Wiadomości Lekarskie
Medical Advances

VOLUME LXXVI, ISSUE 11, NOVEMBER 2023
Official journal of Polish Medical Association has been published since 1928

ISSN 0043-5147
E-ISSN 2719-342X

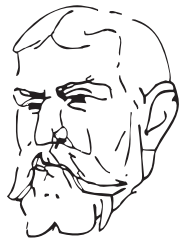
INDEXED IN PUBMED/MEDLINE, SCOPUS, EMBASE, EBSCO, INDEX COPERNICUS,
POLISH MINISTRY OF EDUCATION AND SCIENCE, POLISH MEDICAL BIBLIOGRAPHY

Wiadomości Lekarskie
Medical Advances

VOLUME LXXVI, ISSUES 11, NOVEMBER 2023
Official journal of Polish Medical Association has been published since 1928

ISSN 0043-5147
E-ISSN 2719-342X

ALUNA Publishing



Memory of
dr Władysław
Biegański

Wiadomości Lekarskie is abstracted and indexed in: PUBMED/MEDLINE, SCOPUS, EMBASE, INDEX COPERNICUS, POLISH MINISTRY OF EDUCATION AND SCIENCE, POLISH MEDICAL BIBLIOGRAPHY

The journal Wiadomości Lekarskie is cofinanced under Contract No.RCN/SN/0714/2021/1 by the funds of the Minister of Education and Science

Wiadomości Lekarskie
Medical Advances

Distribution and Subscriptions:
Bartosz Guterman prenumerata@wydawnictwo-aluna.pl

Graphic design / production:
Grzegorz Sztank fajne.work

Publisher:
ALUNA Publishing
ul. Przesmyckiego 29,
05-510 Konstancin – Jeziorna
www.wydawnictwo-aluna.pl
www.wiadomoscilekarskie.pl
www.wiadlek.pl

CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

CASE STUDIES

ORIGINAL ARTICLE

FROM TEXT TO DIAGNOSE: CHATGPT'S EFFICACY IN MEDICAL DECISION-MAKING

DOI: 10.36740/WLek202311101

Yaroslav Mykhalko, Pavlo Kish, Yelyzaveta Rubtsova, Oleksandr Kutsyn, Valentyna Koval
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: Evaluate the diagnostic capabilities of the ChatGPT in the field of medical diagnosis.

Materials and methods: We utilized 50 clinical cases, employing Large Language Model ChatGPT-3.5. The experiment had three phases, each with a new chat setup. In the initial phase, ChatGPT received detailed clinical case descriptions, guided by a "Persona Pattern" prompt. In the second phase, cases with diagnostic errors were addressed by providing potential diagnoses for ChatGPT to choose from. The final phase assessed artificial intelligence's ability to mimic a medical practitioner's diagnostic process, with prompts limiting initial information to symptoms and history.

Results: In the initial phase, ChatGPT showed a 66.00% diagnostic accuracy, surpassing physicians by nearly 50%. Notably, in 11 cases requiring image interpretation, ChatGPT struggled initially but achieved a correct diagnosis for four without added interpretations. In the second phase, ChatGPT demonstrated a remarkable 70.59% diagnostic accuracy, while physicians averaged 41.47%. Furthermore, the overall accuracy of Large Language Model in first and second phases together was 90.00%. In the third phase emulating real doctor decision-making, ChatGPT achieved a 46.00% success rate.

Conclusions: Our research underscores ChatGPT's strong potential in clinical medicine as a diagnostic tool, especially in structured scenarios. It emphasizes the need for supplementary data and the complexity of medical diagnosis. This contributes valuable insights to AI-driven clinical diagnostics, with a nod to the importance of prompt engineering techniques in ChatGPT's interaction with doctors.

KEY WORDS: artificial intelligence, large language models, ChatGPT, clinical decision support, diagnose

Wiad Lek. 2023;76(11):2345-2350

INTRODUCTION

Artificial intelligence (AI) is defined as "the ability of a digital computer or computer-controlled robot to perform tasks commonly associated with intelligent beings" [1]. AI involves creating computer systems or software that can mimic human thinking processes, such as learning from experience, reasoning, problem-solving, and decision-making.

AI systems are designed to analyze and process large amounts of data, extract meaningful patterns, and make predictions or decisions based on that analysis. It enables tasks like speech recognition, natural language understanding, image and video analysis, recommendation systems, and autonomous decision-making.

Large language models (LLM) represent a specific category of AI models that emulate and comprehend human-like text. Built upon deep learning methodologies, these models employ neural networks characterized by multiple layers and a multitude of parameters. The primary objective underlying LLM is to attain an understanding of the structure, syntax, semantics, and contextual nuances of natural language. This comprehension equips them to generate responses that are not only coherent but also contextually relevant. Consequently, they acquire the ability to generate responses covering an expansive array of subjects [2]. The contemporary landscape witnesses an upsurge in the utilization and refinement of LLMs, attributed to the accessibility of extensive datasets and the evolution of AI technologies. This progression has resulted in the significant enhancement of these models' capabilities, enabling them to yield text that closely resembles human-generated content. Furthermore, these models have demonstrated exceptional performance across a spectrum of natural language processing tasks, underscoring their multifaceted potential [3].

In order to be useful for medical application LLMs undergo training on medical data through various methodologies, enhancing their applicability in the medical domain. One fundamental approach involves provisioning pertinent training data, encompassing electronic health records, medical literature, and clinical data. By immersing LLMs in this diverse dataset, they cultivate specialized knowledge tailored to distinct medical disciplines [4]. A pivotal step in the training process entails pre-training LLMs on expansive text

datasets which equips LLMs with an understanding of language structures and patterns. Consequently, they can be adeptly repurposed across a multitude of domains and tasks, a versatility that extends to medical applications [5]. Following pre-training, LLMs undergo fine-tuning on medical data, facilitating adaptation to specific medical functions. For instance, LLMs can be fine-tuned for tasks like clinical decision support or scientific writing assistance. This involves refining the model's capabilities through exposure to a smaller dataset of medical text, thereby enhancing its performance on targeted medical tasks [6,7].

AI has made significant strides within the medical field, reshaping various aspects of healthcare and paving the way for improved patient outcomes and more efficient healthcare processes. One prominent application lies in Biomedical Information Extraction, where AI is harnessed to dissect biomedical texts and extract structured data such as named entities and semantic relationships [8]. Similarly, AI's transformative impact is evident in Drug Discovery and Development, where it plays a pivotal role in tasks like peptide synthesis, virtual screening, toxicity prediction, and drug monitoring. This AI-driven approach not only slashes time consumption and production costs but also introduces efficiencies that address the inefficiencies inherent in traditional drug design methods [9]. The integration of AI into Medical Diagnosis and Treatment is particularly noteworthy. Across various medical disciplines, machine learning algorithms are employed to evaluate radiological images, pathology slides, and electronic medical records [10]. Technologies based on AI and large language models in particular are increasingly penetrating into various areas of the medical industry such as anesthesiology, dentistry, radiation medicine, ophthalmology, cardiology and many others [11-15]. There were attempts to evaluate the performance of LLMs on medical examination.

Usage of AI in supporting clinical decision-making is of great interest as it holds the promise of revolutionizing the healthcare landscape by harnessing the capabilities of artificial intelligence to augment and inform the decision-making processes of medical professionals [16-18].

THE AIM

Evaluate the diagnostic capabilities of the ChatGPT in the field of medical diagnosis

MATERIALS AND METHODS

A selection of 50 clinical cases from the Medscape Case Challenge series, spanning the period between February and July 2023, was utilized for analysis. As the LLM ChatGPT-3.5 was used.

The experiment was structured into three phases. New chat was created for every phase of the study. In the initial phase, comprehensive clinical case descriptions were presented to ChatGPT, encompassing patient complaints, the history of the present illness, past medical records, data derived from physical examinations, outcomes of laboratory tests, and results from imaging studies. At this phase our methodology incorporated a prompt engineering technique referred to as "Persona Pattern". This approach was implemented to guide ChatGPT's responses and to encourage it to simulate the analytical process and decision-making of a proficient medical practitioner when diagnosing the presented clinical cases.

Subsequently, the cases in which the LLM made diagnostic errors were included in the second phase. For such cases, the LLM was proposed a roster of potential diagnoses extracted from the presented cases. We used the same prompt formulation as in the previous phase, ChatGPT was then prompted to choose the most suitable diagnosis from the provided list.

The third and final phase of the experiment focused on assessing ChatGPT's ability to emulate the decision-making process of a medical practitioner in a patient-doctor interaction. The structured prompt was designed to guide ChatGPT in adopting a professional medical perspective and mimicking the diagnostic reasoning of a skilled medical practitioner, while also allowing the model to access supplementary diagnostic information when required. In this context, each clinical case's initial information was restricted to encompass only the description of symptoms, patient complaints, medical history, and physical examination data. Within this constrained framework, ChatGPT was tasked with determining the optimal diagnosis and proposing a set of supplementary investigative methods necessary for precise elucidation. In cases when the required information was available in the clinical case, it was provided to the LLM. Conversely, if such information was absent, the LLM was apprised that the data was unavailable for consideration. This research methodology facilitated a comprehensive evaluation of ChatGPT's diagnostic capacities within a simulated clinical context.

During each phase, a comparative analysis was conducted to assess the accuracy of responses provided by ChatGPT in contrast to medical professionals who resolved the same clinical cases on the Medscape platform. This comparative evaluation allowed us to gauge the alignment between ChatGPT's diagnostic performance and the practicing physicians.

Statistical data are presented as M±SD.

RESULTS

During the initial phase, ChatGPT demonstrated the overall accurate diagnostic capability in 66.00% of cases, whereas the accuracy of physicians on the Medscape platform averaged at 44.82±18.54% which was almost 50% lower than LLM's result.

Of particular significance is a subgroup of 11 clinical cases where the results of supplementary diagnostic methods, including ECG, CT, MRI, and blood smear images, were presented without their corresponding interpretations. The assumption was that physicians have to analyze these images by themselves, upon which the accuracy of the ultimate diagnosis relied heavily. In these instances, the clinical case descriptions were initially provided to ChatGPT without including the interpretation of the aforementioned diagnostic findings. If ChatGPT produced an inaccurate diagnosis, the subsequent step involved offering it the interpretations of these diagnostic studies, performed by the authors of the present study. Notably, across this subset, ChatGPT erred in diagnosis for 3 cases. However, for 4 cases, the correct diagnosis was subsequently achieved after presenting ChatGPT with the additional interpretations of the diagnostic studies. Impressively, in the remaining 4 cases, ChatGPT accurately diagnosed the cases without requiring supplementary interpretations of the diagnostic findings. This subset underscores the intricate interplay between diagnostic expertise and the interpretation of visual diagnostic data, a domain in which ChatGPT demonstrated varying degrees of proficiency.

Among the subset of clinical cases in which ChatGPT accurately identified the diagnosis, real healthcare practitioners achieved an average diagnostic accuracy of 46.55±17.00%. For the clinical cases where ChatGPT did not return correct diagnosis, the average accuracy rate achieved by actual medical practitioners was lower than in the previous subset (41.47±21.38%) but the difference was not statistically significant ($p>0.05$).

In the second phase, a subset of 17 clinical cases was chosen. ChatGPT was presented with comprehensive clinical case descriptions, as in the preceding phase, along with an additional roster of potential diagnoses given in original descriptions on Medscape. Its objective was to choose the most suitable option from the provided variants. Remarkably, the LLM demonstrated a correct decision-making rate of 70.59% within this context. This achievement was nearly twice as high as the average percentage of accurate responses recorded among doctors, which stood at 41.47%.

Among the clinical cases in which ChatGPT correctly identified the diagnosis, the average accuracy rate for real doctors was 45.50±21.94%. However, in instances where ChatGPT's diagnosis was not accurate, the average accuracy of human doctors' responses was 31.80±18.42% ($p>0.05$).

The third phase, meticulously designed to emulate the decision-making process of a real healthcare worker, witnessed ChatGPT achieving accurate disease diagnoses in 23 out of 50 cases, yielding a success rate of 46.00%. In situations where ChatGPT provided accurate diagnoses, the average percentage of correct responses from doctors, who were presented with comprehensive patient data and potential diagnoses to choose from, was very close to ChatGPT's performance at 45.65±18.51%. Moreover, in cases where the LLM, ChatGPT, faced challenges in correctly diagnosing the disease, medical professionals attained an average accuracy rate of 44.11±18.90%.

Within this phase, it is pertinent to underscore distinct subsets of cases that yielded noteworthy observations. In 8 cases, ChatGPT demonstrated a high level of diagnostic acumen by accurately diagnosing diseases without requesting any supplementary information, achieving a correct diagnosis in 5 out of these 8 instances. In 12 cases, the initial diagnostic output of the LLM was erroneous. Although the diagnosis has been changed upon the provision of additional information, it remained inaccurate. In 11 cases, ChatGPT's initial diagnoses were incorrect. However, upon the acquisition of the requested supplemental information, the diagnoses were revised to correct ones. Notably, in 18 cases, the LLM's preliminary diagnostic decisions remained consistent even after the inclusion of the requested supplementary information. Lastly, it's worth noting that in a singular case, an initially accurate diagnosis was altered to an incorrect one following the incorporation of the requested supplementary information.

DISCUSSION

Artificial intelligence is increasingly penetrating into all spheres of human activity and there is no doubt that this process will only intensify over time. Until recently, access to this technology was the prerogative of a limited number of people who used it to solve specific scientific or industrial problems. However, opening access to this technology to the general public has become a real revolution. In this context, large language models deserve special attention, which allow generating texts based on queries entered by the user. Trained on a large amount of data, it can serve as a good assistant in daily activities. However, far from always, the obtained results coincide with expectations and correspond to reality. This is especially important to consider when trying to use this technology in professional activities, especially

in the field of medicine. Nevertheless, this phenomenon should not be completely removed or ignored either. In this situation, it is critical to find a balance and a certain edge, which can only be done empirically. Therefore, it is important to conduct researches and evaluate the possibilities of AI in solving specific practical issues. Such an attempt was made in this study. To do this, clinical cases offered by Medscape platform in the Case Challenge series were used. These cases often cover scenarios that are not commonly encountered by most clinicians but nonetheless occur in actual clinical practice. In this way it gives medical professionals a unique opportunity to test their knowledge, diagnostic and treatment skills in a wide variety of medical fields. The clinical cases are well described and contain all the information necessary to answer the questions posed. In the majority of cases medical practitioners should diagnose the disease based on the given description and choose the correct option from the proposed 4 to seven variants. This approach facilitates the decision-making process for doctors, as they are not required to navigate through the entirety of possible conditions. Instead, their diagnostic considerations are limited to the predefined options, significantly simplifying the task of making an accurate diagnosis. Nevertheless, in this relatively simple situation the average percentage of correct diagnoses made by real doctors in 50 clinical cases chosen for this study was lower than the 50% (44.82±18.54%). This underscores the intricate nature of medical diagnosis and highlights the challenges that healthcare professionals encounter in arriving at correct diagnoses, even when provided with a set of diagnostic options. On the other hand, in the first phase of the current study ChatGPT, being in worse condition, compared to doctors as it was not provided with the variants of answers to choose from, showed almost 50% higher accuracy in making diagnosis. This outcome underscores the unique capabilities of ChatGPT in processing and interpreting clinical information, even in scenarios where it lacks the structured diagnostic choices available to human doctors and highlights the comparative performance of ChatGPT and human doctors across the various diagnostic scenarios.

In the second phase the LLM was actually in the same situation as the doctors, as it was given all the information available to doctors in selected 17 clinical cases. This resulted in further increasement of its productivity. These findings underscore the improved performance of ChatGPT when aided by a list of potential diagnoses and provide valuable insights into its diagnostic capabilities. Furthermore, if to count the overall accuracy achieved by LLM in the first and second phases together it gives an overwhelming 90% of correct diagnoses.

In the third phase, by restricting initial information to encompass only patient complaints, medical history, and physical examination data, and by providing ChatGPT with a prompt to adopt a professional medical perspective, we created a controlled clinical context for evaluation. It's worth mentioning that during this part of the experiment ChatGPT frequently requested additional diagnostic methods that were not originally provided in the clinical case descriptions. As mentioned earlier, in these instances, ChatGPT was informed that the requested results are not available. Conversely, in certain cases, ChatGPT did not request any additional test results. In this challenging for LLM situation it demonstrated the correct diagnosing accuracy noninferior to doctors' one despite they were provided not only with a full range of information related to cases but also variants of answer. The results obtained in different case subgroups within this phase shed light on ChatGPT's diagnostic process, illustrating instances of inherent accuracy, adaptability, and steadfastness across various diagnostic scenarios, especially when presented with additional data. Overall these outcomes emphasize the proficiency of ChatGPT in emulating the diagnostic decision-making process of real doctors, even when faced with complex diagnostic scenarios.

Nonetheless, the integration of large language models (LLMs) within the medical field brings forth several challenges and limitations, necessitating meticulous attention. Among these challenges are potential biases that LLMs might inherit or magnify from the training data. Such biases could yield inequitable outcomes and impede scientific advancement [4,20]. Moreover, the application of LLMs in medicine introduces concerns regarding patient privacy and data security. Responsible usage of LLMs demands robust safeguards to ensure patient information remains protected [4]. While LLMs exhibit promise in medical contexts, their efficacy must be substantiated through rigorous validation and clinical trials within real-world healthcare settings [21,22]. Ethical considerations also surface with the deployment of LLMs, spanning issues like algorithmic bias and the imperative for transparent decision-making processes. Addressing these ethical dimensions is critical to their responsible application [4,20]. In addition, the generalizability of LLMs to novel or unseen data emerges as a limitation. Their competence might wane on tasks lying outside their training data domain [23]. The utilization of LLMs in medicine accompanies a set of challenges and constraints that warrant scrupulous examination. Acknowledging and mitigating these concerns is paramount to ensure the responsible and effective application of LLMs in healthcare. With appropriate attention, LLMs possess the potential to transform

medicine by aiding clinical decision-making, thus fostering enhanced patient outcomes and streamlined healthcare workflows.

CONCLUSIONS

Our findings collectively highlight ChatGPT's high potential and performance as a diagnostic tool in clinical medicine and its value in decision-making process. While it exhibits impressive diagnostic capabilities, particularly in structured scenarios, it also demonstrates the importance of supplementary data and the complex nature of medical diagnosis. This research contributes valuable insights into the evolving landscape of AI-driven diagnostic assistance within clinical contexts. It's worth to note that prompt engineering technics usage plays an important role in the interaction between ChatGPT and a doctor.

REFERENCES

1. Copeland B. Artificial intelligence. Encyclopedia Britannica. 2023. https://www.britannica.com/technology/artificial-intelligence [date access 29.07.2023]
2. Dilmegani C. The Future of Large Language Models. 2023. https://research.aimultiple.com/future-of-large-language-models/ [date access 29.07.2023].
3. Introduction to Large language models; https://attri.ai/blog/introduction-to-large-language-models [date access 29.07.2023].
4. Karabacak M, Margetis K. Embracing Large Language Models for Medical Applications: Opportunities and Challenges. Cureus. 2023;15(5):e39305. doi: 10.7759/cureus.39305.
5. Singhal K, Azizi S, Tu T et al. Large language models encode clinical knowledge. Nature. 2023; 620:172–180. doi:10.1038/s41586-023-06291-2.
6. Shah NH, Entwistle D, Pfeffer MA. Creation and Adoption of Large Language Models in Medicine. JAMA. 2023;330(9):866–869. doi:10.1001/jama.2023.14217.
7. Egli A. ChatGPT, GPT-4, and other large language models - the next revolution for clinical microbiology? Clin Infect Dis. 2023; ciad407. doi: 10.1093/cid/ciad407.
8. Fei H, Ren Y, Zhang Y et al. Enriching contextualized language model from knowledge graph for biomedical information extraction. Brief Bioinform. 2021;22(3):bbaa110. doi: 10.1093/bib/bbaa110.
9. Gupta R, Srivastava D, Sahu M et al. Artificial intelligence to deep learning: machine intelligence approach for drug discovery. Mol Divers. 2021;25(3):1315-1360. doi: 10.1007/s11030-021-10217-3.
10. Mintz Y, Brodie R. Introduction to artificial intelligence in medicine. Minim Invasive Ther Allied Technol. 2019;28(2):73-81. doi:10.1080/13645706.2019.1575882.
11. Hashimoto DA, Witkowski E, Gao L et al. Artificial Intelligence in Anesthesiology: Current Techniques, Clinical Applications, and Limitations. Anesthesiology. 2020;132(2):379-394. doi:10.1097/ALN.0000000000002960.
12. Ossowska A, Kusiak A, Świetlik D. Artificial Intelligence in Dentistry-Narrative Review. Int J Environ Res Public Health. 2022;19(6):3449. doi:10.3390/ijerph19063449.
13. Nensa F, Demircioglu A, Rischpler C. Artificial Intelligence in Nuclear Medicine. J Nucl Med. 2019;60 (2):29S-37S. doi:10.2967/jnumed.118.220590.
14. Keskinbora K, Güven F. Artificial Intelligence and Ophthalmology. Turk J Ophthalmol. 2020;50(1):37-43. doi:10.4274/tjo.galenos.2020.78989.
15. Itchhaporia D. Artificial intelligence in cardiology. Trends Cardiovasc Med. 2022;32(1):34-41. doi:10.1016/j.tcm.2020.11.007.
16. Gilson A, Safranek CW, Huang T et al. How Does ChatGPT Perform on the United States Medical Licensing Examination? The Implications of Large Language Models for Medical Education and Knowledge Assessment. JMIR Med Educ. 2023; 9:e45312. doi:10.2196/45312.
17. Huh S. Are ChatGPT's knowledge and interpretation ability comparable to those of medical students in Korea for taking a parasitology examination?: a descriptive study. J Educ Eval Health Prof. 2023; 20:1. doi:10.3352/jeehp.2023.20.1.
18. Wang X, Gong Z, Wang G et al. ChatGPT Performs on the Chinese National Medical Licensing Examination. J Med Syst. 2023; 47(1):86. doi:10.1007/s10916-023-01961-0.
19. Medscape Case Challenges https://reference.medscape.com/features/casechallenges [date access 29.07.2023]
20. Reddy S. Evaluating large language models for use in healthcare: A framework for translational value assessment. Informatics in Medicine Unlocked. 2023;41:101304. doi: 10.1016/j.imu.2023.101304.
21. Shah NH, Entwistle D, Pfeffer MA. Creation and Adoption of Large Language Models in Medicine. JAMA. 2023;330(9):866–869. doi:10.1001/jama.2023.14217.
22. Singhal K, Azizi S, Tu T et al. Large language models encode clinical knowledge. Nature. 2023;620(7972):172-180. doi:10.1038/s41586-023-06291-2.
23. Safranek C, Sidamon-Eristoff A, Gilson A et al. The Role of Large Language Models in Medical Education: Applications and Implications. JMIR Med Educ. 2023; 1(9):639-650. doi: 10.2196/50945.

