revealed [15]. Ge is one of the trace elements involved in metabolic processes in the human body (the recommended daily dose of Ge is 0,4-1,5 mg) and is present in almost all organs and tissues (muscle tissue, blood, brain, lungs, spleen, stomach, liver, pancreas, thyroid, kidneys, etc.) According to preclinical studies, an insufficient amount of Ge in the diet is accompanied by damage to the bone matrix [13].

The effect of Ge on the human body is highly dependent on the compounds it is included in. In different compounds, it exhibits different functional properties. The most studied is the therapeutic effect of Ge-organic compounds (GOCs). The first water-soluble GOCs are sesquioxides. Bis(2-carboxyethylhemanium) sesquioxide is the most popular. At present, the physiological activity of this compound (also known as Ge-132) is the most studied and widely used in medical practice. The ability of GOCs to enhance the proliferative and reparative function of connective tissue has been proven, and organic Ge compounds have been found to have antitumor properties [15].

The literature reports the bactericidal effect of metallic Ge, and on the other hand, numerous cases of absorption and accumulation of hydrated Ge dioxide by microorganisms have been identified, with the ability to stimulate fibroblast proliferation noted [14-16].

Furthermore, despite the large number of studies on the biological effects of Ge and its compounds, to our knowledge, no data exist on the production of biomaterials based on Ge-doped calcium phosphates and the study of their effect on bone tissue. We only found information on the study of composite coatings of calcium phosphate applied to the surface of implants by microarc oxidation, with nonmetallic Ge in concentrations of 1% and 5% added to the electrolyte [17].

The doping of CFC with Ge can impart new properties to bioactive ceramics, change the nature of biological interaction with bone tissue, and introduce antibacterial, immunomodulatory, antiviral, and antitumor properties.

CONCLUSIONS

The synthetic material from CFCdGe in the experimental study on certified laboratory rabbits of the California breed rabbits demonstrated a significantly better result than the DBM and healing of bone defects under blood clot. It was found that the best bone remodeling on 90th day occurred in group 1, which was manifested by the formation of mature lamellar bone, differentiation of bone tissue, tight fit of granules to the neoplastic bone, partial degradation of granules, the presence of active osteoblasts. On 90th day, in group 2, the granules were completely resorbed and cavities or poorly differentiated bone were observed in their place. So, we revealed the significantly positive Ge component effect in ceramic compound on bone remodeling.

This is the basis for further clinical studies on bone augmentation of jaws as part of preimplantation preparation or usage like a scaffold for stem cells.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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ORIGINAL ARTICLE

Impact of stressors of academic activities under martial law on the cadets' mental health

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ABSTRACT

Aim: The aim is to investigate the mental health indicators of cadets during their stressful academic training under martial law.

Materials and Methods: The research, which was conducted in the academic year 2023-2024, involved 253 male cadets of the 1st-4th training years at the National Academy of Internal Affairs (Kyiv, Ukraine). Research methods: theoretical analysis and generalization of literary sources, mental health testing, and methods of mathematical statistics. Seven psycho-diagnostic methods were used to test the cadets' mental health.

Results: The results obtained indicate a pronounced negative impact of stressors of academic activities under martial law on the cadets' mental health and, in particular, on the level of manifestation of stress disorders, a tendency to develop stress, reduced stress resistance, increased nervous and emotional stress, anxiety, and deterioration of the emotional state. The most pronounced negative changes in these indicators of mental health were found in cadets who have not developed skills to cope with stress during academic activities and the use of effective means of stress prevention and restoration of psycho-emotional state (junior cadets).

Conclusions: The research demonstrates the urgent need to develop cadets' stress resistance during academic training to ensure the effectiveness of their educational activities under martial law and further service activities, as well as to develop skills in the use of available means to prevent stressful phenomena in the process of education during the war.

KEY WORDS: stress, cadets, war, stressors, martial law, mental health

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INTRODUCTION

The service activities of law enforcement officers usually take place in extreme conditions, which require exceptional mental stability. They are constantly under the influence of mental stress, accomplish risky actions in the performance of their service functions, and are subject to high nervous and emotional stress [1]. Law enforcement officers are constantly in contact with people who indirectly or directly commit negative actions, characterized by increased emotionality, rudeness, aggressiveness, inadequacy, etc. Constant service-related stress can cause professional deformation of the individual, increase proneness to conflict in the team, reduce performance, and negatively affect mental health [2, 3].

Scientists [4] note that police service in peacetime, in a somewhat stable time, belonged to the list of difficult and dangerous professions accompanied by significant psychological stress. In the context of the legal regime of martial law, these stresses have increased significantly. Cadets – future law enforcement officers are no exception, as they are most exposed to various types of stress during the war. The academic training process of cadets is full of emotional experiences and takes place against the background of emotional states, including stress. Emotions and feelings that are actualized in the academic training process not only stimulate cognitive activity and regulate learning actions but also significantly affect the content of the material learned, the completeness of its understanding, and the strength of memorization [5]. During academic training activities, negative subjective emotions and feelings are actualized and aimed at certain aspects of the educational process, which act as stressors of learning activities and complicate their quality [6]. A significant psychological factor of cadets' sensitivity to stressors is the peculiarities of their self-awareness and perception of the world: the degree of sensitivity to stressors of learning activities will depend on whether this perception is optimistic or pessimistic [7]. However, cadets' academic training activities during the war lead to negative emotional states and carry a negative connotation due to stressors such as uncertainty, daily routines, social evaluation, risks to life and health during alarms and missile threats, concerns for their families and friends, etc.

Scientists [8] note that negative emotional states are also prerequisites for the emergence and development of stress in cadets. Thus, the study of academic stress shows that fear of future problems provokes anxiety, uncertainty, a sense of helplessness, etc. Stress can also affect cognitive performance: attention disorders (difficulty concentrating, narrowing of the field of attention, increased ability to be distracted from tasks), thinking (disturbance of the logic of thinking, difficulty in making decisions, reduced creative activity), memory (difficulty in reproducing information, deterioration of working memory indicators) [9]. In addition, during academic training under martial law, the cadets' ability to adhere to the principles of a healthy lifestyle has been significantly limited, which also leads to a deterioration in mental health, exacerbation of chronic diseases, and the development of bad habits such as smoking and the use of stimulants [10].

AIM

The aim is to investigate the dynamics of mental health indicators of cadets – future law enforcement officers during their academic training under martial law in Ukraine.

MATERIALS AND METHODS

PARTICIPANTS

The research involved 253 cadets of the National Academy of Internal Affairs (Kyiv, Ukraine) who were studying in Law Enforcement specialty: the 1st training year – n = 69, the 2nd training year – n = 65, the 3rd training year – n = 61, and the 4th training year – n = 58. The research was conducted in the academic year 2023-2024; all respondents were male. There was no unique selection; all male cadets of this specialty were involved in the research. The research began in September 2023 and ended in June 2024.

RESEARCH METHODS

Theoretical analysis and generalization of literary sources, cadets' mental health testing, methods of mathematical statistics. The method of theoretical analysis and generalization of literary sources was used to conduct an analytical review of scientific sources on the outlined range of issues (21 sources from PubMed, Scopus, Web of Science, Index Copernicus and other databases were analyzed).

The testing was used to study the dynamics of cadets' mental health indicators during their martial law training. For this purpose, we used the following methods: the PSM-25 psychological stress scale (Lemur-Tessier-Fillion), the method of determining the tendency to develop stress (T. A. Nemchin, J. Taylor), the test of self-assessment of stress resistance (S. Cowan and G. Williamson), the "Assessment of Nervous and Emotional Stress" method (T. A. Nemchin), the "Stress Resilience" method (F. Gottwald, W. Howald), the reactive anxiety scale (Ch. D. Spielberger, Yu. L. Khanin), the emotional state self-assessment method (A. Wessman, D. Ricks) [11-14]. The PSM-25 psychological stress scale is designed to measure the structure of stress experiences. It contains 25 statements, answering which respondents chose the frequency of their manifestation and rated it in points from 1 to 8, where 1 – never, 8 – constantly. After that, the sum of points for all statements was determined. If the sum was 99 or less, the stress level was considered low; 100-124 points – average; 125 and more – high. The method of determining the tendency to develop stress contains 50 statements; it allows for assessing the cadets' tendency to create stress and anxiety levels and allows for the discussion of their level of stress resistance. The respondents were offered a form with statements with "No" or "Yes" answers opposite them. It was necessary to put "+" if the proposed answer coincided with the respondent's opinion or "–" if it did not. Stress was assessed as follows: if the sum of coincidences was 15 or less, there was no stress; 16-24 – moderate level of tendency to stress; 25-39 – average; 40 and more – high. The test of self-assessment of stress resistance contains 10 questions, answering which the cadets had to choose one of the proposed answers. The answers for questions 1, 2, 3, 7, 9, and 10 were evaluated as follows: never – 0, rarely – 1, sometimes – 2, quite often – 3, frequently – 4; for questions 4, 5, 6, and 8 – never – 4, rarely – 3, sometimes – 2, quite often – 1, usually – 0. If the sum was 6.8 points or less, the level of stress resistance was considered excellent; 6.9-14.2 – good; 14.3-24.2 – satisfactory; 24.3-34.2 – poor; 34.3 and more – very poor. The method for assessing nervous and emotional stress includes 30 signs of this condition, divided into three degrees of severity (a – low degree (complete absence), b – average degree, and c – high degree). The data were processed by summing the points: for answers a – 1 point, b – 2 points, and c – 3 points. The nervous and emotional stress level was considered low if the cadets scored 30-50 points, average – 51-70 points, and high – 71-90 points. The "Stress Resistance" method contains 33 statements with suggested answer options (frequently / strongly, rarely / sometimes, none / never). Each answer option was rated 0, 1, or 2 points. The data were processed by summing the points: 28 and more points – low level of stress resistance; 13-27 – average; 12 and less – high. The reactive anxiety scale contains 20 statements with response options, depending on how the respondents felt during testing: no, it is not true; probably true; true; quite true. The points were calculated using the formula: $RA = \Sigma 1 - \Sigma 2 + 50$, where RA is reactive anxiety, Σ1 is the sum of the numbers on scale items 3, 4, 6, 7, 9, 12, 13, 14, 17, and 18; Σ2 is the sum of the numbers on scale items 1, 2, 5, 8, 10, 11, 15, 16, 19, and 20. The level of anxiety was assessed as low with 30 points or less, moderate with 31-45 points, and high with 46 points or more. The emotional state self-assessment method includes four sets of 10 statements each ("Calm – Anxiety,""Energy – Fatigue," "Elevation – Depression,""Self-confidence – Helplessness"), among which in each set, it was necessary to choose the one that reflected the respondent's emotional state at the time of testing. The formula determined the emotional state: $ES = (I1 + I2 + I3 + I4) / 4$, where ES is an integral indicator of the emotional state; I1, I2, I3, and I4 are individual indicators on the respective scales. The emotional state was assessed as very good at 8-10 points, good – 6-7 points, poor – 4-5 points, bad – 1-3 points.

STATISTICAL ANALYSIS

The methods of mathematical statistics were used to process the data obtained. The compliance of the sample data distribution with the Gauss' law was assessed using the Shapiro-Wilk W test. The reliability of the difference between the indicators was determined using the Student's t-test. The reliability of the difference was set at $p<0.05$. All statistical analyses were performed using SPSS software, version 10.0, adapted for medical and biological research.

The procedure for organizing the study and the topic of the article were previously agreed with the Committee on compliance with Academic Integrity and Ethics of the NAIA. Also this study followed the regulations of the World Medical Association Declaration of Helsinki. Informed consent was received from all participants who took part in this study.

RESULTS

The results of the assessment of cadets' psychological stress, tendency to develop stress, stress resistance, and stress resilience are presented in Table 1.

At the beginning of the research, it was found that the psychological stress of junior cadets on the PSM-25 scale corresponded to an average level and in senior training years – to a low level. During the study period, the stress level in cadets of all training years worsened by 4.4-7.4 points. Still, statistically significant differences were found in the indicators of the 1st and 2nd training year cadets ($p \leq 0.05$). It is stated that at the end of the research, an average level of psychological stress was found in all groups, which emphasizes the negative impact of stressors of academic activities under martial law on the mental health of cadets. The study of indicators of susceptibility to stress development shows that at the beginning of the research, all groups showed a moderate level of stress (18.7-20.3 points), indicating the presence of stressors in cadets' daily and academic activities. During the research period, there was a statistically significant deterioration in the level of stress in all groups ($p \leq 0.05$). The most significant difference between the initial and final test data was found in the junior cadets. Assessment of the level of stress resistance shows that at the beginning of the research, all groups of cadets had a satisfactory level of stress resistance – the average score was in the range of 16.1-17.2 points. In the process of academic training, there was a statistically significant ($p \leq 0.05$-0.01) deterioration in the level of stress resistance in all groups: the least pronounced negative changes occurred in senior cadets, and the most pronounced negative changes occurred in junior cadets (4.9 and 4.4 points). At the end of the research, the cadets of all groups showed a satisfactory level of stress resistance. The analysis

Table 1. Dynamics of indicators of psychological stress, tendency to develop stress, stress resistance, stress resilience in cadets (n = 253) during their martial law academic training, points

Cadets	Research stages		Δ	t / p
	The beginning	The end		
PSM-25 psychological stress scale				
1st training year (n=69)	103.9±2.16	111.3±2.19	7.4	2.41/≤0.05
2nd training year (n=65)	102.4±2.18	108.6±2.20	6.3	2.01/≤0.05
3rd training year (n=61)	98.2±2.09	103.5±2.12	5.3	1.78/>0.05
4th training year (n=58)	97.9±2.15	102.3±2.17	4.4	1.44/>0.05
Method of determining the tendency to develop stress				
1st training year (n=69)	20.3±1.35	25.1±1.38	4.8	2.49/≤0.05
2nd training year (n=65)	19.7±1.33	24.2±1.36	4.5	2.37/≤0.05
3rd training year (n=61)	18.9±1.28	23.3±1.30	4.4	2.41/≤0.05
4th training year (n=58)	18.7±1.26	22.8±1.27	4.1	2.29/≤0.05
Test of self-assessment of stress resistance				
1st training year (n=69)	17.2±0.97	22.1±1.04	4.9	3.45/≤0.01
2nd training year (n=65)	16.9±0.94	21.3±0.98	4.4	3.24/≤0.01
3rd training year (n=61)	16.6±1.01	20.5±1.04	3.9	2.69/≤0.05
4th training year (n=58)	16.1±1.05	20.1±1.08	4.0	2.66/≤0.05
"Stress Resilience" method				
1st training year (n=69)	16.2±1.32	21.3±1.36	5.1	2.69/≤0.05
2nd training year (n=65)	15.8±1.27	20.1±1.29	4.3	2.38/≤0.05
3rd training year (n=61)	15.1±1.25	19.5±1.28	4.4	2.46/≤0.05
4th training year (n=58)	14.6±1.26	18.5±1.30	3.9	2.15/≤0.05

Note: Δ – difference between the studied indicators; t – value of Student's t-test; p – level of statistical significance of differences.

of stress resilience showed that during the academic training, the stress resilience level significantly deteriorated ($p \leq 0.05$) in all groups. The worst stress resilience level was found in the 1st and the 2nd training years.

The results of the assessment of indicators of nervous and emotional stress, reactive anxiety, and emotional state in cadets are presented in Table 2.

The study of the dynamics of nervous and emotional stress indicators shows significant ($p \leq 0.05$- ≤ 0.01) negative changes in cadets of all training years. The deterioration ranges from 4.1 points in the 4th training year to 5.2 points in the 1st training year. It was found that during the academic training, the nervous and emotional stress of cadets was at an average level. The assessment of reactive anxiety shows that at the beginning of the research, the level of anxiety was at a moderate level. During the academic training under martial law, the level of anxiety in cadets of all training years worsened, but significant ($p \leq 0.05$) changes occurred in junior cadets, with the level of anxiety assessed as high. At the end of the research, the worst level of anxiety was observed in the 1st training year cadets. The research on the emotional state shows that during academic training, cadets had a significant deterioration in the emotional state of all training years ($p \leq 0.05$-0.001): the difference is 0.7-1.3 points. At the end of the research, the cadets of all training years showed a worsened level of emotional state.

DISCUSSION

Stress is the greatest threat to human mental health; stress, as a trigger, causes the development of pathological abnormalities in the functioning of all systems of the human body and causes serious psychosomatic diseases resulting from prolonged exposure to stress or repeated stressful events [15].

For the first time in 1915, the American psychophysiologist Walter Bradford Cannon described the body's response to stress, calling it the fight-or-flight response. W. B. Cannon and other doctors, as the first researchers of stress, identified painful conditions that arose due to difficult working conditions and inhuman exploitation of workers. These conditions included increased fatigue, weakness, sleep disorders, and irritability [16]. The body's reaction to external stimuli or traumatic events is a nonspecific response to the effects of stimuli or stress. According to researchers [17], many diseases in most people begin similarly, manifested by weakness, loss of appetite, headaches, etc. Different negative factors lead to the same physiological reactions that eventually cause pathologies.

In studying the problems of stress, American psychologist Richard S. Lazarus combined not only the external effects of stress on the human body, which are available for observation and study but also internal psychological processes that provoke stress reactions. Studying the problems of health psychology, he revealed signs of the emotional state of a person (in terms of anxiety) in stressful situations that pose a danger to the individual [18]. At the same time, Susan Folkman, Professor of Medicine at the University of California, San Francisco [19], studying the problems of psychological stress and ways to overcome it, noted that stress is inherently a complex phenomenon with dynamic and multifactorial manifestations.

