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CORRELATION BETWEEN LYMPHOCYTE-MONOCYTE RATIO AND CYTOKINES IN CHRONIC INFLAMMATION IN RATS TREATED WITH ALLOGENEIC MESENCHYMAL STEM CELLS

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Abstract

A chronic inflammatory process is a pathological condition characterized by an ongoing active inflammatory response and tissue destruction. Many studies show that chronic inflammation can play a severe role in various age-related diseases, including diabetes, cardiovascular, and autoimmune diseases. One of the important but poorly studied factors affecting the regulation of chronic inflammation is regulatory activity of MSCs. In this regard, the study of mesenchymal stem cells preventing chronic inflammation in the experiment is an important area of modern pathology.

On the one hand, increased cytokines, such as α-TNF, IL 6, and CRP, are reliable tools in diagnosis of different inflammatory processes, especially chronic inflammation. On the other hand, we need a more straightforward and not so expensive criterion for this purpose, for instance, a common total blood count and LMR. For the first time, we investigated how trustworthy can be LMR and how possible to use it in chronic inflammation in rats to achieve prognostic goals.

This study investigated the correlation between α-TNF, IL-6, and CRP with LMR in rats' plasma in groups with chronic carrageenan inflammation and chronic inflammation with local injection of MSCs into the affected area. The study involved 132 adult male rats (180–220 g), which were divided into groups. The inflammation model was chronic aseptic myositis caused by an intramuscular injection of 10 mg λ-carrageenan (Sigma-Aldrich GmbH). Our experimental groups of rats were treated with MSCs (the injection into the inflamed site) in the amount of 1–2 million cells once. Blood sampling was performed from 6 hours to 28 days. We calculated our results using Statistica (data analysis software) version 13. For comparison, we used one-way ANOVA, Turkey's post hoc test, where $p < 0.05$ was considered statistically significant.

In our experiment, the correlation between levels of α-TNF, IL-6, and CRP with lymphocyte-monocyte ratio in rats was described for the first time, demonstrating the suppression of chronic inflammation through MSCs.

Keywords: *chronic inflammation, mesenchymal stem cells, lymphocyte-monocyte rate, tumor necrosis factor-alpha; interleukin 6; C-reactive protein.*

Abbreviations:

MSCs – mesenchymal stem cells;
Car – λ-carrageenan;
WBC – white blood cells count;
LMR – lymphocyte-monocyte ratio;
α-TNF – tumor necrosis factor alpha;
IL-6 – interleukin 6;
CRP – C-reactive protein.

1. Introduction

A chronic inflammatory process is a pathological condition characterized by an ongoing active inflammatory response and tissue destruction. A significant number of immune cells, including macrophages, neutrophils, and eosinophils, are involved directly or through the production of inflammatory cytokines in the pathogenesis of chronic inflammation [1].

It is well known from the literature that there is a general concept according to which chronic inflammation is the leading cause of cancer and aging processes [2]. Moreover, many studies show that chronic inflammation can play a role in various age-related diseases, including diabetes, cardiovascular, and autoimmune diseases [3]. One of the important but poorly studied factors affecting the regulation of chronic inflammation is the regulatory activity of MSCs.

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Human MSCs are individual progenitor cells that can be found in most vascularized body tissues. These cells have differentiation potential and are characterized by immunomodulatory and trophic activity [4].

At first, this was met with great skepticism, then the immunomodulatory ability of mesenchymal stem cells was proven and well reproduced in experiments [5, 6] and opened up the possibility of using mesenchymal stem cells for tissue replacement and regeneration and the treatment of immune-mediated and inflammatory diseases [7]. Thus, it was found that the implication of mesenchymal stem cells in the treatment of inflammatory diseases could give the most significant effect [8].

It is the fact that there are many works covering regenerative properties of mesenchymal stem cells [9–17], there are very few studies dedicated to the pathogenetic effect of mesenchymal stem cells on the processes of chronic inflammation [18, 19].

In recent years, there has been a tendency towards an increase in type 2 diabetes, obesity [20], cancer [21, 22], which are a consequence of chronic inflammation and lead to early mortality and disability.

In this regard, the study of mesenchymal stem cells preventing chronic inflammation in the experiment is an important area of modern pathology.

It is well known that increased cytokines, such as α-TNF, IL 6, and CRP, are reliable tools in diagnosis of different inflammatory processes, especially chronic inflammation [23]. (*Fig. 1*) Still, on the other hand, we need a more straightforward and not so expensive criterion for this purpose, for instance, a common total blood count and LMR

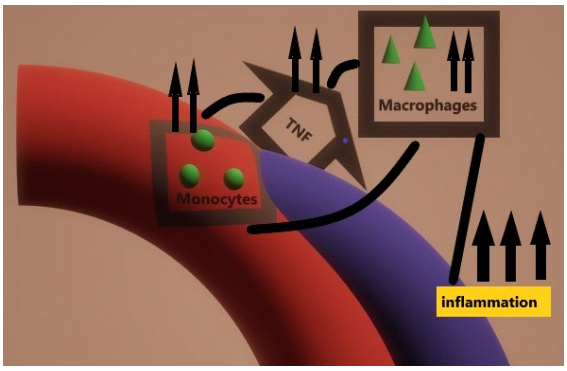

Fig. 1. Scheme of the relationship between TNF, monocytes, and macrophages. The positive feedback of TNF on monocytes for their functioning and then transforming into macrophages, which leads to enhancing the course of chronic inflammation

[24]. LMR is an important prognostic marker of endothelial dysfunction and inflammation. Low LMR correlates with worsening recovery and is probably a prognostic criterion for developing diseases associated with chronic inflammation.

In our study, we investigated how trustworthy can be LMR, and if it is possible to use it in chronic inflammation in rats to achieve prognostic goals.

2. Purposes, subjects and methods:

2.1. Purpose

This study investigated the correlation between α-TNF, IL-6, and CRP with LMR in rats' plasma in groups with chronic carrageenan inflammation and chronic inflammation with local injection of MSCs into the affected area.

2.2. Subjects & Methods

The study involved 132 adult male rats (180–220g), which were divided into groups. The inflammation model was chronic aseptic myositis caused by intramuscular injection of 10mg λ-carrageenan (Sigma-Aldrich GmbH) into the right hip [25].

The studies were carried out under the national "General Ethical Principles for Animal Research" (Ukraine, 2001) [26] (Strasbourg, 18.03.1986 p.), the Declaration of Helsinki, (1964–2000), the charter of the Ukrainian Association for Bioethics and GLP (1992) and used the minimum acceptable for statistical processing and obtaining reliable results, the current number of animals (6 per group). The animals were sacrificed with inhalation of high concentrations of carbon dioxide (CO2), followed by decapitation.

Isolations of MSCs

We isolated MSCs from the rat femur bone marrow using the standard method [27–30]. The bone marrow was withdrawn from the femur epiphyses then washed with Hanks solution (Biowest, France). The cells were centrifuged to pellet (1000 rpm, 10 min). Mononuclear cells (*Fig. 2*)

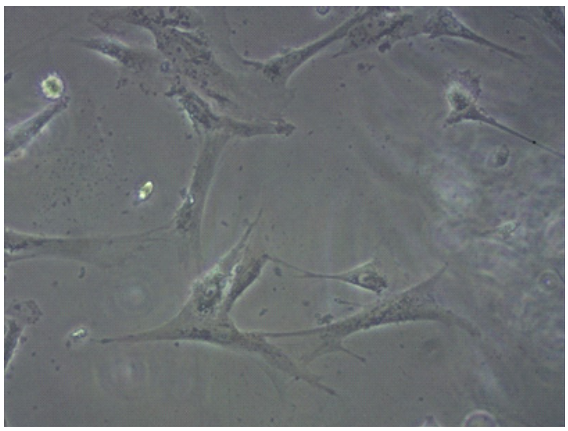

Fig. 2. MSCs of the femoral bone marrow of male rats

were obtained by centrifugation in a Ficoll-Hypaque gradient (density 1.077 g / ml) (Sigma, USA) at 400 g for 25 min and washed twice with Hanks solution (Biowest, France). After that, the cells were resuspended in physiological saline at a concentration of 1.0×106 in 1 ml [31, 32]. We measured the total number of cells with a cytometer by staining with 0.2% trypan blue solution (Janssen Chemica, Belgium). The structure of MSCs was investigated using a phase-contrast microscope; we studied cell cycles by flow cytometry. The immunocytochemical method studied MSCs phenotype.

Primary cultured MSCs were oval, fusiform, or polygonal, adhered to a plastic surface within 24 hours, and reached 90% confluence within eight days. After cleaning and breeding, they were equally long, spindle-shaped, and transmitted every five days. The adhesion rate was complete within 24 hours. Flow cytometry showed that 80% of fourth-generation MSC cells were in the G0 phase. Immunocytochemical analysis showed that MSCs were positive for CD29, CD105, CD166, VLA-4, and P-selectin, but negative for CD34 and CD45.

Experiment and blood collection in rats

In the experiment, sixty rats had edema of the right thigh due to the intramuscular injection of λ-carrageenan. The other sixty rats were simultaneously injected not only with carrageenan but also with a suspension of MSCs. The control group consisted of six intact rats without intervention and six rats that were injected with MSCs without inflammation.

The quantity of MSCs was 2 million cells in 0.4 ml per animal. There were ten terms in the experiment. For each assignment, we analyzed six rats with inflammation of carrageenan and six rats with inflammation plus MSCs. Animals were sacrificed under anesthesia after 6 hours on days 1, 2, 3, 5, 7, 10, 14, 21, 28. Blood samples were obtained by cardiac puncture. The blood smears were performed immediately (*Fig. 3*), and some blood was collected to the sterile tubes containing an anticoagulant (EDTA) for total blood count. Empty sterile tubes were used for plasma preparation. A blood clot appeared in 25–30 min, then the tubes were placed in a centrifuge and processed at 3000 rpm for 10 min. Plasma was obtained and sent to the freezer (-20 °C).

Determination of α-TNF, IL-6, and CRP

Plasma α-TNF, IL-6, and CRP levels were measured using an enzyme-linked immunosorbent assay kit (Sigma-Aldrich GmbH) for quantitative measurement of target markers in biological fluids. We used ELISA for rat TNF-?, ELISA for rat IL-6, ELISA for rat CRP (C-reactive protein).

The lymphocytes monocytes ratio

LMR in rats was calculated in the same way as in humans [33–40]. We took the absolute number of lymphocytes and divided them by the complete number of monocytes. As a result, we found a positive trend of LMR increasing. On day 21, LMR was significantly higher in the group of animals with chronic inflammation and MSCs.

Statistics

All calculations were performed using Statistica (data analysis software) version 13. For comparison, we used one-way ANOVA, Turkey's test, where $p < 0.05$ was considered statistically significant.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results & Discussion

Elevated levels of proinflammatory cytokines accompany the majority of chronic inflammatory conditions. There are several therapeutic options for lowering these levels. These include monoclonal antibodies and cytokine receptor blockers, immunosuppressants, and non-steroidal anti-inflammatory drugs. None of these drugs are entirely safe or effective. Consequently, there is still a need to develop new approaches that can

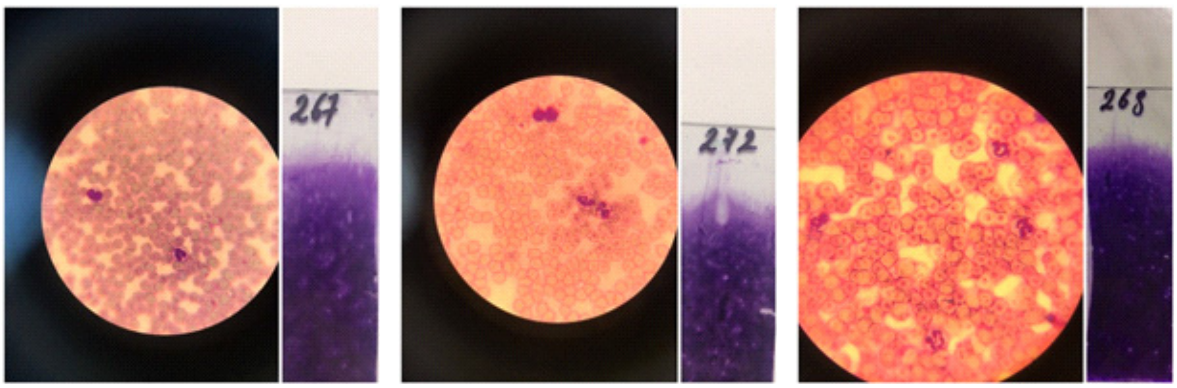

Fig. 3. Peripheral blood smears of rats

target other pathogenetic mechanisms. MSCs may be one such approach for decreasing the production of cytokines.

The main objective of our study showed that the introduction of bone marrow MSCs in the area of chronic inflammation led to a significant decrease in proinflammatory cytokines, such as IL-6, TNFα, and CRP, in the plasma of animals of the inflammatory group plus MSCs (*Fig. 5–7*).

recovers on the 5th day. Since the 7th day, it was always superior during the next days with a peak on the 21st day (*Fig. 4*)

The immunosuppressive activity of MSCs can explain these results. MSCs can support many types of immune cells, including B cells, T cells, dendritic cells (DC), natural killer cells (NK), neutrophils, and macrophages [41]. Interaction mechanisms are based on cell-cell contact,

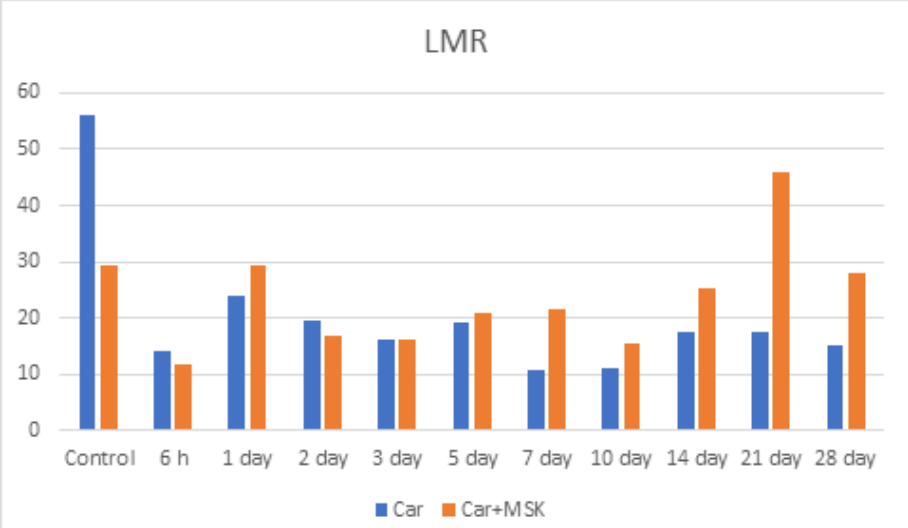

Fig. 4. Lymphocyte-monocyte ratio. The red line is the level of LMR in groups with inflammation treated with MSCs. Blue line – lymphocyte-monocyte rate in the usual course of inflammation

This decrease was statistically significant. LMR was significantly higher in animals with chronic inflammation and MSCs on the 1st day, then

working in conjunction with the secretion of soluble immune factors to induce MSC-regulated immunosuppression [42]. These specific modulators

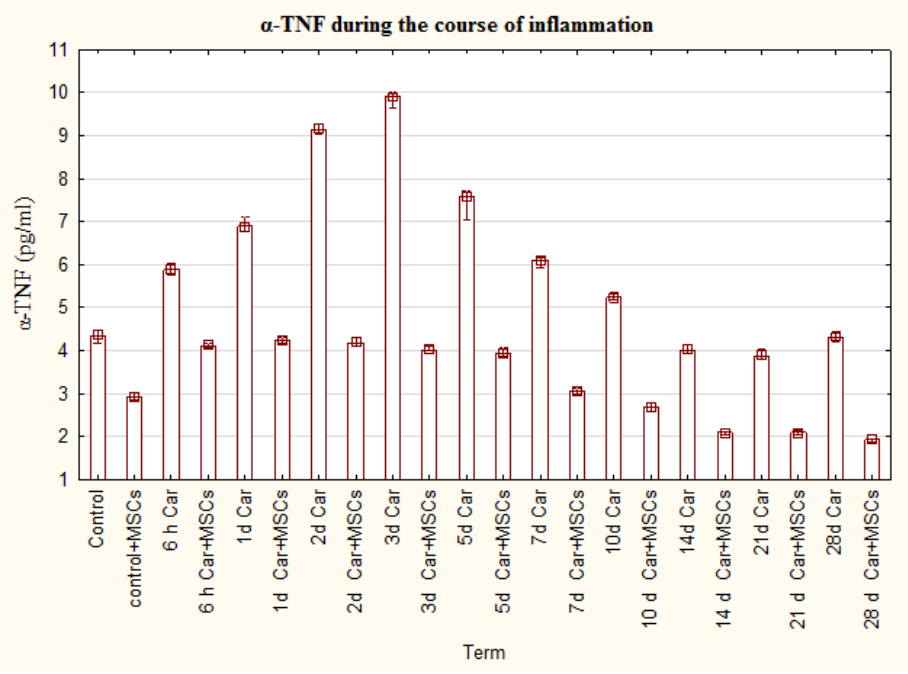

Fig. 5. The levels of α-TNF (natural course of inflammation and inflammation treated with MSCs)