ORCID and contributionship:
Yaroslav Mykhalko: 0000-0002-9890-6665 [A,B,D,F]
Pavlo Kish: 0000-0002-9674-0657 [B,D]
Yelyzaveta Rubtsova: 0000-0001-9395-1822 [E]
Oleksandr Kutsyn: 0000-0001-7902-4598 [B,C]
Valentyna Koval: 0000-0001-8423-9534 [C,E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Yaroslav Mykhalko
Uzhhorod National University
3 Narodna Square, 88000 Uzhhorod , Ukraine
tel: +380660822769
e-mail: yaroslav.myhalko@uzhnu.edu.ua

Received: 30.07.2023
Accepted: 29.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHANGES IN THE GONADS OF CHILDREN 2-6 HOURS AFTER ACUTE UNILATERAL TESTICULAR TORSION

DOI: 10.36740/WLek202311102

Liliya Volos[1], Olga Gorbatyuk[2], Mykyta Veselyi[2], Sergiy Veselyy[3], Dmytro Lavrov[4], Oleksandr Hladkiy[5], Tetiana Usenko[5]

[1]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE
[2]SHUPYK NATIONAL UNIVERSITY OF HEALTH OF UKRAINE, KYIV, UKRAINE
[3]NATIONAL ACADEMY OF MEDICAL SCIENSE OF UKRAINE GOVERNMENT INSTITUTION «INSTITUTE OF URGENT AND RECOVERY SURGERY NAMED AFTER V.K.GUSAK», KYIV, UKRAINE
[4]KREMENCHUK REGIONAL ONCOLOGY HEALTH CENTER OF THE POLTAVA REGIONAL COUNCIL, KREMENCHUK, UKRAINE
[5]REGIONAL MEDICAL CENTRE OF FAMILY HEALTH «DNIPROPETROVSK REGIONAL COUNCIL», DNIPRO, UKRAINE

ABSTRACT

The aim: To determine the morphological and immunohistochemical changes in the testes 2-6 hours after the onset of clinical symptoms of acute unilateral testicular torsion.

Materials and methods: A morphological and immunohistochemical study was conducted on biopsy samples of testicular tissues taken 2-6 hours after the onset of clinical symptoms of acute unilateral testicular torsion during detorsion and orchiopexy surgery in 27 adolescent patients.

Results: In cases of incomplete torsion (180-360°) and a disease duration of up to 2 hours, the seminiferous tubules maintained their normal structure. The convoluted seminiferous tubules showed minor damage during 4 hours of ischemia caused by testicular torsion of 360-450°, which was characterized by mild damage. Glycogen and neutral glycoproteins were preserved in the cytoplasm of spermatogonia, primary spermatocytes, and Sertoli cells, indicating that their intracellular metabolism was relatively preserved. The ischemia that lasted for 4 hours with testicular torsion of 450-720° was characterized by a moderate degree of gonadal damage. However, there was pronounced expression of vimentin and calretinin, and the presence of glycogen and neutral glycoproteins indicated functional activity of the gonads. A six-hour ischemia period with a 360-450° testicular torsion demonstrated 100% gonadal viability, with 50% of the seminiferous tubules preserved and 35% with minor damage. Severe damage to the spermatogenic epithelium was observed in 15% of seminiferous tubules, characterized by dystrophy of spermatogenic epithelial cells with signs of karyopyknosis, karyorrhexis, vacuolization, hyperchromasia of cytoplasmic organelles, shedding of individual cells into the lumen of tubules, and focal necrosis.

Conclusions: 1**.** The degree of torsion and duration of symptoms are prognostic factors for testicular salvage in torsion episodes. Ischemia lasting up to 6 hours is characterized by a moderate degree of gonadal damage, and detorsion of the testicle performed within 6 hours from the onset of pathology allows for preservation of the testicle in 100% of cases. 2. Histological examination of the susceptibility of different cell types to ischemia reveals that Sertoli cells and spermatogonia are the most resistant, while spermatocytes and spermatids are more susceptible and prone to degeneration.

KEY WORDS: testicular torsion, degree of ischemia, ischemia duration, morphology, immunohistochemistry

Wiad Lek. 2023;76(11):2351-2358

INTRODUCTION

Testicular torsion, or torsion of the spermatic cord, is a serious, unexpected, and often dangerous situation that requires immediate action and urgent surgical intervention [1]. This condition primarily affects boys during puberty and can lead to testicular injury and infertility [2]. Testicular torsion accounts for approximately 7% to 30% of urgent pathologies involving the male gonads, according to various sources [3, 4]. Around 30-50% of boys who undergo surgery for testicular torsion have a history of recurrent episodes of acute pain in the testicle, characteristic of recurrent testicular torsion [5]. The severity of structural and functional disruptions in the testicle depends on the duration, degree of torsion, and length of the spermatic cord (twisted and untwisted testicular torsion). This determines the prognosis for the relative viability and functional integrity of the male gonad [6]. The viability of the testicles significantly decreases within 6 hours after the onset of symptoms, so early diagnosis is crucial [1]. Testicular infarction can

occur as early as 4-6 hours after torsion, depending on the duration of symptoms and the degree of twisting of the spermatic cord [7]. Surgical correction within 6 hours of the onset of testicular torsion results in testicle salvage rates of 90-100%, whereas salvage rates decrease to 10% within 12-24 hours [8, 9].

Currently, the study of morphogenesis, which refers to the dynamics of morphological changes in the development of the disease and the morphofunctional disruptions that occur due to alterations in testicular blood supply resulting from torsion, is of great practical interest. There is no consensus regarding the nature and extent of these changes in the testicle. Testicular torsion leads to tissue damage in the testicle and spermatogenesis through various hypothetical mechanisms. However, there is consensus that the consequences of ischemia, ischemia-reperfusion injury, and oxidative stress have the most destructive effects [10].

THE AIM

The aim of the study was to determine the morphological and immunohistochemical characteristics of testicular changes in acute testicular torsion in children within 2-6 hours from the onset of clinical symptoms.

MATERIALS AND METHODS

The material for the morphological study consisted of tissue biopsies taken during detorsion and orchiopexy surgeries from 27 patients within 2-6 hours from the onset of clinical symptoms of acute testicular torsion. Among them, 2 children (7.41%) were observed with testicular torsion of 180-360°, with a duration of torsion up to 2 hours. Complete testicular torsion, lasting less than 2 hours, was observed in 7 children (25.92%), among whom 3 patients (11.11%) had torsion of 360-450°, and 4 patients (14.81%) had 450-720°testicular torsion. Torsion lasting up to 3 hours was observed in 14 patients (51.85%), among whom 8 children (29.63%) had torsion of 360-450°, and 6 children (22.22%) had testicular torsion of 450-720°. With 6 hour testicular torsion, 4 patients (14.81%) presented with torsion of 360-450°. The study was conducted in accordance with the basic bioethical principles of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Helsinki Declaration of the World Medical Association on ethical principles for medical research involving human subjects (1964-2008), and the Order of the Ministry of Health of Ukraine No. 690 dated 23.09.2009.

Histological examination was performed after staining the tissue specimens with hematoxylin and eosin using standard methodology. Additionally, Alcian blue staining was used, and the Schiff's reagent reaction was performed with control sections treated with amylase to detect glycogen.

The visualization of primary antibodies in the immunohistochemical examination of the testicles was performed using a highly sensitive polymer detection system, DAKO (DAKO, Denmark), according to the manufacturer's instructions. Antibodies against vimentin (Clone SP20, Thermo Scientific) were used to identify Sertoli cells, smooth muscle actin (SMA) (Clone Ab-1, Master Diagnostica) to detect actin myofilaments in smooth muscle cells and cells containing α-actin (fibroblasts), and calretinin (Clone SP13, Thermo Scientific) to visualize Leydig cells. The CD117 (c-Kit) antibody, Clone 104D2, can be used in the diagnosis of testicular tumors to differentiate seminomas and embryonal carcinomas. In all our cases, there was no positive expression indicating testicular tumor presence, but we utilized the CD117 antibody to visualize the spermatogenic epithelium and its damage in cases of acute testicular torsion.

We assessed the condition of the spermatogenic epithelium and interstitial tissue. The criterion for determining the degree of damage was based on one of the variations of morphological changes in the seminiferous tubules [11], which is presented below.

1. The normal structure of the tubules - differentiating germ cells is arranged in concentric layers according to the stages of the spermatogenesis cycle, with tubules being round or oval in shape.
2. Mild degree of spermatogenic epithelial cell damage - individual cells showing signs of karyopyknosis, karyorrhexis, vacuolization, hyperchromatic cytoplasm, desquamation of isolated cells into the lumen of the tubules, edema and loosening of the basement membrane, interstitial edema, and changes in tubule shape.
3. Severe degree of damage - a large number of degenerating cells with their desquamation into the lumen of the tubules, disruption of the integrity of the basement membrane, transformation of germ cells into debris, and damage to Leydig cells and interstitium.
4. Complete or partial depletion of seminiferous tubules - only isolated Sertoli cells and individual spermatogonia remain near the tubule walls, with many tubules appearing as shadows due to necrosis.

Visualization and photography were performed using a light-optical universal laboratory microscope, Leica DM 750 (Leica Microsystems GmbH, Germany), equipped with an integrated Leica ICC50 HD digital video camera.

RESULTS

In cases of incomplete torsion (180-360°) and a disease duration of up to 2 hours, minor morphological changes were microscopically observed, including congestion of the microcirculatory vessels, interstitial tissue edema, and

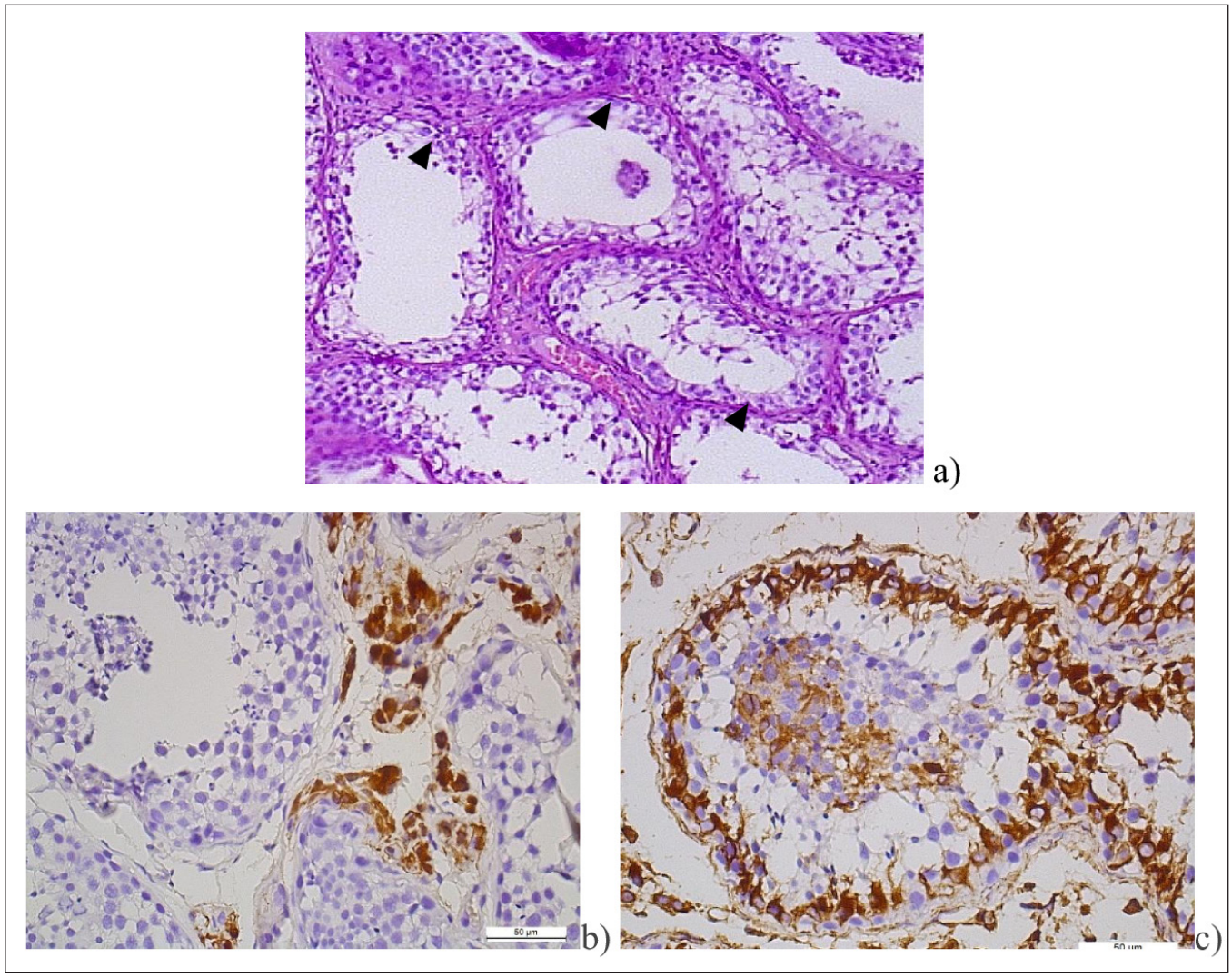


Fig. 1. Condition of seminiferous tubules after a 2-hour ischemia and testicular torsion of 450-720°. Marked disturbances in blood circulation (arrow), loosening of laycrs of the spermatogenic epithelium, and depletion of some tubules (arrowhead) (a). Leydig cells (arrow) (b) and Sertoli cells (arrow) remain unchanged (c). Hematoxylin and eosin staining. x200 (a); Immunohistochemistry staining for calretinin using the MA T clone (SP13, Thermo scientific). x400 (b); Immunohistochemistry staining for vimentin using the MA T clone (SP20, Theremo scientific). x400 (c)

the presence of serous-hemorrhagic transudate. Under conditions of incomplete torsion and minor ischemia, the seminiferous tubules maintained a normal structure.

In the majority of cases, we diagnosed complete testicular torsion (360-720°). Ischemia of the testes was accompanied by histological changes, the severity of which depended on the duration of the damaging effect.

In conditions of a 2-hour ischemia and a torsion of 360-450°, morphological examination of biopsy material revealed that 78% of seminiferous tubules maintained a normal structure. Focal destruction of spermatogonia and primary spermatocytes (leptotene, zygotene, pachytene stages) was observed in 22% of seminiferous tubules. Particularly, changes were evident in primary spermatocytes at the pachytene stage (thick threads), where chromatin often shifted towards the periphery of the nuclei. Individual seminiferous tubules showed round spermatids forming multinuclear aggregates. Additionally, mild damage was characterized by desquamation of some cells from the spermatogenic epithelium, which freely lay in the lumen of the tubule, and vacuolization of spermatocyte cytoplasm. In the interstitial tissue, there were slight edema and hyperemia of the microhemocirculatory vessels. Leydig cells showed no changes, and the size and shape of their nuclei were normal. At the same time, in conditions of a 2-hour ischemia, it was possible to observe compression in the cells of some seminiferous tubules, allowing differentiation between ischemic and unaffected tubules.

In the case of testicular torsion of 450-720° following a 2-hour ischemia, significant disturbances in blood circulation are observed in the testes. The lumen of arterioles is narrowed, venules are dilated, and the endothelium is

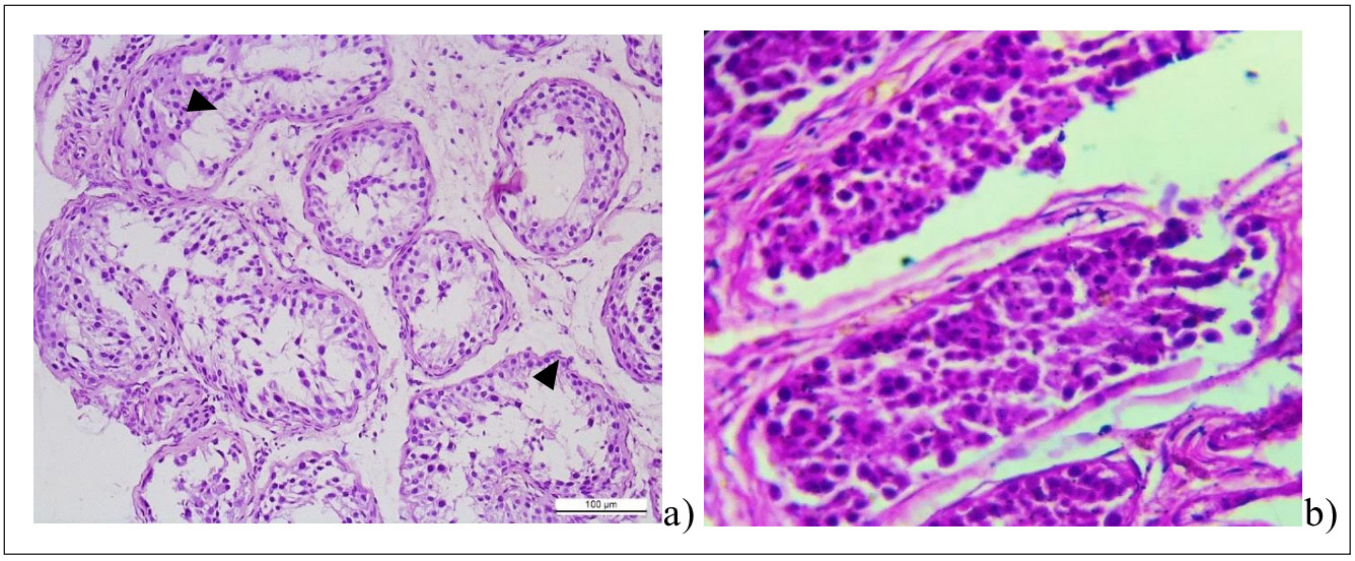


Fig. 2. Condition of seminiferous tubules after a 4-hour ischemia and testicular torsion of 360-450°. Interstitial edema (arrow), stratification of tubule layers, and dystrophy of spermatocytes and spermatids (arrowhead) (a); presence of glycogen in the cytoplasm of spermatocytes and Sertoli cells in tubules with mild damage (arrow) (b). Hematoxylin and eosin staining. (a) x200; Periodic acid-Schiff reaction. (b) x400.

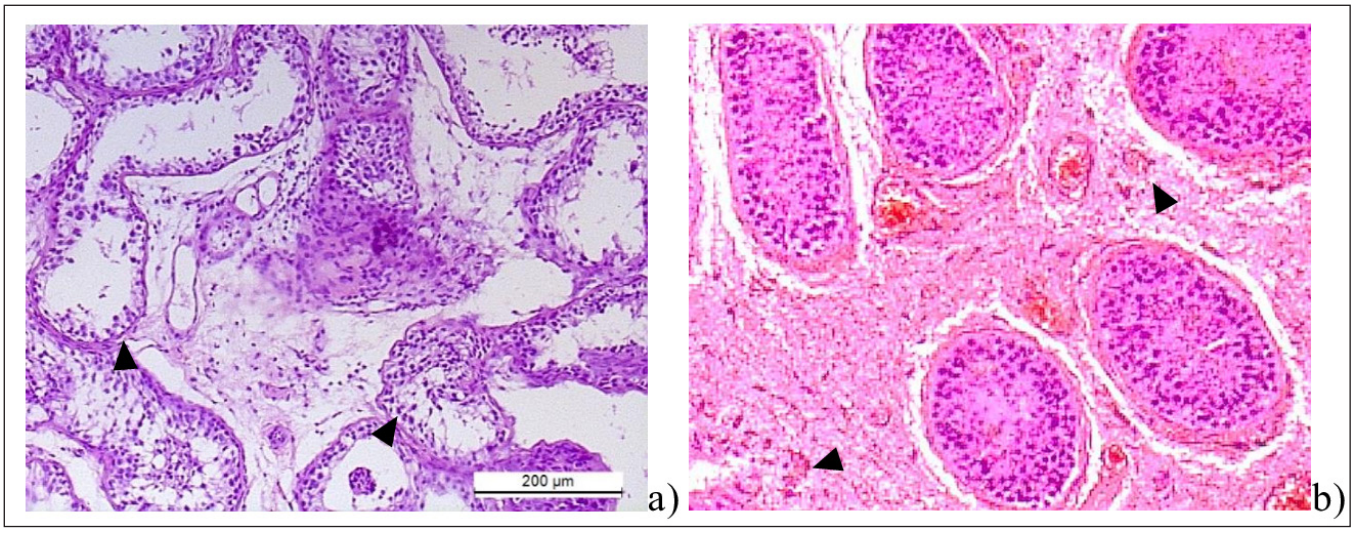


Fig. 3. Condition of seminiferous tubules after a 2-hour ischemia and testicular torsion of 450-720°. Pronounced interstitial edema (arrow) (a), distruption and detachment of spermatogenic epithelial cells from the basal membrane (arrowhead) (a), congestion of blood vessels (arrow), and focal hemorrhages in the intertubular stroma (arrowhead) (b). Hematoxylin and eosin staining. (a) x100, (b) x200.

swollen. Edema increases in the interstitium, and the walls of blood vessels show signs of plasma extravasation. As for Leydig cells, they remain unchanged. 72% of seminiferous tubules have a normal structure, appearing round or oval, with a division of the spermatogenic epithelium into two isolated compartments. However, in 25% of tubules, mild damage is detected, while severe damage is present in 3%, although Sertoli cells maintain a normal structure. In comparison to testicular torsion of 360°, there are dystrophic changes in the testicular parenchyma. The boundaries of spermatocyte nuclei at the pachytene stage become fuzzy, these cells exhibit nuclei with pyknosis, and the cytoplasm of the cells is vacuolated. In spermatids, there are uneven nuclear staining, fragmented chromatin, and loosening of the layers of the spermatogenic epithelium, leading to depletion of some tubules (Fig. 1).