Table 2. Dynamics of indicators of nervous and emotional stress, reactive anxiety, and emotional state in cadets (n = 253) during their martial law academic training, points

Cadets	Research stages		Δ	t / p
	The beginning	The end		
"Assessment of Nervous and Emotional Stress" method				
1st training year (n=69)	54.7±1.10	59.8±1.14	5.1	3.22/≤0.01
2nd training year (n=65)	54.1±1.04	59.1±1.07	5.0	3.35/≤0.01
3rd training year (n=61)	52.7±1.02	56.9±1.05	4.2	2.87/≤0.05
4th training year (n=58)	52.4±1.05	56.5±1.07	4.1	2.73/≤0.05
Reactive anxiety scale				
1st training year (n=69)	43.4±0.94	46.8±0.96	3.4	2.53/≤0.05
2nd training year (n=65)	43.2±0.89	46.5±0.93	3.3	2.56/≤0.05
3rd training year (n=61)	41.8±0.90	43.6±0.92	1.8	1.40/>0.05
4th training year (n=58)	41.1±0.87	42.5±0.88	1.4	1.13/>0.05
Emotional state self-assessment method				
1st training year (n=69)	6.0±0.21	4.8±0.23	1.2	3.85/≤0.01
2nd training year (n=65)	6.4±0.24	5.1±0.25	1.3	3.75/≤0.01
3rd training year (n=61)	6.6±0.20	5.5±0.22	1.1	3.69/≤0.01
4th training year (n=58)	6.8±0.21	6.1±0.21	0.7	2.36/≤0.05

Note: Δ – difference between the studied indicators; t – value of Student's t-test; p – level of statistical significance of differences.

Scientists [9, 20] argue that service in law enforcement agencies is one of the professions with a high stress level, especially during the war when the powers of law enforcement officers have expanded significantly. The activities of law enforcement officers every day require the ability to counteract various negative factors, which are overwhelmingly stressful. This, in turn, leads to various negative emotional states [3]. In the context of the war in Ukraine, there is a tendency for a significant increase in extreme situations in the work of law enforcement agencies, which is associated with an increase in terrorist acts, hostilities, detention and neutralization of criminals, the release of hostages, more frequent use of service weapons, ensuring law and order, etc. [4]. Factors such as irregular working hours, constant contact with antisocial elements, and the need for full commitment of mental and physical strength in preventing crimes reduce the functional reserves of the law enforcement body until they are entirely exhausted [21]. The academic activities of cadets – future law enforcement officers are associated with various professional tasks and their intellectual and emotional saturation. Their academic activities are characterized by specific factors that have a destructive effect on the psychological state of the individual: lack of time to make a decision and implement it, the impact on the personality of extreme stressors, the dominance of negative emotions with a lack of positive ones, increased responsibility and the need for urgent action [5, 7]. The following should be added to the stressors of cadets' academic activities: constant missile attacks, air raids, bombings, blackouts, fear for their lives and the lives of other family members, etc. All of these factors hurt the mental health of cadets – future law enforcement officers. The results of our research have confirmed the findings of many scientists regarding the negative impact of stressors of educational activities on cadets' mental health during their wartime academic training.

CONCLUSIONS

The results obtained indicate a pronounced negative impact of stressors of academic activities under martial law on the mental health of cadets and, in particular, on the level of manifestation of stress disorders, a tendency to develop stress, reduced stress resistance, increased nervous and emotional stress, anxiety, and deterioration of the emotional state. The most pronounced negative changes in these indicators of mental health were found in cadets who have not developed skills to cope with stress during academic activities and the use of effective means of stress prevention and restoration of psycho-emotional state (junior cadets). The research demonstrates the urgent need to develop cadets' stress resistance during academic training to ensure the effectiveness of their educational activities under martial law and further service activities, as well as to develop skills in the use of available means to prevent stressful phenomena in the process of mastering educational material during the war.

PROSPECTS FOR FURTHER RESEARCH

It is planned to investigate the dynamics of both mental and physical health indicators in military personnel depending on the duration of their training and combat activities.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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REVIEW ARTICLE

Schistosomiasis in urology. Outline of the problem

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ABSTRACT

Schistosomiasis very often illness in endemic countries caused by schistosoma haematobium and very rare disease, in Poland caused especially by birds flukes, and in travellers and refugees from endemic countries. It is important for urologist to keep in mind in differential diagnosis also this disease. Symptoms of the disease are primarily due to the body's reaction to the parasite's eggs and dead flukes forms, which cause inflammatory infiltrates. In the early stages, these are reversible and curable, but in later stages, they lead to recidivans haematurie, fibrosis and calcification of the affected organ, resulting in subsequent changes, bladder neck strictures, ureteral strictures, vesico-ureteral reflux, calculi in the urinary tract, squamous cell carcinoma of the urinary bladder, renal failure, and others. In neglected cases, bilharziasis manifestations are fistulas on the scrotum with pseudo-elephantiasis changes.

KEY WORDS: schistosomiasis, urogenital system, complications

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INTRODUCTION

The term "hematuria," represented by the letters "aaa" over 50 times [10] in hieroglyphic writings (such as the Ebers Papyrus, cited 28 times, and the Kahuna and Brugsch) [10], described as an "incurable, fatal disease affecting both women and men" (Pfister E, 1913), was widely recognized in ancient Egypt (Fig. 1) and Mesopotamia [10]. Later named bilharzia or "snail fever," this condition is associated with infection by the blood fluke *Schistosoma haematobium*. This parasite was discovered by Theodor Maximillian Bilharz (1825-1862) and Carl Theodor Ernst (1804-1885) in 1851 while they were working at Kasr El Ainy Hospital (now part of Cairo University). During an autopsy on an Egyptian peasant, they identified the parasite in the portal vein and named it *Distomum haematobium*, publishing their findings in 1852 [2, 10]. Thomas Spencer Cobbold (1828-1886) found this parasite in a lemur from Madagascar and other animals [4]. Later, it was discovered in other primates, rodents, and cattle [4]. Heinrich Meckel von Hemsbach (1821-1856) renamed it *Bilharzia haematobium* in honor of Bilharz, and David Friedrich Weinland (1829-1915) introduced the name *Schistosoma haematobium* [10]. Currently, both names are used interchangeably: bilharzia and *S. haematobium*. The parasite is endemic in Africa (Angola, Cameroon, Gambia, Togo, Guinea, Rhodesia, Rwanda-Burundi, Sierra Leone, Sahara, Senegal, Somalia, Zaire, Zambia), Egypt (Nile Valley), Iraq (Euphrates and Tigris valleys (P. Richter, 1913)), India, Israel, Cyprus, and sporadically in Europe (few foci in Portugal, Corsica [12], and France) [12], as well as South America [14], the Caribbean Islands, southern China, and some regions of Asia. The WHO listed schistosomiasis as an endemic disease in 76 countries in 2015, with approximately 250 million cases [1, 3, 5, 11, 16, 21, 22]. Earlier data from Christian Chatelain in 1977 reported similar numbers of affected individuals, around 200 million [3]. Recent studies show that in the year 2021 at least 251,4 million people required preventive treatment out of which more than 75.3 million people were reported to have been treated and schistosomiasis transmission has been reported from 78 countries (https://www.who.int/news-room/fact-sheets/detail/schistosomiasis). In south of Europe (France, Italy, Germany, Corisica, Sardynia, Portugal and Spain) detected the foci of schistostomiasis with suspicion some of authors to climate change [27]. 0n 16 May 2014, eleven cases (six from France and five from Germany) of uro-genital schistosomiasis have been reported. From above mentioned 11 cases have been confirmed in people from, visiting the river between 2011 and 2013. Four of the six confirmed cases in France were children. All cases were exposed to freshwater in a natural swimming area in southern Corsica in Cavu River [24]. In Poland disease is very rare [7, 25] and is more often diagnose among Polish travelers returning from tropical countries [26], or refugees from these countries. Analysis of Avicenna's (980-1037) writings from the 10th century AD reveals knowledge of this disease [2, 10]. Genoese Giovanni di Carignano (circa 1250-1330) described cases of hematuria in caravaneers crossing the Sahara in 1311 and 1314 [3]. In the 16th and 17th centuries, it was also described in Portugal, which then occupied African coasts. Bacteriologist and pathologist Sir Marc Armand Ruffer (1859-1917) reported in the "British Medical Journal" in 1910 that he found *S. haematobium* infection in two Egyptian mummies from the 20th Dynasty period (1250-1000 BC)

using the ELISA test [10]. During Napoleon Bonaparte's (1769-1821) Egyptian campaign (1798-1801), many cases of hematuria caused by blood fluke infection were noted among soldiers, leading Egypt to be called the "country of menstruating men". Epidemiological studies in 1937 among the population of the northern and eastern Nile Delta showed an 85% infection rate with bilharzia, predominantly among children [28]. In 1968, 50% of Egypt's population, approximately 16 million people, were afflicted with bilharzia [cited in 10]. Wright C. A. noted the presence of this disease in 1961 in Africa and the Middle East (Fig. 2), and a similar distribution was reported by Hermann Hausmann [10], while the contemporary distribution in 2015 is shown on a map (Fig. 3). Bilharzia primarily develops in warm countries and cannot thrive in cold climates. During periods of increased migration from these endemic regions, the disease could potentially spread worldwide. from these countries, safari hunters in Africa, scientific expeditions, cooperative efforts, and the influx of seasonal, untested workers and travelers may all facilitate its spread (Biber a et al. 2022). In endemic countries, bilharzia affects 60-80% of children and 20-40% of adults [5]. Serological studies in these areas have shown

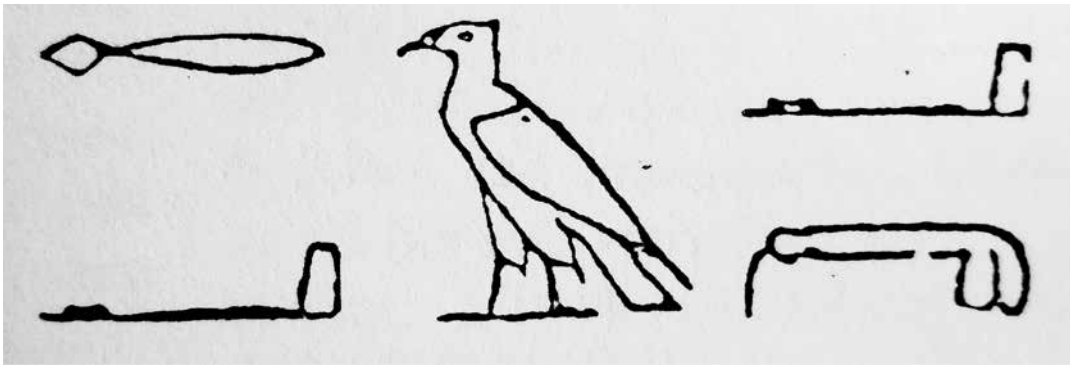

Fig. 1. Hieroglyphic description of „hematuria " according to Egyptian papyri - aaa [10].

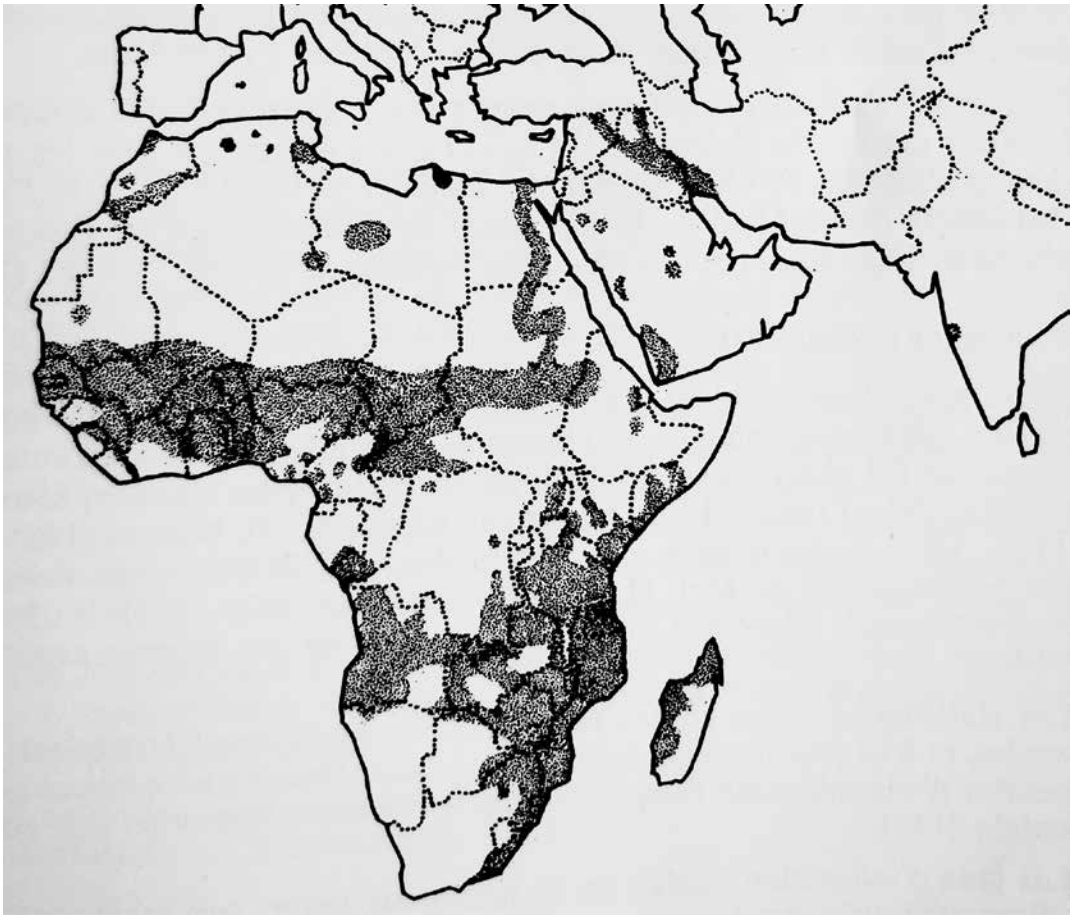

Fig. 2. Geographical distribution of S. haematobium according to W.H Wright. Ass. Franc. d,Urologie 71 Session. Paris 1977,page 39. With permission of AFU [3].

Fig. 3. Recent world distribution of S. haematobium-Source: Wikipedia Commons.

Fig. 4. And egg of S. haematobium urine with characteristic spine on the left in Image from Wikipedia Commons. Schistosoma_haematobium_egg_4842_lores . Public domaine.

nearly 100% infection rates (5). Coexistence of squamous cell carcinoma with bilharzia infection has been observed in 65% of cases in these regions [cited in 16]. The main forms of the disease are intestinal (caused by *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. guinesis*, *E. mekongi*, and *S. malayensis*) and urogenital (*S. haematobium*).

Infection occurs most commonly during bathing in warm freshwater (swimmer's itch, fisherman's fever), where mollusks of the genus *Mollusca* (*Bulinus (Ph) truncatus* or *Bulinus (Ph) globosus*), which serve as intermediate hosts, are present. Infections can also occur during washing clothes in water containing these parasites, as well as during fieldwork. The highest emission of parasites occurs at noon, less in the morning and evening. The primary host for *Schistosoma haematobium* is humans, who excrete the parasite's eggs in urine and feces (Fig. 4). The parasites live in the blood within the portal vein system (*vena portae*) or the hypogastric plexus (*plexus hypogastricus*), attaching to the vessel walls with suckers and persisting for 3-8 years in pairs (male and female). Transmission of the infection from human to human has not been proven. The life cycle of the fluke was described in 1918 by Scottish physician Robert Thomson Leiper (1881-1969).

The female *Schistosoma haematobium* living in a pair with a male (Fig. 5) in a blood vessel lays 20-190 eggs

daily, averaging 50-60 eggs (Fig. 4). In favorable conditions (warm water), miracidia (larval stage, known in Polish as "dziwadełko") hatch from these eggs within 2-3 days and infect the aforementioned mollusks. Inside the mollusks, the larvae mature over 20-30 days (4-6 weeks), transforming through the stages of sporocyst and redia into the next developmental stage, cercariae (Fig. 6), which are the invasive form (known in Polish as "ogonatka"). About 5,000 cercariae are released daily from the snail into the water, where they can survive for up to 2 days. Human infection occurs again through the skin, spreading to the lymphatic vessels and veins, then through the lungs to the heart, and further to the mesenteric arteries (*arteria mesenterica superior et inferior*), hypogastric plexus (*plexus hypogastricus*), and portal vein (*vena portae*). There, they mature over 20-25 days and then migrate to the venous vessels in the pelvis (*plexus venosus perivesicalis*, *plexus haemorrhoidalis*, and the genital veins). They pair up and start laying eggs, thus completing the cycle (Fig. 7).

There are two main forms of schistosomiasis: intestinal and urogenital, caused by five main species of the parasite. Some *Schistosoma haematobium* flukes may lose their tropism and localize in other organs, such as the brain (Imai et al., 2011), spinal cord (Freitas and Angerami, 2013), eyes (Dickinson et al., 1990; Guirou et al., 2021), lungs, spleen, liver (Barsoum et al., 1977; Cheever et al., 1983), kidneys [7], and testes [19], causing corresponding symptoms.