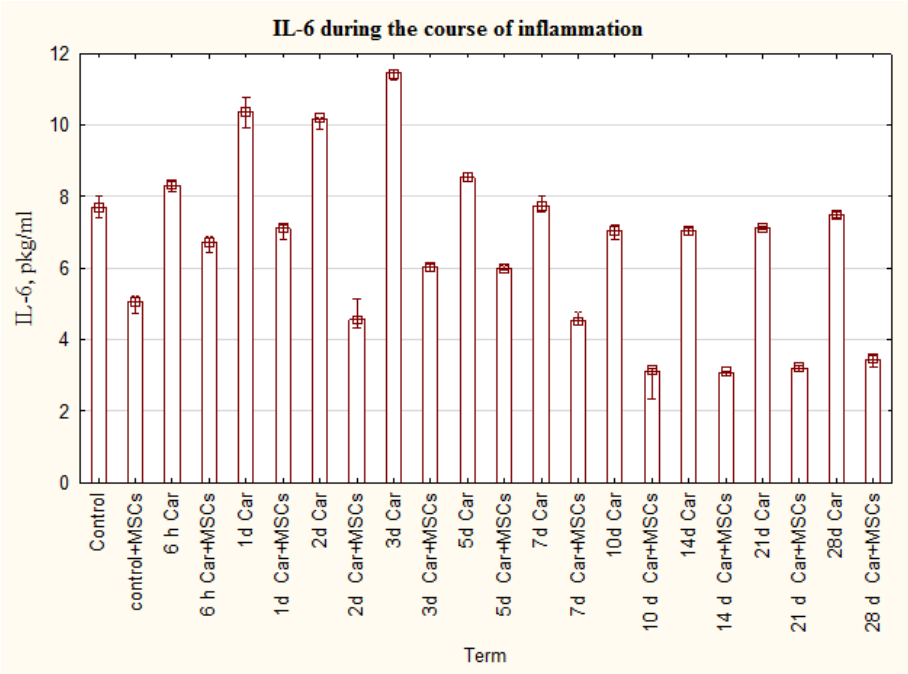

Fig. 6. The levels of IL-6 (natural course of inflammation and inflammation treated with MSCs)

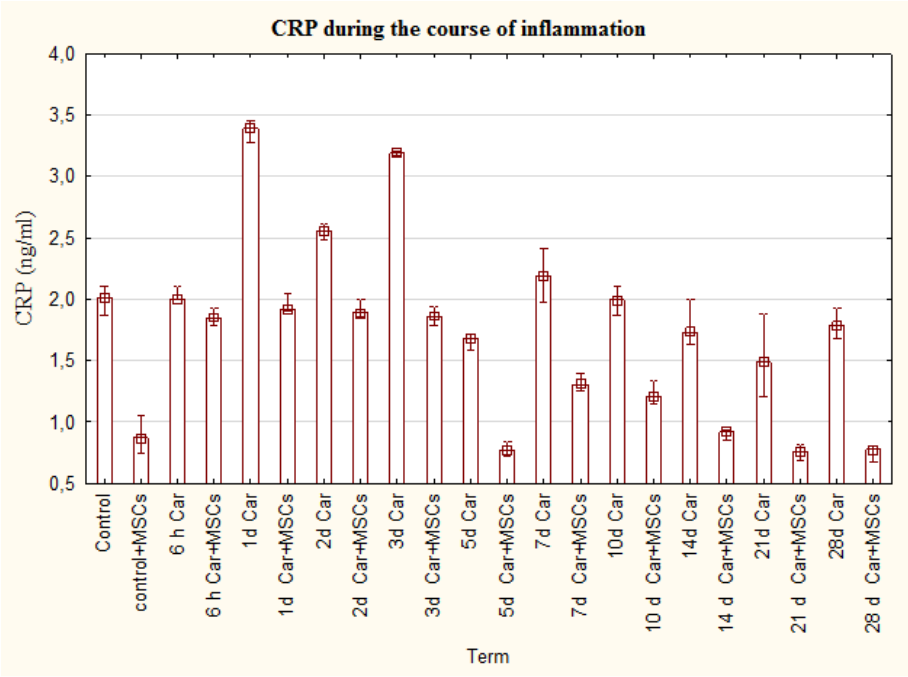

Fig. 7. The levels of CRP (natural course of inflammation and inflammation treated with MSCs)

include immunomodulatory factors, cytokines, growth factors, modulate inflammatory responses, and immune balance profiles. MSCs can also regulate the inflammatory process and repair damaged cells and tissues by attaching to the inflammation area [43]. The integration of MSCs with inflammatory processes enhances and suppress the immune response and depends on the general state of the immune system [44]. Surprisingly, MSCs modulate immunosuppression only when they are initially stimulated by inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin- (IL-) 1 [45]. MSCs respond to inflammatory cytokines and produce immunoregulatory secretors that mediate the process of inflammation [46, 47].

It is crucial to admit that, even though we used allogeneic bone marrow MSCs, there was a significant decrease in the cytokines in the plasma of animals in the control group plus MSCs compared to the control group. Thus, this can be explained by the immunomodulatory ability of MSCs [48]. Such a significant decrease in proinflammatory cytokines may indicate the non-immunogenic properties of allogeneic MSCs. This fact may be necessary in cases where it is impossible to obtain autologous MSCs.

Even though the understanding of the mechanisms of immunomodulation based on MSCs remains incomplete, the growing volume of data prompts further studies of the properties of MSCs and their practical application. We believe that our research could help develop pathogenetic treatments for chronic inflammatory and autoimmune diseases that do not have side effects.

Conclusions

Our study shows that MSCs are able to mitigate severity of the inflammatory response. MSKs also showed good immunomodulatory properties. The LMR can be reliably used as an isolated immunological measurement in chronic inflammation or along with IL-6, α-TNF, highly sensitive C-reactive protein. It can be reliable to use LMR in the course of chronic inflammation in rats to achieve prognostic goals.

There is an excellent potential for further research into preventing chronic inflammation using MSCs, including clinical investigations. We see a great potential in the future study of stem cells as immunomodulatory and anti-inflammatory agents for increasing the quality and longevity of human life.

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BEHAVIORAL REACTIONS AND COGNITIVE FUNCTIONS IN RATS WITH VASCULAR MODEL OF ALZHEIMER'S TYPE DEMENTIA AT DIFFERENT STAGES OF DISEASE BEFORE AND AFTER STEM CELL CORRECTION

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Abstract

Background. The recent investigations offer to conduct the study of Alzheimer disease (AD) mechanisms using diverse experimental models. However, behavioral and cognitive impairment in rats at the different stages of vascular model of dementia of Alzheimer's type developed by us has not been investigated. **Subjects and methods.** The experiment was performed on 32 male WAG rats weighing 180–250 g which were divided into 4 groups. Rats from group 1 and 3 were injected aqueous solution of sodium nitrite at a dose of 50 mg/kg of body mass intraperitoneally during 14 and 28 days respectively. Groups 2 and 4 received 500,000 mesenchymal stem cells in suspension intravenously against a background of experimental nitrite-induced AD. To estimate the behavioral reactions and cognitive functions the Open Field Test (OFT) and Passive Avoidance test (PAT) were used. **Results.** In all experimental groups in most cases significant decrease in vertical and horizontal activity ($p < 0.05$) and an increase in the number of defecation in the OFT were found. Rats from group 3 had the drop in locomotor, research and orientation activity. In the OFT and PAT in groups 2, 4 improvement in research activity and significant cognitive functions recovery was observed ($p=0.012$) after stem cell correction. **Conclusion.** Progression of the protective inhibition and cognitive impairment was found during the experiment. Stem cell administration had positive effects on brain function recovery.

Key words: *Alzheimer disease, sodium nitrite, stem cells, cognition, behavior, brain, rats.*

Introduction

According to the World Health Organization (2019) around 50 million people have dementia worldwide with nearly 10 million new cases every year. Alzheimer disease (AD) is the most common form of dementia and may contribute to 60–70% of cases [1]. Incremental increase of dementia of Alzheimer's type leads to researching the mechanisms of the disease onset and progression. The main amyloid cascade hypothesis involves the excessive production of amyloid plaques with their subsequent accumulation and future nerve cells apoptosis [2]. It is obvious that this theory cannot alone exactly elaborate the all steps of neurodegeneration. The scientists propose other supplemental pathways of progressive neurons injury and loss. It is well known that oxidative stress, neuroinflammation cause endothelial dysfunction that could play the crucial role in development neurodegeneration [3].

The recent investigations offer to conduct the study of AD mechanisms using diverse experimental models. There are plenty of transgenic and non-transgenic animal AD models in vivo and in vitro tissue, cell, molecular simulation models [4]. One of the most common psycho-pharmacological model of Alzheimer's type dementia is induced by scopolamine. It was published that the activity of choline acetyl-transferase was dropped in the cortex of AD patients [5]. It was associated with brain lesions and clinical performance [6; 7]. The cholinergic

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hypothesis of AD was accepted and chronic administration of scopolamine during 28 days was allowed for further AD researching [8; 9].

We have developed another model of dementia of Alzheimer's type where the endothelial dysfunction triggered amyloid formation and cognitive impairment. This model caused by chronic administration of aqueous solution of sodium nitrite intraperitoneal at dose at 50 mg/kg of body mass during 2 weeks [10]. It was not investigated the behavioral and cognitive impairment at the different stages of disease and after stem cells injections.

2. Purpose, subjects and methods

2.1. Purpose

The aim of our study was to assess the changes of behavioral reactions and cognitive functions in rats with vascular model of Alzheimer's type dementia at the different stages of disease before and after stem cells administration.

2.2. Subjects & Methods

The experiment was performed on 32 male WAG rats weighing 180–250 g which were divided into 4 groups. Rats from group 1 (sodium nitrite 2 weeks, n=8) and group 3 (sodium nitrite 4 weeks, n=8) were injected aqueous solution of sodium nitrite at a dose of 50 mg/kg of body mass intraperitoneally during 14 and 28 days (2 and 4 weeks), respectively, resulted in the development of dementia of Alzheimer's type of vascular genesis. Group 2 (sodium nitrite 2 weeks + stem cells, n=8) and group 4 (sodium nitrite 4 weeks + stem cells, n=8) received 500,000 mesenchymal stem cells (MSCs) in suspension intravenously against a background of experimental nitrite-induced AD.

All institutional and national guidelines for the care and use of laboratory animals were strictly followed. The Ethics and Bioethics Commission of Kharkiv National Medical University (October 10, 2018, minutes of the meeting №8) confirmed that the design and manipulations during this experiment were compliant to bioethical requirements of EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of Europe Convection for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123).

Open Field test (OFT) was used to characterize behavioral reactions such as locomotion, anxiety, neophilia (ability to adapt rapidly to extreme change) and exploration. Our OFT apparatus had a circle area with holes into the walls and the field marked with a grid and square crossings. The main variables recorded during the next 300 sec were: crossings (number of times the line of a square is crossed with all 4 legs), head dipping (number of putting rat's head into the hole), rearings (number of times the animal stands on its hind legs), grooming (frequency of grooming activity), number of defecation [11–13]. The animals were tested in the beginning of experiment before sodium nitrite injections, just after sodium nitrite injections and before stem cells injections and in 2 weeks after sodium nitrite and stem cells injections

Cognitive functions were evaluated using Passive Avoidance Test (PAT). In PAT formation of the conditioned reflex was fixed during 180 sec. If animal crossed from the light to the dark compartment with mild foot shock next day after training, the passive avoidance response or conditioned reflex was not formed (0). If rat avoids the entry to the dark compartment and stays at the light compartment the passive avoidance response is formed (1) [14; 15].

Primary culture of MSCs was obtained from bone marrow cell suspension flushed out of rat femurs. The cells were washed in Hanks' balanced salt solution, centrifuged at 450g for 10 min and plated in 75 cm^2 culture flasks at a density of 4x105 cells/cm^2 in DMEM/F12 (1/1) containing 2mM L-glutamine, 10% FBS (SIGMA-ALDRICH, cat.n. F7524) and 2 µl/ml, Antibiotic Antimycotic Solution (SIGMA-ALDRICH, cat.n. A5955). The medium with nonadherent cells was discarded after 24 hours of the culture and fresh medium was added to the adherent fibroblast-like MS cells. They were cultured at 37?C and 5% CO2 in air in an CO2 - incubator for 14 days in the medium changed every 3 days. All reagents for culture were purchased from SIGMA-ALDRICH [16].

To evaluate the behavioral reaction changes inside each group during different periods (before sodium nitrite injections, just after sodium nitrite injections and before stem cells injections, 2 weeks after sodium nitrite injections) one-way ANOVA test was used. To estimate the stem cells efficiency on cognitive functions Pearson's chi-squared test was used. If p values were below 0.05, the difference was statistically significant. All numerical data were analyzed using IBM SSPS Statistics.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results & Discussion

Analyzing the behavioral reactions during one-way analysis of variance the Levene's test

showed a large dispersion of data with identical variation of samples in group 1 (significant point >0.05), which indicates a single adherence to the conditions of the experiment. Throughout the experiment, there was a significant decrease in the number of head dipping into the holes by almost 3 times, which indicates a decrease in research activity. A significant increase in the number of defecations by almost 3 times confirms an increase in anxiety in rats ($p<0.05$). An insignificant decline in the number of rearings and grooming is a non-specific manifestation of a decrease in orientational-research activity, which correlates with a decrease in number of head dipping into the holes. [Poshivalov, V. P. (1978). Jetiologicheskij atlas dlja farmakologicheskih issledovanij na laboratornyh gryzunah. Moscow.]. At the same time, there is a decrease in locomotive activity, which is accompanied by an insignificant decrease in the number of crossings (*Table 1*).

Rats from group 2 showed a significant decrease in locomotion. Comparing the number of head dipping into the holes at different periods of time the explorative activity in 2 weeks after the stem cells administration was less expressed than before the experiment, but higher than before the stem cells correction. It can be assumed that the recovery of explorative activity in animals is

Table 1

Open Field Test (OFT) behavioral reaction results in group 1 (sodium nitrite 2 weeks)

Behavior reactions	Period of time	Mean±CI (confidence interval)	SD (standart deviation)	Levene's test	Significant point	ANOVA, p-value
crossing	before sodium nitrite injection	36.69±9.85	16.31	0.069	0.934	0.622
	after sodium nitrite injection	38.46±9.69	16.03			
	in 2 weeks after sodium nitrite injection	29.5±22.52	14.15			
head dipping into the holes	before sodium nitrite injection	3.85±1.15	1.91	1.931	0.165	0.044
	after sodium nitrite injection	1,85±1,63	2,70			
	in 2 weeks after sodium nitrite injection	1,25±1,52	0,96			
rearing	before sodium nitrite injection	2.5±1.95	2.33	2.993	0. 077	0.059
	after sodium nitrite injection	5.63±3.09	3.7			
	in 2 weeks after sodium nitrite injection	1.75±2.72	1.71			
defecations	before sodium nitrite injection	1±0.74	1.22	0.212	0. 81	0.019
	after sodium nitrite injection	0.92±0.94	1.55			
	in 2 weeks after sodium nitrite injection	3.25±2.39	1.5			
grooming	before sodium nitrite injection	0.69±0.57	0.95	3.345	0.05	0.332
	after sodium nitrite injection	0.69±0.57	0.95			
	in 2 weeks after sodium nitrite injection	0	0			

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

associated with the effect of stem cells. Reducing the number of defecations and the number of crossings hypothesizes the animal stress becomes less. However, according to Kaluev A.V. (2002), Markel "A.L. (1981), this type of reaction signifies the development of defensive inhibition in response to stress factors (pain factor resulting from injections and open field testing) (*Table 2*).

In rats from group 3 there was a drop in all indicators against a background of the increase in the number of defecation (an increase in anxiety levels). found. Thus, the decrease in horizontal and vertical locomotion in contrast to increased anxiety level is not corrected by stem cells. It is possible that protective inhibition in this case is due not only to the administration of sodium nitrite, but also to chronic pain stress (*Table 4*).

The variances of all results of behavioral reactions in control group were not significantly different.