Ischemia of the testicle lasting up to 4 hours with torsion of 360-450° is accompanied by increasing interstitial tissue edema. The lumen of small arteries is narrowed, while veins are dilated. The walls of the blood vessels show signs of plasma extravasation, and the endothelium appears swollen and occasionally desquamated. The Leydig cells are grouped around blood and lymphatic capillaries and have a round or oval shape. Their cytoplasm is light and foamy, staining eosinophilic. Fine and coarse eosinophilic granularity can be observed in the cytoplasm of Leydig cells.

The number of seminiferous tubules maintaining a normal structure decreases to 65%, while the number of

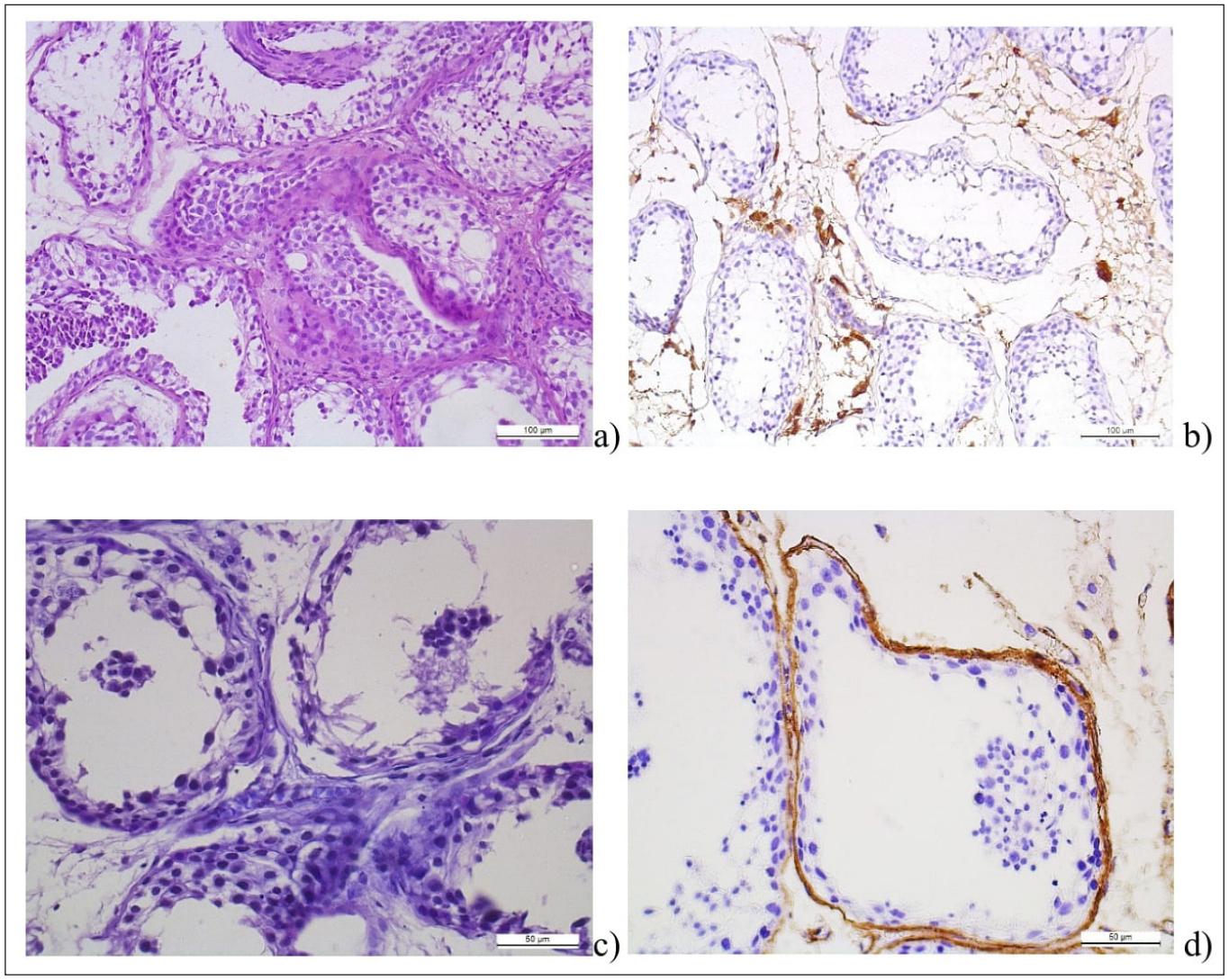


Fig. 4. Condition of seminiferous tubules during 6-hour ischemia and testicular torsion of 360-450°. Interstitial edema, deformation of some seminiferous tubules (arrow) (a), degeneration of Leydig cells (arrow) (b), destruction of spermatogonia and primary spermatocytes, focal desquamation of cells from the basement membrane with their displacement into the lumen of the tubules (arrow) (a-g), visualization of the basement membrane of the tubules during immunohistochemistry (arrow) (g). Hematoxylin and eosin staining (a) x200; Immunohistochemistry (IHC) with calretinin antibody (Clone: SP13, Thermo Scientific) (b) x200; Alcian blue staining (c) x400; Immunohistochemistry (IHC) with smooth muscle actin (SMA) antibody (Clone Ab-1, Master Diagnostica) (b) x400

tubules with severe damage to the spermatogenic epithelium increases to 9%. Some seminiferous tubules exhibit focal loosening of their own membrane components and focal detachment of the spermatogenic epithelium. The cytoplasm of Sertoli cells appears swollen and granular, with the presence of individual vacuoles. In spermatocytes at the preleptotene stage, there are significant changes in nuclear structure. In some cells, granular chromatin is distributed diffusely, while in others, it is compacted. The number of spermatocytes at the preleptotene and pachytene stages decreases compared to the control, and the remaining cells show signs of degeneration. The cytoplasm of most of these cells is vacuolated and granular, and the nuclei appear pyknotic due to abnormal chromatin condensation (Fig. 2). Similar changes are observed in the cytoplasm and nuclei of spermatids at different stages of spermatogenesis. Round spermatids have vacuolated nuclei. Degenerating spermatogonia with fragmented chromatin can be visualized at the base of seminiferous tubules.

Ischemia lasting for 4 hours with testicular torsion of 450-720° is accompanied by pronounced changes in the testicular parenchyma and stroma, with increasing interstitial tissue edema. As a result, the ratio of interstitium to parenchyma volume slightly increases. The lumen of blood vessels is noticeably dilated, and the endothelium appears swollen, with focal hemorrhages in the intertubular stroma. The cytoplasm of Leydig cells is vacuolated and unevenly stained. In some cells, it is basophilic, while in others, it is eosinophilic. The nuclei of the cells vary in size, and the cells themselves have a large volume. The number of seminiferous tubules with mild damage to the

spermatogenic epithelium reaches 30%, while those with severe damage reach 12%. Disruption and detachment of the spermatogenic epithelium from the own membrane of the seminiferous tubules are observed, and desquamated cells are present in their lumen. The cytoplasm of most Sertoli cells and spermatogenic epithelial cells is vacuolated. The number of spermatocytes at the pachytene stage noticeably decreases (Fig. 3).

Thus, a 4-hour ischemia in testicular torsion of 360-450º is characterized by mild damage to the convoluted seminiferous tubules. Some of them show degeneration of spermatogenic epithelium cells with pronounced cytoplasmic vacuolization, pyknosis of nuclei, and focal desquamation of a small number of cells into the lumen of the tubules. Interstitial edema and minor extravasation of blood are observed. Glycogen and neutral glycoproteins are preserved in the cytoplasm of spermatogonia, primary spermatocytes, and Sertoli cells, indicating relative preservation of their intracellular metabolism. Ischemia in testicular torsion of 450-720º is accompanied by pronounced changes in the parenchyma and stroma of the testis due to its diffuse damage. A 720º torsion of the spermatic cord leads to a critical reduction in arterial blood flow and, consequently, ischemia of the testicular tissue. If blood circulation in the testis is not restored in a timely manner, irreversible pathological changes develop.

Our conducted morphological study revealed that a six-hour cessation of blood circulation in the testis during testicular torsion of 360-450º leads to vasomotor paralysis. Blood and lymphatic vessels are dilated, and the interstitial connective tissue is edematous (Fig. 4 a). The Leydig cells are enlarged, with vacuolated cytoplasm and uneven staining. In some fields of view, the nuclei of Leydig cells are reduced, and their cytoplasm is sharply eosinophilic. Immunohistochemistry reveals varying levels of calretinin expression (Fig. 4 b).

A six-hour ischemia leads to deformation of some seminiferous tubules, the diameter of which generally decreases. The ratio between interstitial and tubular tissue increases due to interstitial edema. The basement membrane of the seminiferous tubules becomes folded, loosened, and partially destroyed, especially in cases of severe damage (15%). Seminiferous tubules with mild damage to the germinal epithelium (35%) are characterized by pronounced loosening of its layers, while the basement membrane of the tubules is well visualized, especially with smooth muscle actin immunohistochemistry, indicating the presence of several layers of myoid cells (myofibroblasts) on the basal membrane. Histological staining with alcian blue reveals the presence of acidic mucopolysaccharides (glycosaminoglycans - GAGs), indicating secretory activity of the germinal epithelium and Sertoli cells (Fig. 4 c). There are no disruptions in the synthesis of proteoglycans and glycosaminoglycans in the testicular tissue, indicating the absence of pathological processes in spermatogenesis.

In a portion of the tubules (15%), significant impairment of spermatogenesis was detected. The number of germ cells decreases, and the lumen of the tubules narrows. There is a massive detachment of cells from the basement membrane, with their displacement into the lumen of the tubules (severe degree of damage). Some spermatocytes demonstrate nuclear condensation and fragmentation, while some spermatids form multinuclear clusters (Fig. 4 c, d). There is a noticeable focal reduction of spermatocytes at the pachytene stage and spermatids at various stages of spermiogenesis.

Thus, with a 6-hour duration of testicular torsion, 100% of the gonads were deemed viable. Among them, 50% of the seminiferous tubules remained preserved, while 35% exhibited mild degrees of damage. Severe damage to the germinal epithelium was observed in 15% of the seminiferous tubules. The 6-hour ischemia is characterized by a moderate degree of gonadal damage. Diffuse interstitial edema, focal hemorrhages around the seminiferous tubules, degeneration of spermatogenic epithelium cells, focal desquamation of cells from the basement membrane, and their necrosis are detected.

DISCUSSION

Testicular torsion is a common urological emergency among adolescent boys and young men. The torsion of the testicle and the torsion of the spermatic cord rapidly lead to ischemia, resulting in the loss of germ cells. Therefore, prompt diagnosis and urgent surgical intervention are required [12]. The time interval from symptom onset to surgical intervention for testicular salvage is typically 4 hours, and in some cases, it may extend to 6 or more hours [13]. Subsequent detorsion of the torsion causes reperfusion injury, which further damages the ischemic testicle. Experimental studies on testicular torsion-detorsion are being conducted. Testicular ischemia-reperfusion leads to the formation of reactive oxygen species, proinflammatory cytokines, neutrophil recruitment, lipid peroxidation, anoxia, and apoptosis, all of which carry a significant risk of subsequent infertility [12].

The histopathological findings of testicular changes in acute testicular torsion in children within 2 to 6 hours of the onset of clinical symptoms are present. The obtained data indicate an intensification of destructive processes in the testicles with an increasing duration of the ischemic factor and the degree of testicular torsion. These findings highlight the importance of restoring blood circulation as quickly as possible from the onset of disease symptoms to preserve spermatogenesis.

The results of the study by Sharp VJ. et al. (2013) demonstrate that there is a window of four to eight hours before irreversible ischemic damage occurs. Delay in treatment can lead to reduced fertility or may require orchiectomy [14].

The morphological investigation we conducted on biopsy samples taken during surgical detorsion and orchiopexy of the testicles revealed varying degrees of damage. Acute ischemia lasting up to 4 hours was characterized by mild to moderate gonadal damage, with a viability rate of 100%. In cases of mild damage to the germinal epithelium, only isolated cells showed signs of karyopyknosis, karyorrhexis, cytoplasmic vacuolization, hyperchromatic organelles, desquamation of individual cells into the lumen of the tubules, edema, and loosening of the tubular basement membrane, diffuse interstitial edema, congestion, and hemorrhages around the seminiferous tubules. Histological analysis of the susceptibility of different cell types to ischemia demonstrated that Sertoli cells and spermatogonia were more resistant, while spermatocytes and spermatids were more susceptible and prone to degeneration. Immunohistochemical typing and histochemical analysis of the testicular parenchyma revealed pronounced expression of vimentin and calretinin, as well as the presence of glycogen and neutral glycoproteins in the cytoplasm of spermatogonia, primary spermatocytes, spermatids, and Sertoli cells showed functional activity in response to acute 4-hour ischemia, indicating the viability of the gonads under this condition. Ischemia lasting 6 hours was characterized by a moderate degree of gonadal damage. Severe damage to the germinal epithelium was observed in 15% of the seminiferous tubules.

According to clinical studies by Wiener SL (1990), the inability to correct spermatic cord torsion within 6 hours of testicular ischemia can lead to testicular dysfunction and atrophy [15]. Adam S Howe et al. (2017) developed specific formulas to determine the viability of a twisted testicle based on the duration of symptoms and the degree of torsion. According to their findings, 15 hours of clinical symptoms and torsion of 860 degrees provide a 50% chance of testicular salvage. In children in puberty with torsion less than 360 degrees, the duration of symptoms is relatively long. High postoperative testicular survival is a notable clinical feature of this study, as reported by Xiang Guo et al. [16]. The risk of testicular loss is significantly higher if there is a delay in the initial diagnosis of testicular torsion. The primary goal in the treatment of acute scrotum should be the prevention of testicular loss rather than symptom management [17]. However, the use of Doppler ultrasound should not lead to unnecessary delays in urgent surgical treatment, which includes detorsion and bilateral orchiopexy [18].

Therefore, the comprehensive morphological investigation, including histological and immunohistochemical analysis of the gonads in children with acute torsion, depending on the duration of ischemia (2-6 hours) and the degree of testicular torsion (180-360-450-720º), provided new insights into the nature of changes occurring under acute ischemic conditions in the spermatogenic epithelium cells, components of the hematotesticular barrier, Leydig cells, and Sertoli cells. The degree of torsion and the duration of symptoms serve as prognostic factors for testicular salvage in torsion episodes. Ischemia lasting up to 6 hours is characterized by a moderate degree of gonadal damage, and detorsion performed within 6 hours from the onset of pathology allows for testicular preservation in 100% of cases.

CONCLUSIONS

1. The degree of torsion and the duration of symptoms are prognostic factors for testicular salvage in torsion episodes. Ischemia lasting up to 6 hours is characterized by a moderate degree of gonadal damage, and detorsion performed within 6 hours from the onset of pathology allows for testicular preservation in 100% of cases.
2. Histological investigation of the susceptibility of different cell types to ischemia reveals that Sertoli cells and spermatogonia are more resistant, while spermatocytes and spermatids are more susceptible and prone to degeneration.

REFERENCES

1. Schick MA, Sternard BT. Testicular Torsion. Treasure Island (FL): StatPearls Publishing; 2023. https://www.ncbi.nlm.nih.gov/books/NBK448199/ [date access 06.04.2023]
2. Lacy A, Smith A, Koyfman A, Long B. High risk and low prevalence diseases: Testicular torsion. Am J Emerg Med. 2023; 66:98-104. doi: 10.1016/j.ajem.2023.01.031.
3. Palmer LS, Palmer JS et al. Management of abnormalities of the external genitalia in boys. Campbell-Walsh urology. 11th ed. Philadelphia: Elsevier. 2016, pp. 3384–97.
4. Sheth KR, Keays M, Grimsby GM et al. Diagnosing testicular torsion before urological consultation and imaging: validation of the TWIST score. J Urol. 2016;195(6):1870-6. doi: 10.1016/j.juro.2016.01.101.
5. Okulov AB, Volodko EA, Anikiev AV, Sokolov YY. Familial testicular torsion in 13 years old child. Pediatria. 2016; 95 (6): 213–215.

6. Howe AS, Vasudevan V, Kongnyuy M et al. Degree of twisting and duration of symptoms are prognostic factors of testis salvage during episodes of testicular torsion. Transl Androl Urol. 2017;6(6):1159-1166. doi: 10.21037/tau.2017.09.10.
7. Munden MM, Trautwein LM. Scrotal pathology in pediatrics with sonographic imaging. Curr Probl Diagn Radiol. 2000; 29(6):185-205. doi: 10.1016/s0363-0188(00)90013-6.
8. Keays M, Rosenberg H. Testicular torsion. CMAJ. 2019; 191(28):E792. doi: 10.1503/cmaj.190158.
9. Pogorelić Z, Mustapić K, Jukić M et al. Management of acute scrotum in children: a 25-year single center experience on 558 pediatric patients. Can J Urol. 2016; 23(6):8594–601.
10. Alawamlh OAH, Flannigan R, Hayden R et al. Testicular Torsion and Spermatogenesis. Molecular Mechanisms in Spermatogenesis. Advances in Experimental Medicine and Biology, 2021;1381. doi:10.1007/978-3-030-77779-1_14.
11. Oettle AG, Harrison RG. The Histological Changes Produced in the Testis by Temporary and Permanent Occlusion of the Testicular Artery. J. Path. Bact. 1952;64(2):273-97. doi: 10.1002/path.1700640204.
12. Shimizu S, Tsounapi P, Dimitriadis F et al. Testicular torsion-detorsion and potential therapeutic treatments: A possible role for ischemic postconditioning. Int J Urol. 2016;23(6):454-63. doi: 10.1111/iju.13110.
13. Martin HA, Noble M. Consideration of Testicular Torsion in Young Males with Abdominal Pain Is Essential: A Case Review. J Emerg Nurs. 2021;47(1):186-191. doi: 10.1016/j.jen.2020.09.006.
14. Sharp VJ, Kieran K, Arlen AM. Testicular torsion: diagnosis, evaluation, and management. Am Fam Physician. 2013;88(12):835-40.
15. Wiener SL et al. Testicular Pain. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. Boston: Butterworths; 1990. Chapter 186.
16. Guo X, Sun L, Lei W et al. Management of testicular torsion <360° in children: a single-center, retrospective study. J Int Med Res. 2020;48(4):300060519895861. doi: 10.1177/0300060519895861.
17. Kumar V, Matai P, Prabhu SP, Sundeep PT. Testicular Loss in Children Due to Incorrect Early Diagnosis of Torsion. Clin Pediatr (Phila). 2020;59(4-5):436-438. doi: 10.1177/0009922820903037.
18. Estremadoyro V, Meyrat BJ, Birraux J et al. Diagnostic et prise en charge de la torsion testiculaire chez l'enfant [Diagnosis and management of testicular torsion in children]. Rev Med Suisse. 2017;13(550):406-410.

ORCID and contributionship:
Liliya Volos: 0000-0002-1733-589X [A-C]
Olga Gorbatyuk: 0000-0003-3970-8797 [D,E]
Mykyta Veselyi: 0000-0002-4340-4924 [E,F]
Sergiy Veselyy: 0000-0001-7988-8214 [E,F]
Dmytro Lavrov: 0000-0003-2940-3375 [B,C]
Oleksandr Hladkiy: 0000-0002-3085-9399 [D,E]
Tetiana Usenko: 0000-0001-5477-4739 [A,B]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Liliya Volos
Danylo Halytsky Lviv National Medical University
69 Pekarska St., 79010 Lviv , Ukraine
tel: +380997743810
e-mail: Liliya.volos@gmail.com

Received: 10.05.2023
Accepted: 11.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

PECULIARITIES OF THE FUNCTIONAL STATE OF THE LIVER IN PATIENTS WITH CHRONIC HEPATITIS C IN THE PRESENCE OF CHRONIC PANCREATITIS

DOI: 10.36740/WLek202311103

Mariya A. Derbak, Olha V. Buchok, Taras M. Ganich, Mariya V. Rivis, Yana V. Lazur, Viktoriya M. Polyak-Tovt, Volodymyr V. Timashev
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To study the features of the functional state of the liver in patients with chronic hepatitis C (CHC) in the presence of CP, depending on the enzymatic activity of the pancreas.

Materials and methods: 72 patients were under observation: 52 with CHC and CP with exocrine secretory insufficiency (EI) of the pancreas and 20 - with CHC and CP without EI. In all patients, the degree of liver fibrosis, levels of aminotransferases, total bilirubin, gamma-glutamyltransferase, albumin, stool coproscopy and pancreatic fecal elastase-1 (FE-1) were determined.

Results: It was revealed that in patients with CHC combined with CP+EI of the pancreas, higher activity of the necroinflammatory process and deeper stages of liver fibrosis is more often noted than in patients with preserved exocrine function of the pancreas. A statistically significant association was established between the degree of liver fibrosis and the presence of EI of the pancreas (p=0.03), namely, in patients with CHC and CP with EI of the pancreas, the degree of fibrosis F2-4 was 2.8 times more frequent. Also, higher levels of aminotransferases and lower levels of albumin were noted in this group of patients than in patients with CHC and CP with preserved exocrine function of the pancreatic gland.