REVIEW AND DISCUSSION

SYMPTOMATOLOGY

Symptoms of the disease are primarily due to the body's reaction to the parasite's eggs and dead forms, which cause inflammatory infiltrates. In the early stages, these are reversible,

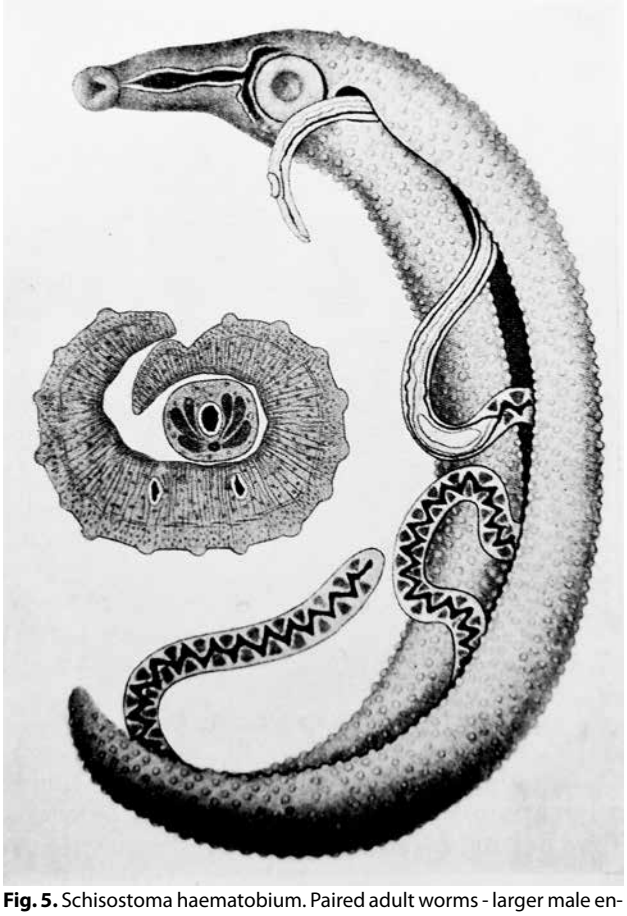

Fig. 5. Schisostoma haematobium. Paired adult worms - larger male enfolding slender female. Hand painting according of E. Pfister from Tropenkrankheiten in Handbuch der Urologie 1927, page. 823. Public domain.

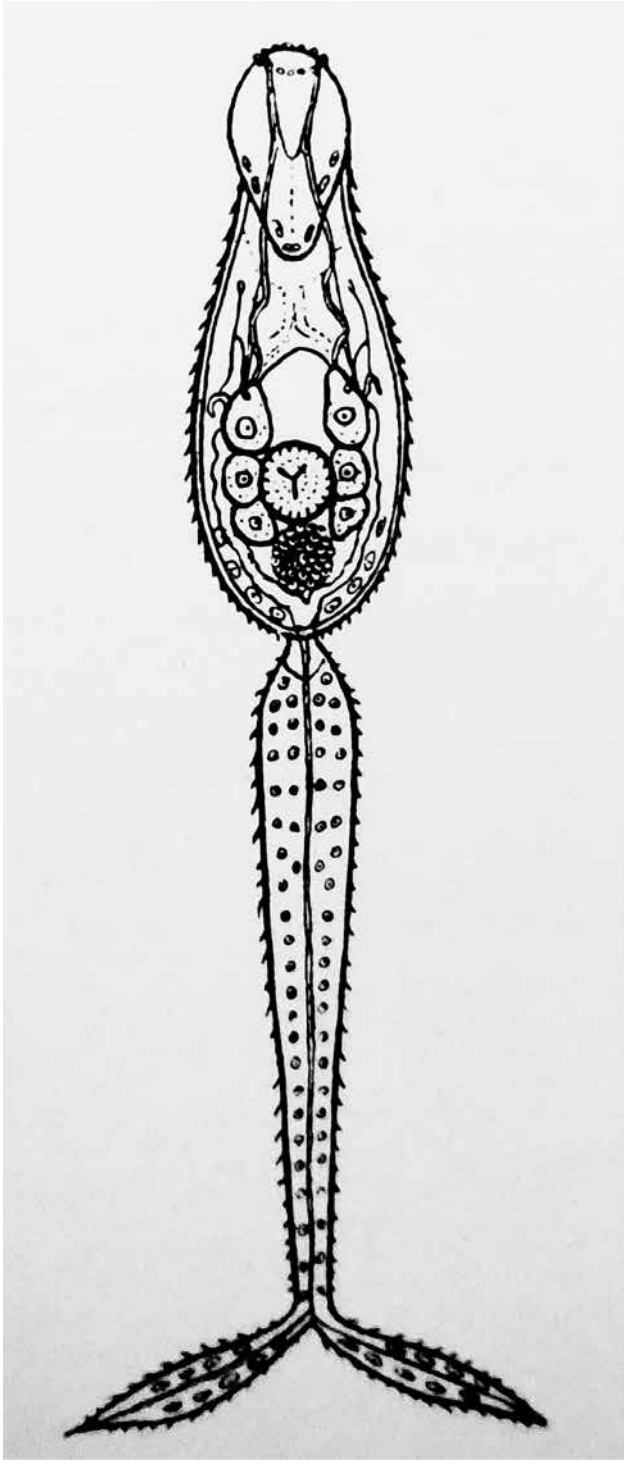

Fig. 6. Hand painting of cercaria according to Manson-Bahr from Tropenkrankheiten in Handbuch der Urologie 1927. Public domain.

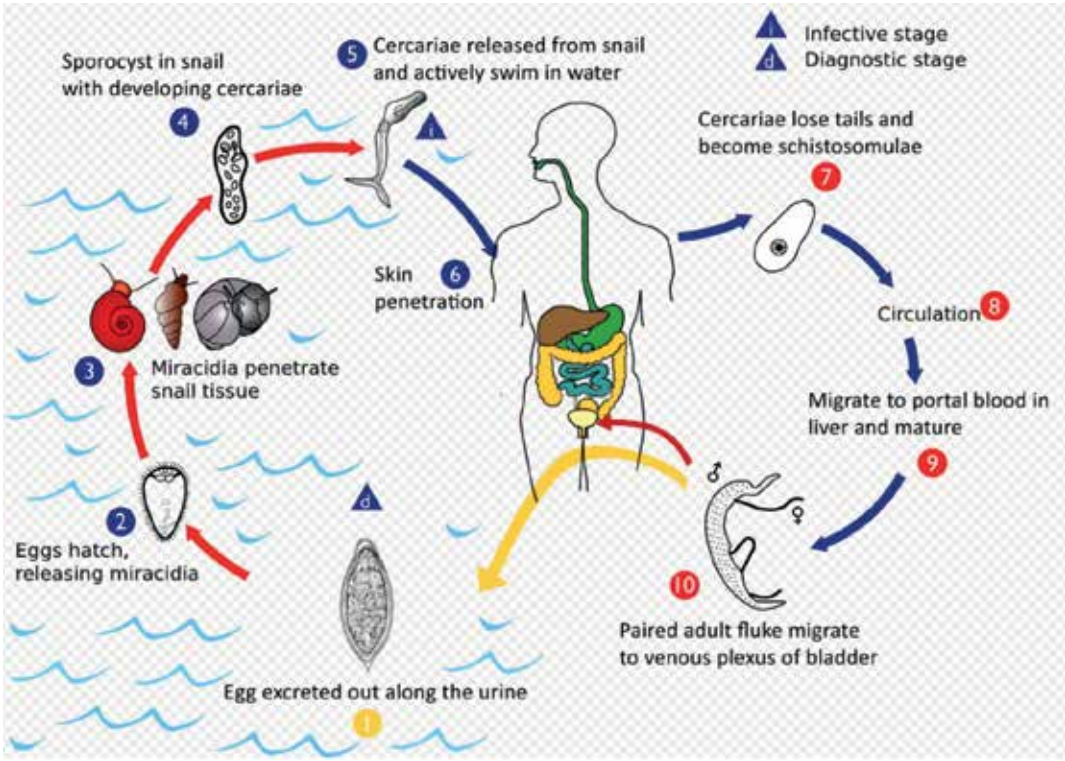

Fig. 7. Life cycle of S.haematobium. From Wikipedia Commons. https://www.cdc.gov/parasites/schistosomiasis/biology.html . According to CDC. PIHL. USA. Public domain.

but in later stages, they lead to fibrosis and calcification of the affected organ, resulting in subsequent changes.

Four stages of the disease are distinguished:

Skin irritation symptoms, sometimes erythema or urticaria, associated with the penetration of the parasite's cercariae into the skin within 10-15 minutes to a few hours after bathing in contaminated water. These symptoms are usually unnoticed or dismissed by the infected individual.

Invasion phase (toxic phase) – associated with the migration and establishment of the parasite. Individuals encountering bilharzia for the first time, such as in the case of "safari fever," may exhibit allergic reactions (urticaria, facial and limb swelling), fever, and eosinophilia (30-45% of cases), as well as irregular fever sometimes accompanied by chills, known as "fievre de safari" (French term). Abdominal, muscular (arthralgia), fatigue, apathy, spasmodic cough, and asthmatic apneas can also occur. Diagnosis in this phase is easier if one is aware of the possibility of this disease and promptly undertakes diagnostic tests.

Onset of organ changes in the urogenital system, where the primary symptom is hematuria in both women and men. However, changes may be nonspecific at this stage, and in some areas where the disease is endemic, transient symptoms may be considered "physiological in young people" – "menstrual men." In about 75% of women, the genital tract is affected, leading to bleeding, egg deposits on the labia, perineum, vagina, and cervix in the form of sandy patches. In reproductive-age women, it frequently causes spontaneous abortions, ectopic pregnancies, menstrual disorders, infertility, and other issues [16, 23], potentially mimicking an ovarian tumor [23].

Late-stage consequences, including bladder neck strictures, ureteral strictures [3, 6, 8, 11, 15, 20], squamous cell carcinoma of the bladder [13, 18], renal failure, and others. The connection between bladder cancer and blood fluke infection was first described by Reginald Harrison (1837-1908) from Royal Infirmary in Liverpool in 1889, followed by Carl Goebel (1867-1946) from Germany in 1903. Ghoneim MA (b. 17.03.1939) and colleagues, in a series of 1026 oncological cystectomies, found the presence of fluke eggs in 85% of cases [8]. Based on these and many other reports (e.g., Mostafa MH et al., 1999, Gouda I et al., 2007), further research was initiated, leading the International Agency for Research on Cancer (IARC) Working Group on the Evaluation of Carcinogenic Risks to Humans WHO in 2009 to classify *S. haematobium*, alongside parasites like *Clonorchis sinensis* and *Opisthorchis viverrini*, as a carcinogenic factor, placing it in Group 1 [13, 18].

DIAGNOSIS

Clinical symptoms described above only indicate the possibility of bilharzia infection. Confirmation, however, always requires finding parasite eggs in urine (cytological examination of urine sediment for parasites) or feces, or microscopic examination of various tissues, such as the bladder wall (Fig. 8), containing *S. haematobium* eggs.

Additional diagnostic methods include the intradermal test with *S. mansoni* antigen, which remains positive even after schistosomiasis is cured (showing redness 15 minutes

after intradermal injection), immunofluorescence tests with 80-90% sensitivity, and complement fixation tests. However, according to the WHO, none of these conclusively diagnose the disease. During the early toxic phase of infection, there is increased eosinophilia (unlike in malaria), which soon drops to 5-15% and disappears entirely after the death of the parasites.

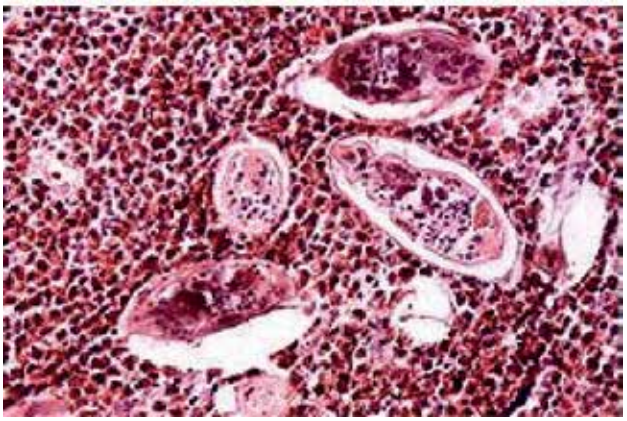

Fig. 8. Eggs of S. haematobium in the wall of the urinary bladder surrounded by infiltrates of eosinophils according to CDC. Dr Edwin P. Ewing Jr. PIHL Nr. # 35. This image is in the public domain and thus free of any copyright restrictions.

Cystoscopic examination reveals changes primarily in the trigone, the dome, and the posterior wall of the bladder, extending to the lateral walls and eventually the bladder apex. Changes are also observed in the neck and posterior urethra, necessitating both cystoscopy and urethrocystoscopy in these cases. Early cystoscopic images of the bladder show whitish (Fig. 9) or whitish-yellow (Fig. 10) granules resembling powdered sugar or semolina. In later stages, these evolve into larger white or yellowish granules with mucosal elevations, 1-2 mm in diameter (resembling rice grains), on a hyperemic mucosal background, which may coalesce into white surfaces.

Further cystoscopic observations include the accumulation of fibrotic nodules and calcified eggs, appearing as "sandy patches" visible through the mucosa, which is pathognomonic. Subsequently, nodular changes up to the size of strawberries may occur, with stromal hyperplasia, which can enlarge, fibrose, or cause necrosis leading to ulcerations, calcifications, and fibrosis of the bladder. These changes can also result in papillomas (bilharzial papilloma), cystitis cystica, cystitis

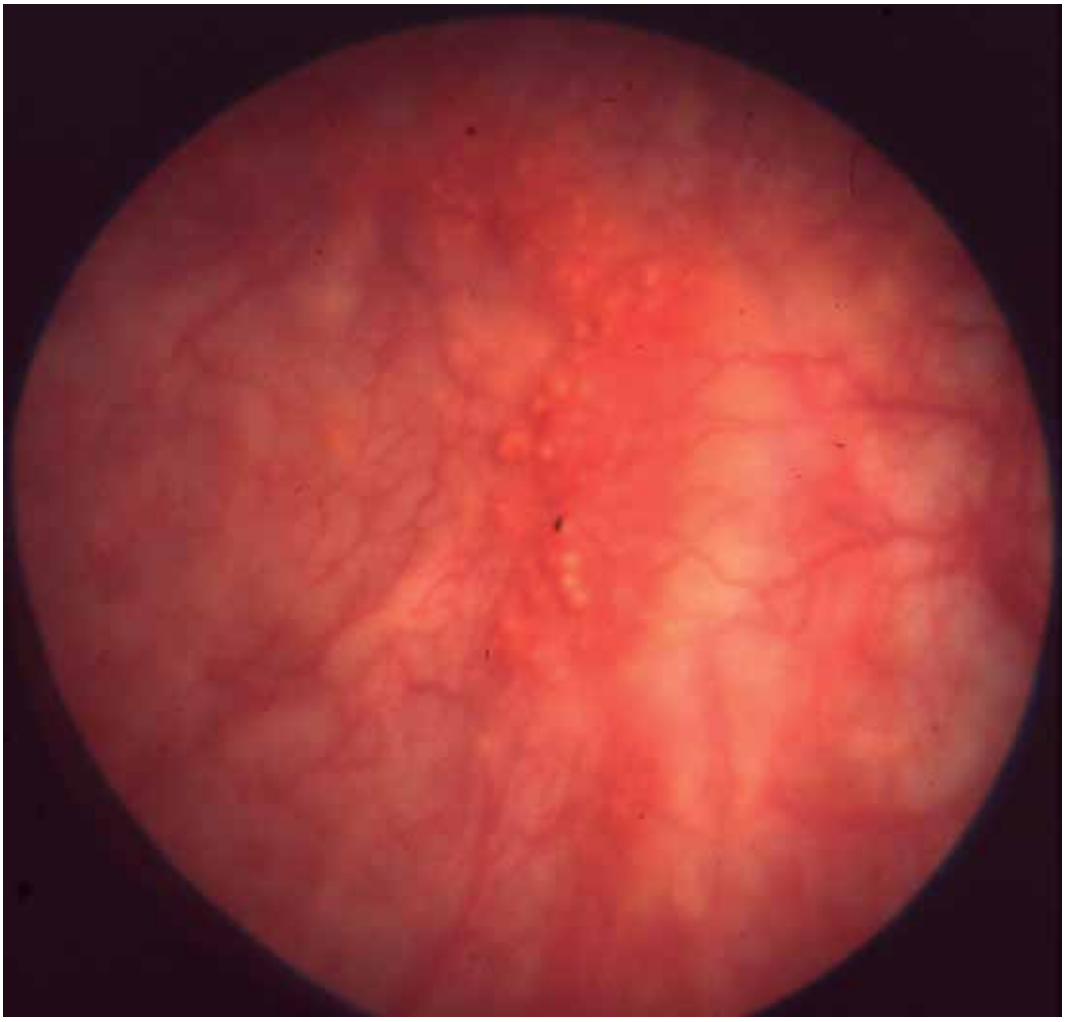

Fig. 9. Cystoscopic appearance of urinary bladder bilharziasis. According to Professor Hans Reuter (1923-2003). Private collection . Courtesy of his son Matthias Reuter MD,PhD.