During PAT conditional reflex was formed in 81.25% of cases in rats from group 2, 4 who received

Table 2

OFT behavioral reaction results in group 2 (sodium nitrite 2 weeks + stem cells)

Behavior reactions	Period of time	Mean±CI (confidence interval)	SD (standart deviation)	Levene's test	Significant point	ANOVA, p-value
crossing	before sodium nitrite injection	41.5±15.9	19.02	1.107	0.349	0.031
	after sodium nitrite injection, before stem cells injections	38.75±12.92	15.45			
	in 2 weeks after sodium nitrite and stem cells injection	19.5±12.76	15.26			
head dipping into the holes	before sodium nitrite injection	5.5±2.72	3.25	2.215	0.134	0.010
	after sodium nitrite injection, before stem cells injections	1.75±1.53	1.83			
	in 2 weeks after sodium nitrite and stem cells injection	2.5±1.26	1.51			
rearing	before sodium nitrite injection	2.88±1.44	1.73	1.661	0.214	0.892
	after sodium nitrite injection, before stem cells injections	3.13±2.16	2.59			
	in 2 weeks after sodium nitrite and stem cells injection	2.63±1.54	1.85			
defecation	before sodium nitrite injection	1.38±1.26	1.51	0 .092	0 .913	0.68
	after sodium nitrite injection, before stem cells injections	1.75±1.244	1.49			
	in 2 weeks after sodium nitrite and stem cells injection	1.13±1.04	1.25			
grooming	before sodium nitrite injection	1.13±1.04	1.25	2.595	0.098	0.667
	after sodium nitrite injection, before stem cells injections	1.38±1,54	1.85			
	in 2 weeks after sodium nitrite and stem cells injection	0.75±0.74	0.89			

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0,05).

Conspicuous is the fact that the number of crossings and head dipping into the holes with heterogeneous data variance are reduced by 4 and 11 times respectively ($p < 0.001$). It is probably associated with large damage of brain tissue (*Table 3*).

In rats from group 4 2 weeks after stem cells injection insignificant raise of the number of head dipping into the holes groups compared to period of time after finishing of sodium nitrite administration and before stem cells injection was stem cells. While in group 1, 3 the conditional reflex was not formed in 37.5% of cases. These results demonstrate positive effects of stem cells on cognitive recovery in rats (*Table 5*).

Conclusion

1. In all experimental groups with chronic stress caused by prolonged intraperitoneal administration of aqueous solution of sodium nitrite and single intravenous administration of stem cells suspension, protective inhibition develops. It is

Table 3

OFT behavioral reaction results in group 3 (sodium nitrite 4 weeks)

Behavioral reactions	Period of time	Mean±CI (confidence interval)	SD (standart deviation)	Levene's test	Significant point	ANOVA, p-value
crossing	before sodium nitrite injection	40.71±10.23	11.06	6.783	0.006	0.000
	after sodium nitrite injection	19.71±15.22	16.46			
	in 2 weeks after sodium nitrite injection	10.43±4.1	4.43			
head dipping into the holes	before sodium nitrite injection	4.86±2.23	3.06	5.166	0.017	0.000
	after sodium nitrite injection	0.71±0.88	2.41			
	in 2 weeks after sodium nitrite injection	0.43±0.49	0.95			
rearing	before sodium nitrite injection	4.29±3.45	3.73	4.825	0.021	0.006
	after sodium nitrite injection	0.43±0.49	0.53			
	in 2 weeks after sodium nitrite injection	0.57±0.73	0.79			
defecation	before sodium nitrite injection	0.57±0.73	0.79	2.482	0.112	0.362
	after sodium nitrite injection	0.86±1.35	1.46			
	in 2 weeks after sodium nitrite injection	1.43±0.9	0.98			
grooming	before sodium nitrite injection	2.83±3.75	2.48	4.507	0.026	0.335
	after sodium nitrite injection	2±0.73	2			
	in 2 weeks after sodium nitrite injection	0	0			

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0.05).

Table 4

OFT behavioral reaction results in group 4 (sodium nitrite 4 weeks + stem cells)

Behavioral reactions	Period of time	Mean±CI (confidence interval)	SD (standart deviation)	Levene's test	Significant point	ANOVA, p-value
crossing	before sodium nitrite injection	34±11.33	13.55	1.475	0.252	0.004
	after sodium nitrite injection, before stem cells injections	18.63±8.2	9.8			
	in 2 weeks after sodium nitrite and stem cells injection	13.63±7.98	9.55			
head dipping into the holes	before sodium nitrite injection	4.13±3.14	3.76	1.982	0.163	0 .201
	after sodium nitrite injection, before stem cells injections	1.63±1.09	1.3			
	in 2 weeks after sodium nitrite and stem cells injection	2.88±2.02	2.42			
rearing	before sodium nitrite injection	3.5±2.36	2.83	2.409	0.114	0 .137
	after sodium nitrite injection, before stem cells injections	1.25±1.07	1.28			
	in 2 weeks after sodium nitrite and stem cells injection	1.75±1.99	2.38			
defecation	before sodium nitrite injection	0.375±0.62	0.74	1.213	0.317	0 .073
	after sodium nitrite injection, before stem cells injections	0.75±0.87	1.04			
	in 2 weeks after sodium nitrite and stem cells injection	1.63±1.09	1.3			
grooming	before sodium nitrite injection	0.75±0.97	1.16	0.398	0.677	1
	after sodium nitrite injection, before stem cells injections	0.75±0.74	0.89			
	in 2 weeks after sodium nitrite and stem cells injection	0.75±1.46	1.75			

Values are mean±confidence interval (CI) for the mean.

Variances for each group do not statistically significantly differ. ANOVA results may be considered correct (Levene's test, significant point > 0,05).

The influence of time on behavior results is statistically significant (ANOVA, p-value<0.05).

Table 5

Passive Avoidance Test (PAT): cognitive function comparison between group with stem cell injections (group 2+group 4) and group without stem cells injections (group 1+group 3)

Test	Sodium nitrite 1, 3 weeks groups without stem cells	Sodium nitrite 2, 4 weeks groups with stem cells	Altogether
Failed	10	3	13
Passed	6	13	19
Altogether	16	16	32

Actual and expected results are statistically different. (Pearson's chi-squared test, p=0.012(<0.05).

accompanied by decrease in vertical and horizontal activity and increase in the number of defecation, as determined by testing in the open field.

2. In all rats with a 4-week disease model, the drop in locomotor, research and orientation activity was found. Probably, it means that the brain tissue is damaged significantly.

3. In the OFT in all animal from groups 2, 4 improvement in research activity in comparison with that before stem cells introduction was observed.

4. In the PAT higher percentage of rats from groups 2, 4 passed this test which indicates the significant cognitive functions recovery after stem cell correction.

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ANATOMICAL PREDICTIONS OF DEVELOPMENT OF RHINOSINUSITIS AND ITS COMPLICATIONS

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Abstract

Background. The anatomical structure of the paranasal sinuses of a person predetermines the risk of development, diversity of presentation, possibility of complications and features of surgical treatment of rhinosinusitis. **Objective:** of our study was to determine the thickness and density of the walls of the maxillary and frontal sinuses, which are potentially dangerous in terms of the development of complications. Materials and methods: Our study involved 121 subjects without any ENT diseases, who underwent SCT examination due to the reasons that were not related to abnormalities of ENT organs. Thickness and density in the region of the lower (orbital) wall and posterior (cerebral) wall of the frontal sinus were calculated. **Results and Discussion.** The maximum density was characteristic of the lower wall of the frontal sinus under physiological conditions and appeared 107.96 ± 201.64 Hu, the minimum for the lower wall was -29.98 ± 208.54 Hu. The thickness of the bone tissue in the frontal sinus was 4.05 ± 2.04 mm. **Conclusion.** The minimum density and thickness of the lower and posterior walls of the frontal sinus and upper and lower walls of the maxillary sinus was established under physiological conditions. The density of the posterior wall was found to be 25.4% lower than the density of the lower wall, and the thickness 22.2% lower.

Key words: *Frontal sinus, Maxillary sinus, Spiral computed tomography, Bone density, Bone thickness.*

Introduction

The anatomical structure of the paranasal sinuses (PNSs) of a person predetermines the risk of development, diversity of presentation, possibility of complications and features of surgical treatment of their inflammation, rhinosinusitis [1–3].

The maxillary sinus is more involved in inflammatory processes than other PNSs [4], which is due to the peculiarities of its anatomical and topographic location, the largest volume, location of the anastomosis above the bottom of the sinus, proximity of teeth. Therefore, maxillary sinusitis is one of the most common forms of rhinosinusitis [5]. This fact is of great interest in the study of the anatomical structure of this area. The most significant in terms of complications is the upper wall, which is at the same time the lower wall of the orbit. Impairment of the integrity of this wall can result in the spread of purulent-inflammatory processes into the orbit. Equally important is the lower wall of the maxillary sinus - a potentially dangerous area for the development of odontogenic maxillary sinusitis.

The anatomical structure of the frontal sinus is also of great importance for development of inflammatory pathological processes [6] and their complications with spread to the neighboring organs and tissues (orbital phlegmon, brain abscess, meningitis). Pathological processes occurring in the frontal sinus due to its topographic and anatomical relationships with nearby structures most often lead to complications. Chronic frontal sinusitis has the greatest specific weight for their occurrence, because they are associated with bone changes in the sinus walls, such as bone demineralization, disappearance of trabeculae, cortical destruction, and focal sclerosis. These changes are manifested as a decrease in bone density, as proven by Dong et al [7]. In addition to density, destructive processes often lead to a change in the thickness of the

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Global Osteitis Scoring Scale, in which the degree of destruction is associated with a decrease in bone thickness [8]. These changes can correlate with the severity of the disease, which must be taken into account when planning surgery for the maxillary sinuses and predicting possible complications.

2. Purposes, subjects and methods:

2.1. Purpose

Given all of the above, the aim of our study was to determine the thickness and density of the walls of the maxillary and frontal sinuses, which are potentially dangerous in terms of the development of complications.

2.2. Subjects & Methods

This study is a part of research work "Optimization of early diagnosis, prevention and treatment of oral tissue diseases with smoking addiction", No. 0120U102057, Kharkiv National Medical University, and is funded by Ministry of Health of Ukraine.

Our study involved 121 subjects without any ENT diseases, who underwent SCT examination due to reasons that were not related to abnormalities of ENT organs (suspected stroke, etc.). The patients were selected with the same distribution by gender and age. The age of the subjects was from 25 to 65 years (see *Fig. 1*).

The study was performed using spiral computed tomography (SCT) findings, a simple, informative, and generally accessible intravital method for determining bone density, which helps to identify structural features, relative position of PNSs, determine the microarchitectonics of bone wall tissue and determine possible prerequisites for the development of rhinosinusitis complications.

Adherence to the principle of non-invasive investigation is so important that often the study of processes in the human body is replaced by modeling or setting up an experiment [9].

Noninvasive detection of internal intravital peculiarities is extremely important for human organism morphology and function [10] with nonobligatory linear-feedback connection [11].

SCT examination makes it possible to identify the sizes and shape of the frontal and maxillary sinuses, which differ in great individual and age-related variability [12].

All patients gave voluntary consent to participate in the studies and were examined by an otolaryngologist. After the SCT examination, the radiologist's conclusion was obtained. To evaluate the bone density by SCT, Hounsfield scale was used, considering that according to M. Hofer, modern devices can cover 4096 shades of gray scale, which represent different density levels in Hounsfield units (HU) (the density of water is taken as 0 HU, and air as 1000 HU) [13].

We studied the indicators of the minimum density in all sections presented. The calculation of the thickness was carried out in the thinnest section of the wall (see *Fig. 2*).

Axial sections and coronary reconstructions were investigated. The thickness and density in the region of the lower (orbital) wall were calculated as the most significant in terms of intraorbital complications development [14], the posterior (cerebral) wall of the frontal sinus was studied, since it is of the greatest importance for performing endoscopic interventions [15].

The obtained digital data were statistically processed using Student–Fisher method, the average value for each variation series (X), standard deviation, and the mean error (m) were determined. Statistical processing was carried out on a personal computer using Microsoft Office Excel 2010 software (USA). The results were considered statistically significant at $p < 0.05$.

The article complies with the requirements of the Declaration of Helsinki. The study was approved by the Bioethics Committee of Kharkiv National Medical University.

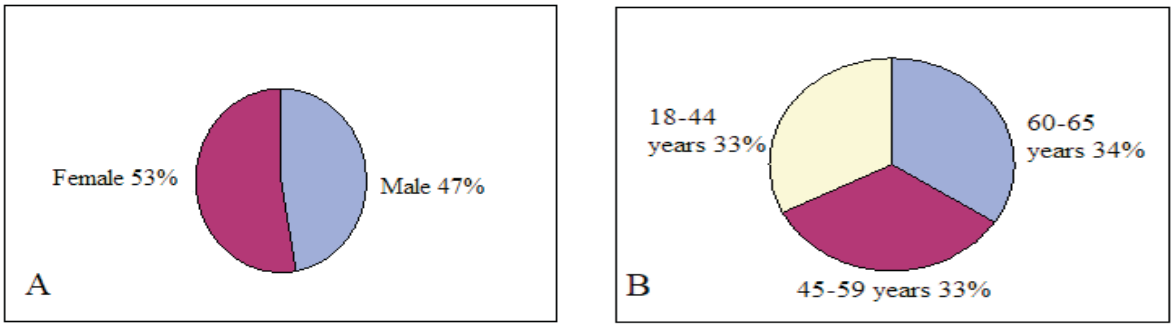

Fig. 1. Patient distribution according to gender (A), age (B)

Conflict of interests

The authors of the article declare no conflict of interest.

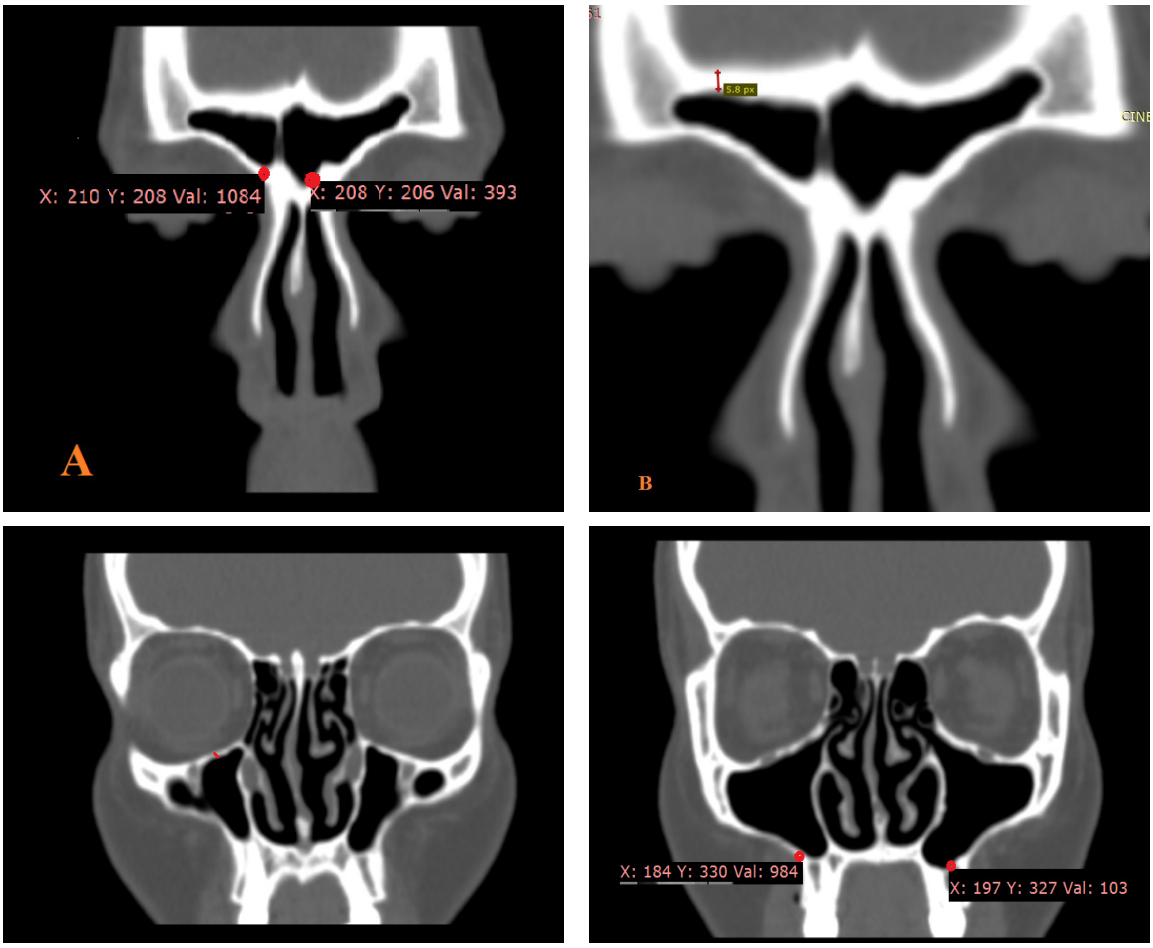

Fig. 2. Example of measuring the density (A) and bone thickness (B) of the frontal sinus and maxillary sinus. SCT, coronary reconstruction

Table 1

Density (Hu) and thickness (mm) of the lower and posterior walls of the frontal sinus

Indicator	Lower wall		Posterior wall	
	Thickness	Density	Thickness	Density
M	4.05	107.96	1.0006	27.42
σ	2.04	201.64	0.538	168.76

Table 2

Density (Hu) and thickness (mm) of the upper and lower walls of the maxillary sinus

Indicator	Upper wall		Lower wall	
	Width	Density	Width	Density
M	2.03	33.21	4.47	-29.98
σ	1.01	109.72	2.11	208.54

3. Results & Discussion

The results of determining the thickness and density of bone tissue (Hu) in SCT study using the Hounsfield scale are presented in *Tables 1–2*.