Conclusions: In patients with CHC combined with CP+EI of the pancreas, higher levels of fibrosis and necroinflammatory activity of the liver are more often detected, as well as a tendency to lower albumin levels, than in patients with CHC and CP without EI.

KEY WORDS: chronic hepatitis C, chronic pancreatitis, exocrine insufficiency, liver fibrosis, fecal elastase-1

Wiad Lek. 2023;76(11):2359-2364

INTRODUCTION

Chronic hepatitis C (CHC) and chronic pancreatitis (CP) are extremely relevant medical problems today. According to the 2021 WHO report, about 1.5 million new cases of CHC are registered every year, and 58 million people live with this diagnosis [1,2]. Spontaneous elimination of HCV in chronically infected patients occurs only in ≈0.02% of patients per year, almost 70% of patients have one or more extrahepatic manifestations of HCV, and about 290,000 people die annually from the consequences of HCV [3,4]. As for CP, it is a disease that actually causes irreversible changes in the morphology and function of the pancreas. Its long-term consequences are type II diabetes (DM) and pancreatic cancer. The incidence of CP in European countries varies from 5 to 10 per 100 thousand population [3-6].

The pancreas and the liver are anatomically in close proximity [7]. Thus, diseases of the pancreas that affect the outflow of bile can lead to concomitant liver damage. However, whether liver disease affects the function of the pancreas is currently not clearly established. Some studies have shown that patients with a diagnosis of acute or fulminant hepatitis also suffer from acute pancreatitis [8–10]. The features of the simultaneous course of CHC and CP and their mutual influence remain unclear. In separate studies, the possibility of replication of the hepatitis C virus in β-cells of the pancreas with subsequent development of diabetes and CP has been demonstrated [11]. Also, CP, both with normal and with impaired excretory function of the pancreatic gland, is considered as a possible extrahepatic manifestation of CHC [12–15]. Therefore, the combination of these diseases and their mutual influence requires further research.

THE AIM

To study the features of the functional state of the liver in patients with chronic hepatitis C in the presence of chronic pancreatitis depending on the enzymatic activity of the pancreas.

MATERIALS AND METHODS

We included in the study 72 patients with CHC with concomitant CP, who were divided into 2 groups, depending on the presence or absence of exocrine insufficiency (EI) of the pancreas. The first group consisted of 52 patients with an existing EI (1st group n=52), and the second group included 20 patients without EI of pancreatic gland (2nd group n=20). All patients were between the ages of 18 and 70 and signed an informed consent to conduct research, the structure of which corresponded to the officially agreed, and the research itself - to the requirements of the Declaration of Helsinki (1975) as amended, the International Code of Medical Ethics (1983) and the relevant laws of Ukraine and WHO regulations. The study was approved by the local ethics commission of the State University "Uzhhorod National University" (protocol No. 6/2 dated September 7, 2021).

The scientific research was carried out within the departmental theme "Combined pathology and correction of homeostasis disorders of residents of the Carpathian region, taking into account adverse factors", state registration number 0121U110808 of the department of faculty therapy of the State University "Uzhhorod National University».

The diagnosis of CHC was made in accordance with the International Classification of Diseases of the 10th revision and verified by the detection of total antibodies of the IgG class to the structural and non-structural proteins of HCV (antiHCV IgG +) by the serological method of ELISA, as well as by the indication HCV RNA + in the blood of the investigated by the PCR method with the determination of the viral load and genotyping. Testing was performed on a thermal cycler with a real-time PCR product detection system "iQ 5", Vio-Rad, USA. General clinical, biochemical, serological, and molecular genetic studies were conducted in certified laboratories of the central city clinical hospital of Uzhhorod, communal non-commercial enterprise "Regional Clinical Infectious Disease Hospital" of the Transcarpathian Regional Council, and commercial laboratories ("Dila" and "Astra-Dia"). Indicators of biochemical blood analysis - total bilirubin and its fractions, total protein and fractions, activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (LF) and γ-glutamyl transpeptidase (GGT) were determined using an automatic biochemical analyzer and original ChemWell reagents. Awareness Technology INC (USA).

The degree of activity of the pathological process was determined by the level of increased activity of ALT, according to the international classification of liver diseases (Los Angeles, 1994). The degree of fibrosis and the activity of the necroinflammatory process in the liver were determined using a non-invasive diagnostic method - FibroMax, which includes: FibroTest, ActiTest, SteatoTest, AshTest, NashTest and is carried out by the company BioPredictive (Paris, France). The patients also underwent an ultrasound examination of the abdominal organs according to the generally accepted method. All patients underwent stool coproscopy, where the appearance of a small amount of neutral fat, altered muscle fibers and extracellular starch made it possible to suspect a violation of the exocrine function of the pancreas and the formation of chronic pancreatitis.

The diagnosis of CP was established in accordance with the Marseille-Rome criteria (1989) with additions and clarifications of the International Classification of Diseases of the 10th revision, as well as in accordance with the Order of the Ministry of Health of Ukraine dated September 10, 2014 No. 638 "On the approval and implementation of medical and technological documents on the standardization of medical of help in chronic pancreatitis" [16]. The exocrine function of the pancreas was evaluated based on the results of fecal coproscopy and pancreatic fecal elastase-1 (FE-1), which was studied by means of ELISA, using the test systems of ScheBo® Biotech AG (Germany). The interpretation of the results was carried out according to the following gradation: the level of FE-1 in feces is more than 200 μg/g of feces - the exocrine secretory function of the pancreatic gland is preserved; 150–200 μg/g of feces - a mild degree of exocrine insufficiency; 100–150 mcg/g of stool - moderate EI; less than 100 mcg/g of feces - severe EI.

The analysis and processing of the results of the examination of patients was carried out using the computer program Jamovi 2.3.21, Microsoft Excel 2016, Statistics for Windows v.7.0 (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the obtained results. The difference was considered statistically significant at $p<0.05$.

RESULTS

When analyzing the obtained data, it was possible to establish that, in contrast to patients with CHC and CP with a preserved exocrine function of the pancreatic gland, defecation disorders are more often noted in patients with CHC in combination with CP with EI. The proportion of patients with defecation disorders (diarrhea, constipation or their alternation) was 2 times higher in 1 group, compared to the group of patients without EI of pancreas (78.8% (41/52) versus 40% (8/20) people). In particular, diarrhea was detected in 21.2% (11/52) in the first group and in 5.0% (1/20) in the second, constipation - in 30.8% (16/52) and 25% (5/20) respectively, and alternating diarrhea and constipation in 26.9% (14/52) in the first group and in 10.0% (2/20) in the second ($p=0.016$). Also, patients of the first

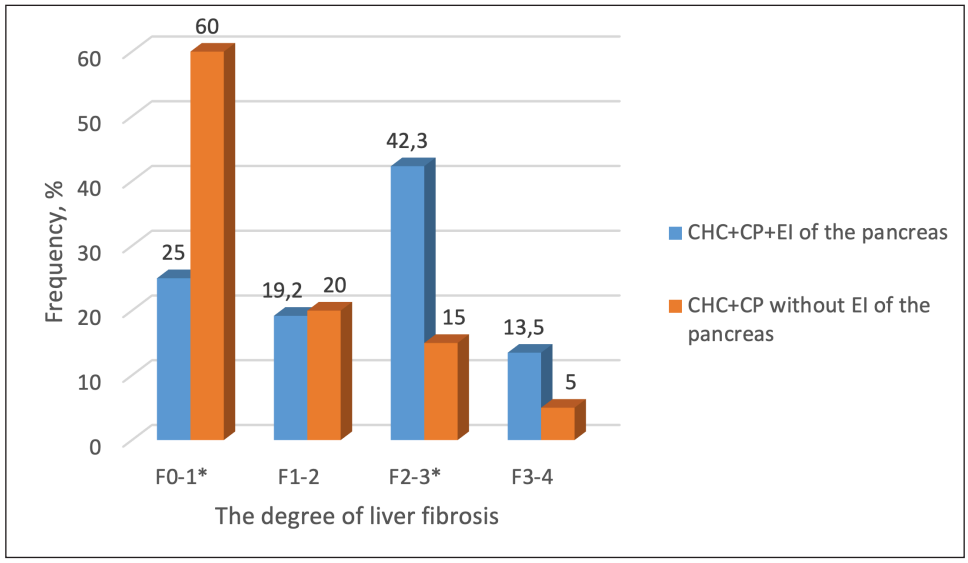


Fig.1. Degree of liver fibrosis in examined patients
Note: * the difference is statistically significant when comparing patients of groups 1 and 2, p<0.05

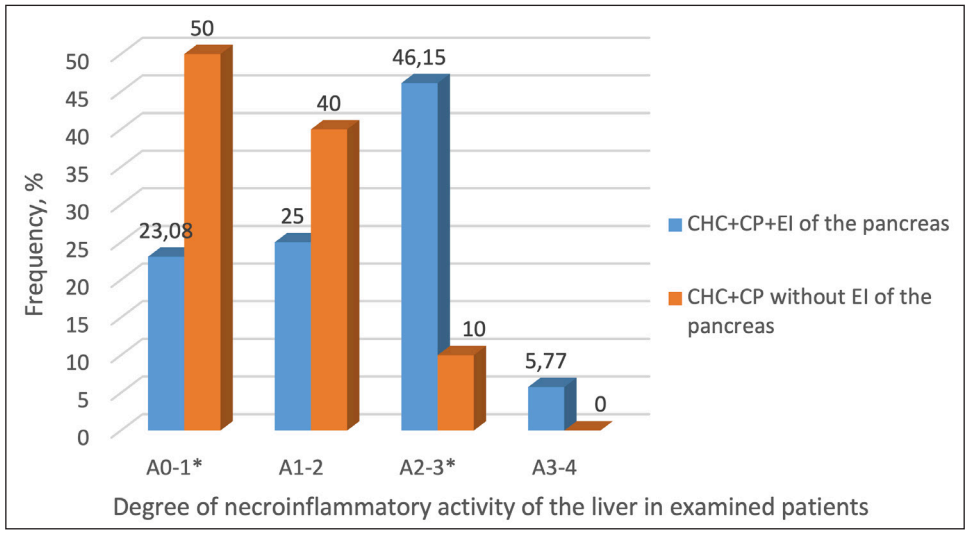


Fig. 2. Degree of necroinflammatory activity of the liver in examined patients
Note: * the difference is statistically significant when comparing patients of groups 1 and 2, p<0.05

group complained more often of abdominal bloating and flatulence - 82.7% (43/52) versus 55.0% (11/20) in the second group (p=0.015). Abdominal pain credibly bothered the patients of the first group - 63.5% (33/52) versus 35% (7/20) in the second group (p=0.03).

During the analysis of the research results, it was found that in patients with CHC with combined CP EI of pancreatic gland, higher activity of the necroinflammatory process and deeper stages of fibrosis of the liver were more often noted than in patients with preserved exocrine function of the panreas. The statistical significance of the association between the degree of fibrosis of the disease and the presence of EI was established (p=0.03). also, the stage of fibrosis F2-3 (42.3% (22/52) vs. 15% (3/20)) was detected 2.8 times more often in patients with CHC+CP with EI and F0 was 2.4 times less often -1 (25% (13/52) vs. 60% (12/20)) (Fig. 1).

It was found that in patients with CHC, comorbid with chronic pancreatitis, LUTS is associated with liver fibrosis: with a higher degree of fibrosis, the probability of secretory insufficiency of the liver is higher (p=0.03).

According to the results of ActiTest, which reflects the level of necroinflammatory activity of the liver, stage A2-3 was detected in patients of group 1 4.6 times more often than in patients of group 2, namely 46.15% (24/52) versus 10% (2/ 20), and 2.2 times less often – stage A0-1, respectively 23.08% (12/52) versus 50% (10/20) (Fig. 2).

It was established that in patients of the first group, the average level of ALT and AST was 69.0 ± 25.2 IU/l and 57.0 ± 22.3 IU/l, respectively, against 42.1 ± 18.0 IU/l and 33, 7 ± 16.2 IU/l in patients of the second group (p<0.001).

Pigment function was unchanged or little changed in both groups. The average level of total bilirubin was 16.7±4.5 mmol/l in the group of CHC+CP+EI and 12.7±4.24 mmol/l in the group of CHC+CP without EI of the pancreatic gland (p<0.001).

Protein synthetic function was evaluated by albumin level. Although its average level was within the normal range in both groups, it was lower in the group of CH-

Table I. Indicators of the functional state of the liver in the examined persons
Note: * the difference is statistically significant when comparing patients of groups 1 and 2, p<0.05

Indicator, unit of measurement	Patients groups	
	1group, n=52 CHC+CP+EI of the pancreas	2 group, n=20 CHC+CP without EI of the pancreas
Albumin, g/l *	38,2 ± 5,14	45,2 ± 4,45
Total bilirubin, mmol/l *	16,7± 4,5	12,7 ± 4.24
ALP, IU/l	234 ± 58,3	226 ± 55,0
GGT, IU/l	44 ±12,6	39,2± 8,0
ALT, IU/l *	69,0 ± 25,2	42,1 ± 18,0
AST, IU/l *	57,0 ± 22,3	33,7 ± 16,2

Note: * the difference is statistically significant when comparing patients of groups 1 and 2, p<0.05

C+CP+SSNPs than in the group of CHS+CP without SSNPs (p<0.001), namely 38.2± 5.14 g/l against 45.2±4.45 g/l.

When examining individual markers of cholestasis (LF and GGT) in the examined patients of both groups, a slight difference in mean values was found, but it was not statistically significant (Table I).

It was found that the level of viral load in CHC is associated with the presence of EI. It was established that patients with impaired exocrine function of the pancreas had a high viral load 4.2 times more often - 42.3% (22/52) against 10% (2/20) in the second group (p=0.002).

DISCUSSION

When evaluating the clinical features of the course of CHC with concomitant CP, we found that dyspeptic symptoms such as defecation disorders, flatulence, flatulence, and abdominal pain are more often detected with reduced enzymatic activity of pancreatic gland. This demonstrates the aggravating effect of the presence of EI on the clinical course of CHC and complements the data obtained by other authors (Babinets et al., 2019) [17]. However, in the presence of stool disorders, we did not establish a clear difference between the frequency of constipation, diarrhea, and unstable stools.

The data obtained by us regarding low levels of albumin in 1 group of patients may be due to insufficient intake of protein from food, as a result of the malabsorption syndrome, which is characteristic of EI. Our data are consistent with the data obtained by Fujita et al. (2019), Diéguez-Castillo et al. (2020), who proved that trophic insufficiency due to malabsorption syndrome often occurs in patients with CP with EI [18,19].

The results obtained by us about the aggravating interaction of a high viral load and inflammatory activity in the liver on the exocrine function of the pancreas are confirmed by the data obtained by other scientists. Thus, such researchers as Jain et al., (2007) and Panic et al. (2020) [8,20]. Blackard (2017) proved the impact of HCV on the endocrine function of the pancreatic gland and suggested the possibility of viral replication in β-cells [11]. It is also known that DM is a frequent extrahepatic manifestation of CHC (Mazzaro 2021; Svegliati-Baroni 2020) [1,21]. When analyzing the obtained data, we found that among patients with a combined course of CHC and CP, a high viral load is more often found in 1 group (patients with CHC and CP with the presence of EI), which may indicate the presence of hepatitis C virus replication in the PC. Previously, Yan et al. (2000) HCV RNA was detected in pancreatic tissue obtained at autopsy [13]. Other scientists, such as Arafa et al. (2020), Fiorino et al. (2019) found that infection with the hepatitis C virus is correlated with the incidence of pancreatic cancer and this may indirectly indicate the previous presence of CP, which, however, was not diagnosed in time [13 ,22, 23]. But the exact mechanism of the connection between HCV infection and the development of pancreatic cancer and CP has not been fully understood. It has been suggested that the anatomical proximity of the liver to the pancreas, as well as the sharing of blood vessels and ducts between the two organs, may make the pancreas a potential reservoir for hepatitis C virus, which can travel through the bloodstream and deposit in tissues unrelated to the liver [23].

CONCLUSIONS

High degrees of fibrosis (higher than F2-4) were detected 2.8 times more often in patients with CHC combined with CP + EI than in patients without EI of the pancreas. At the same time, the frequency of detection and the severity of EI are directly correlated with the levels of aminotransferases and viral load. The higher the degree of inflammatory activity of the liver and the viral load, the more often patients are diagnosed with EI of the pancreatic gland.

In patients with CHC comorbid with CP and presence of the EI, a tendency to lower levels of albumin was registered, while its values were normal in patients with CHC with preserved enzymatic function of pancreatic gland.

REFERENCES

1. Mazzaro C, Quartuccio L, Adinolfi LE et al. A Review on Extrahepatic Manifestations of Chronic Hepatitis C Virus Infection and the Impact of Direct-Acting Antiviral Therapy. Viruses. 2021;13(11):2249. doi:10.3390/v13112249.
2. Pawlotsky JM, Negro F, Aghemo A et al. EASL recommendations on treatment of hepatitis C: Final update of the series. J Hepatol. 2020;73(5):1170–218. doi: 10.1016/j.jhep.2020.08.018.
3. Brunner N, Bruggmann P. Trends of the Global Hepatitis C Disease Burden: Strategies to Achieve Elimination. J Prev Med Pub Health. 2021;54(4):251–8. doi: 10.3961/jpmph.21.151.
4. Yang J, Qi JL, Wang XX et al. The burden of hepatitis C virus in the world, China, India, and the United States from 1990 to 2019. Front Public Health. 2023;11:1041201. doi:10.3389/fpubh.2023.1041201.
5. Bhullar FA, Faghih M, Akshintala VS et al. Prevalence of primary painless chronic pancreatitis: A systematic review and meta-analysis. Pancreatology. 2022;22(1):20–9. doi:10.1016/j.pan.2021.11.006.
6. Beyer G, Habtezion A, Werner J et al. Chronic pancreatitis. The Lancet. 2020;396(10249):499–512. doi: 10.1016/S0140-6736(20)31318-0.
7. Ghurburrun E, Borbath I, Lemaigre FP, Jacquemin P. Liver and Pancreas: Do Similar Embryonic Development and Tissue Organization Lead to Similar Mechanisms of Tumorigenesis? Gene Expr. 2018;18(3):149-155. doi: 10.3727/105221618X15216414278706.
8. Jain P et al. Acute pancreatitis in acute viral hepatitis. World J Gastroenterol. 2007;13(43):5741. 2007;13(43):5741-4. doi: 10.3748/wjg.v13.i43.5741.
9. Imam Z, Simons-Linares CR, Chahal P. Infectious causes of acute pancreatitis: A systematic review. Pancreatology. 2020;20(7):1312–22. doi:10.1016/j.pan.2020.08.018.
10. Simons-Linares CR, Imam Z, Chahal P. Viral-Attributed Acute Pancreatitis: A Systematic Review. Dig Dis Sci. 2021;66(7):2162–72. doi:10.1007/s10620-020-06531-9.
11. Blackard JT, Kong L, Lombardi A et al. A preliminary analysis of hepatitis C virus in pancreatic islet cells. Virol J. 2017;14(1):237. doi:10.1186/s12985-017-0905-3.
12. Kuna L, Jakab J, Smolic R et al. HCV Extrahepatic Manifestations. J Clin Transl Hepatol. 2019;7(2):1–11. doi: 10.14218/JCTH.2018.00049
13. Yan FM et al. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. World J Gastroenterol. 2000;6(6):805. doi: 10.3748/wjg.v6.i6.805.
14. Katakura Y et al. Pancreatic involvement in chronic viral hepatitis. World J Gastroenterol. 2005;11(23):3508. doi: 10.3748/wjg.v11.i23.3508.
15. Pol S, Vallet-Pichard A, Hermine O. Extrahepatic cancers and chronic HCV infection. Nat Rev Gastroenterol Hepatol. 2018;15(5):283–90. doi:10.1038/nrgastro.2017.172.
16. Nakaz MOZ Ukrainy №638 vid 10.09.2014 r. «Pro zatverdzhennia ta vprovadzhennia medyko-tekhnolohichnykh dokumentiv zi standartyzatsii medychnoi dopomohy pry khronichnomu pankreatyti». [Order of the Ministry of Health of Ukraine No. 638 dated September 10, 2014 "On the approval and implementation of medical and technological documents on the standardization of medical care for chronic pancreatitis."] https://zakononline.com.ua/documents/show/35092____35092 [date access 02.05.2023] (In Ukrainian).
17. Babinets LS Shaihen OR, Homyn HI, Halabitska IM. Specific aspects of clinical course in case of combination of chronic pancreatitis and concomitant viral hepatitis C. Wiad Lek. 2019;72(4):595–9.
18. Fujita K, Oura K, Yoneyama H et al. Albumin–bilirubin score indicates liver fibrosis staging and prognosis in patients with chronic hepatitis C. Hepatol Res. 2019;49(7):731–42. doi:10.1111/hepr.13333.
19. Diéguez-Castillo C, Jiménez-Luna C, Prados J et al. State of the Art in Exocrine Pancreatic Insufficiency. Medicina (Mex). 2020;56(10):523. doi:10.3390/medicina56100523.
20. Panic N, Mihajlovic S, Vujasinovic M et al. Pancreatitis Associated with Viral Hepatitis: Systematic Review. J Clin Med. 2020;9(10):3309. doi:10.3390/jcm9103309.
21. Svegliati-Baroni G, Gaggini M, Carli F et al. Mechanisms for increased risk of diabetes in chronic liver diseases. Liver Int. 2020;40(10):2489–99. doi:10.1111/liv.14556.
22. Arafa A, Eshak ES, Abdel Rahman TA, Anwar MM. Hepatitis C virus infection and risk of pancreatic cancer: A meta-analysis. Cancer Epidemiol. 2020;65:101691. doi:10.1016/j.canep.2020.101691.
23. Fiorino S et al. Possible association between hepatitis C virus and malignancies different from hepatocellular carcinoma: A systematic review. World J Gastroenterol. 2015;21(45):12896. doi: 10.3748/wjg.v21.i45.12896.