Fig. 10. Cystoscopic appearance of sessile urinary bladder bilharzial tumor covered by eggs of S. hameatobium . According to Professor Hans Reuter (1923-2003) from his Atlas der Urologischen Endoskopie. Georg Thieme Verlag Stuttgart 1963, Foto number 53. Courtesy of his son Matthias Reuter MD, PhD.

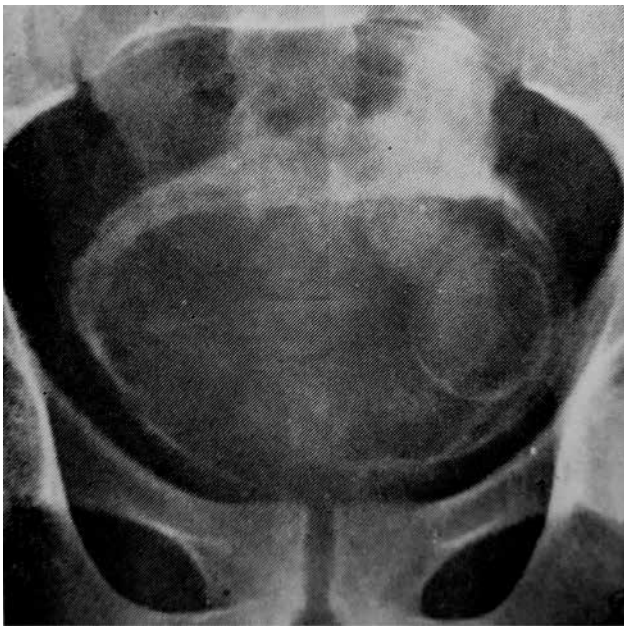

Fig. 12. Calcifications of the urinary bladder and the left orifice um ureteris according to the Department of Urology Al-Azhar University. Cairo after AFU 71 Session . Paris 1977 page 76 (3). With permission of AFU.

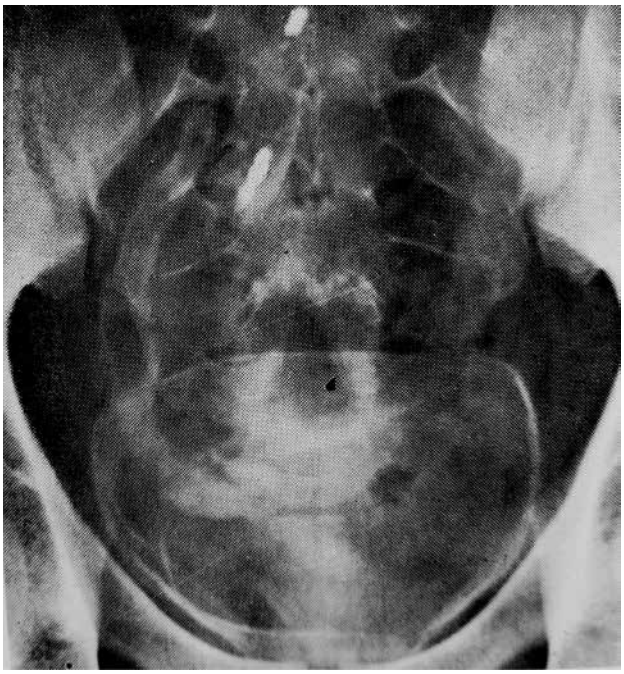

Fig. 11. Calcifications of the urinary bladder and lower part right ureter according to the Department of Urology Al-Azhar University. Cairo after AFU 71 Session . Paris 1977 page 76 (3). With permission of AFU.

Fig. 13. Calcificaitions of the seminal vesicles in a case of bilharzjasis. According to Christian Chatelain Ass. Franc. d,Urol. 71 Session 1977, page 165 (3).With permission of AFU.

glandularis (a precancerous condition) (Fig. 10), ureteritis cystica, and infiltrations at the ureteral orifices causing stenosis or vesicoureteral reflux.

In addition to the aforementioned examinations, imaging studies include ultrasound (currently a standard examination), plain abdominal radiography, intravenous urography, ureteropyelography, computed tomography, magnetic resonance imaging, and colonoscopy. When the disease is located outside the genitourinary system, appropriate imaging studies are conducted.

Plain abdominal radiographs reveal calcifications throughout the genitourinary system, especially in the trigone region, sometimes appearing as a "porcelain bladder" (Fig. 11). Continuous or interrupted calcifications may be seen in the ureters (Fig. 11) or at the bladder orifice of the ureter (Fig. 12), and in the seminal vesicles (Fig. 13). Calcifications may also occur in the kidneys. Differential diagnosis should include tuberculosis.

Urographic images, which are not specific to schistosomiasis, show signs of inflammatory changes in the renal pelvis (*pyelitis*), parenchyma (nodules or schistosomal eggs), and features of obstructive uropathy, primarily resulting from stenotic changes in the ureters, manifesting as dilation of the ureteropelvicalyceal system, or massive hydronephrosis, often bilateral, with concomitant end-stage renal disease (Fig. 14), as well as a bladder tumor appearance (Fig. 15).

The bladder remains relatively elastic in urographic images for a considerable time, but prolonged disease progression leads to fibrotic changes in its walls, muscular atrophy, and reduced bladder capacity (Fig. 16). Vesicoureteral reflux is frequently observed (Fig. 17). In advanced and neglected cases, bilharziasis manifestations (Fig. 18) and fistulas on the scrotum with pseudo-elephantiasis changes (Fig. 19), described by Egyptian physicians in the early 20th century, are visible. These can also be seen in illustrations from the period of the ancient Egyptian state, as reported by Edwin Pfister in 1911 at the 3rd Congress of the German Society of Urology in Vienna (cited by Hermann Hausmann [7]).

TREATMENT

In ancient Egypt, attempts were made to treat this condition using pomegranate root extracts, high-concentration (40%-80%) extracts of wormwood, and Vitriol [10]. Specific pharmacological treatment of schistosomiasis began in 1917 with the intravenous administration of a drug called tartar emetic (Bonnet M, 1921). Subsequently, other treatments included Niridazole (Ciba), methylene blue, and even surgical interventions such as bladder opening and fulguration of lesions [30]. Since these are now historical treatments, the authors will not list further drugs and methods used in those times.

Currently, since the early 1980s, early stages of the disease are effectively treated with Praziquantel and Albendazole for all forms of schistosomiasis. The typical dosage is a single dose of 40 mg/kg or 20 mg/kg in three doses administered every 4-6 hours. Usually, two treatment cycles are performed several weeks apart, achieving good results in almost 90% of cases. To evaluate the efficacy of the treatment, a viability test of the eggs should be conducted several months after

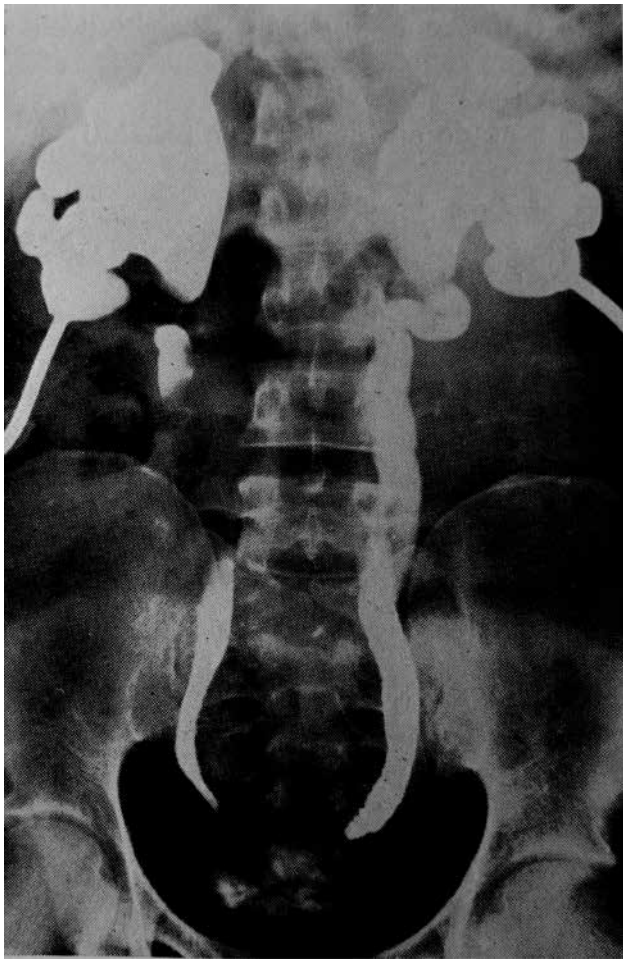

Fig. 14. Bilateral stenosis of the lower part ureters with end stage renal insufficience in a case of bilharzjasis in a case of bilharzjasis. According to Christian Chatelin AFU, 71 Session Paris 1977. Page 158 (3).With permission of AFU.

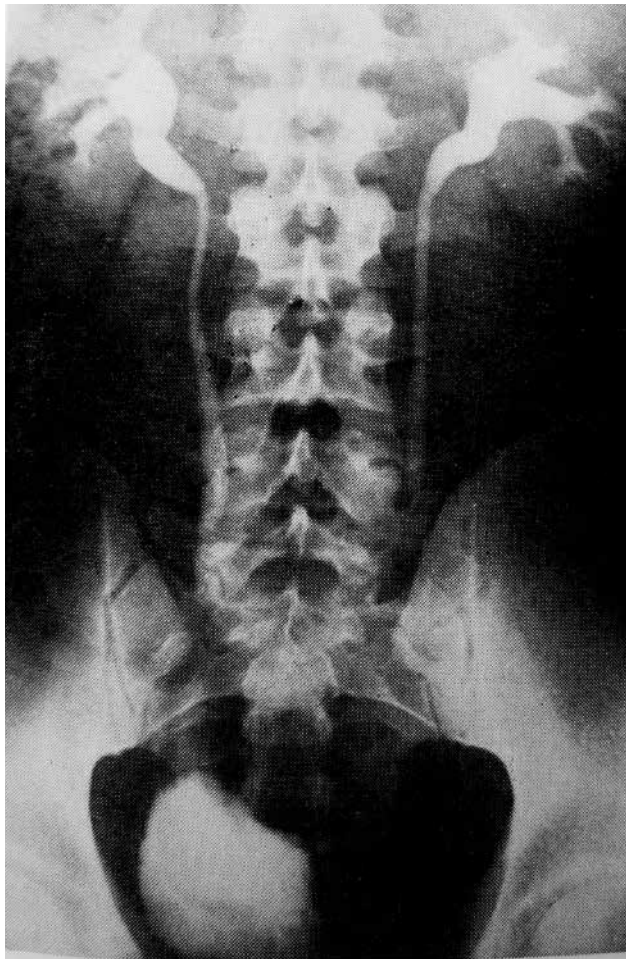

Fig. 15. Large bilharzjal tumor localized in the left part of the urinary bladder (IVP) according to A. Suhler . AFU 71 Session. Paris 1977. Page 97 (3). With permission of AFU.

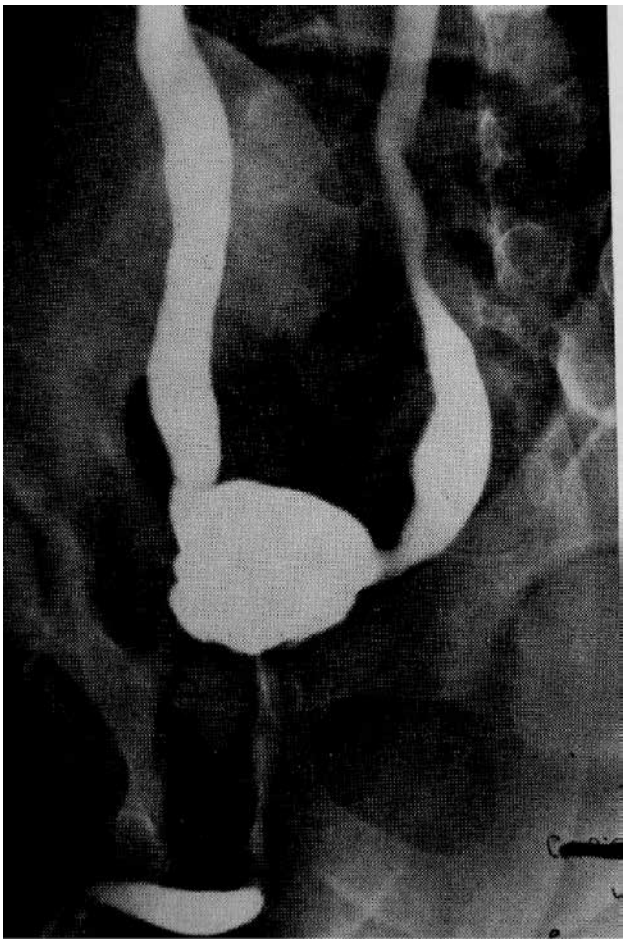

Fig. 16. Small urinary bladder with bilateral vesico-ureteral ruflux(Urethrography) in a case of bilharzjasis. According to Christian Chatelin AFU, 71 Session Paris 1977. Page 95 93). With permission of AFU.

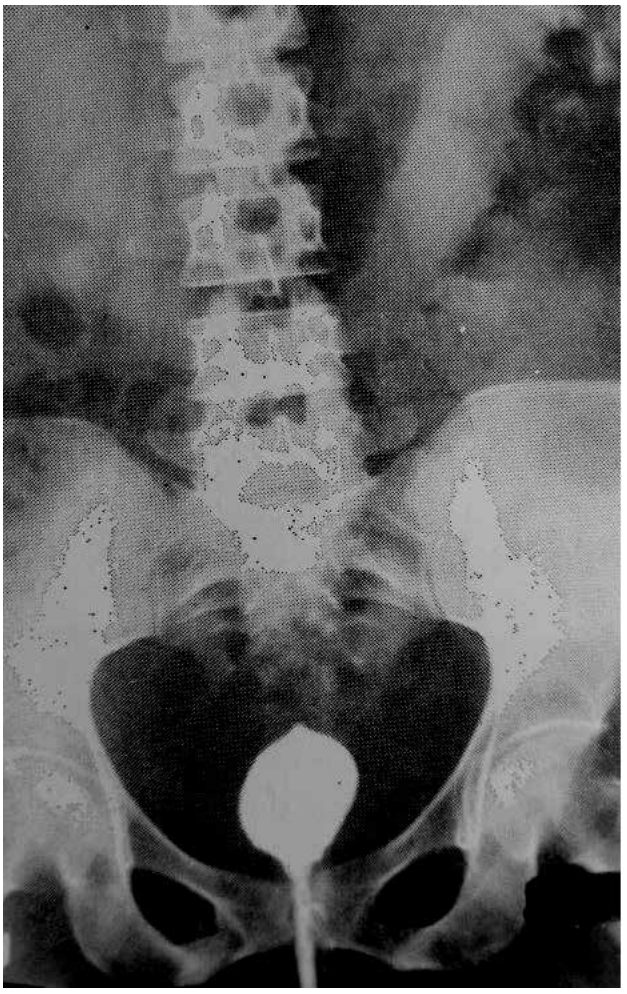

Fig. 17. Image of small urinary bladder in a case of bilharcjasis. According to Christian Chatelain. Paris. Ass. Franc. d, Urologie 71 Session. Paris 1977, page 84 (3). With permission of AFU.

complete recovery. Periodic follow-up examinations are required thereafter.

Late stages with extensive organ damage pose a significant problem, and the most experienced urologists in dealing with these cases are from countries where the disease is endemic (e.g., Egypt, Iraq, African countries). Management includes both ablative and reconstructive surgeries. The most common procedures are surgeries for ureteral strictures, stone disease, bladder fibrosis, and squamous cell carcinoma of the bladder.

CONCLUSIONS

Bilharziosis is diagnosed primary by doctors of infections and tropical medicine and also by family doctors and other specialists with the detailed anamnesis and the use appropriate additional tests (Examination of stool and urine, especially in travelers to countries endemic for bilharziosis as a "gold standard"). Additionally routine urine tests for hematuria, serological and antigen tests are used. In advanced stages specialistic diagnostic methods are required (plain film of the abdomen, IVP (Urography), CT scan, MRI, cystoscopy, microskopic examination).

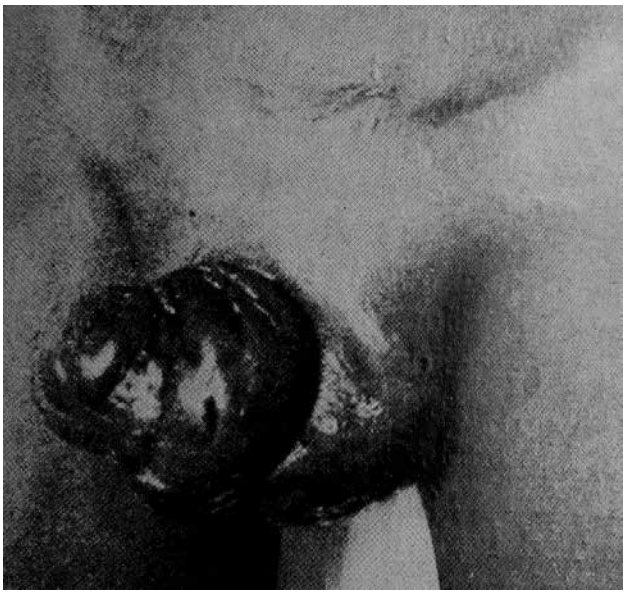

Fig. 18. Bilharzial urethro-cutaneous fistulae with pseudoelephantiasis perineum, penis and scrotum from the Department of Urology University of Cairo according to Christian Chatelain. Ass. Franc. d,Urol. 71 Session 1977, page 135 (3) .With permission of AFU.