During the study, the minimum thickness and density of the posterior (see *Table 2*) and lower (see Table 1) walls of the frontal and maxillary sinus were determined in physiological conditions. The study has shown that the maximum density is characteristic of the lower wall of the frontal sinus under physiological conditions and is 107.96 ± 201.64 Hu, the minimum for the lower wall is –29.98 ± 208.54 Hu. The thickness of the bone tissue in the frontal sinus is 4.05 ± 2.04 mm.

The posterior wall was shown to be much thinner than the lower one, creating the conditions

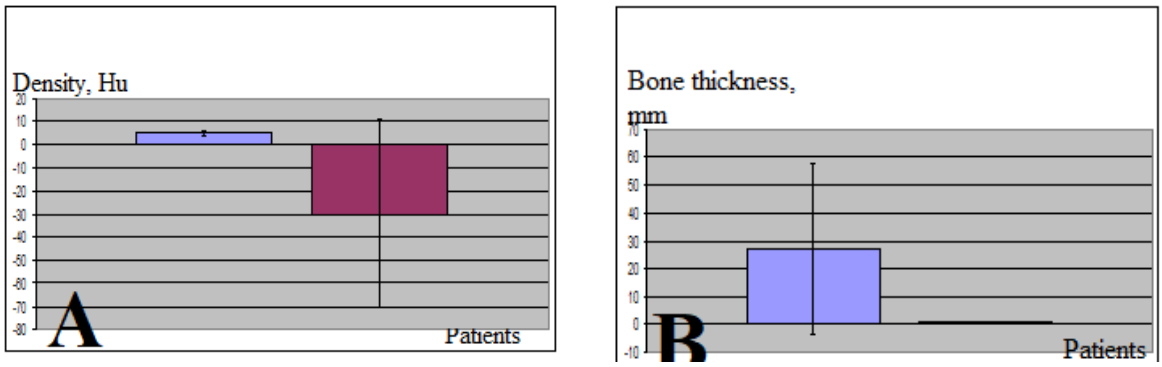

Fig. 3 Chart of average values with confidence intervals of thickness and density of the lower (A) and posterior (B) walls in purulent-polypous frontal sinusitis

for the spread of purulent-inflammatory processes with the development of intracranial complications. The thickness of the lower wall (4.05 ± 2.04 mm) was found to be 22.2% higher than the posterior one (1.006 ± 0.538 mm) under physiological conditions. Based on this, it can be assumed that chronic frontal sinusitis creates more favorable conditions for the spread of the inflammatory process intracranially than intraorbitally. In addition, the likelihood of complications increases due to the lower density in the region of the posterior wall than the lower one. Thus, the density of the posterior wall even under physiological conditions (27.42 ± 168.76 Hu) is 25.4% lower than that of the lower one (107.96 ± 201.64 Hu). In maxillary sinuses, on the contrary, intraorbital complications may prevail over intracranial complications [16].

The minimum thickness of the lower wall of the maxillary sinus was also found to be the thickest among all the studied walls (4.47 ± 2.11 mm). However, it is interesting that with the largest thickness, the density of this wall is rather low (-29.98 ± 208.54 Hu). The low density of this area can probably lead to the spread of a purulent-inflammatory process from the upper teeth to the maxillary sinus [17].

As can be seen from *tables 1* and *2*, bone thickness is a fairly constant indicator – less than the density changes under the influence of inflammatory changes in the sinus. Consequently, in assessing the degree of destructive changes in the sinus walls, perhaps we should rely more on density indicators [18].

In addition, both walls (both the lower and the posterior walls) respond to the inflammatory process by decreasing the density, and the decrease in density is most pronounced with the maximum degree of severity of the inflammatory process in the sinus, which is associated with the appearance of purulent-polypous frontal sinusitis. Thus, bone density depends on the severity of changes in the PNS in patients with different age and physical state [19, 20]. SCT examination can help to determine not only the main morphological aspects of the bone structure, but also to measure its density. Maybe our study will help for the development of artificial intellect or other computed technologies [21–25] for the future studying of medical care workers or doctors [26] in different severity cases of pathological changes in paranasal sinuses or oral cavity [27–29], that allows accepting our results as useful in different field.

Conclusions

1. According to SCT, the minimum density and thickness of the lower and posterior walls of the frontal sinus and upper and lower walls of the maxillary sinus was established under physiological conditions. The density of the posterior wall was found to be 25.4% lower than the density of the lower wall, and the thickness 22.2% lower.

2. The wall thickness values were shown to be 1.0006 ± 0.538 mm for the posterior wall of the frontal sinus and 4.47 ± 2.11 mm.

3. The posterior wall was the thinnest among all PNSs in all the studied groups. In addition, it had a minimum density, creating the conditions for the spread of purulent-inflammatory process in the skull.

4. The lower wall of the maxillary sinus is quite thick, but has a minimum density, which can create favorable conditions for the development of odontogenic maxillary sinus.

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NON-ALCOHOLIC FATTY LIVER DISEASE AND HYPERTENSION: CLINICAL VARIABILITY OF COMORBIDITY

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Abstract

Introduction. Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases; and considerable attention is paid to the comorbidity of NAFLD with hypertension (HT), which affects around one-third of the world's population. The combination of NAFLD with hypertension has been suggested to have a mutual potentiating effect, and hypertension affects the severity of NAFLD. **The purpose:** to study the features of the clinical manifestation of NAFLD in patients with hypertension. **Materials and methods.** The study included 115 patients with NAFLD at the stage of nonalcoholic steatohepatitis. The main group consisted of 63 patients with NAFLD and HT, the comparison group included 52 patients with isolated NAFLD, and the control group was composed of 20 healthy volunteers. The patients underwent anthropometric measurements, evaluation of biochemical markers of liver functional activity, lipid profile and carbohydrate metabolism changes, C-reactive protein (CRP) levels. **Results.** A significant increase in the proportion of patients with active complaints in the group of patients with NAFLD with HT (subjective signs of liver damage, manifestations of dyspeptic and asthenic syndrome) was detected. Significant differences were found in almost all anthropometric indicators in both groups of patients with NAFLD in comparison with the control group. The level of CRP had significant differences and was 7.90 mg/l (95% CI = 7.96–8.75 mg/l), 6.55 mg/l (95% CI = 6.47–7.57 mg/l) and 2.07 (95% CI = 1.83–2.85 mg/l) in patients with NAFLD and HT, isolated NAFLD and the control group, respectively ($p < 0.001$). Fasting glucose levels were significantly higher in both groups of examined patients with NAFLD compared with controls. Significant differences were found in the levels of total cholesterol, VLDL cholesterol, HDL cholesterol and atherogenic factor in patients with NAFLD depending on concomitant HT. There was no significant difference between LDL cholesterol and triglycerides in the two groups of patients with NAFLD. **Conclusions.** Based on the obtained data, it can be stated that GC in patients with NAFLD determines important deviations in the clinical manifestation of the disease and can be considered as a trigger factor for the progression of NAFLD.

Keywords: *non-alcoholic fatty liver disease, NAFLD, hypertension, NAFLD with HT, NAFLD comorbidity, clinical variability, clinical manifestation.*

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common metabolic pathologies. Manifestation of NAFLD is the accumulation of lipids in the liver with the development of steatosis and fibrosis of the liver parenchyma. NAFLD differs in stages, from simple steatosis pathological processes can progress to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis of the liver. In the final stages, the pathology can lead to hepatocellular carcinoma development [16].

The incidence of NAFLD is about 70% among chronic liver diseases, affecting 17 to 40% of the adult population in different countries and 20–30% of the European population [4]. Considerable attention is paid to the comorbidity of NAFLD with cardiovascular diseases, especially hypertension (HT). High blood pressure is found in about 30% of the world's population, it is determined by one of the most significant risk factors for cardiovascular mortality [12].

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According to several studies, NAFLD and hypertension have the effect of mutual potentiation in combination. Hypertension affects the severity of NAFLD and the rate of fibrosis in the liver parenchyma, and NAFLD contributes to the progression of hypertension and causes secondary damage to target organs [5].

It is known that NAFLD is a hepatic manifestation of the metabolic syndrome, one of the components of which is hypertension. In this regard, hypertension doubles the risk of liver fibrosis progression [14; 13; 17]. The association between NAFLD and HT has also been shown to be independent of other manifestations of the metabolic syndrome, such as overweight and insulin resistance. Changes in eating behavior, hypokinesia, overweight and smoking were identified among the main common risk factors for the two pathologies [15]. In the pathogenesis of NAFLD, systemic inflammation plays an important role [20]. A significant increase in C-reactive protein and other inflammatory markers is observed in patients with NAFLD, especially at the stage of NASH [6].

Research data indicate the activation of inflammatory processes associated with HT. Endothelial dysfunction, tissue hypoxia, and immune response changes play a key role in the inflammation pathogenesis in such patients [19].

Lipid profile changes has been drawing considerable attention in the study of patients with both NAFLD and HT. The risk of mortality in patients with hypertension is defined as increased in the presence of hypercholesterolemia and hypertriglyceridemia [21]. Other studies indicate an increased risk of NAFLD progression with elevated levels of triglycerides (TG), low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol and concomitant reduction of high-density lipoprotein (HDL) cholesterol [7; 9].

Common pathogenic pathways associated with the activation of the renin-angiotensin-aldosterone system, systemic inflammation, oxidative stress, apoptosis, and endotoxemia determine higher morbidity and mortality in patients with a combination of NAFLD and HT. Several studies note that even elevated normal blood pressure figures predict the development and progression of NAFLD, and confirmed NAFLD significantly increases the risk of developing hypertension [19; 3].

2. Purposes, subjects and methods

2.1. The purpose of the research was to study the features of the clinical manifestation of non-alcoholic fatty liver disease in patients with hypertension.

2.2. Subjects and methods

115, of them 57 men and 58 women, aged 38 to 59 years (n = 115; M = 48.3; 95% CI = 47.4–49.3) s with verified NAFLD at the stage of non-alcoholic steatohepatitis in combination with and without hypertension were examined. According to the presence of hypertension, the patients were divided into two groups. The main group included patients with NAFLD in combination with HT (n = 63; 32 men and 31 women), aged 38 to 59 years (M = 48.4; 95% CI = 47.2–49.6). The comparison group consisted of patients with isolated fatty liver (n = 52; 25 men and 27 women) aged 39 to 59 years (Me = 48.3; 95% CI = 46.8–49.8). The control group was composed of 20 healthy volunteers, including 12 women and 8 men aged 38 to 56 years (Me = 47.1; 95% CI = 45.1–49.1).

The duration of NAFLD in patients with comorbid NAFL ranged from 2 to 16 years (Me = 6.6; 95% CI = 5.8–7.3), and hypertension in this group of patients was diagnosed from 2 to 19 years ago (Me = 8.4 years; 95% CI = 7.3–9.5). The duration of NAFLD in the group of patients with an isolated fatty liver was also in the range from 2 to 16 years, but on average was insignificantly longer than in the group of comorbid course (Me = 7.8; 95% CI = 6.7–8.8; p = 0.086).

NAFLD was diagnosed in the previous stages of managing the patients according to the Order of the Ministry of Health of Ukraine as of 06/11/2014, No. 826 "Unified clinical protocol of primary, secondary (specialized) medical care: non-alcoholic steatohepatitis" and EASL-EASD-EASO Clinical Practice Guidelines, 2016. Hypertension was diagnosed in the previous stages of managing patients according to the Order of the Ministry of Health of Ukraine No. 384 as of 24/05/2012, "Unified clinical protocol for medical care in arterial hypertension" and criteria of European (ESH / ESC) clinical guidelines for arterial hypertension, 2018.

The overall study included anthropometric measurements – height, weight, waist (WC) and thigh circumference (TC) were determined by standard methods. Body mass index (BMI) and thigh to waist ratio (TWaR) were also calculated according to generally accepted formulas. Biochemical parameters of liver functional activity were determined by spectrophotometric and colometric methods (including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT)). In addition, the

levels of of lipid metabolism (total cholesterol, triglycerides, VLDL cholesterol, HDL cholesterol) were determines by the enzymatic method on a biochemical analyzer. Low-density lipoprotein cholesterol (LDL cholesterol) was calculated by Friedewald W.T. (1972) and atherogenic coefficient was determined by the following formula, proposed by A. N. Klimov (1982): Total cholesterol – HDL cholesterol / HDL cholesterol. In order to assess carbohydrate metabolism, glucose levels were determined using the glucose oxidant method. The level of acute-phase marker of inflammation, C-reactive protein (CRP), was determined using a highly sensitive method (hs-CRP ELISA) (Biomerica, USA). All statistical processing of obtained results was performed using computer software packages "Excel 2019" (Microsoft), "Statistica 8.0. for Windows" (StatSoft Inc.). Continuous variables are presented as mean (M) or median (Me) depending on the correspondence with the normal distribution and confidence intervals with established reliability $\gamma = 0.95$ (95% CI). Non-parametric methods were used to determine the relationships between risk factors, clinical and laboratory parameters. Spearman's rank correlation coefficient was determined and Chaddock scale was used to determine the strength of the relationship. The statistical significance of differences in relative indicators was determined using Pearson's chi-squared test. The Manng Whitney U-test was used to determine the difference between the two independent samples by the trait level. The maximum allowable probability of committing a type I error (p-value) was established as the value of the level of statistical significance less or equal than 0.05.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results

Typical complaints of patients with NAFLD with or without hypertension were general weakness, fatigue, loss of appetite, right upper quadrant discomfort and pain, signs of emotional instability (emotional lability, sleep disturbances). The analysis of complaints revealed a significant increase in the proportion of patients with active complaints in the group of patients with NAFLD against a background of hypertension (*Table 1*).

The levels of systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patients

Table 1

The frequency of the main complaints in NAFLD patients depending on the presence of HT, n, %

Complaints	NAFLD and HT (n = 63)		NAFLD (n = 52)		Probability of difference (Pearson's chi-squared test results)
	n	%	n	%	
General weakness	43	68.25	13	25	df = 1 $\chi^2 = 21.333$ $p < 0.001$
Rapid fatigue	51	80.95	14	26.92	df = 1 $\chi^2 = 33.839$ $p < 0.001$
Impaired appetite	50	79.37	25	48.08	df = 1 $\chi^2 = 12.294$ $p < 0.001$
Early satiety	38	60.32	18	34.62	df = 1 $\chi^2 = 7.532$ $p = 0.007$
Right upper quadrant discomfort	42	66.67	22	42.31	df = 1 $\chi^2 = 6.849$ $p = 0.009$
Right upper quadrant *pain*	12	19.05	6	11.54	df = 1 $\chi^2 = 1.217$ $p = 0.271$
Telangiectasia	15	23.81	6	11.54	df = 1 $\chi^2 = 2.874$ $p = 0.091$
Sleep disorders	42	66.67	13	25	df = 1 $\chi^2 = 19.820$ $p < 0.001$
Emotional lability	47	74.6	12	23.08	df = 1 $\chi^2 = 30.273$ $p < 0.001$

with NAFLD and HT naturally had significantly difference in comparison with NAFLD patients without HT and the control group. The figures of DBP in the comparison group were significantly lower than in the control group (*Table 2*).

values that was 2.07 mg/l (95% CI = 1, 83–2.85 mg/l) in 3.8 (p <0.001) and 3.2 (p <0.001) times, respectively (*Figure*).