The research was performed within the departmental topic of the Department of Faculty therapy of the Uzhhorod National University « Combined pathology and correction of homeostasis disorders of the residents of the Carpathian region, taking into account the effects of adverse factors» № state registration 0121U110808.

ORCID and contributionship:
Mariya A. Derbak: 0000-0003-4791-4080 [A,E,F]
Olha V. Buchok:0000-0001-5502-5158 [B,D]
Taras M. Ganich: 0000-0002-5278-7576 [E,F]
Mariya V. Rivis: 0000-0002-5282-6061 [A]
Yana V. Lazur: 0000-0002-7892-4946 [E]
Viktoriya M. Polyak-Tovt: 0009-0004-0929-8533 [C]
Volodymyr V. Timashev: 0000-0001-9432-3318 [C]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mariya A. Derbak
Uzhhorod National University
20 Hryboiedova St., 88000 Uzhhorod, Ukraine
tel: +380506275075
e-mail: morika1415@gmail.com

Received: 25.05.2023
Accepted: 09.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

PREDICTION OF EXTERNAL PANCREATIC FISTULA DEVELOPMENT IN PATIENTS WITH ACUTE INFECTED NECROTISING PANCREATITIS

DOI: 10.36740/WLek202311104

Olexii I. Dronov, Inna O. Kovalska, Andrii I. Horlach, Ivanna A. Shchyhel
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

ABSTRACT

The aim: To search for risk factors for the development of external pancreatic fistula (EPF) in patients with acute infected necrotizing pancreatitis.

Materials and methods: A prospective single-center observational study was conducted with the participation of 160 adult patients with infected acute necrotizing pancreatitis (ANP) who required intervention at different stages of the disease. Depending on the amylase activity of the drainage secretions, the groups with and without diagnosed EPF were compared according to 15 demographic, clinical, laboratory and instrumental parameters of the disease. To identify predictors of the risk of developing EPF in ANP, a regression logistic analysis was performed and logistic regression models were built for each factor attribute.

Results: We did not find statistically significant differences in the indicators characterising patients on the first day of illness, as well as in the comparison of indicators characterising local complications of AP, the timing of AP infection, the incidence of sepsis and the level of PON in sepsis. When comparing the groups by the frequency of the type of microbial agent of infected APB, no statistically significant differences were found. Comparative pairwise analysis between the groups by morphological characteristics revealed statistical differences in the frequency of focal superficial and transmural PN ($p < 0.001$). Next, we performed a regression logistic analysis and built logistic regression models for each factor attribute, namely age, gender, BMI, BISAP score and Charlson comorbidity index, morphological characteristics of necrotizing pancreatitis. After univariate regression analysis, a statistically significant association between the depth of PN and the risk of developing PFN was found (OR 2.7 (1.4-5.2), p=0.001).

Conclusions: We found that the risk of developing an external pancreatic fistula was associated with the depth of pancreatic necrosis.

KEY WORDS: pancreatic fistula, acute pancreatitis, infected pancreatic necrosis

Wiad Lek. 2023;76(11):2365-2371

INTRODUCTION

Treatment methods for late-phase infected pancreatic necrosis (IPN) vary depending on the manifestations of the disease [1-4]. The course of acute pancreatitis (AP) accompanied by PN and its infection can lead to the destruction of the pancreatic duct system with the formation of a pancreatic fistula with a certain flow rate, direction of drainage (external and internal fistula), duration and clinical outcome [5-7]. The diagnosis of external pancreatic fistula (EPF) as a consequence of infected NP is based on the receipt of enzyme-active secretions from the established drainage [5, 8], similar to the definition of postoperative pancreatic fistula [9, 10]. The development of EPF rarely leads to fatal complications, but it can disable patients with AP for a long time and lead to repeated surgical interventions, increasing the duration and cost of treatment [11-15]. With the identification of risk factors for the development of EPF, it becomes possible to find ways to influence them and prevent the occurrence of the latter.

THE AIM

To search for risk factors for the development of external pancreatic fistula in patients with acute infected necrotising pancreatitis.

MATERIALS AND METHODS

A prospective single-centre observational study was conducted at the Department of general surgery of the Bogomolets National Medical University. The study included 160 adult patients with acute necrotising infected pancreatitis who required intervention at different stages of treatment. Inclusion criterion - the presence of a positive bacterial culture obtained from the aspirate of acute pancreatic fluid collections (APFC) during a fine-needle aspiration or discharge from drained fluid collections in patients with ANP. Exclusion criterion - patients who died in the early postoperative period (7 days after the intervention). The diagnosis and severity of AP were assessed ac-

cording to the revised Atlanta criteria [16]. Depending on the timing of the disease and indications, all study patients underwent sonographically controlled percutaneous interventions or "open" necrosecrectomies. The level of amylase was monitored daily in the secretions from the drains using the qualitative Wolgemuth method. An increase in the level of amylase in the drainage aspirate by more than three times compared with the reference value, in the absence of amylasemia, was considered as EPF [5, 8, 9], these patients were referred to the EPF "+" group. Patients who did not have an increase in amylase in the drainage content of more than three times the reference value during inpatient treatment were included in the control group EPF "-". In patients with diagnosed EPF, we entered into the database the date of diagnosis of EPF (from the onset of the disease), its duration, maximum amylase level and daily flow rate. All patients were assessed for 15 gender-demographic, clinical, laboratory and instrumental parameters of the disease. Among the clinical, laboratory and instrumental parameters, we analysed the comorbidity index, body mass index (BMI), BISAP score, aetiology; clinical and morphological features reflecting systemic and local signs of the disease - mean MODS, CTSI and its components: Balthazar index, necrosis index; factors associated with the development of infectious complications - timing of infection, aetiology of pancreatic infection (PI), presence of sepsis and its severity according to SOFA. Local complications in patients with AP were ranked using the computed tomographic severity index, which was calculated by the sum of Balthazar score and pancreatic necrosis score. The clinical and morphological classification of Olexii I. Dronov (patent No. 26755, 2002) was used to characterise the distribution and depth of pancreatic necrosis.

When comparing data among patients with and without EPF, depending on the characteristics of the data and after analysing the data distribution for normality, the Mann-Whitney U test, the $\chi 2$ test, and the $\chi 2$ test with Bonferroni correction were used when comparing three or more subgroups. To establish the relationship between the predictors of acute necrotic pancreatitis and the risk of developing EPF, a logistic regression analysis was performed and logistic regression models were constructed for each factor attribute with the 95% CI of the Odds Ratio. The reason for constructing a multivariate logistic regression model was the preliminary confirmation of the predictor in a single-factor analysis. A value of $p<0.05$ was considered statistically significant. The EZR package (R-statistics) was used to calculate and analyse the data [17].

RESULTS

The study included 88 (55%) women and 72 (45%) men, with a median age (Q I - Q III) of 43 (37.5 - 60) years. The median (Q I - Q III) comorbidity index was 3 (1 - 4) points. Comorbidities were mainly represented by cardiovascular disease and liver disease - arterial hypertension was observed in 132 (82.5%), chronic hepatitis in 123 patients (76.9%), obesity was also common in the cohort - 105 (65.6%) cases, diabetes mellitus was observed in 23 (14.4%) patients. The median (Q I - Q III) BMI was 32.1 (28.1 - 35.9) kg/m^2. External pancreatic fistulas were diagnosed from day 18 to 38 of the disease, their median (Q I - Q III) was 29 (24 - 31) days. The proportion of patients who were diagnosed with EPF at the stage of sonographically controlled percutaneous puncture-drainage interventions was 19.7% - 15 out of 76, on average 6.8 (1.4) days after the intervention. The time interval between the onset of the disease and the diagnosis of the fistula was 20.6 (1.4) days on average. Drainage of one retroperitoneal zone - the anterior pararenal space - was performed in 10.5% of cases (8 out of 76) and two retroperitoneal zones - the anterior pararenal and perirenal spaces - in 89.5% of cases (68 out of 76). Drainage of the posterior pararenal space was not performed in any case. In cases of drainage of the anterior pararenal zone by several drains located in the omental sac and the right or left paracolon, an increased content of amylase was determined in each of the drains. In cases of combined drainage of the two zones in patients with EPF, the level of amylase in the perirenal space remained within the normal range, the median (Q I - Q III) of which was 23 (17 - 38) U. In 80.3% - 61 of 76 patients, EPF developed after necrosequestrectomy (NSE), on average 3.7 (1.4) days after surgery. The mean time from the onset of the disease to fistula formation was 29.8 (3.2) days. It was inexpedient to analyse the retroperitoneal lesion zones, since during NSE, the anatomical landmarks of the ileum lost their relevance due to the pathological process. The drained area containing pancreatic juice was limited to the post-sequestral cavity. External pancreatic fistulas after ANP closed on average in 33.8 (15.5) days, at least in 10 days, and at most in 68 days. The level of amylase in the drainage contents of patients with EPF ranged from 510 to 3856 U, the median (Q I - Q III) was 1218 (875 to 1551) U. Low-drainage fistulas were recorded in 36 patients (47.4%), medium-drainage in 32 patients (42.1%), and high-drainage fistulas were observed in 8 patients (10.5%).

A comparative analysis of the characteristics of the APN determined on the first day of hospital admission in patients with existing EPF and in patients where EPF was not determined is shown in Table I. In a compara-

Table I. Comparative characteristics of the factors determined in the studied patients on the first day of the disease

Parameter EPF «-»		Indexes EPF «+»		p
Age, years, Me (Q_I - Q_{III})		47,5 (39 – 61)	42 (37 – 57,5)	0,09[a]
Gender, n (%)	Men	50 (59,5)	49 (64,5)	0,63[b]
	Women	34 (40,5)	27(35,5)	
BMI, kg/m2, Me (Q_I - Q_{III})		30,4 (27,8 – 36,8)	32,2 (28,3 – 34,2)	0,95[a]
Comorbidity index, points Me (Q_I - Q_{III})		3 (2 – 4)	2 (1 – 3,5)	0,07[a]
BISAP-score, points, Me (Q_I - Q_{III})		3 (3 – 3)	3 (3 – 3)	0,98[a]
Etiology, n (%)	Alcoholic	38 (50)	22 (26)	<0,05[c]
	Biliary	26 (34)	31 (37)	
	HTG-associate	4 (5)	16 (19)	
	EPST-induced	6 (8)	8 (10)	
	Idiopathic	2 (3)	7 (8)	

[a] - Mann-Whitney U test
[b] - the χ2 criterion
[c] – χ2 with Bonferroni correction (p=0.056)

Table II. Comparative characteristics of systemic and local complications in patients with existing and without external pancreatic fistulas

Parameters	Me (Q_I – Q_{III}) EPF «-»	Me (Q_I – Q_{III}) EPF «+»	P
Average MODS, (points)	8,2 (6 – 10,3)	8,6 (7,6 – 11,1)	0,13
CTSI (points)	8 (6,5 – 10)	8 (6 – 10)	0,83
Balthazar index, (points)	4 (4 – 4)	4 (4 – 4)	0,07
Necrosis index (points)	4 (3 – 6)	4 (2 – 6)	0,96
Volume of extrapancreatic necrosis (ml)	1250 (814 – 1522)	1216 (825 – 1523)	0,34

Table III. Comparative characteristics of the spread of pancreatic necrosis in patients with and without external pancreatic fistulas

Configuration of pancreatic necrosis	EPF "+", frequency	EPF "-", frequency	p
Total superficial	11%	17%	0.37
Subtotal transmural	33%	30%	0.8
Subtotal superficial	27%	28%	0.96
Focal transmural	29%	7%	< 0,001
Focal superficial	0%	18%	< 0,001

tive analysis of the indicators listed in Table I, we were unable to identify statistically significant differences in any of the indicators that characterised patients on the first day of illness who subsequently developed or did not develop EPF.

Next, we compared between the groups (according to the Mann-Whitney U test) the factors reflecting the level of systemic complications and the morphological characteristics of local necrotic complications, which is shown in Table II. When comparing the indicators of any of the parameters characterizing local complications of ANP, namely according to the levels CTSI, Balthazar index and necrosis index, as well as in the volume of extrapancreatic lesions, no statistically significant difference was found. The level of systemic complications, which was reflected in the form of the average MODS in the first week of the disease, also did not establish statistical differences in the groups of studied patients.

The configuration of PN in the studied patients varied from focal to total in distribution and from superficial to trasmural in depth. Frequency and comparative characteristics of types of pancreatic necrosis according to the clinical and morphological classification of O.I. Dronov is shown in Table III.

As indicated above (Table III), a comparative pairwise analysis between groups (according to the χ2 criterion) did not establish a statistical difference in the frequency of total superficial (p = 0.37), subtotal trasmural (p = 0.8),

Table IV. Comparative characteristics of the course of the infectious process in the studied patients

Parameter	Indexes		p
	EPF «+»	EPF «-»	
Periods of infection, days, Me(Q_I - Q_{III})	10 (8 – 15)	10 (8 – 13)	0,22 [a]
Frequency of sepsis, n (%)	39 (46,4)	42 (55,3)	0,33 [b]
max SOFA, points, Me (Q_I - Q_{III})	6 (2 – 12)	6 (4 – 8)	0,57 [a]

[a] – Mann-Whitney U test

[b] -the $\chi 2$ criterion

Table V. Taxonomic characteristics of the causative agents of pancreatic infection in the studied patients and pairwise comparison

Type of microorganism	EPF "+", frequency	EPF "-", frequency	p
C.albicans	3%	4%	0.85
P.morganii	0%	1%	0.94
P.aeruginosa	3%	5%	0.87
A.lwoffi	1%	0%	0.94
A.baumannii	9%	9%	0.95
S.haemolyticus	1%	2%	0.92
Enterobacter spp.	10%	11%	0.9
S.aureus	12%	10%	0.8
E.feacium	16%	18%	0.9
K.pneumoniae	16%	19%	0.68
E.coli	29%	21%	0.27

Table VI. Results of univariate analysis of the influence of factors of acute necrotizing pancreatitis on the development of external pancreatic fistulas

Factor sign		Model Coefficient (b±m)	p	Odds ratio (95% CI)
Age		-0,03 ± 0,01	0,058	0,96 (0,94 – 0,99)
Sex	Men	Reference		
	Women	0,3 ± 0,31	0,22	1,4 (0,7 – 2,7)
BMI		-0,037 ± 0,03	0,2	0,96 (0,9 – 1,0)
BISAP		0,07 ± 0,35	0,84	1,07 (0,5 – 2,17)
Charlson comorbidity index		-0,12 ± 0,09	0,17	0,88 (0,73 – 1,06)
CTSI		0,02 ± 0,09	0,8	1 (0,8 – 1,24)
Necrosis index		-0,007 ± 0,1	0,9	0,9 (0,8 – 1,2)
The depth of necrosis	Deep	Reference		
	Superficial	1,01 ± 0.001	0.001	2,7(1,4-5,2)

subtotal superficial (p = 0.96) of necrotic pancreatitis and revealed statistical differences in the frequency of focal transmural PN (p < 0.001) and focal surface PN (p < 0.001). Total transmural ANP was not observed in both groups, patients with this type of necrotic lesion did not live to the time interval that corresponded to the late postoperative period, where the EPFs were analysed after NSE.

Taking into account the results of pairwise comparison by depth and spread of PN, we assumed that the depth of necrosis is clinically relevant, so we performed a pairwise comparative analysis. Among the superficial PNs, 53 (64.6%) did not develop EPF, and 29 (35.4%) were diagnosed with EPF. In the group of deep PNs (which included transmural spread), 31 (39.7%) patients did not have complications with the development of EPF, and 47 (60.3%) were diagnosed with EPF. According to the results of the analysis, it was found that the groups were different in the frequency of superficial and deep PN (p=0.02 by $\chi 2$ criterion)

The next factors of comparison were those reflecting the characteristics of infectious complications. Table IV shows a comparison of the terms of infection of patients with ANP, the frequency of sepsis among groups of patients, and the level of sepsis-induced multiorgan insufficiency with the maximum SOFA indicator in those patients who had generalised infectious complications. We did not find a statistically significant difference in terms of infection with PN (p = 0.22), frequency of sepsis (p = 0.33) and level of PN among patients with sepsis (p = 0.57).

Taking into account the variability of PI pathogens that caused the development of infected ANP, the next step was to study the influence of PI etiology on the risk of developing EPF in the studied patients. Therefore, 198 microbial isolates were obtained from 160 patients, of which 106 were from 76 patients with EPF and 92 isolates were from 84 patients without EPF. The microbial landscape was represented by eleven types of microorganisms, which were dominated by gram-negative ones, of which, in turn, *E. coli* and *K. pneumoniae* prevailed. So, in the group with the presence and absence of EPF, *E. coli* was respectively identified in 26/92 and 22/106 cases, *K. pneumoniae* – in 15/92 and 20/106, respectively, *E. feacium* – in 15/92 and 20/106 respectively, *S. aureus* - 11/92 and 11/106, respectively, *Enterobacter spp.* - 9/92 and 12/106, respectively, S. *haemolyticus* - 1/92 and 2/106, respectively, *A. baumannii* - 8/92 and 10 /106, respectively, *P. aeruginosa* – 3/92 and 5/106, respectively, *C. albicans* – 3/92 and 4/106, respectively. The comparative etiological characteristics of the causative agents of PI in patients with and without EPF are presented in Table V.

A pairwise comparative analysis of the frequency of the type of microorganism that caused the development of infected ANP in patients who had post-operative PN and those who did not have this complication did not establish statistically significant differences among any of the identified types of microorganisms according to the $\chi 2$ test. Thus, it can be asserted that the aetiology of PI did not affect the development of EPF in patients with infected ANP.

In order to establish the relationship between the predictors of ANP and the risk of developing EPF, logistic regression analysis was performed and logistic regression models were built for each factor characteristic, namely, age, gender, BMI, BISAP scale and Charlson comorbidity index, morphological characteristics of necrotizing pancreatitis (Table VI). After a univariate regression analysis, a statistically significant association was found between the depth of PN and the risk of developing EPF (OR 2.7 (1.4-5.2), p=0.001)

DISCUSSION

Although the clinical course of an external pancreatic fistula, even with a disconnected pancreatic duct, may end with spontaneous closure, the duration of treatment is measured in months [18-21]. We identified the depth of pancreatic necrosis as a key risk factor for the development of EPF, or rather, the existing post-sequestral cavity, into which the different diameters pancreatic duct of the functioning residual distal, relative to the necrosis, pancreatic parenchyma, drains. Accordingly, every patient with acute pancreatitis, the course of which was complicated by deep PN, may subsequently develop EPF.

Along with the consequences of pancreatic necrosis, the occurrence of EPF is considered as a complication associated with pancreatic resection, which is currently being studied more [22-26]. Thus, some researchers, when searching for predictors, prefer visualisation methods, recommending to take into account preoperative data of instrumental studies [27-29]. Further search for predictors of EPF in patients with infected ANP should expand the field of possible risk factors, taking into account proven predictors of EPF after pancreatic surgery and trauma.

CONCLUSIONS

We did not find any statistically significant predictor that could influence the development of EPF in the postoperative period in patients with infected ANP among those that characterise the patient on the first day of the disease, reflect systemic and local signs of the disease and are determined in the first week, and among the factors associated with the development of infectious complications (p>0.05). However, among the clinical and morphological signs, we found that the risk of developing an external pancreatic fistula was associated with the depth of pancreatic necrosis. (OR 2.7 (1.4-5.2), p=0.001)

REFERENCES

1. Thomson JE, Van Dijk SM, Brand M et al. Managing Infected Pancreatic Necrosis. Chirurgia (Bucur). 2018;113(3):291-299. doi:10.21614/chirurgia.113.3.291.
2. Purschke B, Bolm L, Meyer MN, Sato H. Interventional strategies in infected necrotizing pancreatitis: Indications, timing, and outcomes. World J Gastroenterol. 2022;28(27):3383-3397. doi: 10.3748/wjg.v28.i27.3383.