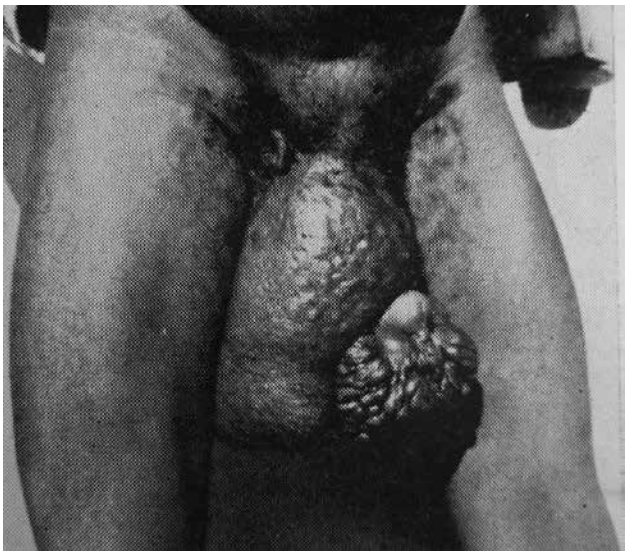

Fig. 19. Bilharziasis of the penis according to F. Madden from University of Cairo. According to Handbuch der Urologie. Springer Verlag 1927,page. 841.Public domain.

Prevention through proper prophylaxis in endemic countries is considered the most appropriate measure for eliminating the disease. This involves avoiding drinking unboiled or non-bottled water, avoiding bathing in local waters, and controlling snails by eliminating them. The World Health Organization (WHO) in its 2021-2030 strategy for combating schistosomiasis emphasizes special protection for preschool and school children, preventive actions among adults who have contact with water in endemic countries, and recommends treating at-risk children multiple times each year for many years.

ATTENSION

This publication aims to highlight a problem that urologists may encounter in the era of increased migration, particularly involving individuals, often unexamined, from regions endemic for schistosomiasis. It provides a concise overview of this widespread disease, which is increasingly observed in Germany [10, 23], yet practically non-existent or incidentally diagnosed in Poland [7].

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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CREATIVE COMMONS 4.0

REVIEW ARTICLE

Overcoming the consequences of psychological trauma with the help of "The Murray Method" and becoming a healthy balanced personality

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ABSTRACT

Aim: The aim of the study is to analysis of "The Murray Method", which is aimed at overcoming the consequences of adverse childhood experiences, the consequences of various psychological traumas of the individual during life and is aimed at the formation of a healthy balanced personality, the study of the fundamentals of the emergence of the method, the disclosure of the psychological mechanisms of how a person experiences a traumatic event, consideration of the main basic concepts of the concepts of "The Murray Method".

Materials and Methods: To solve the tasks and achieve the goal of the article, we used general scientific methods of the theoretical level (analysis, synthesis, comparison, systematization, generalization of scientific and theoretical data), in relation to the psychotherapeutic method of becoming a healthy balanced personality through overcoming trauma, violence and deprivation according to "The Murray Method".

Conclusions: "The Murray Method" is based on sufficiently strong immersion in the «Pool of Pain» and its «devastation» in an atmosphere of support and love, reducing the role of the protective mechanism as a result of working with images, manifesting, talking about painful emotions, analyzing traumatic experiences, as well as searching and the formation of personal talents, the formation of a healthy, balanced person, capable of sympathizing, showing empathy, protecting oneself without harming others, enjoying life.

KEY WORDS: adverse childhood events, egg of trauma, pool of pain, original feeling child, controlling child, healthy balanced person

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INTRODUCTION

Modern living conditions of a person often lead to traumatization of his psyche. As a result, her well-being, emotional and psychological state deteriorates, there is a negative impact on all spheres of a person's life. New discoveries in neurobiology and psychology confirm following statement: Negative childhood experiences (NCE), psychological traumas lead to a disturbance in the balance of neurotransmitters and biological changes in our body [1, 2]. Identifying the link between traumatic experiences and later dysfunctions and illnesses offers a chance for healing. The study of traumatic experiences (especially war) and related stress and post-traumatic stress disorder (PTSD) provides new «lenses» through which one can better understand the traumatized individual. Armed with knowledge, people traumatized by war heal on a deeper level. M. McCarthy is sure that the flexibility of the brain reveals many ways for its own immune rehabilitation. The brain has the ability to restore itself. The researcher convinces that a psychologically traumatized person can heal old wounds and become what he could be if it were not for the traumas he had to endure.

That is why nowadays it is very important to search for the latest psychotherapeutic methods, with the help of which it is possible to overcome the consequences of the traumas experienced, avoid their negative results, help a person to be reborn to a new life, to be a harmonious and healthy person again.

Domestic and foreign scientists have studied the impact of negative experiences on the further development of a person's risky behavior [3]. Thus, they focused on the fact that almost all drug-addicted adults in the past had an adverse childhood experience in the form of abuse, which caused mental trauma and led to the abuse of psychoactive substances (PAS) in the future [4-6]. Thus, it was proved that in women the probability of using PAS was directly related to the severity of childhood abuse. Women suffering from drug addiction were twice as likely to report childhood sexual abuse (70%) compared to women in the general population (35%). PAS use disorders in men are less associated with early sexual abuse, but male respondents report a high level of physical abuse in childhood.

In a study conducted in the state of California, USA, among university students (2,953 people), 75% of respondents stated that they use PAS. Among the factors of adverse childhood experiences, 25% of them most often mentioned cases of psychological violence, 16% of sexual violence, 14% of physical violence, and 9% of violence from parents or intimate partners. At the same time, 50% of respondents reported family violence or dysfunctions, 23% reported about multifactorial adverse childhood experiences [6].

A similar study was conducted with students (1580 people) of higher education institutions (HEI) and colleges with an average age of 20 years. During the study, the facts of adverse childhood experiences were discovered, which were distributed among the study participants as follows: sexual abuse was noted in 5.7%, physical abuse in 14%, emotional abuse in 37.9%, physical abuse in 53.3%, and emotional neglect in 57.9%. Of them, 84.6% of respondents reported at least one type of violence, 28.2% of respondents experienced two types of violence in the family, and 17.5% of respondents reported four or more types of violence. At the same time, the results of the study show that the probability of such forms of behavior that harm health (smoking, alcohol and drug use, having multiple sexual partners and suicide attempts) increases with an increase in the number of childhood violence.

Also, the study, in which teachers and students of the Faculty of Psychology of Kyiv National University named after Taras Shevchenko aging from 21 to 60 years old, a total of 73 people, took part, indicates that 40% of the study participants encountered five traumatic events in their childhood, 30 % have preserved memories of 6-10 such cases and 15% of respondents have from 11 to 15 such memories. The study indicated the dependence of negative childhood experiences and behavioral characteristics in adulthood [7].

According to research data, compared to the absence of adverse childhood events (ACE), the presence of four or more adverse childhood events is associated with an increase in the following risks in the future: smoking – 2.1 times, alcohol abuse – 10.4 times, drug use – 11 times. 9 times, risky sexual behavior – 1.7 times, unwanted pregnancy – 7.9 times and suicide attempts – 23.2 times. For patients with a history of childhood abuse, the onset of use of PAS at an early age is characteristic. People, who started using PAS, have a reduced ability to analytically and synthetically process information. Cognitive comparison of events whose images are stored in memory and events occurring at the current moment leads to a lack of social confidence in such individuals, which is reinforced by emotional self-doubt. As a result, behavior with a low level of arbitrariness prevails due to insufficient self-control, which is characterized by impulsive responses to external stimuli of the environment, with a predominance of orientation to situationally significant objects, which PAS often become.

The nature of the use of PAS in persons who experienced violence in childhood takes a more severe form, causing such serious disorders as overdose, heavy drinking, severe withdrawal disorders. It has been shown that patients with adverse childhood experiences have a higher risk of developing health problems related to alcohol use (7.4 times higher than in control respondents) and problems arising from drug use (from 4.7 to 10.3 times higher than in the control group) [8]. Patients of this group more often use illegal drugs, sedatives and tranquilizers. They are also more likely than their non-abused peers to use PAS to relieve somatic pain, avoid family problems, or relieve psycho-emotional stress [9].

In children who have experienced violence, the use of PAS is a psychological aid to escape from an abusive and unacceptable environment; a form of self-medication through which the child tries to gain control over his negative life experience; a method of self-affirmation that increases a child's self-esteem; departure from the feeling of isolation and loneliness [10].

Thus, ACE acts as a factor that harms the child in the process of his growth and development, and is the cause of a set of consequences: cognitive problems (inattentiveness and deficit of executive functions); problems of interpersonal and social interaction (mutual rejection by peers); internal and external symptoms (depression, anxiety, oppositional disorders, conduct disorders, aggressiveness); post-traumatic stress disorder (PTSD), etc.

AIM

The aim of the study is to analysis of "The Murray Method", which is aimed at overcoming the consequences of adverse childhood experiences, the consequences of various psychological traumas of the individual during life and is aimed at the formation of a healthy balanced personality, the study of the fundamentals of the emergence of the method, the disclosure of the psychological mechanisms of how a person experiences a traumatic event, consideration of the main basic concepts of "The Murray Method".

MATERIALS AND METHODS

To solve the tasks and achieve the goal of the article, we used general scientific methods of the theoretical level (analysis, synthesis, comparison, systematization, generalization of scientific and theoretical data), in relation to the psychotherapeutic method of becoming a healthy balanced personality through overcoming trauma, violence and deprivation according to "The Murray Method". The literary works of the author of "The Murray Method" and other authors who implement the method in their theoretical and practical activities, researchers who study the impact of negative childhood experiences on a person's future life, were analyzed.

An empirical study was conducted to verify the effectiveness of "The Murray Method" for overcoming the consequences of psychological traumatization using the following research methods: the research method of negative childhood experience (according to Felitti, Andom), the symptomatic distress questionnaire (according to Derogatis), the method «Style of self-regulation of behavior» (according to Morosanova); diagnostics of socio-psychological adaptation (according to Rogers, Diamond), «Spiritual potential of the individual-2» method (according to Pomytkin); methods of statistical processing of the obtained data.

REVIEW AND DISCUSSION

According to Principle 9 of the Declaration of the Rights of the Child (DRC), adopted by the UN General Assembly on November 20, 1959, «the child must be protected from all forms of neglect, cruelty and exploitation». In order to overcome the consequences of adverse childhood experiences (psychological trauma, all types of violence, emotional deprivation) and various forms of addictions (narcotics, in particular), into which an immature personality runs, an appropriate psychotherapeutic method of assistance is needed. This is the method of becoming a healthy balanced personality by overcoming trauma, abuse and deprivation "The Murray Method" has proven [11, 12].

"The Murray Method" has proven its effectiveness and is used in psychological practice to overcome psychotraumatic events in children and adolescents, for the prevention of addictive behavior, to overcome the negative consequences of childhood psychotraumas on the personality of an adult, for the rehabilitation of teenagers who are in conflict with the law, abuse South Africa, and employees of internal affairs bodies who participated in hostilities within the framework of the «Side by Side» project [13, 14].

"The Murray Method" of developing a healthy balanced person through overcoming injuries, violence and deprivation covers almost all of the indicated aspects of working with a person, from internal experiences, overcoming addictions, overcoming childhood traumatization of personal growth, to violations of interaction with other people, etc. [15]. The method was developed by the American psychotherapist Marilyn Murray in 1981, and over the past years it has been improved and spread in more than 45 countries of the world [16, 17].

In the life of Marilyn herself, at the age of 8, a tragic traumatic situation occurred: she was raped by a group of drunken soldiers. The psychological trauma was so great that this terrible event was pushed out of her memory. As a result of the emotional shock, serious physiological disorders of the body began: she was constantly tormented by bronchial asthma, migraine attacks, etc., she behaved as a codependent who took care of many adults who can take care of themselves.

Only at the age of 44, having achieved significant success in business and being the director of her own art gallery, she is faced with the urgent need to undergo a course of psychotherapy and takes it at Dr. Osborne's clinic in Burlingame (USA, California). The expected two-week period of treatment dragged on for a long seven months. In the process of such long-term therapy, memory recovery occurred, and it seemed to regress for a while, until the age of eight. Subsequently, based on the analysis of her own recovery process, she develops the author's "The Murray Method".

This method was created with the aim of helping people who experienced childhood violence, sexual, in particular. The author developed a theory that reveals the mechanism of how a person experiences a traumatic event. Applying it in psychotherapy practice, she discovered that the method can be applied to other negative factors, after which the theory was developed and applied in a wider context. It included not only issues of trauma, but also violence and deprivation. In the future, the author begins to use this method as an effective means of developing a person's personality, through the therapy of the consequences of trauma, violence and deprivation [12].

"The Murray Method" offers a transparent and complete explanation of the consequences of pain experienced in childhood and reveals the process of trauma, violence, contempt and deprivation, as well as protective mechanisms to distort a person's mental perception and emotional response, which, as a result, affects his health and behavior in later life.

The goal of therapy, according to "The Murray Method", is the formation of a harmonious personality, balanced and balanced in five main areas of life: physical, emotional, intellectual, social and spiritual. «Healthy balanced person» is a balanced combination of positive qualities of the «Original feeling child», along with the strengths of the «Sobbing hurting child» and the «Controlling Child». This method is aimed at working with psychological injuries of any severity, it covers almost all aspects of working with a person, from internal experiences to interaction with other people.

"The Murray Method" is especially useful for working with people who have experienced violence in the past, psychological trauma or grew up in dysfunctional families. The long-term effect is observed after working with the consequences of physical, sexual, emotional, psychological or spiritual violence; contempt and emotional deprivation; problems in the relationships. Also, the method has been tested in working with various kinds of addictions [18].

One of the key concepts of "The Murray Method" is the «Original feeling child». This is the child who from birth has a complex of predispositions, which later turn into: intelligence, talents and gifts, creative abilities, spirituality, healthy sexuality, it is characteristic for her to feel and express all feelings. Under the influence of negative external factors, such as trauma, insult, contempt, illness, etc., the integrity of the «Original feeling child» is destroyed, then the «Sobbing hurting child» appears. The painful feelings experienced by the «Original feeling child» in childhood created her «Pool of Pain», which accumulates sadness, fear, anger, loneliness, helplessness, resentment, etc., the very feelings that the child was unable to express during the trauma. This inhibition of self-expression can occur due to the influence of family, cultural, ethnic customs, traditions, norms, rules of morality. Unreacted emotions accumulate in the «Pool of Pain» and if in the present tense there is a stimulus with a strength of 1, 2 or 3 points on a 10-point scale, then a person reacts to it inadequately, namely, at the level of 8, 9, 10 points [12].

The problem is not only that the child has experienced trauma, violence or deprivation. The problem is that trauma, violence, and deprivation caused painful feelings that were not reacted to in a healthy way in an atmosphere of love, respect, and support, and this, in turn, began to change the child's thinking and attitude toward himself, others, and life, and formed the ACE.

To protect the «Sobbing hurting child», the «Controlling Child» joins. Due to injuries and various types of violence and the inability to react in a healthy way, the «Sobbing hurting child» accumulated painful experiences, there were disturbances in the neurochemistry of the brain, and a protective mechanism was formed «Controlling Child». The «Controlling Child» defense mechanism manifests itself differently in different people. These can be pain relievers (food, alcohol, drugs, nicotine, sex, caffeine, gambling, etc.) or distractions (codependency, work, study, sports, TV, computer, church, caring for others, constant employment, etc.). A person becomes addicted to what helps him not to feel pain. As a result, a person does not develop as a person, does not live the life that is intended for him, but survives, «playing the wrong role». The «survival-oriented brain» resists changes that cause real or imagined fears, if at the same time there is no means to protect itself. As a defense mechanism, the «Controlling child» should act as a temporary aid to reduce pain during times of stress. If such protection is used constantly, it can lead to the development of pathological addictions, forming various types of addictions, in particular, addiction to PAS. However, «Original feeling child», «Sobbing hurting child» and «Controlling child» all have their strengths. So, the advantages of the «Original feeling child» include: spirituality, spontaneity, playfulness, dreaminess, creativity, ingenuity, curiosity, sexuality, sense of humor. The «Sobbing hurting child» is distinguished by such positive features as: sensitivity, the ability to understand the feelings of others, sympathize, worry, empathize, be gentle. The strengths of the «Controlling child» are: the ability to build and protect their own «borders», which prevents others from turning a person into a victim, and does not allow treating others as their own victims; responsibility, diplomacy, discipline.

A balanced, harmonious combination of the strengths and positive aspects of the «Original feeling child», «Sobbing hurting child», «Controlling child» is a «Healthy balanced person». A «healthy balanced person» means a person who not only combines these advantages, but also develops harmoniously in physical, emotional, intellectual, social and spiritual terms [15].

In addition, there may also be two unbalanced combinations, but no longer «positive», but «negative» children: «Angry rebellious child» and «The stubborn selfish child».