Evaluation of metabolic profile revealed changes in one of the main indicators of

Table 2

Blood pressure numbers in NAFLD patients depending on the presence of HT, mm Hg

Indicator	NAFLD and HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
SBP, mm Hg	140 (95% CI = 137.86-140.55)	120 (95% CI = 120.83-122.24)	123 (95% CI = 121.94-126.56)	p_{1-2} <0.001 p_{1-3} <0.001 p_{2-3} = 0.059
DBP, mm Hg	85 (95% CI = 82.72-86.17)	70 (95% CI = 70.54-73.30)	75 (95% CI = 73.75-79.25)	p_{1-2} <0.001 p_{1-3} <0.001 p_{2-3} = 0.004

Note: p < 0,05 – the difference is statistically significant between groups;
p_{1-2} – the difference between the NAFLD + HT group and the isolated NAFLD group;
p_{1-3} – the difference between the NAFLD + HT group and the control group;
p_{2-3} – the difference between the isolated NAFLD group and the control group.

No significant differences in anthropometric parameters of patients with comorbid and isolated NAFLD were found. Meanwhile, significant differences were found in comparison with the control group in almost all indicators of both the main and the comparison group, except for the thighs circumference. This was a reflection of the regular prevalence of abdominal obesity in patients with NAFLD (*Table 3*).

carbohydrate metabolism: fasting glucose levels were significantly higher than in patients with NAFLD and HT, which was 5.87 mmol/l (95% CI = 5.76–5.98 mmol/l) (p <0.001) and in the comparison group, where the figure reached 5.62 mmol/l (95% CI = 5.43–5.60 mmol/l) (p < 0.001) in relation to the control values 4.52 mmol/l (95% CI = 4.48–4.61 mmol/l). Comparative analysis of glucose levels in the examined groups of

Table 3

Anthropometric parameters in NAFLD patients depending on the presence of HT, cm

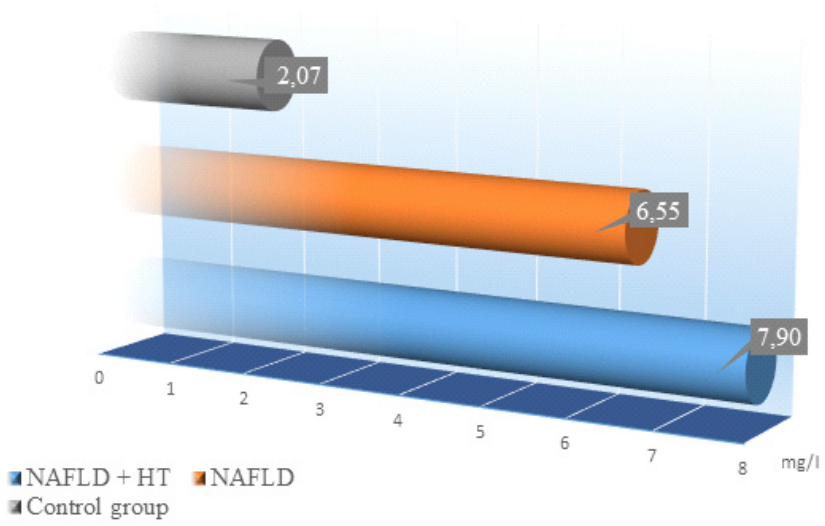
Indicator	NAFLD and HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
BMI, cm	26.9 (95% CI = 24.45-29.34)	25.1 (95% CI = 25.38-26.56)	22.7 (95% CI = 22.41-23.46)	p_{1-2} = 0.477 p_{1-3} < 0.001 p_{2-3} < 0.001
WC, cm	86.0 (95% CI = 82.9-87.4)	82.5 (95% CI = 81.01-85.87)	71.40 (95% CI = 67.57-75.13)	p_{1-2} = 0.302 p_{1-3} < 0.001 p_{2-3} < 0.001
TC, cm	98.0 (95% CI = 95.95-98.40)	97.0 (95% CI = 95.70-97.72)	96.5 (95% CI = 90.79-97.61)	p_{1-2} = 0.267 p_{1-3} = 0.177 p_{2-3} = 0.436
Thigh to waist ratio (TWaR)	0.89 (95% CI = 0.86-0.90)	0.86 (95% CI = 0.84-0.89)	0.76 (95% CI = 0.74-0.77)	p_{1-2} = 0.395 p_{1-3} < 0.001 p_{2-3} < 0.001

Note: p < 0,05 – the difference is statistically significant between groups;
p_{1-2} – the difference between the NAFLD + HT group and the isolated NAFLD group;
p_{1-3} – the difference between the NAFLD + HT group and the control group;
p_{2-3} – the difference between the isolated NAFLD group and the control group.

Determination of C-reactive protein levels as a non-specific marker of inflammatory processes also revealed a significant difference in its content among the groups. In patients of the main group C-reactive protein level was 7.90 mg/l (95% CI = 7.96–8.75 mg/l), in patients with isolated course of NAFLD was 6.55 mg/l (95% CI = 6.47–7.57 mg/l) (p <0.001). These results exceeded the control

patients also showed statistically significant difference (p < 0.001).

Significant differences between the groups were found in determining the lipid profile to assess the possibility of combining the mechanisms of dyslipidemia in patients with comorbid NAFLD and hypertension. Almost all indicators of the lipid profile of both examined groups had a significant difference



The levels of C-reactive protein in NAFLD patients depending on the presence of HT, mg/l

in comparison with the control indicators, except for HDL cholesterol in the comparison group. Comparison of lipid profile in patients with NAFLD against a HT background with isolated NAFLD individuals revealed significant differences in levels of total cholesterol, LDL cholesterol, HDL cholesterol and atherogenic coefficient. There was no significant difference between LDL cholesterol and triglycerides in the two groups (*Table 4*).

The obtained data confirm the idea of the prevalence of the type of hyperlipoproteinemia phenotype "IIb" in patients with HT, determine the serum lipid profile in patients with NAFLD as proatherogenic, and indicate the effect of concomitant hypertension on lipid metabolism in patients with NAFLD.

The study of the levels of markers of liver damage revealed similar deviations: significant differences between the groups of examined patients were found for all indicators. There was no statistically significant difference only in the group with isolated NAFLD in the assessment of alkaline phosphatase and De Ritis ratio in comparison with the parameters of almost healthy individuals. At the same time, only 12 patients from the main group had an AST/ALT index in

Table 4

Lipid profile results in NAFLD patients depending on the presence of HT, mmol/l

Indicator	NAFLD and HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
Total cholesterol, mmol/l	6.78 (95% CI = 6.60-6.97)	5.93 (95% CI = 5.77-6.08)	4.00 (95% CI = 3.76-4.24)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
LDL cholesterol, mmol/l	4.11 (95% CI = 3.99-4.44)	3.14 (95% CI = 2.97-3.45)	2.28 (95% CI = 2.25-2.65)	$p_{1-2} = 7.38$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
LDL cholesterol, mmol/l	0.78 (95% CI = 0.74-0.82)	0.61 (95% CI = 0.57-0.66)	0.50 (95% CI = 0.45-0.56)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.014$
HDL cholesterol, mmol/l	1.01 (95% CI = 1.01-1.09)	1.19 (95% CI = 1.14-1.24)	1.165 (95% CI = 1.13-1.28)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} = 0.821$
Triglycerides, mmol/l	1.9 (95% CI = 1.84-2.02)	2.03 (95% CI = 1.90-2.07)	1.27 (95% CI = 1.16-1.38)	$p_{1-2} = 0.124$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$
Atherogenic coefficient	5.57 (95% CI = 5.27-5. 87)	4.12 (95% CI = 3.86-4.37)	2.37 (95% CI = 2.11-2.64)	$p_{1-2} < 0.001$ $p_{1-3} < 0.001$ $p_{2-3} < 0.001$

Note: $p < 0,05$ – the difference is statistically significant between groups;
p_{1-2} – the difference between the NAFLD + HT group and the isolated NAFLD group;
p_{1-3} – the difference between the NAFLD + HT group and the control group;
p_{2-3} – the difference between the isolated NAFLD group and the control group.

the range > 1, which can be considered a marker of fibrotic and cirrhotic changes in the liver (*Table 5*).

4. Discussion

The effect of concomitant hypertension on the clinical manifestation of NAFLD has been determined in various studies. Ampuero J. et al. showed that HT was independently linked to significant fibrosis in patients with NAFLD (p=0.028). Also, investigation showed significantly low HDL in 9.6 % and hypertriglyceridemia in 23.6% of patients with comorbid course of NAFLD on the background of HT was found [1].

The possibility of the two-way relationship between these diseases remains under study. Prospective cohort study of Liu P. et.al. included 6704 eligible hypertension-free subjects and 9328 NAFLD-free subjects and bidirectional association between NAFLD and hypertension was also shown. Among 6704 participants free of HT at baseline, 2561 (38.2%) developed hypertension and 2289 (24.5%) participants developed NAFLD on the background of HT. Patients with NAFLD and HT had significantly higher mean waist circumference and BMI, levels of serum lipids (TC, TG, and LDL-c). This study provided evidence that development and persistence of hypertension were the high risk factors for incident and more severe course of NAFLD in patients without T2D2 or obesity [10].

These conclusions contradict the results of anthropometry obtained in this study, but they confirm the state of lipid metabolism in the patients who were examined.

The study of Ma J. included 1051 participants with and without NAFLD, and showed that baseline NAFLD was associated with increased odds of incident HT (OR 1.42; 95% CI 1.15–1.76; p=0.001) and patients with hypertension at baseline had higher odds of NAFLD development (OR 3.34; 95% CI 2.04-5.49) (p<0.03).

Table 5

Markers of liver damage in NAFLD patients depending on the presence of HT, IU/l

Indicator	NAFLD and HT (n = 63)	NAFLD (n = 52)	Control group (n = 20)	Reliability between groups
ALT, IU/l	79.00 (95% CI = 80.00-86.98)	69.00 (95% CI = 65.29-70.79)	20.00 (95% CI = 18.77-23.92)	p_{1-2} < 0.001 p_{1-3} < 0.001 p_{2-3} < 0.001
AST, IU/l	75.05 (95% CI = 68.13-75.17)	54.00 (95% CI = 53.16-56.99)	16.50 (95% CI = 15.36-20.04)	p_{1-2} < 0.001 p_{1-3} < 0.001 p_{2-3} < 0.001
De Ritis ratio (AST/ALT)	0.90 (95% CI = 0.88-0.92)	0.80 (95% CI = 0.80-0.83)	0.82 (95% CI = 0.79-0.87)	p_{1-2} < 0.001 p_{1-3} = 0.005 p_{2-3} = 0.439
AF, IU/l	1840.00 (95% CI = 1764.83-1872.79)	1150.00 (95% CI = 1059.91-1213.17)	1160.00 (95% CI = 1032.76-1222.24)	p_{1-2} < 0.001 p_{1-3} < 0.001 p_{2-3} = 0.826
GGTP, IU/l	64.00 (95% CI = 63.31-70.53)	57.83 (95% CI = 55.08-60.57)	28.15 (95% CI = 23.84-32.46)	p_{1-2} < 0.001 p_{1-3} < 0.001 p_{2-3} < 0.001

Note: p < 0,05 – the difference is statistically significant between groups;
p_{1-2} – the difference between the NAFLD + HT group and the isolated NAFLD group;
p_{1-3} – the difference between the NAFLD + HT group and the control group;
p_{2-3} – the difference between the isolated NAFLD group and the control group.

It was also found significant differences in the glucose level, as well as the state of lipid metabolism in patients with NAFLD and accompanied HT. The study showed possibility of insulin resistance influence on linking the bi-directional association of fatty liver and CVD risk factors throw overproduction of VLDL and increased influx of free fatty acids into the liver from adipose tissue [13].

According to Aneni E. C. et al., more prevalent NAFLD may occur early in the development of HT, even in conditions of the absence of other metabolic risk factors. The authors concluded that controlling blood pressure levels among even non-obese hypertensive patients may be important in preventing or limiting NAFLD [2].

Hypertensive patients in this research had significantly higher waist circumference values (98.52±12.52 cm) and body mass index (29.99±1.41 kg/m2), higher glycaemia level (124.14±45.33 mg/dL), higher level of triglycerides (195.27±74.52 mg/dL), and hs-CRP

(0.53±0.44 mg/dL) [18]. In fact, this is in line with the results of this study, which also point to the importance of blood pressure control due to the negative impact of hypertension on the manifestation of NAFLD.

Ilan, Y in showed that the prevalence of NAFLD in cases of normal blood pressure, prehypertension, and HT reaches 16.5, 37.5, and 59.3%, respectively. In multivariate analyses, prehypertension and hypertension have been associated with elevated risk of presence NAFLD. High hs-CRP values was found in hypertensive patients and it was considered as an independent risk factor for HT. Authors concluded that this biomarker can aggravate hypertension by participating in local and systemic inflammatory responses [8]. Significant differences in the CRP level obtained during the examination of patients with comorbid and isolated NAFLD course also confirm this statement.

Summary from 19 prospective studies by Lonardo A. at al. suggest that HT can lead to differences in NAFLD course and condition the rapid deterioration of fatty liver patients and combination of this pathologies could be a precursor of the metabolic syndrome [11].

Conclusions

The obtained data suggest that the presence of concomitant hypertension in patients with NAFLD introduce certain deviations in the clinical manifestation of the disease, which is manifested by a significant increase in the incidence of subjective signs of liver damage, dyspeptic and asthenic syndrome. The results indicate a significant impact of HT on the quality of life of patients, which can be manifested by significant changes in the patient's subjective perception of their physical and psychological condition. The course of both isolated NAFLD and comorbidity NAFLD with HT is accompanied by changes in carbohydrate and lipid profile and levels of nonspecific marker of inflammation, C-reactive protein, which confirmed the pathophysiological role of metabolic disorders and chronic systemic low-grade inflammation in NAFLD. The combination of these problems leads to a significant increase in the severity of deviations in these indicators, which makes it possible to consider HT as a trigger factor for the progression of NAFLD in patients with this "nosological tandem".

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CHANGES IN CARDIOVASCULAR AND RESPIRATORY SYSTEMS IN YOUNG ATHLETES

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Abstract

Changes in cardiorespiratory complex of adult athletes are the subject of scientific researches. However, there is a lack of researches that would assess such changes in children and adolescents involved in intensive physical training. **The purpose** of the study was to evaluate functional and morphological changes of cardiovascular and respiratory systems in boys of primary school age engaged in football. **Materials and methods.** 109 male children aged 10–11 years were enrolled in the study, among which 81 children have been attending sports football schools, 28 children didn't have regular physical activity. The study design included a general clinical examination, a spirometry, the condition of cardiovascular system was assessed with echocardiography, exercise stress test with cycle ergometer (VEM), and ambulatory blood pressure monitoring (ABPM). **Results.** The boys 10–11 years of age who play sports have higher values of minute respiratory volume and cardiac indices, which may support the hypothesis of the myocardial hypertrophy development, although they correspond to normal Z-score values. The incidence of complaints in boys who do not practice football is significantly higher during the exercise test. According to ABPM results The duration of physical activity may influence on transition from vasodilation to vasoconstriction. **Conclusions.** We obtained statistically significant quantitative differences in the functional state of the cardiovascular and respiratory systems in children-athletes. The changes found in young athletes may result to further supervision of a pediatric cardiologist, but decision should be made individually.

Keywords: *cardiovascular system; football; primary school children; respiratory system.*

Introduction

Physical activity is an important determinant of the general health in children [1]. Modern recommendations of such organizations as the American Academy of General Medicine, the American Academy of Pediatrics, the American College of Sports Medicine, the American Medical Society for Sports Medicine, the American Orthopedic Society for Sports Medicine, and the recommendations of the American Heart Association and the European Society of Cardiology (2019) relate to the fact that, first, before a child is involved in sports, he or she must be examined for respiratory and cardiovascular diseases. Nevertheless, it is necessary to remove unnecessary restrictions on participation in sports or exercise program [2]. Unnecessary restrictions usually apply not due to abnormal condition of respiratory and cardiovascular systems, but rather arise from concerns about complications that could be avoided by preventive measures [3]. The concern is that one of the current problems in all countries of the world is the problem of mortality during sports competitions [4]. Sudden death in young athletes occurs with a frequency of 1 in 50,000 per year [5]. Despite the fact that sudden cardiac death at a young age is quite rare, each of these cases is a tragedy for society and family members, as athletes are considered the healthiest cohort of the population [6]. In 2015, a proposal was issued by the Sport Cardiology Section of the European Association for Cardiovascular Prevention and Rehabilitation "Sudden cardiac arrest in sport - the need for uniform registration", which states that there are large differences in morbidity, registration methods and causes of

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sudden cardiac arrest/sudden cardiac arrest. death in athletes. Such factors of inequality in registration of sudden deaths include age, sex, presence of comorbid disease, geographical region and participation in sports [7]. The sudden cardiac death is often associated with "hidden" cardiorespiratory pathology. Therefore, today it is recommended to develop strategies based on evidence and expert consensus, health policy aimed at protecting young athletes from such dramatic events [8].