3. Baron TH, DiMaio CJ, Wang AY, Morgan KA. American Gastroenterological Association Clinical Practice Update: Management of Pancreatic Necrosis. Gastroenterology. 2020;158(1):67-75.e1. doi: 10.1053/j.gastro.2019.07.064.
4. Maurer LR, Fagenholz PJ. Contemporary Surgical Management of Pancreatic Necrosis. JAMA Surg. 2023;158(1):81-88. doi: 10.1001/jamasurg.2022.5695.
5. Unegbu FC, Anjum F. Pancreatic Fistula. In: StatPearls. Treasure Island (FL): StatPearls Publishing. 2023. https://pubmed.ncbi.nlm.nih.gov/32809706/ [date access 26.04.2023]
6. Rana SS. An overview of walled-off pancreatic necrosis for clinicians. Expert Rev Gastroenterol Hepatol. 2019;13(4):331-343. doi: 10.1080/17474124.2019.1574568.
7. Maatman TK, Roch AM, Ceppa EP et al. The continuum of complications in survivors of necrotizing pancreatitis. Surgery. 2020;168(6):1032-1040. doi: 10.1016/j.surg.2020.07.004.
8. Sunderland GT, Imrie CW. Pancreatic Fistulas in Acute Pancreatitis. Pancreatic Fistulas. Springer, Berlin, Heidelberg. 1992. doi: 10.1007/978-3-642-77418-8_4.
9. Bassi C, Marchegiani G, Dervenis C et al. The 2016 update of the International Study Group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 Years After. Surgery. 2017;161(3):584-591. doi: 10.1016/j.surg.2016.11.014.
10. Marchegiani G, Andrianello S, Salvia R, Bassi C. Current Definition of and Controversial Issues Regarding Postoperative Pancreatic Fistulas. Gut Liver. 2019;13(2):149-153. doi: 10.5009/gnl18229.
11. Singh A, Aggarwal M, Garg R et al. Spontaneous Internal Pancreatic Fistulae Complicating Acute Pancreatitis. Am J Gastroenterol. 2021;116(7):1381-1386. doi: 10.14309/ajg.0000000000001282.
12. Holsing K, Anton B, Timmerman G. Delayed Internal Pancreatic Fistula and Pseudocyst Formation Three Years Status-Post Distal Pancreatectomy in the Setting of Acute Pancreatitis. S D Med. 2020;73(11):520-522.
13. Kone LB, Maker VK, Banulescu M, Maker AV. Should Drains Suck? A Propensity Score Analysis of Closed-Suction Versus Closed-Gravity Drainage After Pancreatectomy. J Gastrointest Surg. 2021;25(5):1224-1232. doi: 10.1007/s11605-020-04613-7.
14. Pearson EG, Scaife CL, Mulvihill SJ, Glasgow RE. Roux-en-Y drainage of a pancreatic fistula for disconnected pancreatic duct syndrome after acute necrotizing pancreatitis. HPB (Oxford). 2012;14(1):26-31. doi: 10.1111/j.1477-2574.2011.00397.x.
15. Khiatah B, Huynh T, Frugoli A, Lyche KD. Acute Pancreatitis with an Ongoing Pancreatic Duct Leak Complicated by Refractory Pleural Effusion: A Case Report. Am J Case Rep. 2021;22:e931330. doi: 10.12659/AJCR.931330.
16. Banks PA, Bollen TL, Dervenis C et al. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102-111. doi: 10.1136/gutjnl-2012-302779.
17. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452–458. doi:10.1038/bmt.2012.244.
18. Rana SS, Sharma R, Kang M, Gupta R. Natural course of low output external pancreatic fistula in patients with disconnected pancreatic duct syndrome following acute necrotising pancreatitis. Pancreatology. 2020;20(2):177-181. doi: 10.1016/j.pan.2019.12.011.
19. Tarantino I, Carrozza L, Rizzo GEM et al. Radiologic and endoscopic treatment for a disconnected pancreatic duct syndrome associated with percutaneous pancreatic fistula: achieving the "internalization of the fistula". Endoscopy. 2023;55(S01):E777-E778. doi: 10.1055/a-2088-8921.
20. Erdogan O, Teke Z, Ulku A et al. Intractable external pancreatic fistula post-necrosectomy treated successfully with a fistulojejunostomy. Ann Ital Chir. 2020;9:S2239253X20031898.
21. Larsen M, Kozarek R. Management of pancreatic ductal leaks and fistulae. J Gastroenterol Hepatol. 2014;29(7):1360-1370. doi: 10.1111/jgh.12574.
22. Rykina-Tameeva N, Samra JS, Sahni S, Mittal A. Drain fluid biomarkers for prediction and diagnosis of clinically relevant postoperative pancreatic fistula: A narrative review. World J Gastrointest Surg. 2022;14(10):1089-1106. doi: 10.4240/wjgs.v14.i10.1089.
23. Marchegiani G, Bassi C. Prevention, prediction, and mitigation of postoperative pancreatic fistula. Br J Surg. 2021;108(6):602-604. doi: 10.1093/bjs/znab125.
24. Bonsdorff A, Sallinen V. Prediction of postoperative pancreatic fistula and pancreatitis after pancreatoduodenectomy or distal pancreatectomy: A review. Scand J Surg. 2023;112(2):126-134. doi: 10.1177/14574969231167781.
25. Han IW, Cho K, Ryu Y et al. Risk prediction platform for pancreatic fistula after pancreatoduodenectomy using artificial intelligence. World J Gastroenterol. 2020;26(30):4453-4464. doi: 10.3748/wjg.v26.i30.4453.
26. PARANOIA Study Group; Writing committee, Pande R, et al. External validation of postoperative pancreatic fistula prediction scores in pancreatoduodenectomy: a systematic review and meta-analysis. HPB (Oxford). 2022;24(3):287-298. doi: 10.1016/j.hpb.2021.10.006.
27. Yue Y, Li M, Zhang X et al. Prediction of clinically relevant pancreatic fistula after pancreatic surgery using preoperative CT scan: A systematic review and meta-analysis. Pancreatology. 2020;20(7):1558-1565. doi: 10.1016/j.pan.2020.09.009.
28. Zhang W, Cai W, He B et al. A radiomics-based formula for the preoperative prediction of postoperative pancreatic fistula in patients with pancreaticoduodenectomy. Cancer Manag Res. 2018;10:6469-6478. doi: 10.2147/CMAR.S185865.
29. Kolbinger FR, Lambrecht J, Leger S et al. The image-based preoperative fistula risk score (preFRS) predicts postoperative pancreatic fistula in patients undergoing pancreatic head resection. Sci Rep. 2022;12(1):4064. doi: 10.1038/s41598-022-07970-2.

ORCID and contributionship:
Olexii I. Dronov: 0000-0003-4033-3195 [B, E, F]
Inna O. Kovalska: 0000-0002-6264-2928 [A, B, E, F]
Andrii I. Horlach: 0000-0003-4330-4904 [A-D]
Ivanna A. Shchyhel: 0000-0002-1672-8149 [A-D]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Ivanna A. Shchyhel
Bogomolets National Medical University
26/4b Taras Shevchenko Boulevard, 01030 Kyiv, Ukraine
tel: +380681180580
e-mail: Ringoo3110@gmail.com

Received: 20.05.2023
Accepted: 11.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

NON-INVASIVE METHODS OF DIAGNOSTICS OF GASTRO-ESOPHAGEAL REFLUX DISEASE IN PATIENTS WITH ISCHEMIC HEART DISEASE

DOI: 10.36740/WLek202311105

Mariya A. Derbak[1], Romana-Mariia I. Tovtun[1], Olesya M. Horlenko[1], Oksana T. Hanych[1], Volodymyr V. Timashev[1], Paul M. Lukach [2], Mykhaylo Hechko[1]

[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

[2]COMMUNAL NONPROFIT ENTERPRISE «CENTRAL CITY CLINICAL HOSPITAL» OF UZHHOROD CITY COUNCIL, UZHHOROD, UKRAINE

ABSTRACT

The aim: To determine the diagnostic value of non-invasive methods of GERD diagnosis based on questionnaire data and a diagnostic test with a proton pump inhibitor (PPI) with Rabeprazole in patients with coronary heart disease (CHD).

Materials and methods: 90 patients were under observation, namely, 68 patients with coronary heart disease with concomitant essential arterial hypertension (EAH), 6 patients with coronary artery disease + arrhythmias, and 18 - others diseases. All patients were surveyed according to the GerdQ questionnaire, followed by PPI testing with Rabeprazole, and body mass index (BMI) was calculated for all the patients. A BMI of 18.5-24.9 kg/m2 was considered as normal body weight, and a BMI > 24.9 kg/m2 was considered overweight.

Results: Based on the results of the questionnaire, it was established that the most common complaints typical for GERD were noted by patients with CHD in combination with EAH. There were 48 of such patients and, depending on BMI, they were divided into 2 groups: 1st group (n=14) - patients with CHD+EAH+GERD with normal body weight and 2nd group (n=34) – overweight patients with CHD+EAH+GERD. In patients of group 1, typical symptoms of GERD prevailed (in 71.4% of patients), and in patients group 2, the distribution of typical and extra-esophageal symptoms did not differ significantly (52.9% vs. 47.1%). Among the extraesophageal manifestations, pain behind the sternum (in the projection of the esophagus) was significantly more often recorded in patients of group 1, and rhythm disturbances in patients of group 2 (43.8% and 75.0% of patients, respectively, $p<0.05$). The results of the GerdQ questionnaire showed a direct relationship between GERD, body weight and symptom score. Patients with GERD+normal body weight had a mean score of 6 for classic gastroesophageal reflux symptoms, while patients with GERD+increased body weight had a mean score of 7. The sensitivity of the questionnaire was 78.7%, and the specificity - 92.9%. According to the PPI test, in the 1st and 2nd groups, already in the first three days, 28.5% and 23.5% of patients noted the disappearance of heartburn and after 10 days - 85.7% and 64.7%, respectively. Over the entire period, that is, after 14 days of observation, 85.7% of patients in the 1st group and 73.5% in the 2nd group noted improvement.

Conclusions: It has been established that questionnaires based on the GerdQ questionnaire followed by a PPI test with Rabeprazole in patients with coronary heart disease combined with essential arterial hypertension have a high diagnostic value and can be used for early diagnosis and effective treatment of GERD.

KEY WORDS: coronary heart disease, essential arterial hypertension, gastroesophageal reflux disease, increased body weight, proton pump inhibitor, non-invasive methods, GerdQ questionnaire

Wiad Lek. 2023;76(11):2372-2377

INTRODUCTION

Extensive epidemiological studies in the countries of Western Europe and the USA indicate that 40% of people constantly suffer from heartburn - the main symptom of gastroesophageal reflux disease (GERD) [1-3].

At the same time, GERD can lead to functional disorders of the cardiovascular system and be the trigger for a cascade of pathological processes that initiate destabilization of coronary blood flow and myocardial ischemia. 35-70% of patients with esophageal disease are misdiagnosed with coronary heart disease (CHD), and vice versa, esophageal disease remains undiagnosed in 50% of patients with CHD [4]. The significant similarity in character, localization, irradiation is explained by the close anatomical location and common innervation of the heart and esophagus, which in case of pathology of the esophagus due to viscero-visceral reflexes can provoke arrhythmias and play the role of a trigger factor in the occurrence of new angina attacks [2,5].

In patients with coronary artery disease, fibroesophagogastroduodenoscopy (FEGDS) is performed only based on vital signs, and often the pathology of the gastroduodenal zone in these patients remains outside the attention of the doctor. Taking into account the

mutually aggravating influence of the combination of GERD and coronary heart disease, a more in-depth study of the extraesophageal manifestations of GERD and the search for simpler and unified methods of its diagnosis are necessary. In such cases, in the absence of alarming signs, non-invasive methods such as patient questionnaires and the use of empiric acid-suppressive therapy in the form of a diagnostic test with a proton pump inhibitor (PPI) come to the fore. Today, the GerdQ questionnaire (Gastroesophageal Reflux Disease Questionnaire) exists as the most promising, as it was created on the basis of three other statistically based questionnaires for the assessment of reflux syndrome (GIS, GSRS, RDQ) [6,7,8]. If the sum of Gerd-Q scores is 8 or more, GERD is diagnosed. Indicators of quality of life or dynamics of well-being under the influence of treatment are evaluated separately: the sum of points 3 or more - severe GERD; less than 3 points - moderate [9].

THE AIM

The aim of the study was to determine the diagnostic value of non-invasive methods of GERD diagnosis based on questionnaire data and a diagnostic test with a proton pump inhibitor (PPI) with Rabeprazole in patients with coronary heart disease (CHD).

MATERIALS AND METHODS

In 2023, 90 patients who have been treated in the cardiology department of the Uzhhorod central city clinical hospital and have been screened for GERD were under observation. This included patients with coronary heart disease with concomitant essential arterial hypertension (EAH) - 68 people, coronary heart disease + arrhythmia - 6, others - 18. All examined were divided by gender, age, duration of the disease and body mass index. There were 36.7% (33) men, 63.3% (57) women. The average age of patients is 64.5±1.5 years.

For further research, the main group of examinees was singled out, which included 48 patients with coronary artery disease with concomitant EAH and in whom GERD was diagnosed.

The study was conducted at the department of faculty therapy with the consent of the patients, and the methodology of their conduct corresponded to the Helsinki Declaration of 1975 and its revision of 1983. The study was also approved by the local ethics committee (protocol No. 7/5 dated 05/16/2023).

All patients underwent a general clinical examination and special examination methods, which included: electrocardiography (ECG) on the HeartScreen 112 Visit electrocardiograph, echocardioscopy, ultrasound examination of extracranial vessels, abdominal organs (ultrasound of the abdominal cavity) using the CX50 ultrasound system, Philips Ultrasound, blood glucose fasting, glycated hemoglobin, blood insulin and insulin resistance index, total cholesterol (C), low and high density lipoproteins (LDL and HDL), triglycerides, uric acid, blood potassium level. All studies were carried out in certified laboratories. Also, body mass index (BMI) was calculated for all patients, which was calculated according to the formula: BMI = weight (kg)/height (m)2 and measured in kg/m2. A BMI of 18.5-24.9 kg/m2 was considered normal body weight (BMI). A BMI > 24.9 kg/m2 was considered overweight. Obesity was diagnosed with BMI ≥ 30.0 kg/m2: I degree of obesity - BMI from 30-34.9 kg/m2, II degree - 34.9-39.9 kg/m2, III degree - 39.9 and above kg/m2.

All patients were interviewed according to the GerdQ questionnaire. During the questionnaire, typical GERD complaints (heartburn, acid belching) were determined. The questionnaire contained 6 questions, each of which is valued at a maximum of 3 points. If the final score was equal to 8 or more, it means that the patient has a high probability of GERD.

The diagnosis of GERD was confirmed according to the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine No. 943 of October 31, 2013) [10]. Gastrointestinal videoscope Olympus GIF-XP170N, Olympus GIF-H170 (2018) was used to perform FGDS. The diagnosis of CHD and EAH was made on the basis of protocols No. 2857 (order of the Ministry of Health of Ukraine dated December 23, 2021) [11] and No. 384 (order of the Ministry of Health of Ukraine dated May 24, 2012) [12].

The patients were prescribed therapy of the main disease, according to the existing Standards of medical care, which included anti-anginal/anti-ischemic drugs, beta-blockers and/or calcium channel blockers, antithrombotic drugs, hypolipidemic drugs, ACE inhibitors, inhibitors of the renin-angiotensive system and others if necessary. All patients with coronary heart disease combined with hypertension underwent a PPI test with Rabeprazole 20 mg twice a day 40 minutes before meals, which lasted for two weeks as part of inpatient treatment. During this period, the initial symptoms of the disease were monitored every day. Then, if the patient previously has got only the typical GERD symptoms, when the complaints regressed, he was recommended to receive rabeprazole 20 mg twice daily 40 minutes before meals on an outpatient basis for 8 weeks. If, in addition to the typical symptoms of GERD, the patient also had extraesophageal symptoms, he was recommended Rabeprazole at a dose of 20 mg twice a day 40 minutes before meals for a longer pe-

Table I. Clinical manifestations of GERD in patients with CHD+EAH

Sign	Group (abs./%)	
	1st group n=14 CHD+EAH+GERD+ normal body weight	2nd group n=34 CHD+EAH+GERD+ increased body weight
Presence of clinical manifestations of GERD (n=48)	14/100	34/100
Esophageal symptoms of GERD (n=28)	10/71,4*	18/52,9
Heartburn	7/70,0	10/55,5*
Sour eructation	5/50,0*	2/11,1
Feeling of heaviness in the upper part of the abdomen	3/30,0	4/22,2
Nausea	3/30,0	2/16,7
Extraesophageal symptoms of GERD(n=20)	4/28,6	16/47,1*
Pulmonological symptoms	-	2/12,5
Dry barking cough	0/0	2/12,5
Cardiological symptoms		
Chest pains (along the esophagus)	1/25,0	7/43,8*
Interruptions in the heart work	3/75,0*	8/50,0
Other complaints (n=48)		
Flatulence	3/6,25	6/27,3*
Weakness, fatigue	8/16,6	14/63,6

Note. Significance of the difference: * - in comparison with the indicator between the 1st and 2nd groups (the indicator is calculated according to the Mann-Whitney test, $p<0.05$)

riod, namely for 12 weeks with further monitoring of symptoms and follow-up examination [13].

The analysis and processing of the results of the examination of the patients was carried out using the Statistics for Windows v.7.0 computer program (StatSoft Inc, USA) using parametric and non-parametric methods of evaluating the results. The difference was considered to be significant at $p<0.05$. Statistical processing and analysis of the obtained results was performed using Jamovi software.

RESULTS

We found that 53.3% (48 out of 90) of patients with cardiovascular diseases had complaints typical for GERD. It was established that GERD is significantly more often registered in patients with coronary heart disease with overweight than with normal body mass (70.8% vs. 29.2%, $p<0.05$). The distribution according to diagnoses and body weight was next. There were 48 patients with CHD+EAH+GERD, 20 people with CHD+EAH, 6 arrhythmias, and 18 - others. There were 33 (36.7%) men and 57 (63.3%) women. When dividing by the weight, it was found that 31 (34.4%) patient had a normal body weight , and 59 (65.6%) had an increased body weight.

Based on the results of the questionnaire, it was established that the most common complaints typical for GERD were noted by patients with coronary artery disease in combination with EAH. There were 48 such patients and, depending on BMI, they were divided into 2 groups: 1st group (n=14) are patients with CHD+EAH+GERD with normal body weight and 2nd group (n=34) are overweight patients with CHD+EAH+GERD. So, among comorbid patients, there were more often persons with increased body weight. This is explained by the fact that the onset and progression of GERD occurs more often in obesity, which is associated with increased intra-abdominal pressure and acid exposure in the esophagus, and acid reflux rates in these individuals correlate with body mass index [14].

Characterizing the manifestations of GERD in patients with different body weights, we found that typical manifestations of GERD prevailed in patients with normal BMI (in 71.4% of patients against 28.6% of atypical ones, $p<0.05$), while in patients with increased BMI distribution of typical and extraesophageal symptoms was not significantly different (52.9% vs. 47.1%). In patients of groups 1 and 2, among the typical symptoms of GERD, heartburn was significantly more often registered, namely in 70.0% and 55.5%, respectively.

Extraesophageal manifestations of GERD were observed in 41.7% (20 out of 48) of patients, among whom 20.0% (4/20) of patients had normal BMI and 80.0% (16/20) of patients had increased BMI ($p<0.05$). In patients with overweight, pain behind the sternum (in the course of the esophagus) was significantly more

Table II. The number of patients in whom symptoms of GERD disappeared during the PPI test

Number of days of taking PPIs	Groups (abs./%)	
	1st group n=14 CHD+EAH+GERD+ normal body weight	2nd group n=34 CHD+EAH+GERD+ increased body weight
0-3 days	4/28,5	8/23,5
4-7 days	6/42,8	4/11,8
8-10 days	2/14,3	10/29,4
Up to 10 days	12/85,7*	22/64,7
12-15 days	0	3/8,8
During the whole period	12/85,7*	25/73,5

Note. Significance of the difference: * - in comparison with the indicator of group 2 (the indicator is calculated according to the Mann-Whitney test, $p<0.05$)

often registered, namely in 43.8%, while in patients with normal body weight, rhythm disturbances were significantly more often registered - 75.0% (3/4) of patients ($p< 0.05$). Patients also noted other complaints, such as flatulence, weakness, and rapid fatigue, the frequency of which significantly ($p<0.05$) prevailed in people with increased body weight (Table I).

After analyzing the questionnaire data of the surveyed patients, we obtained the following results: to the question "How often do you have heartburn?" patients of group 1 answered as follows: 3 times a day - 14.2% (2/14) of patients; once every 2-3 days – 14.2% (2/14) of patients; 5 times for 4-7 days – 7.1% (1/14) of patients; patients of 2 groups answered as follows: 3 times a day - 17.6% (6/34) of patients; once every 2-3 days – 14.7% (5/34) of patients; 5 times for 4-7 days - 14.7% (5/34) of patients. The answer to the second question: "How often do you feel food or liquid being thrown into the throat?" in 1 group - within 1 day, 14.2% (2/14) of patients; within 2-3 days – 21.4% (3/14) of patients; within 4-7 days – 7.1% (1/14) of patients; Group 2 answered as follows: within 1 day - 11.7% (4/34) of patients; within 2-3 days – 8.8% (3/34) of patients; within 4-7 days - 5.8% (2/34) of patients. To the third question: "How often do you feel pain in the center of the upper abdomen?" responses of group 1: within 1 day – 21.4% (3/14) of patients; within 2-3 days - 14.2% (2/14) of patients; within 4-7 days – 14.2% (2/14) of patients; responses of 2 groups: within 1 day – 5.8% (2/34) of patients; within 2-3 days – 8.8% (3/34) of patients; within 4-7 days - 5.8% (2/34) of patients. On the fourth question: "How often do you feel nausea?" 1 group answered: within 1 day - 14.2% (2/14) patients; within 2-3 days – 28.5% (4/14) of patients; within 4-7 days – 14.2% (2/14) of patients; the answers of 2 groups were as follows: within 1 day - 8.8% (3/34) of patients; within 2-3 days – 14.7% (5/34) of patients; within 4-7 days - 5.8% (2/34) of patients. To the fifth question "How often did heartburn or belching prevent you from sleeping at night?" 1 group of patients answered: 1 day – 7.1% (1/14) of patients; within 2-3 days - 14.2% (2/14) of patients; The 2nd group answered as follows: 1 day – 11.7% (4/34) of patients; within 2-3 days - 8.8% (3/34) of patients. To the sixth question, "How often do you use medicines for the treatment of heartburn or regurgitation, which the doctor advised you?" respondents of 1 group answered: within 1 day - 7.1% (1/14) of patients; within 2-3 days - 14.2% (2/14) of patients; patients of 2 groups: 1 day – 14.7% (5/34) of patients; within 2-3 days - 8.8% (3/34) of patients.

The results of the GerdQ questionnaire in our study demonstrated a direct relationship between body weight and symptom of GERD score, which is consistent with the data of other researchers [6,14,15]. Patients with GERD+normal BMI had a mean score of 6 on the classic symptoms of gastroesophageal reflux, while patients with GERD+increased BMI had a mean score of 7 on the corresponding items, which allows us to argue that the frequency of symptoms depends on weight gain. The sensitivity of the questionnaire was 78.7%, and the specificity was 92.9%.