When the «Sobbing hurting child» needs are not met, and the «Controlling child» has already lost patience and is tired of trying to ease the «Sobbing hurting child» pain, then the negative qualities of these children combine to form the «Angry rebellious child». «Angry rebellious child» is openly hostile and active-aggressive, demanding, impatient, she is distinguished by a tendency to justify her behavior, an unwillingness to consider the consequences of her actions and a refusal to bear responsibility for them. She usually does what she wants, even if she knows that these actions will harm her and other people.

It often happens that when it does not turn out to be a «Healthy balanced person» and it is dangerous to openly express aggression, or education does not allow, then the negative traits of the «Sobbing hurting child» and «Controlling Child» are joined by negative traits (selfishness, fickleness, infantilism, etc.) of the «Natural child» with his charm, charisma, attractiveness, and then a person expresses aggression not openly, but covertly. This combination of negative traits of three «children» according to "The Murray Method" is called «The stubborn selfish child».

«The stubborn selfish child» is passive-aggressive, behaves intriguing, manipulative, indecipherable in sexual relations, tends to present himself as grandiose, narcissistic. She considers herself a victim, but in fact she herself constantly becomes an offender, causing harm to herself and others, tends to see others as the reasons for her own failures.

The program for the rehabilitation of persons injured in war, according to "The Murray Method", reveals the process of the action of trauma, violence, contempt, as well as the protective mechanisms of a person on the distortion of a person's mental perception and emotional response, which affects the health and behavior of a person. The result of the application of the rehabilitation program based on "The Murray Method" is the «Development of a healthy balanced person» through the conscious and complete explanation of the consequences of the pain experienced in childhood and the acquisition of knowledge and the development of skills for healthy behavior patterns in the present and future.

Regarding the individual who was forced to experience trauma and did not process it, according to "The Murray Method", five groups of dysfunctions are distinguished: addictions, codependency, the state of the victim, manifestations of narcissistic traits, active and passive aggression. It is they who are an obstacle on the way to a full-fledged life of persons affected by injuries, and the subject of work in the rehabilitation program.

Based on the integration and generalization of the provisions of "The Murray Method" regarding the formation of a «Healthy balanced person» by overcoming the consequences of psychological trauma, violence and deprivation, we have developed a rehabilitation model.

CONCLUSIONS

Thus, "The Murray Method" is aimed at the formation of a «Healthy balanced person» by overcoming psychological trauma, violence and deprivation. One of the basic concepts of "The Murray Method" is the «Original feeling child», which has talents and gifts, intelligence, creative abilities, healthy sexuality, spirituality, it is characteristic for her to harmoniously feel and express all feelings. Under the influence of adverse external factors, such as contempt, illness, injury, insult, etc., the essential integrity of the «Original feeling child» is destroyed, then the «Sobbing hurting child» appears. The painful experiences that the «Original feeling child» felt during the trauma created her «Pool of Pain», where helplessness, fear, loneliness, sadness, anger, resentment, etc. accumulate. These are exactly the feelings that a person is not trained to express during trauma. Then a «Controlling Child» is added to protect the «Sobbing hurting child». A person becomes dependent

on what helps them not feel pain (drugs, food, alcohol, gambling, nicotine, sex, caffeine, work, constant employment, codependency, etc.). A harmonious combination of the strengths of «Original feeling child», «Sobbing hurting child» and «Controlling child» is «Healthy balanced person».

"The Murray Method" is based on sufficiently strong immersion in the «Pool of Pain» and its «devastation» in an atmosphere of support and love, reducing the role of the protective mechanism as a result of working with images, manifesting, talking about painful emotions, analyzing traumatic experiences, as well as searching and the formation of personal talents, the formation of a healthy, balanced personality, capable of sympathizing, showing empathy, protecting oneself without harming others, enjoying life. The perspective of further research is to study the rehabilitation of persons traumatized by war and the formation of a healthy balanced personality according to "The Murray Method".

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CONFLICT OF INTEREST
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CREATIVE COMMONS 4.0

REVIEW ARTICLE

Applications of magnetic resonance imaging in the diagnosis of posttraumatic stress disorder

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ABSTRACT

The application of magnetic resonance imaging (MRI) techniques in diagnosing posttraumatic stress disorder (PTSD) represents a significant research focus, providing deeper insights into trauma-related psychological disorders. PTSD often arises from chronic stress, a state in which the body struggles to adapt to overwhelming stimuli. Patients frequently experience persistent flashbacks (intrusive memories or sensory recollections of the traumatic event) and nightmares, which evoke intense emotional responses and disrupt daily life. These recurring episodes of stress and anxiety severely impact behavior and functioning. Understanding the brain changes associated with PTSD has become possible through advancements in MRI studies. This article aims to review the scientific literature on the use of magnetic resonance imaging techniques in diagnosing posttraumatic stress disorder. The review is based on scientific literature from medical databases, including Web of Science and Scopus, covering publications up to October 30, 2024. The findings from this analysis may contribute to a better understanding of the neurobiological mechanisms underlying PTSD.

KEY WORDS: magnetic resonance imaging, posttraumatic stress disorder, psychiatric disorders

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a psychological condition that develops in individuals who struggle to process and adapt to trauma. A hallmark of this disorder is the persistent, uncontrollable recurrence of thoughts related to the traumatic experience [1]. This often manifests as intrusive and distressing memories of the event, accompanied by overwhelming emotional suffering, nightmares, and flashbacks. These experiences contribute to the erroneous perception that the trauma is ongoing, and the threat remains imminent. Prolonged exposure to stress associated with PTSD leads to both physical and mental exhaustion. This may present as physical weakness, a lack of energy, reduced immunity to infections, or a constant state of mental alertness, anticipating negative outcomes. Common symptoms include hyper-vigilance, heightened reactivity, and severe stress or physical arousal in situations that evoke traumatic memories. Individuals with PTSD often withdraw from social connections, sever ties with loved ones, and struggle to resume their pre-trauma activities [2]. They may engage in avoidance behaviors, suppressing fragments of traumatic memories through retrograde amnesia. Pessimistic thoughts about themselves and others are pervasive, accompanied by negative emotions such as fear, anger, shame, and guilt. Their ability to experience positive emotions like joy, pleasure, or contentment is markedly diminished. At the core of these symptoms lies the challenge of processing trauma-related memories, which disrupts their emotional and cognitive stability [3].

Neurophysiological research on post-traumatic stress disorder (PTSD) explores the mechanisms underlying fear memory and associative learning [4, 5]. Fear memory has been studied through contextual and tone-based fear conditioning procedures, as well as an instrumental procedure called one-trial inhibitory avoidance [6]. These mechanisms involve key brain regions such as the hippocampus (contextual conditioning and inhibitory avoidance), the basolateral amygdala (inhibitory avoidance), and the lateral amygdala (conditioning to a tone). Additionally, the circuitry extends to the pre- and infralimbic ventromedial prefrontal cortex, central amygdala subnuclei, and the dentate gyrus. Fear learning models, particularly inhibitory avoidance, have proven valuable for studying the biochemical mechanisms of memory consolidation. These findings build upon in vitro research on long-term potentiation and other forms of neural plasticity [7,8]. Brain plasticity, defined as changes in the number and function of synaptic connections, reflects the brain's response to stress and is fundamental to associative learning [9]. Associative learning links previously unrelated elements in the brain through conditioning and underpins three basic learning types: classical conditioning (unconscious processes), instrumental conditioning (conscious processes), and observational learning [10]. In PTSD, trauma alters cortisol secretion and disrupts hippocampal function, impairing the formation of chronological memories. Conversely, the amygdala, responsible for encoding non-chronological memories, becomes hyperactive during threats, prompting responses such as fight, flight, or freeze [11]. This leads to incomplete conscious memories of the trauma but intact

unconscious "body memories." Dissociation, or the disconnection of components of an experience, often occurs, resulting in fragmented recollections [12]. Trauma memories frequently manifest as intrusive flashbacks, wherein individuals relive past traumatic events with vivid intensity and realism. Flashbacks blur the distinction between past and present and can be triggered by external or internal cues linked to the original trauma [13].

Magnetic resonance imaging (MRI) plays a critical role in the structural and functional neuroimaging of PTSD. It is particularly effective for evaluating brain structures implicated in fear memory and advancing our understanding of the neurobiological underpinnings of the disorder.

AIM

The purpose of our article is to analyze papers devoted to the topic of MRI in the diagnosis of post-traumatic stress disorder.

MATERIALS AND METHODS

The review is based on scientific literature from medical databases, including Web of Science and Scopus, covering publications up to October 30, 2024.

REVIEW AND DISCUSSION

MAGNETIC RESONANCE IMAGING IN PTSD

Magnetic resonance imaging (MRI) is an invaluable imaging technique that provides detailed, multi-plane images of brain structures with exceptional spatial resolution. It is particularly useful in diagnosing post-traumatic stress disorder (PTSD). MRI leverages the properties of elemental nuclei, such as protons, which, under specific conditions, generate signals that allow the visualization of anatomical brain structures [14]. Studies have demonstrated that MRI is effective in identifying structural brain abnormalities associated with trauma-related disorders [15, 16]. Additionally, functional MRI (fMRI) enables the study of brain activity during specific tasks or stimuli, offering valuable insights into brain function in individuals who have experienced traumatic stress [17].

The literature [18, 19] reveals MRI studies conducted on PTSD in both children and adults. In children, trauma often leads to the persistent re-enactment of the traumatic event through play or drawings [20]. Symptoms can include hyperactivity, irritability, spontaneous crying, difficulty concentrating, memory issues, and nightmares [21]. PTSD in young children may lead to developmental regression and the loss of acquired skills. Furthermore, children often exhibit anxiety over the potential loss of parents, manifesting as anxiety attacks during prolonged absences [22]. Adolescents may demonstrate lowered self-esteem, engage in self-destructive or risky behaviors, and turn to psychoactive substances [23]. A study by Uematsu et al. [24] showed early maturation of the amygdala and other subcortical regions related to threat reactivity in children with PTSD, while Gee et al. [25] found maturation in emotion-regulatory regions such as the dorsal/lateral prefrontal cortex. Cross-sectional studies on emotion regulation processing indicate reduced anxiety and improved regulation of the amygdala-prefrontal cortex (mPFC) connection in older children compared to younger ones [26]. Other studies on PTSD in children show structural and functional brain abnormalities similar to those found in adults, including reduced ventromedial prefrontal cortex (vmPFC) volume, hippocampal volume, and increased amygdala and insula activity [27,28]. These changes, however, were not observed in all children studied, which may reflect the neurodevelopmental diversity of the group. Research also highlights that children and adolescents are more sensitive to stress than adults, underscoring the need for special care in treating PTSD in these populations. PTSD can result in abnormal connections in the neuronal network, particularly between the prefrontal cortex and amygdala, potentially perpetuating trauma in adulthood [29].

Modern neuroimaging techniques provide critical insights into the effects of trauma on the brain [31]. Adults with PTSD show significant brain abnormalities, which can be identified using MRI. Brain morphometry in PTSD often focuses on the hippocampus, amygdala, and dorsolateral prefrontal cortex [32]. Morphometric measurements include the volume and microstructure of subcortical and cortical gray matter. Some researchers have pointed out that PTSD sufferers have difficulties with attention, which is linked to overactivity in the dorsolateral prefrontal cortex [33, 34].

Other studies suggest that trauma-related overactivity in the medial prefrontal cortex, which is responsible for self-identification, is also evident [35, 36]. PTSD onset has been associated with abnormalities in anxiety learning, threat detection, executive functions, emotional regulation, and contextual processing [3, 37, 38]. MRI plays a key role in both structural and functional neuroimaging of the brain. In PTSD, MRI can reveal focal gray matter atrophy, altered fractional anisotropy, and changes in neuronal activity and connectivity. These findings suggest that areas such as the medial and dorsolateral prefrontal cortex, orbitofrontal cortex, insula, lentiform nucleus, amygdala, hippocampus, parahippocampus, anterior and posterior cingulate cortex, precuneus, cuneus, fusiform and lingual gyri are involved in the pathophysiology of PTSD [3]. Among these, lesions in the anterior cingulate cortex, amygdala, hippocampus, and insula are the most commonly observed in MRI studies. Additional studies have indicated that regions such as the hippocampus, temporal cortex, thalamus, frontal cortex, and even the cerebellum play significant diagnostic roles in PTSD. In contrast, research by Cobb et al. and Postel et al. in individuals with anxiety disorders has shown reductions in gray matter in the insula, striatum, and anterior cingulate cortex [14, 39]. Kamiya et al. found similar gray matter losses in the hippocampus, amygdala, insula, thalamus, and frontal regions in individuals with mood disorders, anxiety, or substance abuse [40]. Analysis of MRI studies in PTSD has shown atrophic changes, especially in the frontal insula and the dorsal part of the anterior cingulate cortex, known as the salience network (SN) in neurocognition. Voxel-based morphometry (VBM) and volumetric studies provide comprehensive brain scans, identifying gray and

white matter abnormalities without the need for predefined regions of interest (ROI), using fully automated techniques [17, 24, 41].

Magnetic resonance imaging (MRI) reveals structural changes in the brain that indicate reduced integrity in regions associated with executive functions. In patients with PTSD, decreased cortical volume has been observed in the anterior cingulate cortex (ACC) and frontal cortex, as well as in subcortical structures such as the hippocampus and amygdala.

Additionally, MRI findings show reduced white matter integrity in the uncinate fasciculus, corticospinal tract, and corpus callosum, while increased white matter integrity has been noted in the inferior temporal gyrus and inferior fronto-occipital fasciculus. Similar changes may also be present in other anxiety and trauma-related disorders. However, when combined with biochemical and genetic testing, these neuroimaging findings have the potential to serve as biomarkers for PTSD. Neuroimaging plays a crucial role in monitoring disease progression and mitigating the effects of stress. It aids in the planning of pharmacotherapy and personalized psychotherapy, making treatment more effective and motivating patients to adopt healthier lifestyles. While MRI is not yet a standard diagnostic tool for PTSD, integrating it with other assessments may bring clinicians closer to establishing standardized care for individuals affected by trauma.

CONCLUSIONS

In conclusion, Magnetic Resonance Imaging is non-invasive and is effective in identifying structural brain abnormalities associated with traumatic stress.

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CONFLICT OF INTEREST

The Author declares no conflict of interest

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REVIEW ARTICLE

Special pedagogy of people with intellectual disability and contemporary psychiatry in Poland – mutual complementarity or lack of understanding?

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ABSTRACT

Intellectual disability is the subject of interest of psychiatry and special education. Both sciences in Poland adopt an inclusive model of care for people with disabilities. The path of each of them from a directive model to an inclusive model has been different.

The aim of the study is to compare the attitude towards people with intellectual disabilities and the evolution of changes that have taken place in Polish special education and in Polish psychiatry in the last few decades. The comparison was carried out using the narrative method through a review of key publications on the understanding of the intellectual disability phenomenon by special education in Poland and by psychiatry. In 1989, Polish special education departed from dialectical materialism as a binding doctrine in the social sciences. A postmodern understanding of the phenomenon of disability, culminating in the formulation of a humanistic paradigm of special education consisting of four microparadigms, developed instead. Special education moved away from quantitative research in favor of qualitative research, negating the biomedical aspects of disability. Qualitative and conceptual research studies carried out in Poland have not entered the global circulation of scientific information and are known locally in Poland. Polish psychiatry has undergone a less revolutionary path of change than special education. The development of community psychiatry in Western Europe slowly began to be implemented in Poland. It has been implemented in recent years in the form of mental health centers. Since the beginning of the political transformation, Polish psychiatry has been present in international journals in published English with the Impact Factor. Despite the postmodern, inclusive understanding of mental disorders, but in contrast to Polish special education, Polish psychiatry is based on quantitative research studies. Their results are published in international journals. Polish special pedagogy of people with intellectual disabilities, as well as Polish psychiatry, have undergone a significant paradigmatic reconstruction in the last few decades. Both sciences adopted an inclusive model of understanding intellectual disability. The different ways of development of the two sciences are reflected in publications and scientific research, as well as in certain terminological differences.

KEY WORDS: special education, psychiatry, paradigms, intellectual disability

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INTRODUCTION

The change of the socio-political system in Poland in the 1990s changed the approach towards people with mental disorders and towards people with disabilities, including intellectual disabilities [1, 2]. Dialectical materialism, as an official and binding scientific doctrine, ceased to be binding in Poland after 1989. This fact influenced a paradigm shift in both psychiatry and special education. The juxtaposition of the evolution of these two scientific disciplines is interesting, because both sciences deal with people with disabilities, including intellectual disabilities [1-3]. A common subject of interest and a common object of scientific research should, at least in theory, bring researchers and practitioners in the field of psychiatry and special education closer to each other. However, both these disciplines in Poland developed independently of each other after 1989, in a different paradigmatic environment, in different academic centers. Special education was developed at universities and at the Academy of Special Education, psychiatry at medical universities and the Institute of Psychiatry and Neurology. The activities of special educators included broadly understood education, while psychiatrists focused on treatment and prevention. Therefore, both the academic centers and the places of social activity of the representatives of the two sciences discussed were separate. Psychiatrists did not work in education and oligophrenopedagogues rarely visited health care facilities. Although contemporary social psychiatry and special pedagogy have developed very similar standards

of conduct, they are based on different assumptions and different terminology is still in force in both sciences.

AIM

The aim of the study is to discuss the similarities and differences between psychiatrists and special educators in paradigmatic thinking on intellectual disability.