The role of airway obstruction in the development of sudden death in athletes is still unclear [7, 8].

In recent decades, the pages of medical publications were dedicated to the search for methods for predicting the occurrence of exercise-induced bronchial obstruction in athletes [9, 10]. It has been shown that the subjective symptoms of exercise-induced bronchial obstruction before and after beta2-agonist administration do not correlate with the changes in airway caliber in athletes. Therefore, subjective assessments of respiratory symptoms after treatment with inhaled beta2-agonists should not be used as the only diagnostic tool to detect this condition [11].

The "gold standard" for determining aerobic fitness for exercise is measurement of respiratory gas exchange, but physiological responses to exercise during the growth and development of children are still poorly understood, especially in children athletes [2, 12].

Recent literature reviews suggest that there are difficulties faced by family physicians in diagnosing exercise-induced respiratory disorders. These difficulties arise not only due to lack of awareness in this matter, but also due to lack of access to special tests for their diagnosis [13, 14]. However, it is not yet clear how family physicians diagnose exercise-induced bronchial obstruction. Moreover, the diagnosis of bronchial obstruction caused by exercise in adult athletes is difficult, and in children it may not be defined at all [15, 16].

2. Purpose, subjects and methods:

2.1. The purpose of the research is to define morphological and functional changes of the cardiorespiratory complex in boys of primary school age engaged in football.

2.2. Subjects & Methods

109 boys at the age of 10–11 years were enrolled in study, of them 81 children attended sports football schools, 28 children did not have regular physical activity. The children were divided into groups according to duration of football school attendance: group 1 – children who were engaged in football for more than 4 years, n = 26; Group 2 consisted of the children engaged in football from 2 to 4 years, n = 34; Group 3 included the children who played football for less than 2 years, n = 21. The control group (group 4) consisted of clinical data from 28 boys of the same age, who do not play sports and have no health problems. Inclusion criteria were: 10–11 year old boys involved in football. Exclusion criteria were: female children, age less than 10 years or 12 and more years, history of confirmed genetic pathology, and any acute condition within 3 months prior to recruiting.

The study design included a general clinical examination, spirometry (PFT), condition of cardiovascular system assessed with echocardiography, exercise stress test with cycle ergometer (VEM), and ambulatory blood pressure monitoring (ABPM).

PFT studies were performed using the method of computerized pneumotachography on the devices "Custo-Vit" (Germany) and SpiroCom ("XAI-Medica", Ukraine).

Echocardiography was performed on the devices "Toshiba-Nemio" (Japan) and "Radmir Ultima PA" (Ukraine).

ABPM was performed with "CardioSens ECG+BP" (XAI-MEDICA, Ukraine).

Statistical analysis of the data was performed using statistical packages "Excel for Mac" and "Statistica 7.0. for Windows". Verification of electoral groups for compliance with the Gauss law was performed using Shapiro-Wilk test, which proved the need for nonparametric methods. The median (Me), lower (Lq) and upper (Uq) quartiles of distribution were determined. To compare the characteristics of incidence we used the method of angular transformation with the evaluation of the F-criterion. Non-parametric analysis of variance Kruskal-Wallis ANOVA [KW] was used to compare samples of more than two. Non-parametric Mann-Whitney criterion [MW] was used to compare two independent samples. The difference in parameters was considered statistically significant at $p < 0.05$.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results & Discussion

The demographic characteristics of children in all groups as well as early neonatal and general history did not reveal any significant difference. There were no family reported cases of sudden cardiac death.

Not all of PFT results were acceptable according to ATS/ERS guidelines [17]. Therefore, we excluded from statistical analysis 25 PFTs due to poor start effort or absence of expiratory plateau. The main results of PFT testing are listed in *table 1*.

greatest values of TV and MVL, however in this group there was a subset of children with decrease in FEV1 that probably would require further PFT monitoring.

Doppler echocardiography did not reveal any

Table 1

PFT results in boys aged 10–11 years depending on duration of physical activity (Me; (Lq;Uq))

Parameter, units	Groups of observation				p (KW)
	Group 1 n=24	Group 2 n=20	Group 3 n=20	4 группа n=20	
VC, l	2.61 (2.49; 2.92)	2,78 (2,26; 2,91)	2,58 (2,32; 2,88)	2,68 (1,86; 2,82)	0,5711
FVC, l	2.95 (2.65; 3.25)	3,09 (2,82; 3,32)	3,26 (2,95; 3,41)	2,95 (2,77; 3,05)	0,2541
MVL, l/min	91.5 (55.25; 106)	95 (86; 115,5)	88,5 (84,5; 106)	93 (84; 107,5)	0,6364
% FEV1 of predicted value,	106.0 (99.0; 109.0)	108.0 (95.5; 117.5)	109.0 (97.5; 114.0)	97.5 (90.5;103.5)	0.4066
% TV	119,0 (95.0; 126.0)	103.0 (86.5; 118.5)	102.0 (97.5; 130.0)	94.0 (81.5; 100.5)	0.0555
MW ($p_{1,2}$=0.4611; $p_{1,3}$=0.9483; $p_{1,4}$=0.0150; $p_{2,3}$=0.5961; $p_{2,4}$=0.1100; $p_{3,4}$=0.0457)					
% MVL	135.5 (127.5; 157.5)	136.0 (125; 174.5)	116.0 (109.75; 166.75)	112.0 (101.0; 120.5)	0.0243
MW ($p_{1,2}$=0.6212; $p_{1,3}$=0.1933; $p_{1,4}$=0.0041; $p_{2,3}$=0.1800; $p_{2,4}$=0.0051; $p_{3,4}$=0.4031)					

VC – vital capacity, FVC – forced vital capacity, MVL – minute lung ventilation volume, FEV1 – forced expiratory volume for 1 second, TV – tidal volume; KW - Kruskal-Wallis ANOVA test ; MW – Mann-Whitney test.

The changes of the respiratory system, according to PFT results, in boys 10–11 years old who play football, depended on the duration of sports: boys 10–11 years old have an increase in TV and MVL and compared to boys of the same age who did not play sports, even if they were engaged for a short time – up to two years. In the boys 10–11 years who were involved in football for more than 4 years, there were the

structural abnormalities in the subjects enrolled in the study. However, there were significantly different values of linear characteristics and left ventricular mass indexed to body surface area (*table 2*).

All parameters were within normal values according to z-score. According to multifactorial nonparametric analysis, we discovered that in 10–11 years old boys of different sport activity

Table 2

Echocardiographic characterstics in boys aged 10–11 years depending on duration of physical activity (Me; (Lq;Uq))

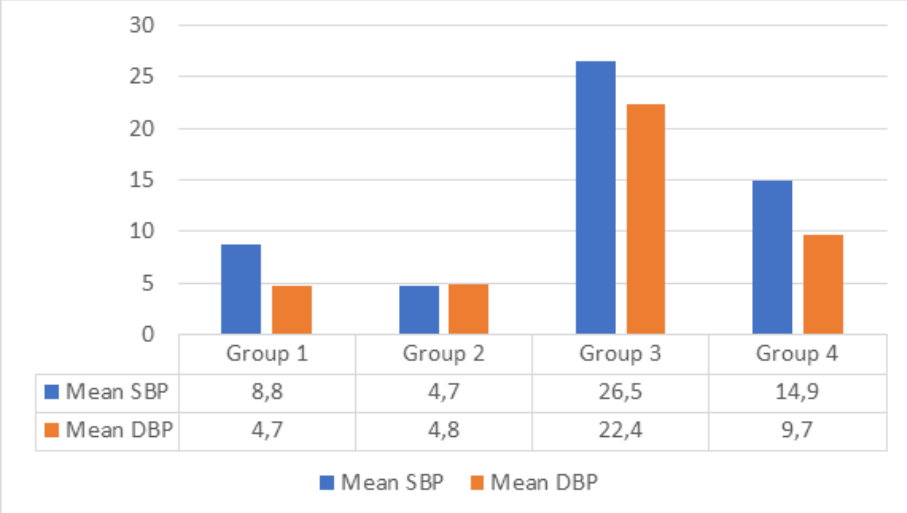
Parameter, units	Groups of observation				KW p
	Group 1 n=26	Group 2 n=34	Group 3 n=21	Group 4 n=28	
IVSd, mm	7.6 (7.3; 8.3)	7.85 (7.1; 8.3)	7.9 (7.5; 8.1)	7.9 (7.5; 8.1)	0.0018
MW: $p_{1,2}$=0.9586; $p_{1,3}$=0.5886; $p_{1,4}$=0.0038; $p_{2,3}$=0.8033; $p_{2,4}$=0.0033; $p_{3,4}$=0.8847					
LVPWd, mm	7.8 (7.; 8.5)	7.75 (6.9; 8.3)	7.9 (7.6; 8.1)	6.9 (6.3; 7.4)	0.0029
MW: $p_{1,2}$=0.7502 $p_{1,3}$=0.6182; $p_{1,4}$=0.0036; $p_{2,3}$=0.5303; $p_{2,4}$=0.0087; $p_{3,4}$=0.0956					
LVMMi, г/м^2	81.05 (70.05; 86.97)	77.75 (71.84; 93.44)	82.15 (72.64; 90.01)	64.17 (53.86; 68.90)	0.0001
MW: $p_{1,2}$=0.7841; $p_{1,3}$=0.8239; $p_{1,4}$=0.0011; $p_{2,3}$=0.7637; $p_{2,4}$=0.0001; $p_{3,4}$=0.0487					
RVDd, mm	17.65 (16.8; 18.50)	16.80 (16.20; 17.20)	16.55 (15.95; 18.15)	16.30 (16.11; 17.00)	0.0193
MW: $p_{1,2}$=0.0270; $p_{1,3}$=0.0112; $p_{1,4}$=0.0047; $p_{2,3}$=0.9111; $p_{2,4}$=0.6621; $p_{3,4}$=0.6391					

IVSd – Interventricular septum thickness at end-diastole, LVPWd- left ventricular posterior wall at end-diastole, LVMMi – left ventricle mass index, RVDd- right ventricular dimension at end-diastole; KW - Kruskal-Wallis ANOVA test ; MW – Mann-Whitney test.

duration there were indices of heart morphology that reflects development of myocardial hypertrophy. LVMMi was significantly higher in all boys involved in sport comparing to the group of control. Left ventricular posterior wall was bigger in those who have been training for more than 2 years. And the right ventricular dimension was increased in boys who played football over 4 years without significant difference in other groups.

ABPM evaluations did not reveal any significant difference of elevated blood pressure. But in the beginning of training (children of group 3), the lowest values of DBP occur during the day (p=0.0495) (*figure*).

Nocturnal changes in BP by non-dipper type (<10%) were registered in 8 (13.1%) children engaged in sports and not registered in children not engaged in sports (p = 0.1088); by type of over-dipper (> 20%) was registered in 9 (14.7%) children engaged in sports and in 2 (9.0%) children not engaged in sports (p = 0.4810), night peak is very low blood pressure index was registered in 1 (5%) child with sports experience over 4 years.



The prevalence of decreased systolic blood pressure (SBP) and diastolic blood pressure (DBP) during daytime in boys aged 10–11 years depending on the physical activity duration (%)

Discussion

The results of the relationship between anthropometric data and PFT values in child populations of many, even low-income countries have already been published [98–102]. However, there is no such data in Ukraine.

PFT values recommended as normal by the American Thoracic Society/European Respiratory Society (ATS/ERS) for the general population, cannot be used in the athlete population [17]. Although it is well known that exercise can affect the lung volume, the effect of sports activity on lung function testing has never been studied [23]. In 2016, a study was published that examined differences in functional respiratory parameters in athletes in different sports by measuring lung volume and the influence of the factors that most affect respiratory function. But this study, in contrast to ours, included men aged 18–35 years. The authors concluded that although all anthropometric characteristics differed significantly between groups, they correlated with respiratory parameters and participation in sports is associated with respiratory adaptation, and the degree of adaptation depends on the type of activity. Endurance sports athletes have higher lung volumes in comparison with skill, mixed and power group of sport [103].

Our study of 10–11 year old boys who were playing football showed an increase in LVPWd, IVSd, LVMMi and RV dimension caused by sports training duration, but these increases did not go beyond the upper limits of the centile distribution for age, height and body weight. Therefore, we cannot imply on the development of an athletic heart syndrome. But the number of works covering the study of the formation of the athletic heart in childhood, depending on the sport, remains small.

There is little information on the adaptation of the right ventricle to exercise in children. One study looked at the effect of 5 months of intensive training on the morphology and function of the right ventricle in 94 swimmers aged 10.8 ± 0.2 years. The researchers showed that after 5 months of intensive training, the end-diastolic dimension of the right ventricle increased (24.9 ± 4.1 vs 23.6 ± 3.0 mm/m^2, p = 0.15) at its normal function. The authors considered this not as a manifestation of cardiomyopathy, but a normal reaction to exercise. We obtained similar data [24].

The hypothesis of cardiac muscle remodeling was tested in a study of the adaptation of the left and right atria in 5-month training of 94 children (57 athletes and 37 sedentary children) aged 10.8 ± 0.2 and 10.2 ± 0.2 years. The size of these heart chambers and their contractile capacity in athletes are increased, which suggests that morphological adaptation occurs in the early stages of sports careers in children, which is similar to the results of our study [25].

Another recent study of children similar to ours enrolled skiers aged 12.1 ± 0.2 years, showed morphological and functional changes in the heart. In young athletes comparing with those who do not do sports, increased end-diastolic volume (79 ± 7 vs. 68 ±7 ml/m^2, p <0.001), LVMMi (69 ± 12 vs. 57 ± 13 g/m^2, p <0.001), diameter of the right ventricle (28.3±3.0 vs. 25.4±3.5 mm/m^2, p <0.001) and diameter of the right atrium (10.6 ± 1.4 vs. 9.7 ± 1.2 cm, p < 0.01). There was no difference in left ventricle ejection fraction [26].

Another problem of medicine, which was subject of our study, but that remain unsolved is the adaptation of blood vessels to exercise and hypertension in athletes [27]. Static and dynamic components of physical activity (overload), i.e. cardiovascular stress, vary depending on the needs of a particular sport or activity. As an example, a football midfielder who runs to the goal is engaged in dynamic exercises. Systolic blood pressure rises during endurance and dynamic exercise; both systolic and diastolic blood pressure increase during resistance or static exercise. These increases in blood pressure reflect the body's efforts to increase cardiac output to meet the metabolic needs of working muscles [27]. The epidemiological prevalence of the hypertension in athletes has not been established.

In a study of 3697 athletes (men and women aged 19 to 49 years), the authors noted that the value of blood pressure in athletes who played sports "dynamic type" (speed, endurance, ball games) is higher than in sports "static type". It was noted that water sports athletes have a higher blood pressure [27].

According to a retrospective cohort study of male athletes from 636 football teams, it was concluded that the prevalence of hypertension among football players is higher than among non-football players [29]. There is no known increased risk of developing hypertension in weightlifters or other strong athletes (eg, discus throwers, shooters) [30].

The limitation of our study is a small sample of boys 10–11 years of age who are engaged in football and lack of information of their history of trainings.

Conclusions

The boys 10–11 age who are engaged in football do not present with significant decrease in PFT that may be considered as bronchoconstriction. The duration of athletic activity affects minute respiratory volume. We obtained statistically significant quantitative differences in the heart morphology and mean blood pressure. The identified changes should be estimated with personalized approach in each case and should not be considered as criteria for sport discontinuation.