According to the PPI test, the following results were obtained: 28.5% (4/14) of patients from group 1 and 23.5% (8/34) of patients from group 2 noted the disappearance of heartburn in the first three days. After 7 days, GERD symptoms disappeared in 42.8% (6/14) of patients from group 1 and 11.8% (4/34) from group 2, and after 10 days improvement was noted in 14.3% (2 /14) and 29.4% (10/34) of patients, which was a total of 85.7% and 64.7%, respectively, in groups 1 and 2. Over the entire period, that is, after 14 days of observation, 85.7% (12/14) of patients in the 1st group and 73.5% - in the 2nd group noted improvement (Table II).

DISCUSSION

The data we received on the diagnostic value of the questionnaire showed the sensitivity of the questionnaire - 78.7%, and the specificity - 92.9%, which was

established on the basis of the disappearance of the detected GERD symptoms using the questionnaire, after the appointment of Rabeprazole. Similar data were obtained by such scientists as V.Yu. Prykhodko, D.Yu. Moreva, I.H. Palii, S.V. Zayka. [2, 4, 5, 13]. According to the obtained data, the questionnaire can be used as a convenient tool for a personalized approach to the diagnosis and treatment of GERD [8,9]. It is also important to note that although the GerdQ represents a useful diagnostic tool, it should not be viewed as a unique diagnostic test. It can be used as a baseline test in the absence of anxiety symptoms [6,7].

The obtained high rates of GERD symptom reduction during the PPI test in a short period (from 3 to 10 days) gives us reason to assert the high effectiveness of Rabeprazole and the diagnostic value of this test. Since heartburn, which is the main symptom of GERD, disappears with the use of rabeprazole, this gives us the opportunity to put the PPI test alongside other methods of GERD diagnosis, especially in patients with cardiovascular diseases, where the patient's condition often makes FGDS impossible.

CONCLUSIONS

It has been established that questionnaires based on the GerdQ questionnaire followed by a PPI test with Rabeprazole in patients with coronary heart disease combined with hypertension have a high diagnostic value and can be used for early diagnosis and effective treatment of GERD.

REFERENCES

1. Sandhu DS, Fass R. Current Trends in the Management of Gastroesophageal Reflux Disease. Gut Liver. 2018;12(1):7-16. doi:10.5009/gnl16615.
2. Prykhodko VYu, Moreva DYu. Osoblyvosti perebihu ta likuvannia arterialnoi hipertenzii u poiednanni z khronichnoiu ishemichnoiu khvoroboiu sertsia u khvorykh z hastroezofahealnoiu refliuksnoiu khvoroboiu [Features of the course and treatment of arterial hypertension in combination with chronic coronary heart disease in patients with gastroesophageal reflux disease]. Simeyna medytsyna. 2016.1:65–70. http://nbuv.gov.ua/UJRN/simmed_2016_1_15. [date access 08.06.2023] (In Ukrainian).
3. Zheng Z, Shang Y, Wang N et al. Current Advancement on the Dynamic Mechanism of Gastroesophageal Reflux Disease. Int J Biol Sci. 2021;17(15):4154-4164. doi: 10.7150/ijbs.65066.
4. Palii IG, Zaika SV. Rol rabeprazolu v uspishnii antykhelikobakternii ta kyslotosupresyvnii terapii u patsiientiv iz kyslotozalezhnymy zakhvoriuvanniamy [The role of rabeprazole in successful anti-helicobacter and acid-suppressive therapy in patients with acid-dependent diseases]. Hastroenterolohiya. https://health-ua.com/multimedia/userfiles/files/2015/Gastro_2_2015/Gastro_2_2015_46-50.pdf. [date access 08.06.2023] (In Ukrainian).
5. Prykhodko VYu, Moreva DYu. Osoblyvosti perebihu hastroezofahealnoi refliuksnoi khvoroby u patsiientiv z sertsevo-sudynnoiu patolohiieiu. Rol empirychnoi kyslotosupresyvnoi terapii rabeprazolom v diahnostytsi ta likuvanni HERKh [Peculiarities of the course of gastroesophageal reflux disease in patients with cardiovascular disease. The role of empiric acid-suppressive therapy with rabeprazole in the diagnosis and treatment of GERD]. Specialized medical journal «Health-ua.com». 2016;1(39):53-54. https://health-ua.com/wp-content/uploads/2016/04/52-54.pdf [date access 08.06.2023] (In Ukrainian).
6. Palii IG, Zayka SV, Ksenchyn OO, Skichko NS. Diahnostychni mozhlyvosti opytuvalnyka GerdQ na osnovi rezultativ dobovoho ezofaho-impedans-rH-monitorynhu [Diagnostic possibilities of the GerdQ questionnaire based on the results of daily esophageal-impedance-pH-monitoring]. Vnutrishnya medytsyna. 2021;2(2):22-29. doi:10.31793/2709-7404.2021.2-2.21. (In Ukrainian).
7. Hurr TJ. The six-question Gastroesophageal Reflux Disease Questionnaire (GerdQ) cannot accurately quantify reflux and reflux-associated symptoms frequency. Gastroenterology Report. 2022;1-2:1-2. doi:10.1093/gastro/goac043.
8. Zaika S, Paliy I, Chernobrovyi V, Ksenchyn O. The study and comparative analysis of GerdQ and GSRS Questionnaires on gastroesophageal reflux disease diagnostics. Gastroenterology Rev. 2020; 15 (4): 323–329. doi: 10.5114/pg.2020.101561.
9. Shcherbynina MB, Gladun VM, Emelianenko TG. Anketuvannia yak instrument vyiavlennia potentsiinykh patsiientiv z hastroezofahealnoiu refliuksnoiu khvoroboiu [Questionnaire as a tool for identifying potential patients with gastroesophageal reflux disease]. Suchasna hastroenterolohiya. 2019;6(110):13-19. doi:10.30978/MG-2019-6-13. (In Ukrainian).
10. Nakaz MOZ Ukrayiny № 943 vid 31.10.2013 Unifikovanyy klinichnyy protokol pervynnoyi ta vtorynnoyi (spetsializovanoyi) medychnoyi dopomohy «Hastroezofaheal'na reflyuksna khvoroba» [Order of the Ministry of Health of Ukraine No. 943 dated 10/31/2013 Unified clinical protocol of primary and secondary (specialized) medical care "Gastroesophageal reflux disease"]. 2013. https://www.dec.gov.ua/wp-content/uploads/2019/11/2013_943_ykpmd_gerx.pdf [date access 08.06.2023] (In Ukrainian).
11. [Nakaz MOZ Ukrayiny № 2857 vid 23.12.2021 Unifikovanyy klinichnyy protokol pervynnoyi, vtorynnoyi (spetsializovanoyi) ta tretynnoyi (vysokospetsializovanoyi) medychnoyi dopomohy «Stabil'na ishemichna khvoroba sertsya»]. Order of the Ministry of Health of Ukraine No. 2857 dated 23.12.2021 Unified clinical protocol of primary, secondary (specialized) and tertiary (highly specialized) medical care "Stable coronary heart disease". 2021. https://www.dec.gov.ua/wp-content/uploads/2021/12/2021_2857_ykpmd_stabihs.pdf [date access 08.06.2023] (In Ukrainian).

12. Nakaz MOZ Ukrayiny № 384 vid 24.05.2012 Unifikovanyy klinichnyy protokol pervynnoyi ta vtorynnoyi (spetsializovanoyi) medychnoyi dopomohy «Hipertonichna khvoroba»[Order of the Ministry of Health of Ukraine No. 384 dated 05/24/2012 Unified clinical protocol of primary and secondary (specialized) medical care "Hypertension"]. 2012. https://www.dec.gov.ua/wp-content/uploads/2019/11/384_2012ykpmd_ag.pdf [date access 08.06.2023] (In Ukrainian).
13. Bakheit AH, Al-Kahtani HM, Albraiki S. Chapter Four - Rabeprazole: A comprehensive profile / Profiles of Drugs Substances, Excipients and Related Methodology. 2021.46:137-183. doi:10.1016/bs.podrm.2020.07.003.
14. Thalheimer A, Buete M. Excess Body Weight and Gastroesophageal Reflux Disease. Visceral Medicine. 2021.37:267–272. doi: 10.1159/000516050.
15. Derbak M, Boldizhar O, Koval G et al. [clinical and endoscopic characteristics of the gastroduodenal mucosal lesions in patients with chronic hepatitis c infection with different body mass status]. Georgian Med News. 2019;(288):73-77. https://pubmed.ncbi.nlm.nih.gov/31101780/ [date access 08.06.2023

The research was performed within the departmental topic of the Department of Faculty therapy of the Uzhhorod National University « Combined pathology and correction of homeostasis disorders of the residents of the Carpathian region, taking into account the effects of adverse factors» № state registration 0121U110808.

ORCID and contributionship:
Mariya A. Derbak: 0000-0003-4791-4080 [A,E,F]
Romana-Mariia I. Tovtun: 0009-0006-4476-5357 [B,D]
Oksana T. Hanych: 0000-0001-8213-1829 [B,F]
Olesya M. Horlenko: 0000-0002-2210-5503 [E,F]
Mykhaylo Hechko: 0000-0003-2793-5044 [C,F]
Volodymyr V. Timashev: 0000-0001-9432-3318 [C]
Paul M. Lukach: 0009-0007-6033-8491 [F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mariya A. Derbak
Uzhhorod National University
20 Hryboiedova St., 88000 Uzhhorod, Ukraine
tel: +380506275075
e-mail: morika1415@gmail.com

Received: 22.06.2023
Accepted: 28.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

ASSESSMENT OF CARDIOVASCULAR DISEASE RISK FACTORS IN PATIENTS WITH CORONARY HEART DISEASE COMBINED WITH NONALCOHOLIC FATTY LIVER DISEASE

DOI: 10.36740/WLek202311106

Yaroslav Y. Ihnatko, Maria A. Derbak, Paul M. Lukach, Kseniya I. Chubirko, Oleksandr O.Boldizar, Olesia I. Ihnatko
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To study the risk factors of cardiovascular diseases in patients with coronary heart disease with stable angina pectoris II functional class in combination with NAFLD.

Materials and methods: The study included 245 patients with a diagnosis of CHD, stable angina pectoris II functional class (FC), who were being treated at the Communal Nonprofit Enterprise «Central City Clinical Hospital» of Uzhhorod City Council. We singled out 2 groups of patients: group 1 (n=145) – patients with CHD with stable angina pectoris II FC in combination with NAFLD and group 2 (n=100) – patients with CHD with stable angina pectoris II FC.

Results: Analysis of the frequency of occurrence of CVD risk factors in patients with CHD showed that among patients of group 1 there are 50% more people with abdominal obesity, excess body and dyslipidemia. The reliability between the groups in the occurrence of hypertension and type 2 diabetes was not revealed. The obtained results confirm the data that the prevalence of NAFLD increases with increasing body weight and a high degree of obesity increases the risk of its development.

Conclusions: The most frequent risk factors for CVD in patients with coronary artery disease in combination with NAFLD are hypertension, obesity, and dyslipidemia.

KEY WORDS: overweight, NAFLD, risk factors, diabetes mellitus, dyslipidemia, coronary heart disease, stable angina pectoris

Wiad Lek. 2023;76(11):2378-2382

INTRODUCTION

Cardiovascular diseases (CVD) have for a long time taken the first place in the structure of morbidity and mortality all over the world and are an important medical and social problem, due to the impact ony employable people [1]. The prevalence of all forms of coronary heart disease (CHD) among adults in Ukraine is 24%, including 10% among the working population. [2]. Annually, according to national statistics, more than 50,000 new cases of acute myocardial infarction (MI) are registered, and more than 500,000 citizens die from CVD in general, and this indicator continues to grow [3].

The most common form of CHD is stable tension angina, its frequency varies in different regions from 1.8 to 6.5%, while the prevalence gradually increases with age in both sexes [4].

In a third of patients, the development of coronary heart disease occurs against the background of provoking factors, namely excess body weight or obesity, which complicates the course of the main disease, and is also combined with such conditions as hypertension, non-alcoholic fatty liver disease (NAFLD), dyslipidemia, insulin resistance (IR), hyperinsulinemia and diabetes mellitus (DM) [5].

NAFLD is the most common liver disease in the world. It occurs in all age groups [6]. In the general population of developed countries, NAFLD is found in 14–27% [7]. However, the true prevalence of the disease is unknown, since a significant part of patients do not seek medical help. NAFLD has a mild course of symptoms, in most cases it is detected accidentally, during examination for other diseases, such as obesity, diabetes, CHD, when elevated levels of transaminases are detected, etc. [8].

Epidemiological studies have found that NAFLD is associated with CVD risk. Their presence during liver diseases increases the total mortality by 57%, mainly due to cardiovascular pathology [9].

NAFLD is not just a marker of cardiovascular pathology, but also a factor in its pathogenesis [10]. Potential pathogenetic mechanisms include endothelial dysfunction, systemic inflammation, oxidative stress, atherogenic dyslipidemia, genetic features, etc. [11].

Fatty liver index (FLI) is considered a surrogate marker of NAFLD. FLI, associated with insulin resistance, thickness of the intima-media complex, increased risk of CHD, is an independent predictor of the development of diabetes. High FLI values are associated with a high risk of mortality from both CVD and liver disease [12].

The problem of the development and progression of NAFLD in combination with CVD is one of the important issues of internal medicine, as it contributes to the deterioration of the prognosis and course of the underlying disease, and also leads to a decrease in the quality of life of patients [11].

Despite the presence of a large number of studies on the relationship between NAFLD and cardiovascular pathology, the mechanism of influence of NAFLD on cardiovascular risk has not been fully elucidated, which determines the relevance of the study.

THE AIM

The aim of the research was to study the risk factors of cardiovascular diseases in patients with coronary heart disease with stable angina pectoris II functional class in combination with NAFLD.

MATERIALS AND METHODS

The study included 245 patients with a diagnosis of CHD stable angina pectoris II functional class (FC), who were being treated at the at the Communal Nonprofit Enterprise «Central City Clinical Hospital» of Uzhhorod City Council in cardiology department with intensive care units (ICU). We singled out 2 groups of patients: group 1 (n=145) – patients with CHD stable angina pectoris of II FC in combination with NAFLD and group 2 (n=100) – patients with with CHD stable angina pectoris of II FC.

All subjects signed an informed consent, the methodology of which was in line with the Helsinki Declaration of 1975 and its revision in 1983 and was approved by Uzhhorod National University's Commission on Bioethics (Protocol №2/20 of 04.11.2022).

Criteria for inclusion in the study: informed consent of the patient, presence of CHD and NAFLD.

Exclusion criteria: alcoholic disease or liver cirrhosis, autoimmune and viral hepatitis; decompensated heart failure; acute coronary syndrome or acute cerebrovascular accident less than three months before the start of the study; congenital or acquired heart defects; systemic, oncological, autoimmune pathology.

The diagnosis of CHD stable angina pectoris of II FC was established according to the recommendations of the European Society of Cardiology (2013) and the order of the Ministry of Health of Ukraine No. 436 of 03.07.2006, based on the presence of angina attacks, a myocardial infarction suffered no earlier than 6 months ago, the results of cycle ergometry and coronary angiography (coronary artery stenosis was > 70%).

The diagnosis of NAFLD was established according to the unified clinical protocol «Nonalcoholic steatohepatitis» (2014) and according to the recommendations of the European Association for the Study of the Liver (EASL) [13,14].

Upon admission to the hospital, all patients with coronary artery disease underwent a comprehensive examination according to the generally accepted algorithm of the Ministry of Health. The following methods were used to solve the research tasks: clinical - collection of complaints and anamnesis, physical examination - to assess subjective and objective manifestations of the disease; anthropometric measurement - height, body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), conicity index - ratio of waist circumference to hip circumference. BMI was calculated according to the formula: BMI = body weight (kg) / height (m2). Determination of the level of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL) was carried out using a set of Biolatest reagents from PLIVA-LACHEMA (Czech Republic) using an automatic biochemical photometer analyzer. The level of low-density lipoprotein cholesterol (LDC) was calculated according to Friedewald's formula (1972): LDL= TC - (HDL + TG/2.2). The following formula was used to calculate the atherogenic index (AI): AI = (TC - HDL) / HDL. The level of blood glucose, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the concentration of total bilirubin were studied according to generally accepted methods. Fibrinogen concentration was determined by the gravimetric method of R.A. Rutberg. (1961). The prothrombin index (PTI) was determined according to the method of V. I. Tugolukov. (1952).

Data analysis was performed using Janovi version 2.3.28. The average values of the numerical data were represented as M ± SD. The normality of the distribution was evaluated by the Shapiro-Wilk test. The critical level of reliability was considered to be $\alpha = 0.05$.

RESULTS

According to the clinical and anamnestic data, the following CVD risk factors were identified (Table I).

Analysis of the frequency of occurrence of CVD risk factors in patients with CHD showed that among patients of group 1 there are 50% more people with abdominal obesity (χ 2 =7.479; df=1; p0.05), excess body weight (χ 2 = 6.67; df=1; p0.05) and dyslipidemia (χ 2 =6;34 df=1; p0.05). The reliability between the groups in the occurrence of hypertension (χ 2 =1.472; df=1;

Table I. The risk factors of cardiovascular diseases in patients according to anamnestic data

Indicator	Group 1 n=145 abs./%	Group 2 n=100 abs./%
Excessive body weight	13/9*	4/4
Obesity	36/25*	15/15
Dyslipidemia	15/10*	6/6
Arterial hypertension	71/49	69/69
Type 2 diabetes mellitus	10/7	6/6

Note: The significance of the difference:* - with the indicator between groups 1 and 2 (p<0,05).

Table II. Clinical and laboratory indicators in patients

Indicator (units of measurement)	Group 1 n=145	Group 2 n=100
Systolic blood pressure	160±9,6	155±7,6
Diastolic blood pressure	90±10,1	85±10,3
BMI, kg/m2	31,5±7,7*	28,5±6,2
WC, see	95,7±6,1*	90,1±5,4
TC, mmol/l	6,3±4,1	6,1±2,7
HDL, mmol/l	1,1±0,6	1,3±1,2
LDL, mmol/l	3,2±0,9	3,9±0,8
TG, mmol/l	2,8±1,6*	1,8±1,2
AI	3,04±0,5	2,8±0,6
Blood glucose, mmol/l	4,9±2,1*	3,8±1,9
ALT, Unit	42±13,6	38±10,4
AST, Unit	40±15,1	36±3,4
Bilirubin, mmol/l	13,4±2,4	13,1±2,1
PTI, %	99,4±5,2*	86,1±4,3
Fibrinogen, g/l	3,4±2,5*	2,8±1,5

Note: The significance of the difference:* - with the indicator between groups 1 and 2 (p<0,05).

p0.05) and type 2 diabetes mellitus ($\chi 2=1.197$; df=1; p>0.05) was not revealed. The obtained results confirm the data that the prevalence of NAFLD increases with increasing body weight and a high degree of obesity increases the risk of its development.

During the analysis of clinical and laboratory indicators between the groups, the following data were found (Table II).

Statistically significant differences in anthropometric indicators were found in the patients of the examined groups. Thus, BMI and WC in patients with CHD with NAFLD were significantly higher than in patients with CHD (p<0.05).

The level of triglycerides is also significantly higher in group 1 compared to group 2 (p<0.05). A higher level of glucose in patients of group 1 may be due to a higher percentage of patients with type 2 diabetes mellitus (p<0.05).

When studying the functional state of the liver, it was established that the indicators of ALT and AST activity in patients did not differ between groups. In terms of bilirubin levels, the difference between the examined groups was not reliable.

When analyzing coagulation indicators, a tendency to increase the level of fibrinogen in blood serum was revealed in patients of group 1 compared to group 2 (p<0.05). Patients of group 1 had a significantly higher PTI (p<0.05), which may indicate the presence of hypercoagulation syndrome in this category of patients as a factor in the progression of coronary artery disease.

DISCUSSION

Thus, in patients with coronary heart disease with NAFLD, probably higher BMI, WC, increased levels of triglycerides and PTI were more often observed. These changes indicate more pronounced disorders of the lipid spectrum of the blood, as well as prothrombotic changes in the blood in patients with coronary heart disease with diffuse liver diseases.

To assess the relationship between the functional state of the liver and laboratory parameters in patients with CHD with NAFLD, a correlation analysis was performed. Relationships between AST and BMI (r=+0.59; p AI (r=+0.78; p<0.05); blood glucose (r=+0.69; p<0.05) were revealed .

Therefore, the data obtained by us coincide with the data of scientists regarding the large specific gravity of NAFLD in patients with excess body weight [15,16]. According to a study by Spanish scientists, from 70 to 100% of patients with NAFLD suffer from obesity [17]. Also in the work of Hassen et al. it is shown that among lipid disorders, NAFLD is more often associated with hypertriglyceridemia, which, according to modern data, is considered an important independent risk factor for CHD [18]. The authors explain this by the fact that when adipose tissue loses sensitivity to insulin, the level of TG and free fatty acids in the blood increases. A vicious circle is formed in which obesity, hepatic steatosis, and insulin resistance are related factors that stimulate mutual progression.

CONCLUSIONS

1. The most frequent risk factors for CVD in patients with coronary artery disease in combination with NAFLD are hypertension, obesity, and dyslipidemia.
2. In patients with coronary artery disease in combination with NAFLD, probable correlative relationships of the functional state of the liver with indicators of carbohydrate and lipid metabolism, anthropometric parameters, and prothrombotic changes of blood have been established.

Prospects for further research. Study of the clinical course of CHD in combination with NAFLD and development of optimal managament for this category of patients.