MATERIALS AND METHODS

The literature review is of a narrative nature. It takes into account the most important achievements of contemporary Polish special pedagogy and community psychiatry from the mid-twentieth century to the present. Due to the lack of English-language publications on Polish special pedagogy, Polish-language publications were used.

REVIEW AND DISCUSSION

General and special pedagogy, from the period of real socialism in Poland, were characterized in the ideological sphere by references to dialectical materialism, whereas in the research sphere they employed the modernist model of scientific quantitative research. The results of these studies constituted a collection of results and information rather disordered in character, although great scientists and social activists such as Maria Grzegorzewska, the founder of Polish special education, were active during that period [4]. The works of Bogdan Suchodolski [4], psychometric research in pedagogy of Bolesław Niemierko [5], and the controversial experiment on the education of a socialist man carried out in Poznań by Heliodor Muszyński [3] have gone down in the history of general pedagogy. The loss of the leading role of dialectical materialism in 1989 on the one hand caused a paradigmatic void, and on the other hand, it opened pedagogy and special pedagogy to the modern trend of postmodern pedagogy, which was then popular in the United States and Western Europe. The pioneers of the postmodern approach in general pedagogy in Poland were Tomasz Szkudlarek [6], Zbyszko Melosik [7] and Bogusław Śliwerski [8]. Szkudlarek brought from America a fresh breath of modern pedagogy, so different from the ossified and outdated pedagogy of real socialism. Following general pedagogy, special education also adopted postmodern paradigms as the basis around which knowledge in the field of special education is built [9, 10]. In parallel to special pedagogy understood in the spirit of postmodernism, even since the times of communism, personalist special pedagogy, referring to the pedagogy of Kazimierz Twardowski [11] had been developing in Poland at the Catholic University of Lublin and at the Academy of Catholic Theology. It has been fully implemented in the typhlopedagogical center in Laski near Warsaw – the Róża Czacka Memorial School and Education Center for Blind Children. Despite the universality of Catholicism in Poland, personalistic special pedagogy did not have a major impact on the academic trend of pedagogy nor on general and special pedagogy in Poland, except for the Catholic University of Lublin, the Cardinal Stefan Wyszyński University in Warsaw (former Academy of Catholic Theology) and at non-public Catholic academic level schools. The postmodern understanding of the phenomenon of disability in Polish special education has taken the form of numerous studies conducted at the individual universities. However, they lacked a common iunctim. These efforts were only summed up and systematized by Amadeusz Krause, which allowed Polish postmodern special education to build its identity and a coherent conceptual network. Amadeusz Krause formulated the so-called humanistic paradigm of special education, consisting of four microparadigms [9, 12]. These are as follows:

- the social microparadigm of disability – denoting the social construction of the phenomenon of disability,
- the normalization microparadigm postulating the normalization of the social environment of a person with a disability, and not the forceful normalization of people with disabilities to the able-bodied majority,
- the emancipatory microparadigm suggesting the need for the emancipation of people with disabilities and a shift from an excessive focus on medical and therapeutic procedures to participation in social life,
- the qualitative microparadigm, i.e. an interpretative microparadigm postulating a departure from quantitative research (performed on large groups, using psychometric methods) to exclusively qualitative research [9, 12].

In particular, Melosik, Krause and Szkudlarek sharply criticize undertaking research in the field of special pedagogy in any medical context. They often refer to anti-psychiatrists [13, 14], postmodernists or thinkers such as Fritjow Capra [15], Eric Parens [16] or Petr Skrabanek [17]. Skrabanek, an Irish researcher of Czech origin, in particular, criticizes the obsessive fixation of modern man on super health, maximum extension of the life span, and the compulsion of citizens to achieve the ideals of good health. Skrabanek negates the legitimacy of the campaigns against tobacco, obesity, alcohol consumption, and for health promotion. This author noticed totalitarian tendencies in the pro-health policy, excessive interference of the state with human freedom [17, 18].

Krause and Melosik use the narrative of medicalization and pharmacologization of society, juxtaposing imperialism with modernist, Cartesian medical and postmodern discourse of flickering meanings. Unfortunately, it should be noted that negation of the biomedical aspects of disability does not solve the problem of people with various forms of disability, which are always caused by medical problems [19, 20]. Disability is a transactional concept that originated at the interface of medical sciences and social sciences [21, 22]. The one-sided, postmodern narrative on disability blurs the real problems of people with various mental and behavioral problems, including intellectual disabilities [23]. Krause, Ćwirynkało and Żyta postulate de-medicalization and debiologization of intellectual disability. They point out that disability is not only a biological aspect, but above all a social and sociological one [9,12]. The key problems of people with disabilities are always medical problems and any attempt to negate this fact is, unfortunately, a distortion.

Beata Cytowska [24], Katarzyna Ćwirynkało [12], Władysław Dykcik [25], Zenon Gajdzica [26], Remigiusz Kijak [27], Czesław

Kossakowski [28], Barbara Marcinkowska [29], Jan Pańczyk [30], Janina Wyczesany [31], Agnieszka Żyta [12, 32], Teresa Żółkowska [33], Hanna Żuraw [34] and many others not mentioned in the paper have contributed to the development of the humanistic paradigm in work with people with intellectual disabilities. Amadeusz Krause summed up these tendencies by formulating paradigms of special education and comparing them with analogous paradigms in the pedagogy of the German-speaking area [9, 12]. He was not their creator, although he was the only one who skillfully summarized and described them. The shift of special pedagogy towards postmodern demedicalization has certainly contributed to some extent to the social emancipation of people with intellectual disabilities and their exit from a certain ghetto, a reserve of public space. However, the question arises whether it was the position of special educators that favored such emancipation, or whether this emancipation took place spontaneously thanks to grassroots environmental initiatives and global tendencies. Or, possibly, Polish special pedagogy captured and codified these processes in the form of theoretical dissertations in the right way and at the right time.

However, there are many negatives of such an extreme, anti-empirical and anti-medical position of special educators that may be mentioned. The negation of the importance of biomedical aspects of disability and the almost complete lack of quantitative research have marginalized the importance of publications in the field of Polish special pedagogy in the global trend of disability research. Polish academic centers dealing with special pedagogy are not visible in the world's leading journals in the field of education with a high impact factor. The publications of Polish researchers are known only locally, among Polish academic staff. However, these publications do not exist in the global circulation of scientific information. This fact strongly marginalizes Polish special education, including special pedagogy of people with intellectual disabilities.

The fact that there is no intensive cooperation between special educators and psychologists in the field of intellectual disability research is also somewhat surprising. Polish psychology, with a psychometric and experimental attitude, does not fit into the integrative paradigm of special education [35]. Special pedagogy, focused on qualitative research, opposes psychometric research of intelligence as a method of population segregation, which was clearly described by Amadeusz Krause [10]. Nevertheless, psychometric measurement of intelligence and functioning of an individual is a basic research tool in the psychology of people with intellectual disabilities. In Poland, many psychometric tests to measure intelligence have been adopted, including the Wechsler test for adults and children [35]. An important contribution of Polish psychology to world psychology is the research on temperament [35]. It does not depend on the measurement process itself, but on the non-substantive intentions of the researchers whether the measurement in psychology serves to segregate and discriminate, or to determine resources. The metaphor of atomic energy can be cited here. Properly used, it is the most ecological and cheapest source of energy. If used badly, it can lead to the destruction of humanity in a nuclear conflict. The fact that someone can use psychometric measurement for socially unacceptable purposes does not mean that psychology should not be developed as a science. Psychology and pedagogy are to serve the human being, which is successfully implemented in Poland by psychological and pedagogical counseling centers. However, they are conducted by practicing psychologists and practicing pedagogues, at a certain distance from academic centers dealing with special education.

A certain exception is the work of a psychologist Małgorzata Kościelska, considered by special educators as pioneering for this discipline [36, 37]. Research on intellectual disability is also conducted by psychologist Ewa Zasępa from the Academy of Special Education in Warsaw [38, 39]. At the junction of special pedagogy and psychology, in order to better cooperate, such a construct as psychopedagogy was created. However, it is neither precisely defined nor is it a universally recognized subdiscipline of any of the social sciences. Psychopedagogy is usually understood as the application of developmental psychology in pedagogy. In Poland, the so-called psychopedagogues are educated in pedagogical faculties at some universities. A diploma of a pedagogue with a specialization in psychopedagogy does not give any rights assigned to psychologists. The attempt to distinguish psychopedagogy as an interdisciplinary subdiscipline has failed to bring Polish special educators and psychologists closer together.

Polish psychology of people with intellectual disabilities has developed from the so-called defectological psychology, which was then a subdiscipline of general psychology [40, 41]. From the very beginning, the term defect raised objections among Polish psychologists, because it is objectifying and stigmatizing when used in relation to a human being. Currently, the term revalidation psychology is used in psychology. Polish contemporary psychology has remained faithful to the tradition of psychological experiments and psychometric research.

From the beginning of the political change, Polish psychiatry has strived to appear on the global publishing market. Polish psychiatrists have published in English-language journals with the Impact Factor, and its achievements are known in the global circulation of scientific information. An example can be the publications of Janusz Rybakowski or Stanisław Pużyński. Two Polish journals: Polish Psychiatry led by Dominika Dudek and Advances in Psychiatry and Neurology led by Łukasz Mokros have long had an Impact Factor, which cannot be said about any Polish journals in the field of special pedagogy. Therefore, the achievements of Polish psychiatry have long been present in the global circulation of scientific information, in contrast to the regional character of Polish special education.

Polish psychiatry, on the other hand, has followed a similar path (as special pedagogy) in terms of relating to people with intellectual disabilities from stigmatization to full integration and inclusion. A textbook of psychiatry published in 1960, edited by the distinguished researcher and physician Tadeusz Bilikiewicz, used such terms as imbecility, moronism, idiocy, cretinism and amentia [42, 43]. These terms have now become

vulgarisms due to their stigmatizing aspect, and using them as designators for people with intellectual disabilities is considered an affront to human dignity. The clinical picture of people with intellectual disabilities presented in Bilikiewicz's textbook was negative, the authors warned against the unpredictability, ingratitude, aggressiveness and danger of people with low intelligence quotient [42,43]. These people were portrayed as mean, debauched and ungrateful. In the 1970s, a more neutral term "mental retardation" was used, included, among others, in the ICD-10 classification [44]. This term, initially neutral, has become slightly stigmatizing and unacceptable to the community of people with intellectual disabilities itself. In the second decade of the 21st century, many people began to use the term intellectual disability in psychiatry, which was largely due to the monograph on intellectual disability published by the Continuo publishing house edited by Kinga Bobińska, Tadeusz Pietras and Piotr Gałecki [45]. This book discusses the integration and social inclusion of people with disabilities in the model of community psychiatry. However, the chapters on pedagogy were far from the postmodern understanding of contemporary Polish special education, which met with some criticism from the pedagogical community. Despite these critical comments, that monograph is the only comprehensive study of issues related to intellectual disability present on the Polish publishing market [46]. It should be expected that the community of pedagogues would one day be tempted to write a new monograph in the spirit of postmodern inclusion, inviting psychologists and psychiatrists to cooperate. In the ICD-11 classification, the term intellectual disability is to be replaced by the term intellectual development disorder, which is currently considered to be much less stigmatizing. By analogy, the term will also become stigmatizing over time. The importance of research on intellectual disability for psychiatry is evidenced by the fact that the Scientific Section of Mental Disorders in Patients with Intellectual Disabilities was established in the Polish Psychiatric Association.

The anti-psychiatry movement has contributed to the development of the so-called community psychiatry. Its assumption is to treat mental disorders in the patient's social environment with the least possible participation of a total institution in the Gofmanian sense, such as a psychiatric hospital [47]. Long-term stay in a psychiatric hospital induces in the patient a syndrome of adaptation to a total institution. The hospital environment is significantly different from the natural social environment, so the social revalidation of patients in such an environment is a fiction. Moreover, many mental disorders have begun to be perceived not as disorders, but as differences in the development of the central nervous system and called neuroatypicality. A construct of a non-neurotypical minority against a neurotypical majority has been created. Neuroatypical people also include people with intellectual disabilities [48] together with people on the autism spectrum. Neuroatypical are considered to be people with a different organization of the nervous system, which results in behavior different from the general public. Such an unusual organization of the brain and thus cognitive, emotional and behavioral functioning results in such widely accepted phenomena as non-binary, non-heterosexual orientation, left-handedness, sensory and emotional hypersensitivity, outstanding abilities, and artistic talent. Thus, the stigma of mental disorders has been removed from intellectual disability as such, leaving it for psychotic or behavioral disorders accompanying intellectual disability [48]. Intellectual disability has therefore been equated with other behavioral phenomena typical of various minorities and to some extent it has been deprived of the attribute of a disorder.

It is difficult to identify the founders of community psychiatry in Poland who have contributed to the normalization of the environment of people with mental disorders. Community psychiatry has often developed from the bottom up thanks to the initiative of people with mental disorders and their families. Foundations, associations, pressure groups dealing with people with mental disorders and lobbying from the bottom up to change the social perception of psychiatric disability began to emerge. Among the many names of people meritorious for the development of community psychiatry, the following should certainly be mentioned: Jacek Wciórka, Andrzej Cechnicki, Joanna Meder, Maria Załuska, Marek Balicki, Anna Depukat, Beata Galińska-Skok, Joanna Krzyżanowska-Zbucka [49, 50]. We are aware that there are many more of such people and it is impossible to mention all of them in the paper. Among the non-governmental organizations meritorious for the development of community psychiatry are the Society of Friends of the Disabled in Łódź, the Polish Association for People with Intellectual Disabilities, the eFkropka Foundation, the Association for the Development of Community Psychiatry and Care, and many others. Contemporary psychiatry has constructed a conceptual system similar to that of Polish special pedagogy, with the difference that psychiatrists perform quantitative scientific research published in international journals, which cannot be said about Polish special educators [51]. The different and independent ways of development of the two fields in Poland are evidenced by certain terminological differences. For example, the term multiple disability used in pedagogy is replaced by the term co-morbidity in psychiatric literature.

Special educators are rarely invited to psychiatric conferences, just as there are usually no psychiatrists at pedagogical conferences. Psychiatrists such as Tadeusz Pietras and Krzysztof Krysta, who often participate in conferences organized by pedagogues, and a special educator and psychologist – Dorota Podgórska-Jachnik, involved in cooperation with psychiatrists, are a certain exception here.

LIMITATION OF THE STUDY

The study is limited by its narrative nature. On the other hand, an article discussing paradigmatic changes cannot be a quantitative meta-analysis using the PRISMA method [52, 53]. Key book publications devoted to these changes were selected to compare paradigmatic changes in Polish social psychiatry and special education. Choosing important publications and omitting less important ones is always a subjective choice. However, most of the co-authors of the publication are specialists in the field of special education and psychiatry and clinical psychology. All the

authors of this publication are present on the international publishing market. Therefore, we believe that our analysis of paradigmatic changes in both fields of knowledge reflects the real process of changing the way of thinking in Polish science. The change of paradigms in the context of the rapid progress of knowledge and the revolution in physics, biology and cultural anthropology also forces a change in the way of thinking in medicine and special education.

CONCLUSIONS

A comparison of the path from stigmatization and exclusion to integration and inclusion of Polish special pedagogy and Polish psychiatry enables us to draw the following conclusions:

Polish special pedagogy of people with intellectual disabilities has adopted the postmodern model of conduct and research formulated by Amadeusz Krause in the form of the humanistic paradigm of special education. In this model, qualitative research is preferred, hence the lack of representation of Polish special education of people with intellectual disabilities in the world literature.

Polish psychiatry has undergone a similar development path as special pedagogy, with the difference that care has been taken to publish the results of empirical research in international literature.

Minor differences are observed in the conceptual systems of pedagogy of people with intellectual disabilities and psychiatry.

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CASE STUDY

Painful swelling of the skull as the first symptom of multiple myeloma – a case report

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ABSTRACT

This article presents the case of a 53-year-old patient who reported to the neurology emergency room because of painful swelling of the left side of the forehead, temple and parietal region that had been persisting for 4 days. In the interview, the patient is under constant cardiology care due to pharmacologically treated hypertension, additionally diagnosed with nephrolithiasis and left intercostal neuralgia as a result of chronic cough that had been persisting for 2 months. Neurological physical examination revealed slight facial asymmetry, with the left eyeball set deeper and the zygomatic bone less prominent on the left side. Noteworthy was the swelling of the soft tissues of the left side of the head, covering half of the forehead, temple and parietal region, without involvement of the back of the head or the right side. The patient underwent an imaging examination of the head using computed tomography (CT). Due to ambiguous changes in the bones of the skull cap, an extension of the diagnostics was ordered with magnetic resonance imaging (MRI). Very numerous osteolytic foci were found, which enhanced after contrast administration, scattered throughout the skull bone, and at least one foci in the spine – in the apex of the dentary tooth. The most probable origin of the changes was indicated as multiple myeloma, for differential diagnosis with other metastatic changes. The patient underwent a hematological consultation, during which a bone marrow aspirate and protein tests were taken. The histopathological result revealed numerous plasma cells and a monoclonal protein peak (M). A date was set for the patient to be admitted to the Hematology Department for further treatment.