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THE VALUE OF THE MICROBIAL FLORA OF THE NASAL AND OROPHARYNGEAL MUCOSA IN FORMATION OF CLINICAL AND IMMUNOLOGICAL FEATURES OF INFECTIOUS MONONUCLEOSIS IN CHILDREN

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Abstract

Objective. The article presents the results of studies determining significance of the microbial flora of the nasal mucosa and oropharynx in the formation of the clinical course and immune response in children with infectious mononucleosis (IM). **Materials and methods.** The study involved 93 children aged three to nine years, with mononucleosis. In 32 children (group 1), Streptococcus pyogenes at concentrations of 10^{-5} and higher was isolated during bacteriological examination of the mucosa of the nasopharynx and oropharynx. 30 (group 2) – 10^{-4} degrees or less. In 31 (group 3), Staphylococcus aureus, Spirochetae buccalis, E. Coli and other bacteria, except streptococcus, were shown in smears from the mucous membrane of the nasoropharynx. The immune status of patients was assessed by indicators of levels of lymphocytes $CD3^+$, $CD4^+$, $CD8^+$, $CD22^+$ and the content of interleukins 1β, 4, TNF-α. **Results.** The acute period of the mononucleosis in children of group 1 was characterized by more severe symptoms of intoxication, more severe morphological changes in the tissues of the tonsils, lymph nodes, liver and spleen. Also a significant decrease in the relative amount of $CD3^+$, $CD4^+$, $CD8^+$ was observed compared with the indicators of children of the second and third groups. The increase in blood $CD22^+$ content was more significant in children of the first group. The content of pro-inflammatory IL-1β and TNF-α in patients of all groups was significantly higher than in healthy children. The IL-4 increased in children of the second and third groups. In the period of early convalescence in children of the second and third groups, the relative content of $CD3^+$, $CD4^+$, $CD8^+$ cells approached the corresponding indices of the control group. This was not observed in children of the first group. $CD22^+$ levels in all observation groups decreased by the convalescence period, but remained high compared with the control group. In children of the studied groups, by the period of reconvalescence, a decrease in the levels of IL-1β, TNF-α was noted, more significant in children of the second and third groups. At the same time, in children of the first group, the level of pro-inflammatory interleukins by the period of reconvalescence remained at high numbers. The content of IL-4 was a significant difference in the indicators of its content in comparison with the digital characteristics of healthy ones in children of the second and third groups. **Conclusion.** An analysis of the results of the study found that the presence of streptococcus in its high concentration on the mucosa of the nosopharynx of children with mononucleosis already contributes to the formation of cellular immunosuppression and a pronounced reaction of pro-inflammatory interleukins at the initial stage of the disease, which, in general, leads to aggravation of the clinical manifestations of the disease and, in our opinion, may be a causative factor of a possible unfavorable course of the disease.

Keywords: *Children, infectious mononucleosis, Epstein-Barr virus, microbial flora, immunity.*

Introduction

Diseases of herpes virus etiology, including infectious mononucleosis (IM), in which Epstein–Barr virus (EBV) (herpes type 4 virus) is a causative agent, are currently a widespread pathology among the child population, both in Ukraine and abroad [1]. According to the WHO, more than 5 million children die every year from these diseases and their consequences in the world [2].

The results of laboratory studies of recent years convincingly prove infection with Epstein–Barr virus in almost 98% of people living on the

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Earth [3]. However, EBV diseases with the presence of appropriate symptoms are recorded much less frequently [4]. It is believed that in children under the age of 2 years, the disease can occur in the clinical form resembling acute respiratory viral infections, which is apparently due to their initial contact with the virus and, possibly, a feature of the immune response [5]. At the same time, in older people, a typical clinical picture of infectious mononucleosis develops [6].

The clinical picture of IM is characterized by fever, tonsilopharyngitis, lymphadenopathy, hepato- and splenomegaly, specific changes in the peripheral blood parameters [7].

Scientists argue that the clinical picture of the disease and the complications that can develop with IM depend on the microbial flora of the nasal and oropharynx mucosa of the patients [8, 9]. However, there are very few works devoted to the study of this problem in the literature, and their results are very contradictory [10].

Some authors prove that tonsillitis in IM has a viral and bacterial origin, while the role of the microbial flora is dominant [11, 12]. Others indicate the leading role of the virus in formation of pathological changes in the lymphoid formations of the nose and oropharynx of patients, and the participation of pathogenic microorganisms in the occurrence of tonsillitis is considered secondary [13, 14].

The difficulties in establishing the role of certain microorganisms in the defeat of the oropharynx are also due to the fact that healthy people have an extremely diverse microbial flora in the oral cavity and tonsils [15].

Staphylococus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae and other Streptococcus, Enterococcus, Lactobacillus Actinomices, Neisseria, Actinomyces, Clostridium, Pseudomonas, Staphylococus aureus are most often detected in the oral cavity and nasopharynx. Streptococcus dominates of all these bacteria and makes up 30–60% of the entire microflora. [16].

Owing to the recent studies, it was found that in 60–80% of healthy children with mucous membranes of the nasopharynx and oropharynx, Streptococcus is shown, which has a high pathogenic potential and can cause the development of a disease [17].

However, many scientists claim that the presence of Streptococcus is not in all concentrations pathogenic [18].

It is known that the clinical picture and outcome of any disease depends on the timeliness and adequacy of the immune responses of the human body. This is especially observed with herpesvirus pathology, including mononucleosis, a disease that is considered a disease of the immune system [19]. Active proliferation of EBV in all lymphoproliferative organs leads to their structural changes, which is reflected in the immune response (cellular and humoral) [20].

At the same time, in the available literature there are no works considering the effect of the microbial flora of the nasopharynx on formation of the immune response of patients, and hence the clinical picture of the disease, its course and outcomes. In our opinion, studies in this direction will improve prediction of the course of IM in children, outcomes and more reasonably outline ways to increase the effectiveness of treatment of patients.

2. Purpose, subjects and methods:

2.1. The purpose of the study was to determine the significance of the microbial flora of the nasal and oropharyngeal mucosa in the formation of the clinical course and immune response of children with infectious mononucleosis.

2.2. Subjects & Methods

The study involved 93 children aged three to nine years with mononucleosis of moderate severity who were treated at the Regional Children's Infectious Clinical Hospital in Kharkiv, Ukraine. The diagnosis of IM was verified by positive results of the investigation for the disease markers by ELISA (anti-EBV IgM and IgG) and PCR (detection of EBV DNA in the blood). In 32 children (group 1), Streptococcus pyogenes at concentrations of 10^{-5} and higher was isolated during bacteriological examination of the mucosa of the nasopharynx and oropharynx and in 30 children (2^{nd} group) – in 10^{-4} degrees or less. In 31 children (group 3) Staphylococcus aureus, Spirochetae buccalis, E. Coli and other bacteria, except streptococcus, were shown in smears from the mucous membrane of the nasoropharynx. The immune status of patients was assessed by indicators of levels of populations and subpopulations of peripheral blood lymphocytes, which were determined by indirect immunofluorescence using monoclonal antibodies to surface antigens of lymphocytes $CD3^+$, $CD4^+$, $CD8^+$, $CD22^+$ as well as the content in their blood of interleukins 1β, 4, TNF-α. The studies were carried out in the acute period (1–2 days of illness) and in the period of early convalescence (8–13 days). As a comparison, we took the corresponding indicators of 30 healthy children of the same age and gender.

Statistic processing of the results was carried out using computer programs Excel and Statistica 6.0. The reliability of the difference in values was

revealed using Student's test and Fisher's method. Differences were considered significant at a significance level of $P<0.05$.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results & Discussion

When comparing the clinical and laboratory parameters of the children of the compared groups, it was found that the children, whose Streptococcus pyogenes was isolated on the rhinopharyngeal mucosa at concentrations of 10^{-5} and higher, the clinical picture was characterized in the onset of the disease by a higher temperature reaction of the body, more severe morphological changes in the tissues of the tonsils, significantly more pronounced increase in submandibular and cervical lymph nodes, liver and spleen. In the blood of children of group 1, higher numbers of the relative content of neutrophils and low lympho-monocytes were determined (*Table 1*).

nasal and oropharyngeal mucosa in the acute period of the disease, a significant decrease in the number of CD3+, CD4+ CD8+ was observed compared with the indicators of children of groups 2 and 3 (P_2, $P_3<0.05$). The increase in blood $CD22^+$ content was more significant in children of group 1 ($P_1<0.05$, $P_2<0.05$, $P_3<0.05$).

Some authors argue that violation of the cellular-humoral reactivity of the body with a tendency to suppress cell-mediated mechanisms and enhance the humoral mechanisms of the immune response affects the clinical and biochemical manifestations of the disease and leads to its long-term undulating course. [21].

At the same time, other studies have revealed an increase in the activity of the cellular component of the immune response in children with IM in the acute period of the disease. In our opinion, the immune response in mononucleosis depends on many factors, including the patient's age, activity of the process, viral load, the initial

Table 1

Clinical and laboratory characteristics of the acute period of mononucleosis in children of the compared groups

Clinical and laboratory manifestations of the disease	Compared groups (M±m)		
	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)
1.Temperature of the body, °C	39.2±0.21 [1,2]	38±0.31	37.7±0.27
2. Tonsillitis:			
- catarral (%)	11.28±1.91[1,2]	29.56±2.03	33.32±1.43
- purulent (quinsy) (%)	88.72±2.04[1,2]	71.44±1.87	67.67±0.09
3. Lymph node size centimeters (cm)			
- submandibular	2.5±0.13[1,2]	1.5±0.32	1.5±0.12
- cervical	1.5±0.31[1,2]	1.2±0.24	0.9±0.32
4. Enlargement of the spleen (cm)	3.3±0.26 [1,2]	2.1±0.24	2.0±0.13
5. Enlargement of the liver (cm)	2.1±0.24[1,2]	1.03±0.17	1.03±0.36
6. Analysis of the blood:			
- count of the neutrophils (%)	62.08±1.62[1,2]	41.33±1.97	42.26±1.13
- count of the lymphocytes (%)	34.03±1.22[1,2]	49.17±2.03	50.13±1.17

Note:
P1 – probability of the characteristic of group 1 relative to group 2;
P2 – probability of the characteristic of group 1 relative to group 3;
P3 – probability sign between groups 2 and 3.

In the children of group 1, the course of the disease was longer and amounted to 17.56 ± 1.56 days, group 2 – 13.24 ± 1.37 and in the children of group 3 – 10.24 ± 1.54 days.

The differences in the severity of clinical manifestations and the results of paraclinical examination which was given, as well as taking into account the importance of immune factors in this, we conducted studies to determine the immune status of children in all groups (*Table 2*).

It should be noted that in children with a high degree of insemination of streptococcus of the

background of the patient and the presence of comorbidities, etc., and needs further investigation [22].

However, these studies concerned children with mononucleosis in the form of mono-infection without taking into account the presence of coccal flora on the mucous membranes of the nasopharynx and its amount, which may affect the immune response of children.

All children have characteristic signs of activation of anti-infection protection in the acute period of mononucleosis. The content of pro-

Table 2

Indicators of the immune status of patients in the acute period of mononucleosis (M ± m)

Indicator	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)	Controls (n=30)
CD 3+, %	47.16±0.74[1,2,3]	57.30±0.52[1]	58.10±0.72[1]	60.60±1.20
CD 4+, %	30.57±0.42[1,2,3]	33.86±0.34[1]	34.02±0.22[1]	36.30±0.75
CD 8+, %	19.37±0.34[1,2,3]	23.40±0.39[1]	23.60±0.12[1]	25.70±0.68
CD 22+, %	35.60±0.25[1,2,3]	32.56±0.42[1]	32.63±0.28[1]	17.30±0.79
Interleukins: pg / ml IL-1β	18.66±1.19[1,2,3]	9.80±0.91[1]	7.90±0.84[1]	5.32±1.73
IL-4	3.66±0.24[1,2,3]	5.40±0.30[1]	5.10±0.21[1]	2.06±0.94
TNF-α	11.20±1.43[1,2,3]	6.90±1.12[1]	6.30±1.08[1]	2.69±1.67

Note here and further:
P1 – reliability of the difference in the digital values of indicators of healthy and sick children;
P2 – probability of the characteristic of group 1 relative to group 2;
P3 – probability of the characteristic of group 1 relative to group 3;
P4 – probability sign between groups 2 and 3.

inflammatory IL-1β in blood serum in the onset of the disease in patients of all groups was significantly higher than in healthy children (P1<0.05). In children with additional infection of the nasal and oropharynx mucosa with streptococcus with a high degree of seeding, the concentration of IL-1β was 18.66 ± 1.19 pg/ml and was significantly higher than the corresponding indicators of the second group - 9.8 ± 0.91 pg/ml (P2<0.05) and the third – 7.90 ± 0.84 pg ml (P3<0.05).

The acute period of mononucleosis is accompanied by a significant increase in the blood level of patients with TNF-α compared with its content in healthy children (P1<0.05), while the level of pro-inflammatory TNF-α in patients of the first group was significantly higher than similar indicators in children of the second and third groups (P2<0.50; P3<0.05).

When studying the levels of IL-4 in the blood serum of children of the studied groups, an increase in its content was revealed in comparison with healthy children, however, a significant difference in its content was determined only in the indicators of children of the second, third and control groups (P2<0.05, P3<0.05). And although in patients of the first group there was an increase in the level of IL-4 in the blood, it was less significant than in children of the second and third groups to healthy children (P1<0.05).

In the period of early convalescence in children of groups 2 and 3, the relative content of $CD3^+$, $CD4^+$, $CD8^+$ cells approached the corresponding indices of the controls (P2,3≥0.05), which indicated a tendency to normalize the cellular immunity of patients. This was not revealed in children which were seeding streptococcus with a high degree on the mucosa of the nasal and oropharynx. in children of group 1, the content of $CD3^+$, $CD4^+$, $CD8^+$ increased in the period of IM convalescence compared with the acute period, but was significantly lower (P1<0.05) compared with the control group. As in the acute period, in the period of IM convalescence in children of group 1, signs of a cellular immune response deficiency were found, which must be taken into account in the dynamics of correction of the therapy (*Table 3*).

CD22+ levels in all observation groups decreased by the convalescence period, but

Table 3

Indicators of the immune status of patients in the early reconvalescence period of mononucleosis (M±m)

Indicator	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)	Controls (n=30)
CD 3+, %	47.16±0.74[1,2,3]	57.30±0.52[1]	58.10±0.72[1]	60.60±1.20
CD 4+, %	30.57±0.42[1,2,3]	33.86±0.34[1]	34.02±0.22[1]	36.30±0.75
CD 8+, %	19.37±0.34[1,2,3]	23.40±0.39[1]	23.60±0.12[1]	25.70±0.68
CD 22+, %	35.60±0.25[1,2,3]	32.56±0.42[1]	32.63±0.28[1]	17.30±0.79
Interleukins: pg / ml IL-1β	18.66±1.19[1,2,3]	9.80±0.91[1]	7.90±0.84[1]	5.32±1.73
IL-4	3.66±0.24[1,2,3]	5.40±0.30[1]	5.10±0.21[1]	2.06±0.94
TNF-α	11.20±1.43[1,2,3]	6.90±1.12[1]	6.30±1.08[1]	2.69±1.67

remained high compared with the controls (P1<0.05).

In children of the studied groups, by the period of reconvalescence, a decrease in the levels of IL-1β, TNF-α was noted, which was more significant in children of groups 2 and 3, in which there was no mathematical difference in the levels of these interleukins in blood compared with healthy children (P2,3> 0.05).

At the same time, in children of group 1, the level of pro-inflammatory interleukins by the period of reconvalescence remained at high numbers (P1<0.05).

The content of anti-inflammatory interleukin-4 in the blood of children by the period of their recovery exceeded these indicators of the acute period. However, in the reconvalescence period only in children of groups 2 and 3 there was a significant difference in IL-4 in comparison with the digital characteristics of healthy ones (P2,3<0.05).

Conclusions

1. Microorganisms that are present on the mucous membrane of the nasal and oropharynx have a different effect on the formation of the immune response of children with mononucleosis. This explains the differences in the severity of clinical symptoms and the duration of the disease.

2. Streptococcus is the most aggressive microbial structure that negatively affects the immune response of patients with mononucleosis. Moreover, the degree of the indicated effect is proportional to the level of streptococcus contamination of the nasopharyngeal mucosa.

3. The most significant deviations from the norm of the indicators of the immune response of patients with mononucleosis are observed in children in whom streptococcus 10-5 and higher is sown on the mucous membrane of the nasal and oropharynx. In our opinion, the immunosuppressive state is a factor in the prolongation of the disease.

4. The differences that were identified as a result of the study can serve as an additional criterion for predicting the course of mononucleosis and the choice of therapy, which will reduce adverse outcomes and improve treatment.

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SYNDROMAL CHARACTERISTICS OF DEPRESSIVE MANIFESTATIONS IN PATIENTS WITH COGNITIVE IMPAIRMENTS AT DEPRESSIVE DISORDERS

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Abstract

362 patients with cognitive impairment at depressive disorders were examined, of them 123 patients with recurrent depressive disorder (RDD), 141 patients with bipolar affective disorder (BAD) and 98 patients with prolonged depressive reaction (PDR). Differentiated clinical and psychopathological features of patients with cognitive impairment at depressive disorders were established: 1) combination of apathetic-adynamic, astheno-energetic and anxious symptom complexes; predominance of moderate and major depressive episodes; severity of apathy, subjective and objective signs of depression, decrease in concentration and ability to feel were determined in patients with RDD; 2) combination of astheno-energetic, apathetic-adynamic, and melancholic symptom complexes; predominance of moderate and major depressive episodes; the severity of apathy, subjective signs of depression, suicidal thoughts, insomnia and decrease in concentration in patients with BAD; 3) combination of anxious and apathetic-adynamic symptom complexes; the predominance of moderate and minor depressive episodes; the severity of internal stress, apathy, suicidal thoughts and loss of appetite in patients with PDR.

Keywords: *patients with cognitive impairment, depressive disorders, clinical and psychopathological features, recurrent depressive disorder, bipolar depressive disorder, prolonged depressive reaction.*

Introduction

The relevance of the clinical study of depressive disorders is associated with the high prevalence of depression in both psychiatric and general somatic practice, involving 10 % of the population [1]. 20 % of women and 10 % of men experience a depressive episode during their lifetime [1–3].

According to the WHO, in the recent decade depression has become not only a medical but also a social problem that needs to be addressed due to such adverse effects as a significant reduction in the social functioning of these patients, suicide problems, and finally significant economic disability losses. [1, 4, 5]. The increase in the number of affective, in particular, depressive states, is associated with a number of factors: an increase in the number of affective disorders, pathomorphosis of mental illnesses occurring at milder psychopathological levels, tendencies of mental disorders to somatization with complex autonomic and visceral disorders and maladaptation [6].

It should be noted that there are significant difficulties in providing medical and rehabilitation care for the patients with depression. Thus, duration of depressive episodes today is from 3 to 5 years [2, 5, 7]. Proper diagnosis and administration of antidepressants takes place on an average two years after the onset of depression [1, 8–10]. The share of depression in patients who seek help in general medical institutions of outpatient and hospital network, ranges from 10 % to 22–33 %, and only in 10–30 % of cases depressive disorders are recognized as such by general practitioners [4, 7, 9, 11]. In most cases, depression has a chronic long-term course. According to the level of disability, the population of patients with depression exceeds the population of patients with schizophrenia [5, 12]. The difficulties in recognizing depression at the initial stage of the disease can be explained by variety of somatic complaints that

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hide affective pathology. Adequate clinical evaluation is important both for the prognosis of depressive disorder and for the choice of effective therapy. Therefore, investigation aimed at clarifying the differential clinical and psychopathological features of anxiety and depressive manifestations in patients with cognitive impairment (CI) at depressive disorders (DD) is relevant and can improve diagnostic criteria and effectiveness of treatment of different types of DD.

2. Purposes, subjects and methods:

2.1. Purpose of the study was to identify the clinical and psychopathological features of the leading symptom complexes and severity of depressive manifestations in patients with cognitive impairment at different types of depressive disorders.

2.2. Subjects & Methods

The study involved 362 patients with CI at DD: 123 patients with recurrent depressive disorder (RDD), 141 patients with bipolar affective disorder (BAD) and 98 patients with prolonged depressive reaction (PDR).

The study used an integrated approach, which consisted of the use of clinical-psychopathological, psychometric (Montgomery–Asberg Depression Rating Scale (MADRS)) and statistical methods [13]. Statistical data processing was used to determine the average values of quantitative parameters, their standard errors (in the format % ± m %), the reliability of differences (Student-Fisher criteria [t], Kolmogorov–Smirnov [λ]). Statistical processing of the results was performed using Excel-2010 and STATISTICA 6.1.

Conflict of interests

There is no conflict of interests. We certificate that we do not have any financial or personal relationships that might bias the content of this work.

3. Results & Discussion

The group of the patients with RDD consisted of 57 men (46.34 ± 2.78) % and 66 women (53.66 ± 2.99) %, that with BAD – 76 men (53.90 ± 2.61) % and 65 women (46.10 ± 2.42) %, with PDR – 43 men (43.88 ± 3.39) % and 55 women (56.12 ± 3.83) %, which is generally consistent with the typical distribution by sex in DD. That is, women predominated (51.96 %, DC = 0.66, MI = 0.02, p = 0.046), only in the group of patients with BAD there were more men (53.90 %, DC = 0, 66, MI = 0.02, p = 0.046). The predominant number of patients with CI in DD was characterized by the age of 30–44 years (38.12 %). There were more young people (18–29 years) among patients with PDR (21.43 %, DC = 8.19, MI = 0.74, p = 0.0001) and among patients with BAD (31.21 %, DC = 9.82, MI = 1.37, p = 0.0001), and middle-aged people (45–59 years old) and elderly people (60–65 years old) among patients with RDD (37.40 %, DC = 1.54, MI = 0.09, p = 0.016 and 17.07 %, DC = 4.78, MI = 0.27, p = 0.002).

Differentiated analysis of syndromal variants of depressive disorder in patients with cognitive impairment is presented in *Figure 1*.

Invetigation of the leading type of affect revealed that apathetic-dynamic (27.64 ± 1.93) %, astheno-energetic (26.83 ± 1.88) %, anxious (13.01 ± 0, 99) % and senesto-hypochondriac (11.38 ± 0.88) % symptom complexes dominated in the patients with RDD. The prevalence of dreary (5.69 ± 0.45) %, dysphoric (7.32 ± 0.58) % and obsessive-depressive (8.13 ± 0.64) % of symptom complexes was significantly lower.

Analysis of the syndromal structure of depressive disorders in BAD allowed to determine

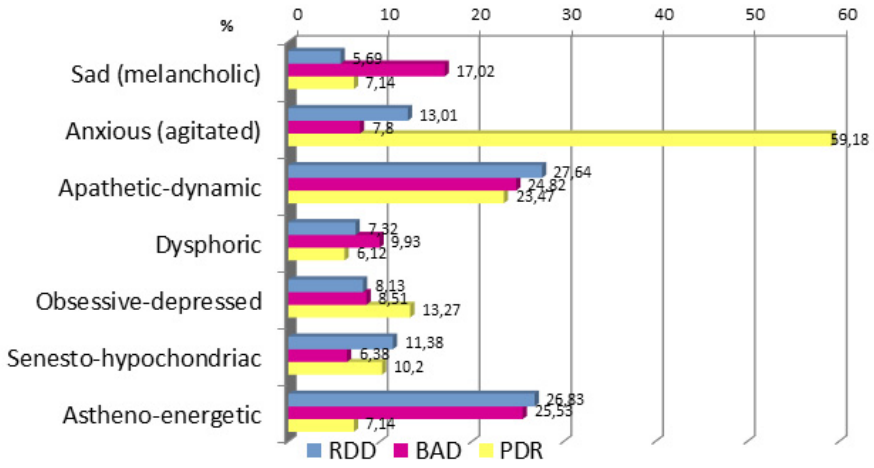

Fig. 1. Syndromal variants of depressive manifestations in patients with cognitive impairment at depressive disorders

the predominance of astheno-energetic (25.53 ± 1.57) %, apathetic-dynamic (24.82 ± 1.54) % and melancholic (17.02 ± 1.11) % syndromes. depressive episode (36.59 ± 2.39) %; 34.96 % of persons had a major depressive episode and 28.46 % of patients had a small depressive episode (*Fig. 2*).

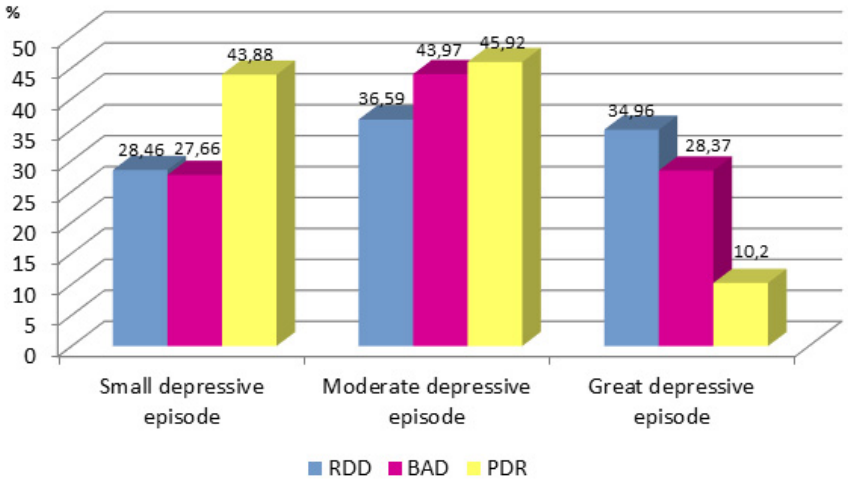

Fig. 2. Severity of depression in patients with cognitive impairment at depressive disorders (MADRS)

Senesto-hypochondric (6.38 ± 0.44) %, agitated (7.80 ± 0.54) %, obsessive-depressive (8.51 ± 0.58)% and dysphoric (9.93 ± 0.67) % symptom complexes were significantly less common.

In patients with PDR, the first place among the typical affective syndromes is occupied by anxiety (59.18 ± 3.90) %, as well as apathetic-dynamic (23.47 ± 2.12) %, obsessive-depressive (13.27 ± 1.27) % and senesto-hypochondriac (10.20 ± 1.00) % syndromes. The least common were dysphoric (6.12 ± 0.61) %, sad (7.14 ± 0.71) % and astheno-energetic (7.14 ± 0.71) % syndromes.

Statistical analysis of the syndromal structure of depressive disorders showed that the patients with BAD had a more pronounced sadness syndrome (17.02 ± 1.11) %, compared with the patients with RDD and PDR (5.69 %, DC = 4.76, MI = 0.27, $p < 0.0023$ and 7.14 %, DC = 3.77, MI = 0.19, $p < 0.0124$, respectively). The patients with PDR had a more pronounced anxiety syndrome (59.18 ± 3.90) % compared with patients with RDD and BAD (13.01 %, DC = 6.58, MI = 1.52, $p < 0.0001$ and 7.80 %, DC = 8.80, MI = 2.26, $p < 0.0001$) and the least pronounced astheno-energetic syndrome (7.14 ± 0.71) %, which was more characteristic of the patients with RDD (26.83 %, DC = 5.75, MI = 0.57, $p < 0.0001$) and BAD (28.57 %, DC = 5.53, MI = 0.51, $p < 0.0001$).

The clinical and psychopathological method was supplemented by psychometric scales for assessing depression MADRS, the results of which allowed determining that among patients with RDD dominated the persons with a moderate

Among patients with BAD, the vast majority of people had a moderate severity of depressive episode (43.97 ± 2.35) %, 28.37 % of patients had a major depressive episode and 27.66 % had a minor depressive episode. The patients with moderate (45.92 ± 3.48) % and minor (43.88 ± 3.38) % depressive episodes predominated among patients with PDR, only 10.20 % of them were diagnosed with major depressive episode. The conducted statistical analysis allowed to establish that the patients with a small depressive episode predominated among persons with PDR (43.88 ± 3.38) % than among patients with RDD (28.46 %, DC = 1.88, MI = 0, 15, $p < 0.0068$) and BAD (27.66 %, DC = 2.00, MI = 0.16, $p < 0.0039$), in which there were more patients with a major depressive episode (34.96 % and 28.37 %, respectively) compared with the patients with PDR (10.20 %, DC = 5.35, MI = 0.66, $p < 0.0001$ and DC = 4.44, MI = 0.40, $p < 0.0003$ respectively).

The patients with a moderate depressive episode predominated in the group with PDR (45.92 ± 3.48) % and BAD (43.97 ± 2.35) % than in the group with with RDD (36.59 %, DC = 0.99 , MI = 0.05, $p < 0.041$ and DC = 0.80, MI = 0.03, $p < 0.047$, respectively).

The study analyzed in more detail the features of manifestation of depressive disorders in patients with CI at different types of depression (*Fig. 3*). Thus, according to MADRS, it was determined that in patients with RDD the most pronounced depressive manifestations were impaired concentration (5.89 ± 2.05 points), subjective and objective signs of depression (5.88 ± 2.11 and 5.67 ± 2.09 points, respectively),

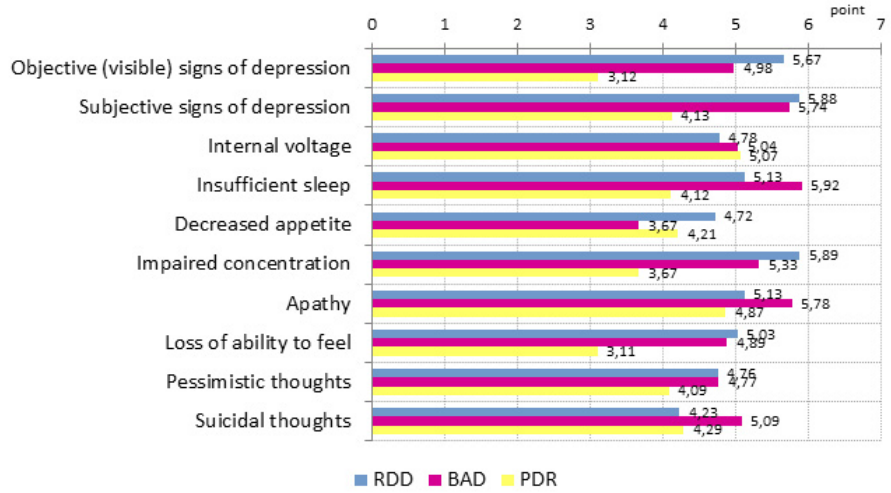

Fig. 3. Features of depressive manifestations in patients with cognitive impairment at depressive disorders (according to the MADRS scale)

apathy (5.13 ± 2.42 points) and loss of ability to feel (5.03 ± 3.07 points). In patients with BAD, the most pronounced manifestations of depressive disorders were insufficient sleep (5.92 ± 2.89 points), apathy (5.78 ± 3.06 points), subjective signs of depression (5.74 ± 3.11 points), impaired concentration (5.33 ± 2.86 points) and suicidal thoughts (5.09 ± 3.06 points). In patients with PDR depressive manifestations were expressed primarily by the presence of internal stress (5.07 ± 1.77 points), apathy (4.87 ± 1.46 points), suicidal thoughts (4.29 ± 2.46 points) and decreased appetite (4.21 ± 1.80 points).

Statistical analysis of the results revealed that the patients with RDD and BAD differed from those with PDR by greater severity of such depressive manifestations as subjective (5.88 ± 2.11 points, $p < 0.005$ and 5.74 ± 3.11 points , $p < 0.0025$, respectively) and objective (5.67 ± 2.09 points, $p < 0.001$ and 4.98 ± 3.08 points, $p < 0.0025$, respectively) signs of depression, more pronounced sleep disturbances (5.13 ± 2.02 points, $p < 0.025$ and 5.92 ± 2.89 points, $p < 0.001$, respectively), decreased concentration (5.89 ± 2.05 points, $p < 0.0001$ and 5.33 ± 2.86 points, $p < 0.005$, respectively) and loss of ability to feel (5.03 ± 3.07 points, $p < 0.01$ and 4.89 ± 2.03 points, $p < 0.025$, respectively). It was also found that the decrease in appetite was more pronounced in patients with RDD (4.72 ± 1.87) than in patients with BAD (3.67 ± 1.94 points, $p < 0,043$).

According to numerous studies, the presence of depressive disorders is associated with the pathomorphosis of mental disorders, tendencies to somatization with complex autonomic and visceral disorders and maladaptation of patients [4, 5]. The obtained results coincide with the literature data and indicate that depressive disorders in patients with cognitive impairments have a complex syndromal structure, which differs depending on the type of depressive disorder. The obtained data will help to expand the scientific understanding of the syndromal characteristics of depressive manifestations in patients with cognitive impairment in depressive disorders and to improve the strategy of providing treatment and rehabilitation care to patients with depression.

Conclusions

Our findings demonstrate that:

– in patients with RDD, a combination of apathetic-dynamic (27.64 %), astheno-energetic (26.83 %, $p < 0.05$), anxiety (13.01 %) symptom complexes; predominance of moderate and major depressive episodes (36.59 % and 34.96 %, respectively); predominance of the following clinical signs of depression: apathy (5.13 points), subjective and objective signs of depression (5.86 and 5.67 points, respectively), decreased concentration (5.89 points), loss of ability to feel (5 .03 points) can be determined;

– in patients with BAD, a combination of astheno-energetic (25.53 %, $p < 0.05$), apathetic-dynamic (24.82 %), melancholic (17.02 %, $p < 0.05$) symptom complexes; predominance of moderate and major depressive episodes (43.97 % and 28.37 %, respectively); predominance of the following clinical signs of depression: apathy (5.78 points), subjective signs of depression (5.74 points), suicidal thoughts (5.09 points), sleep disturbances (5.92 points), decreased concentration (5.33 points) can be determined;

– in patients with PDR, a combination of anxiety (59.18 %, $p < 0.05$), apathetic-adynamic (23.47 %)

symptom complexes; predominance of moderate and minor depressive episodes (45.92 % and 43.88 %, respectively); predominance of the following clinical signs of depression: internal tension (5.07 points), apathy (4.87 points), suicidal thoughts (4.29 points), loss of appetite (4.21 points) can be determined.

Thus, the study identified clinical and psychopathological features of depressive manifestations and leading symptom complexes in patients with CI in different types of DD, which can be used as diagnostic criteria and should be considered at implementation of psychocorrectional and rehabilitation measures.

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