KEY WORDS: plasma cell myeloma, multiple myeloma, transient soft tissue swelling of the face and head

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INTRODUCTION

Multiple myeloma (MM) – ICD-10: C90.0 – together with plasmacytoma and Waldenström's macroglobulinemia belongs to the group of neoplasms derived from mature B lymphocytes [1]. The pathogenesis of MM consists of uncontrolled proliferation of plasma cells in the bone marrow with excessive production of monoclonal immunoglobulin [2]. In reference to WHO statistics based on Global Cancer Statistics 2020 – GLOBOCAN 2022, in relation to data for 36 cancers from 185 countries, plasma myeloma is responsible for nearly 190,000 new cases per year, which corresponds to 21 positions [3]. As part of this study, mortality statistics were also provided, in which MM was classified in 17th place, responsible for over 121,000 deaths per year. Thus, it accounted for 14% of all cases of lymphoma, leukemia and multiple myeloma [4]. According to statistics, the greatest increase in incidence is observed in people over 50 years of age, especially in men. Despite the persistent increase in the number of cases in recent years, a decrease in mortality has been noted [5]. The diagnosis of multiple myeloma is made based on the number of plasma cells in a bone marrow biopsy and the determination of the amount of M protein in serum and urine electrophoresis [2, 5]. Other important tests used to assess the condition of the skeleton include low-dose computed tomography (LDCT), positron emission tomography (PET) and magnetic resonance imaging [5-7]. The most frequently mentioned clinical and laboratory symptoms include those related to the characteristic damage of the end organs under the acronym CRAB – hypercalcemia, renal failure, anemia and bone damage [5]. The most common causes of neurological symptoms include motor and sensory disorders of the spinal and cranial nerves resulting indirectly from pathological fractures or directly from compression by the tumor [2, 5, 8]. The prognosis depends on numerous individual factors, including the stage of the disease at the time of diagnosis, the severity of symptoms, the effectiveness of the treatment and individual factors such as age, sex or genetic factors [5-7].

CASE REPORT

The patient is a 53-year-old man with no previous history of neurological diseases. Due to the occurrence of a severe headache and painful swelling of the left side of the forehead, temple and left parietal region lasting for 4 days, he reported to the neurological emergency room of the local hospital. The patient had been under constant cardiology care, pharmacologically treated for hypertension. However, according to a verbal message, he has not been taking prescribed medications recently. The history revealed the presence of nephrolithiasis and left-sided intercostal neuralgia caused by chronic persistent cough for two months. The patient had no family history of neurological diseases, did not report any other past illness, denied allergies and stimulants, and potential causes of pain such as trauma, infection or insect bites. The subject neurological examination revealed a slight asymmetry in the facial area, with the left eyeball set deeper and the zygomatic bone less prominent on the left side. The soft tissue swelling of the left side of the head was noticeable, covering half of the forehead, temple and parietal region, without involvement of the back of the head or the right side. No other abnormalities were found. The patient underwent an imaging examination of the head using computed tomography (CT) (Fig. 1b and 2c). According to the description, the image showed thickening of the soft tissue of the left frontal-parietal region up to 6 mm, particularly intense in the left temporal region up to 11 mm, without focal changes. The changes in the bones of the skull cap were ambiguous: the first suggested changes were dilated venous channels of the lacuna/venous extrusion type. Due to the non-diagnostic image, extended diagnostics were ordered and the patient was referred to the Neurology Department. A blood test package was performed, including: morphology, APTT clotting times, PT (INR), D-dimer level, plasma albumin concentration, C-reactive protein (CRP), glucose level, creatinine level, and determination of sodium and potassium concentration in plasma. Abnormalities in laboratory tests were noted: slightly reduced haemoglobin level (HGB) – 13.5 g/dl and mean corpuscular haemoglobin weight (MCH) – 26.5 pg. Additionally, increased D-dimer level (0.54 µg/ml), increased C-reactive protein (CRP) values – 61.06 mg/L, and decreased creatinine concentration (0.59 mg/dl) (Table 1). The patient underwent a general urine test – the result was without any deviations from the norm.

Due to ambiguous changes in the skull cap bones, an extension of the diagnostics was ordered to include a Magnetic Resonance Imaging (MRI) scan. It revealed numerous osteolytic foci scattered throughout the skull bones, which enhanced after contrast administration, and at least one foci in the spine – at the apex of the dentary (Fig. 1-3). Swelling and thickening of the subcutaneous connective tissue of the left frontal-parietal region were observed – an image consistent with that seen in the CT scan performed during the patient's stay at the Neurological Emergency Department (Fig. 2a, 2b, 3a, 3b). No other abnormalities were found.

Due to the features of infection and the elevated CRP index, empirical antibiotic therapy with a third-generation cephalosporin (ceftriaxone) was initiated at a dose of 2 g, once daily, intravenously. A haematological consultation was ordered for the following day. Due to the maintenance of optimal blood pressure values and the patient not taking any prescribed medications recently, pharmacotherapy for hypertension was not introduced. The patient underwent a haematological consultation, during which a bone marrow aspirate, protein tests and a urine Bence-Jones protein test were performed. The biopsy revealed numerous plasma cells in the bone marrow and a monoclonal protein peak (M) in the serum proteinogram. No Bence-Jones protein was detected in the urine. The final diagnosis was multiple myeloma – ICD-10 – C90.0. On the fifth day of stay, based on the internal medicine consultation, the disappearance of facial swelling and the results of control laboratory tests: a decrease in CRP concentration (25.68 mg/l), negative procalcitonin and no symptoms of infection, antibiotic therapy was discontinued. A referral to the Hematology Department was issued within the established deadline. After 5 days of hospitalization, the patient was discharged home in a general good condition, neurological – without significant deviations from the norm.

DISCUSSION

In relation to scientific reports and the present case, special attention is paid to the oncological vigilance of medical personnel. The first symptoms of multiple myeloma, especially in the case of the oligosecretory variety, may be nonspecific

Table. 1 Presents abnormal values of the results obtained in blood laboratory tests, along with the range of reference values used

Parameter in a blood laboratory test with an incorrect result obtained	The result obtained	Reference Value Range
HGB	13.5 g/dl	min: 14 max: 18 (g/dl)
MCH	26.5 pg	min: 27 max: 34 (pg)
D-dimers	0.54 µg/ml	min: 0 max: 0.5 (µg/ml)
CRP	61.06 mg/L	min: 0 max: 5 (mg/L)
Serum creatinine concentration	0.59 mg/dl	min: 0.7 max: 1.2 (mg/dl)

HGB – hemoglobin; MCH – mean corpuscular hemoglobin weight; CRP – C-reactive protein

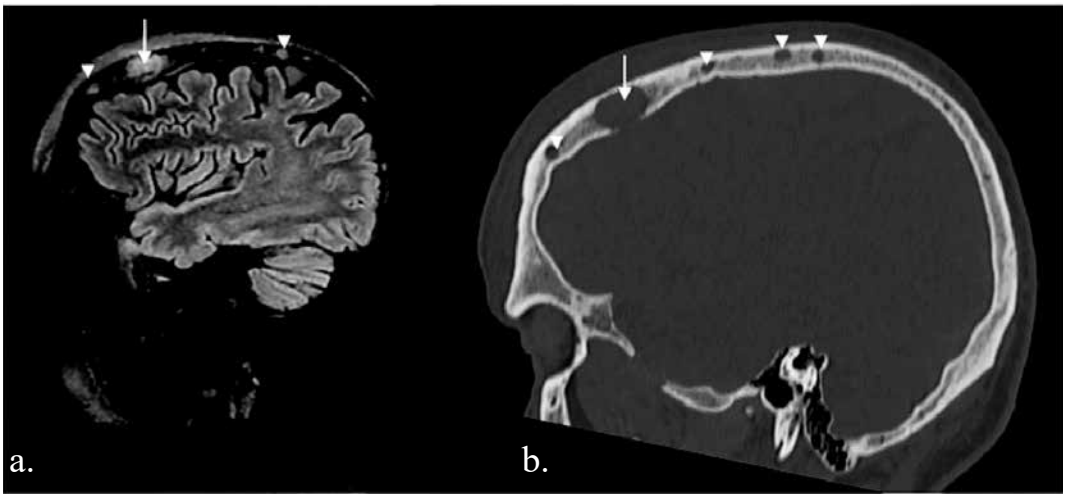

Fig. 1. Osteolytic changes of the skull cap - single dominant in the left frontal bone with destruction of the inner and outer plates (white arrows) and smaller changes in the vicinity (arrowheads). 1a. MRI T2 FLAIR images in the sagittal plane 1b. sagittal CT reconstruction in the bone window.

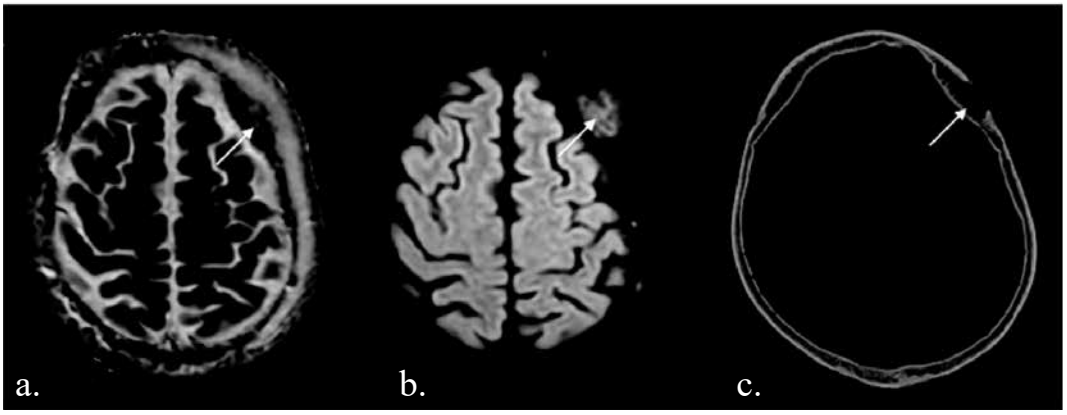

Fig. 2 Osteolytic lesion of the frontal bone on the left side (white arrow) **2a** and **2b** MRI ADC maps and DWI images showing restriction of extracellular water particles with infiltration and edema of the subcutaneous tissue **2c.** CT osteolytic lesion destroying the external plate of the frontal bone.

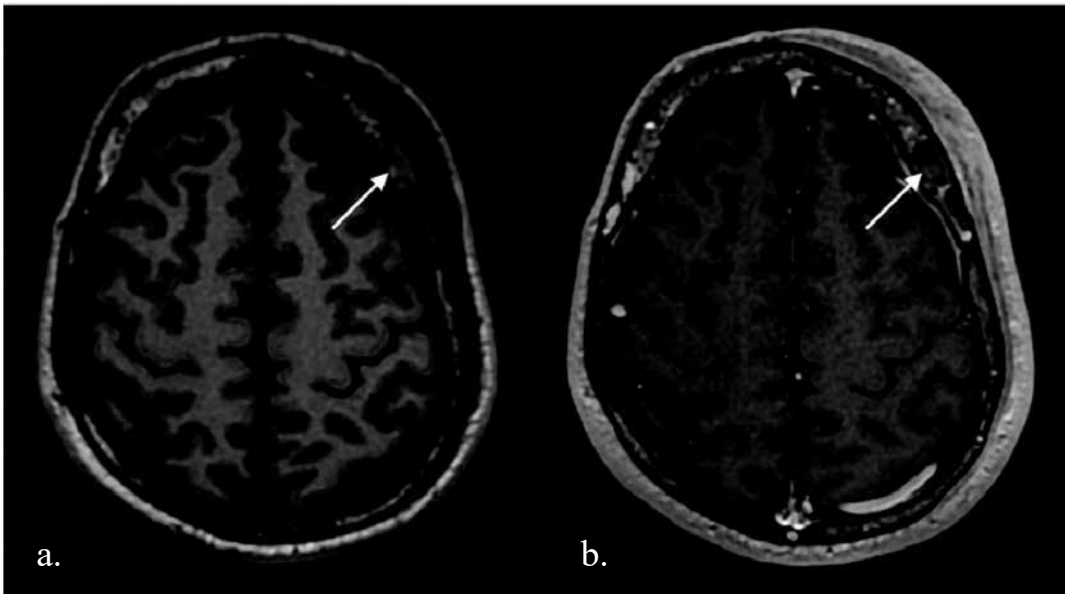

Fig. 3 MRI T1-weighted images **3a** before and **3b** after intravenous contrast agent administration – isointense osteolytic lesion with weak enhancement after contrast agent administration (white arrow). Subcutaneous tissue edema.

symptoms of a neurological nature [6, 9]. These include those related to the peripheral nervous system, most often resulting from compression and impairment of nerve function indirectly due to pathological fractures or as a result of direct impact of the tumor mass in the case of plasmacytoma. These changes may also cause impairment of cranial nerve function [8,10-12]. In the case described above, no such symptoms were observed. The first symptom that the patient noticed was headache and swelling of the soft tissues of the head. No description of a similar case was found in the available literature.

Varieties of multiple myeloma include plasma cell leukemia, plasmacytoma – an intra- or extramedullary tumor, and the classic form [2, 13]. In relation to the above, in the case of diagnostic imaging studies, non-contrast computed tomography usually shows osteolytic changes, often with concomitant fractures. The test result may also suggest spinal canal involvement and stenosis, but in this case MRI remains the test of choice, which allows for precise determination of myelopathy and compression [2, 14]. Raymond et al. also noted the occurrence of neurological symptoms in some patients as the first symptoms of leukemia, lymphoma, or multiple myeloma [15]. Based on the conducted systematic review, they distinguished hearing loss, otalgia, and facial nerve palsy [15]. In connection with the occurrence of hypoproteinemia due to multiple myeloma, attention is also drawn to the occurrence of hyperviscosity syndrome, which may be a direct life-threatening condition. The triad of the syndrome, in addition to mucosal hemorrhages, includes visual disturbances and neurological symptoms [16].

In the context of considering the course of multiple myeloma in relation to neurological symptoms, side effects of the treatment are also indicated. In the case of autologous hematopoietic stem cell transplantation (autoHSCT), numerous neuropsychiatric symptoms are observed depending on the time of its performance. The occurrence of pain of various characteristics, nausea, sleep disorders and emotional disorders is indicated [17]. Attention is also drawn to physical abnormalities, such as weakened vision, limited or loss of taste, as well as limb tremors [17]. In patients undergoing autoHSCT, its potential relationship with statistically more frequent autoimmune encephalitis is also indicated, including with antibodies directed against membrane receptors binding γ-aminobutyric acid, type A (GABA-A) [18]. Neurotoxicity as one of the side effects is also attributed to biological treatment, including the use of Talquetamab. According to the results of the study by Chari et al., it may constitute up to 10% of all adverse events [19]. Depending on the treatment regimen and dosage, individual cases of anosmia, confusion, aphasia and encephalopathy have also been reported [19].

CONCLUSIONS

Multiple myeloma is a disease that can often present with nonspecific symptoms. Its development in the initial stages can be asymptomatic and have a nonspecific course. For this reason, this case report, together with the reports presented, suggests an important role for oncological vigilance of physicians and all medical personnel. It also indicates that nonspecific neurological symptoms, such as headache in the described patient, as well as other general symptoms, such as swelling, may be the first symptoms of multiple myeloma, as well as other hematological diseases.

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The main aim of the conference is to present trends and results of the latest scientific research in the field of complementary disciplines of physiotherapy, emphasizing their practical dimension/application in strengthening the professional competences of physiotherapists. We cordially invite representatives of medical, social, natural, technical and other sciences who, through their activities, can participate in the comprehensive rehabilitation process and strengthen patients' functional reserves.

Conference Topics:

- Biological sciences in the development of physiotherapy
- Social activation of people with disabilities
- Animal-assisted therapy and physiotherapy of animals
- Psychotherapy and occupational therapy in physiotherapy
- Professional profile of a physiotherapist – professional profile vs. social expectations
- Technical/engineering support in physiotherapy
- Varia – other topics related to new challenges of physiotherapy in clinical medicine of humans and animals

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The conference will include the following workshops:

- Low Pressure Fitness (a form of core muscle training using specific breathing techniques);
- Augmentative and Alternative Communication (AAC).

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The organizer will provide all registered participants with conference materials, the opportunity to take part in workshops, two lunches and coffee breaks; additionally for active participants (speech, poster): dinner on 22nd May 2025, overnight stay on 22nd-23rd May 2025, breakfast on 23rd May 2025.

Research and review works will be presented in Polish during the thematic sessions and the poster session. The poster session, addressed to young scientists (students and PhD students), will include a competition with prizes – the award for the best work.

The summary written in Polish should not exceed 150-250 words. In the case of research works, the summary should include the following sections: aim of the research, material and methods, results, conclusions and keywords. In the case of review works: description and keywords.

Registration* with an abstract should be sent electronically by 11th April 2025. The Scientific Committee will qualify the works for presentations at the Conference.

It is planned to prepare a reviewed post-conference monograph in 2025 published by the Publishing House of John Paul II University in Biała Podlaska. The deadline for submitting works for review, as well as the guidelines related to the layout and formatting of the works will be provided in the 2nd Announcement.

The publication fee of 100.00 PLN (applies only to active participants who will submit their work as a chapter in a post-conference monograph) must be paid after the work has been approved for printing (no later than 14 days from the date of receiving the decision).

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Registration card

**Limited number of participants. We will inform you about your qualification via e-mail*

Conference office
John Paul II University in Biała Podlaska – Department of Physiotherapy
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2nd National Scientific Conference

"COMPLEMENTARY DISCIPLINES OF PHYSIOTHERAPY"

22nd - 23rd May 2025

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