REFERENCES

1. Oksak HA. Medyko-sotsialne obgruntuvannia optymizovanoi modeli nadannia tretynnoi medychnoi dopomohy khvorym z hostrym infarktom miokarda. [Medical and social justification of the optimized model provision of tertiary medical care to patients with acute heart attack myocardium.] [dissertation] Poltava: Ukrainska medychna stomatolohichna akademiia, Ministerstvo Okhorony Zdorovia Ukrainy. 2021, p.281. http://repository.pdmu.edu.ua/bitstream/123456789/15586/5/Oksak_dys.pdf [date access 03.05.2023] (in Ukrainian).
2. Sertsevo-sudynni zakhvoryuvannya — holovna prychyna smerti ukrayintsiv. Vysnovky z doslidzhennya hlobal'noho tyaharya khvorob u 2019 rotsi. https://phc.org.ua/news/sercevo-sudinni-zakhvoryuvannya-golovna-prichina-smerti-ukrainciv-visnovki-z-doslidzhennya. Tsentr hromads'koho zdorov'ya MOZ Ukrayiny. [date access 03.05.2023] (in Ukrainian).
3. Kapshytar NI. Udoskonalennia diahnostyky, prohnozuvannia ta likuvannia Q-infarktu miokarda, uskladnenoho hostroiu sertsevoiu nedostatnistiu na tli hiperhlikemii.[Improvement of diagnosis, prognosis and treatment Q-myocardial infarction complicated by acute heart failure dissertation against the background of hyperglycemia.] [dysertatsiia]. Zaporizhzhia: Zaporizkyi derzhavnyi medychnyi universytet, Ministerstvo Okhorony Zdorovia Ukrainy. 2020, p.240. http://dspace.zsmu.edu.ua/bitstream/123456789/15779/1/09062020_dis_kapshytar.pdf [date access 03.05.2023] (In Ukrainian).
4. Andonieva NM, Berezin Ole, Berezin OO et al. Arterialna hipertenziia ta komorbidnist : monohrafiia. [Arterial hypertension and comorbidity: monograph / by ed. O. M. Bilovola.] – Kharkiv : KhNMU. 2019, p.176. https://repo.knmu.edu.ua/bitstream/123456789/28070/1/%D0%91%D1%96%D0%BB%D0%BE%D0%B2%D0%BE%D0%BB%2_%D0%93%D0%A5%20%D1%82%D0%B0%20%D0%BA%D0%BE%D0%BC%D0%BE%D1%80%D0%B1%D1%96%D0%B4%D0%BD%D1%96%D1%81%D1%82%D1%8C_%D0%BC%D0%BE%D0%BD%D0%BE%D0%B3%D1%80%D0%B0%D1%84%D1%96%D1%8F.pdf [date access 03.05.2023] (in Ukrainian).
5. Labinska Ole. Vplyv reperfuziinoi terapii na yakist zhyttia patsiientiv iz hostrym infarktom miokarda za naiavnosti ozhyrinnia.[The effect of reperfusion therapy on the quality of life of patients with acute myocardial infarction in the presence of obesity.] Praktykuiuchyi likar. 2022;11(2–3):29–34. https://plr.com.ua/index.php/journal/article/download/712/588 [date access 03.05.2023] (in Ukrainian).
6. Yefimenko TI, Mykytiuk MR. Nealkoholna zhyrova khvoroba pechinky: chas dlia zmin. [Non-alcoholic fatty liver disease: time for changes International journal of endocrinology.] Mizhnarodnyi endokrynolohichnyi zhurnal. 2021;17(4):334-¬345. doi: 10.22141/2224-0721.17.4.2021.237350. (In Ukrainian).
7. Burra P, Becchetti C, Germani G. NAFLD and liver transplantation: Disease burden, current management and future challenges. JHEP reports. 2020;2(6):100-192. doi:10.1016/j.jhepr.2020.100192.
8. Chung GE, Cho EJ, Yoo J¬J et al. Young adults with nonalcoholic fatty liver disease, defined using the fatty liver index, can be at increased risk of myocardial infarction or stroke. Diabetes, Obes Metab. 2022;24(3):465–72. doi:10.1111/dom.14597.
9. Bentsa TM. Nealkoholna zhyrova khvoroba pechinky ta sertsevo-sudynni zakhvoriuvannia: osoblyvosti komorbidnoho perebihu. [Nonalcoholic fatty liver disease and cardiovascular diseases: features of the comorbid course.] Liky Ukrainy. 2020;237(1):44-47. http://lu-journal.com.ua/article/download/214176/214285/0 [date access 03.05.2023] (in Ukrainian).
10. Bentsa TM. Terapevtychni aspekty nealkoholnoi zhyrovoi khvoroby pechinky.[Therapeutic aspects of non-alcoholic fatty disease liver Medicines of Ukraine.] Liky Ukrainy. 2022;(8(264)):18–21. http://lujournal.com.ua/article/view/271835/ [date access 03.05.2023] (In Ukrainian).

11. Stepanov YuM, Filippova Olu. Evoliutsiia uiavlen pro nealkoholnu zhyrovu khvorobu pechinky: vid ryzyku do katastrofy. [The evolution of ideas about non-alcoholic fatty liver disease: from risk to disaster] Zaporozhskyi medytsynskyi zhurnal. Zaporozhye Med J. 2020;2:267–74. doi: 10.14739/2310-1210.2020.2.200637. (In Ukrainian).
12. Kushnir IE. Nealkoholna zhyrova khvoroba pechinky: suchasni metody diahnostyky ta stratehii likuvannia. Suchasna hastroenterolohiia. [Non-alcoholic fatty liver disease: modern methods diagnostics and treatment strategies]. Suchasna hastroenterolohiya. 2020;3(113):51–61. doi: 10.30978/MG-2020-3-51. (in Ukrainian).
13. Nakaz MOZ Ukrainy № 826 vid 06.11.2014 r. Pro zatverdzhennia unifikovanoho klinichnoho protokolu pervynnoi, vtorynnoi (spetsializovanoi) medychnoi dopomohy «Nealkoholnyi steatohepatyt». Kyiv. [Order of the Ministry of Health of Ukraine No. 826 of 06.11.2014 on approval unified clinical protocol of primary, secondary (specialized) medical care "Non-alcoholic steatohepatitis".] 2014. https://ukrgastro.com.ua/edu-snc/protocols/unifikovaniy-klinichniy-protokol-pervinnoyi-vtorinnoyi-specializovanoyi-medichnoyi-dopomogi-nealkogolniy-steatogepatit [date access 03.05.2023] (In Ukrainian).
14. Poda OA. Suchasni kryterii veryfikatsii ta osnovni pryntsypy vedennia nealkoholnoi zhyrovoi khvoroby pechinky. [Modern verification criteria and basic management principles non-alcoholic fatty liver disease.] Problemy ekolohiyi ta medytsyny. 2021;25(1– 2):50–6. doi: 10.31718/mep.2021.25.1-2.13 (In Ukrainian).
15. Brunner KT, Henneberg CJ, Wilechansky RM, Long MT. Nonalcoholic fatty liver disease and obesity treatment. Curr Obes Rep. 2019;8(3):220–8. doi: 10.1007/s13679-019-00345-1.
16. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. Metabolism. 2019;92:82–97. doi: 10.1016/j.metabol.2018.11.014.
17. Hernandez-Baixauli J, Quesada-Vázquez S, Mariné-Casadó R et al. Detection of early disease risk factors associated with metabolic syndrome: a new era with the NMR metabolomics assessment. Nutrients. 2020;12:806. doi:10.3390/nu12030806.
18. Hassen G, Singh A, Belete G et al. Nonalcoholic fatty liver disease: An emerging modern-day risk factor for cardiovascular disease. Cureus. 2022;14(5). doi: 10.7759/cureus.25495.

Relationship of the article with the planned research works. The research was performed within the departmental topic of the Department of Therapy and Family Medicine of the Uzhhorod National University « Innovative methods of diagnosis and treatment of pathology of internal organs in obese patients» № state registration 0121U111773.

ORCID and contributionship:
Yaroslav Y. Ihnatko: 0000-0003-1618-8952 [A-D]
Mariya A. Derbak: 0000-0003-4791-4080 [B,D,E]
Paul M. Lukach: 0009-0007-6033-8491 [F]
Kseniya I.Chubirko: 0000-0002-4379-0538 [F]
Oleksandr O. Boldizhar: 0000-0002-9553-5782 [F]
Olesia I. Ihnatko: 0000-0002-4379-5407 [B-D]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Yaroslav Y. Ihnatko
Uzhhorod National University
20 Hryboiedova st., 88000 Uzhhorod, Ukraine
e-mail: yaroslav.ihnatko@uzhnu.edu.ua

Received: 16.05.2023
Accepted: 10.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

A STUDY OF THE INFLUENCE OF JUVENILE ADJUVANT ARTHRITIS ON DENTAL HARD TISSUES CONDITION IN EXPERIMENTAL ANIMALS

DOI: 10.36740/WLek202311107

Valentyna M. Kulygina[1], Olha Yu. Pylypiuk[2], Iurii V. Turchyn[3], Nataliia G. Gadzhula[2], Mariia M. Shinkaruk-Dykovytska[2], Anastasiia V. Povsheniuk[2], Lina O. Kovalchuk[2]

[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

[2]NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

[3]TURCHYN DENTAL CLINIC, KYIV, UKRAINE

ABSTRACT

The aim: To study the intensity and depth of carious tooth lesions in rats with experimental juvenile adjuvant-induced arthritis.

Materials and methods: An experimental study on a model of juvenile adjuvant arthritis (JAA) in 10 one-month-old rats induced by method of A.M. Bendele was carried out. 10 rats of the same age were intact. Injection of adjutant in rats of experimental group led to the development of acute local reaction and then caused generalized joint reaction of autoimmune origin. The performed basic therapy of JAA promoted transition of acute autoimmune process to chronic. Rats were withdrawn from the experiment in 58 days and the dental-jaw blocks were made, in which the intensity and depth of carious lesions of the masticatory group of teeth were determined.

Results: The course of JAA was accompanied by the development of dental caries in 100% of experimental animals. It was found that the intensity of carious teeth lesions in terms of the number of carious teeth and cavities is probably higher than in intact rats (respectively 4.3 ± 0.3 vs. 2.2 ± 0.6 and 4.5 ± 0.3 vs. 2.3 ± 0.7, $p < 0.001$). In rats with JAA, mostly middle and deep carious cavities were revealed, at the same time in intact rats – superficial and middle carious cavities were observed.

Conclusions: It has been established that adjuvant arthritis is accompanied by 100% prevalence of dental caries, high intensity of carious process, presence of middle and deep carious cavities, that confirm the negative influence of autoimmune disease on the condition of the hard tooth tissues.

KEY WORDS: dental caries, experimental study, rats, juvenile adjuvant arthritis

Wiad Lek. 2023;76(11):2383-2388

INTRODUCTION

Despite particular achievements in medicine, adequate treatment of dental caries in children with somatic pathology is important in pediatric dentistry [1, 2]. It is well known that the growth and development of all systems of the child's body, including the dentognathic system, occurs under the influence of many factors of internal and external environment. There is a high susceptibility of teeth to cariogenic factors in children in the presence of autoimmune diseases, including juvenile rheumatoid arthritis (JRA) [3]. Increasing of prevalence and activity of dental caries, the development of multiple foci of enamel demineralization in children with JRA have been clinically proven [4]. These changes, according to the authors [5], occur due to the complex pathogenesis of JRA, metabolic disorders, in particular phosphorus-calcium metabolism, owing to the use of hormonal drugs of corticosteroid class. However, the obtained results by scientists about development of dental caries in this group of sick children were not confirmed in experimental studies.

Currently, there are various models of experimental rheumatoid arthritis, but the closest to human autoimmune disease are the models of adjuvant arthritis for adults [6] and juvenile adjuvant arthritis (JAA) for children [7]. Therefore, for a deep understanding of the development of carious tooth lesions in children with JRA, there is the need to study these issues in experimental animal studies.

THE AIM

The aim of this work was to study the intensity and depth of carious tooth lesions in rats when modelling experimental juvenile adjuvant arthritis.

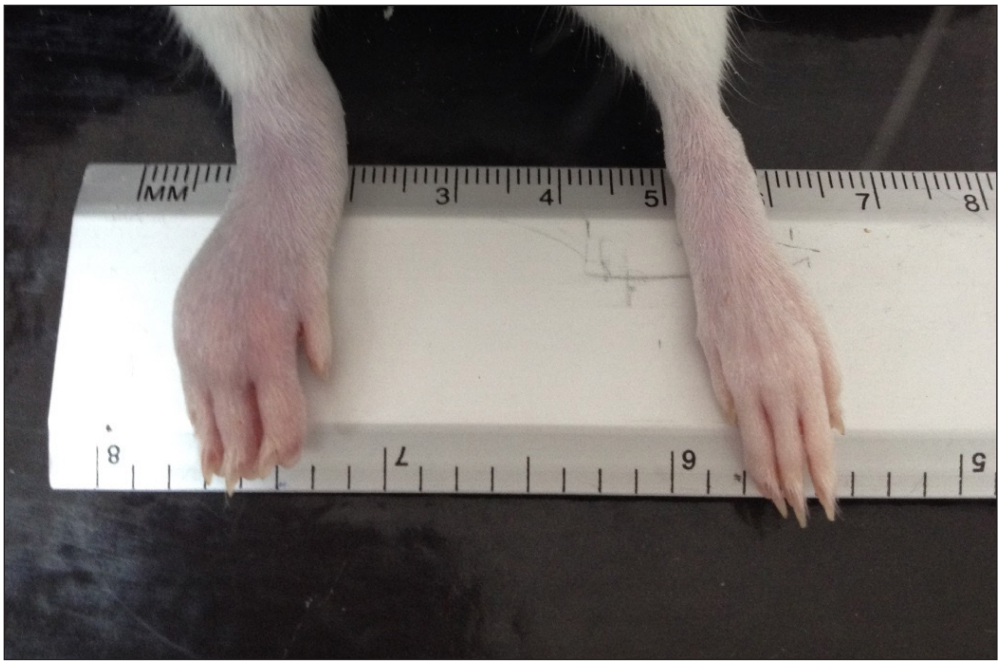


Fig. 1. Picture of a rat number 3 (group II), 3rd day of experimental research. Inflammatory reaction of rat's right hind paw after injection of Freund's adjuvant: limb enlargement, edema of the ventral part of the paw, skin redness of the damaged hindlimb

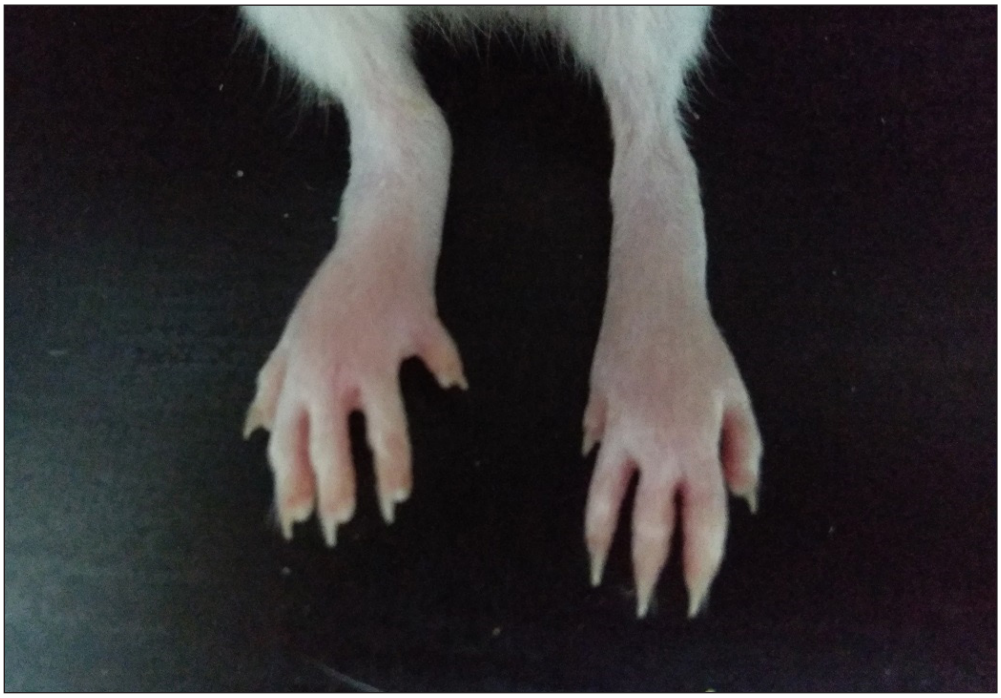


Fig. 2. Picture of rat number 4 (group II), peak of the inflammatory process (14th day of the experimental study): generalized reaction and progression of JAA, the spread of the inflammatory reaction to the intact left rat's paw, skin redness and swelling in the area of the joints of both extremities

MATERIALS AND METHODS

To achieve this goal, 20 white laboratory rats of both sexes of 1-month-mature with an average weight of 49.0 ± 0.916 g were used. Two groups of rats with 10 animals in each were formed: the control (group I) and the experimental (group II). All rats of the control (intact) and experimental (main) groups were kept in the standard conditions of the vivarium: air temperature, humidity, diet and water intake.

In rats of group II the JAA was induced by the method of A.M. Bendele [8]: 0.1 ml of Freund's adjuvant was injected once subcutaneously in rat's right hind paw (subplantarly). Composition of Freund's adjuvant is antigen inactivated and dried mycobacteria (Bacillus Calmette-Guérin, BCG) in oil emulsion. According to the author [8], this agent is the most adequate for reproducing JAA and extrapolating the results to humans.

The experiment lasted 58 days with daily visual examination and assessment of the clinical condition of rats upon reaching the peak of the inflammatory process (14th day), the formation of chronic immune inflammation (28th day) and the end of the experiment

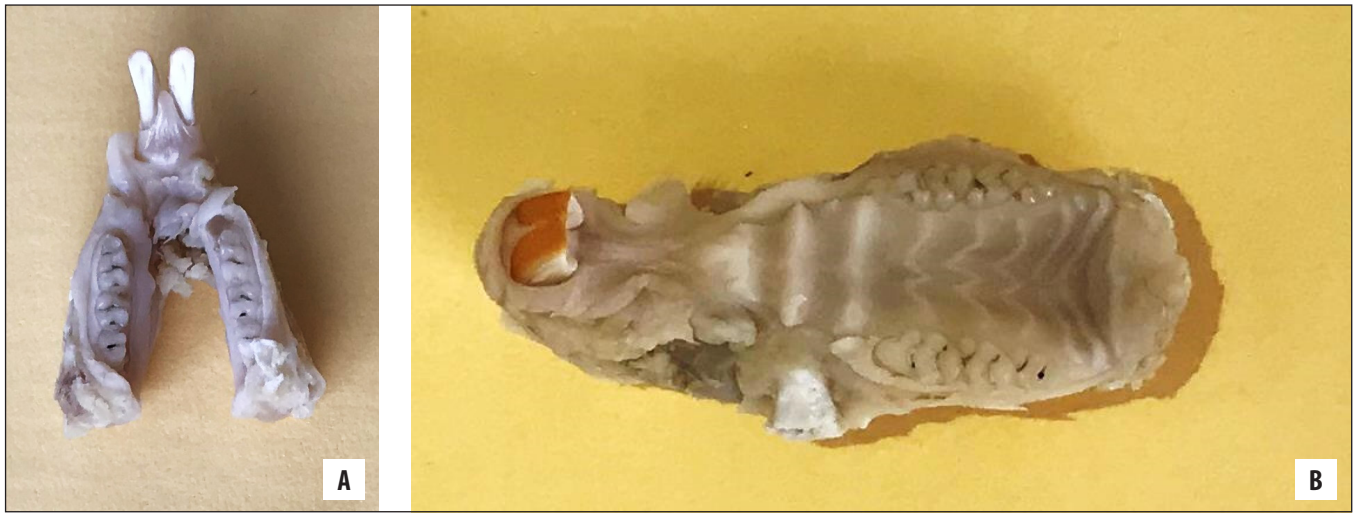


Fig. 3. Dental-jaw blocks of the upper and lower jaws of rat number 7 (group II), 58th day of experiment, Dental-Missing-Filled-Teeth (DMFT) scores = 9

(58th day). The appearance of animals, changes in behavior, reaction to external stimuli, the condition of the joints of rats, motor activity were evaluated. Local (swelling in the right hindlimb) and generalized reaction (swelling in the left hindlimb) to Freund's adjuvant were observed. Hematological parameters were used as criteria of the development of chronic autoimmune inflammation, which were determined on the 28th day of the experimental study. Treatment of JAA in rats of the experimental group was performed for 30 days (from 14th day of the experiment) according to the protocols of basic therapy of autoimmune disease by administering drugs intragastrically in doses according to the recommendations of Yu. R. Rybolovlev and R. S. Rybolovlev [1979]. Basic therapy included the use of disease-modifying antirheumatic drugs, glucocorticoids, nonsteroidal anti-inflammatory and antirheumatic drugs, calcium supplements, vitamins in accordance with the Order of the Ministry of Health of Ukraine dated April 11, 2014 No. 263 "Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical care and medical rehabilitation". In 58 days, all animals were withdrawn from the experiment by total bloodletting under propofol anesthesia (60 mg / kg).

To compare the condition of the hard tooth tissues of intact and experimental animals, dental-jaw blocks were made, in which the intensity and depth of carious lesions of the masticatory group of teeth were determined using a binocular magnifying glass. The intensity of the carious process was studied by counting the number of carious teeth and cavities, on average, per rat. The depth of carious lesion was determined in scores: superficial – carious cavity within the enamel (1 score), middle – carious cavity within the upper layers of dentin (1.5 scores), deep – a large carious cavity reaching the pulp chamber (2 scores).

Experimental studies were carried out in accordance with the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Declaration of Helsinki (2000), according to the policies of general ethical principles of experiments on animals (Kyiv, 2001). The study protocol was approved by the Biomedical Ethics Committee of National Pirogov Memorial Medical University, Vinnytsya. Ethics and morality were not violated during the study. Rats were housed in the vivarium room with a constant temperature, humidity and 12-hour dark-light cycle.

Statistical analysis of the study results was carried out using computer programs Microsoft® Excel 2017 for Mac (corporate license, Product ID: 02984-001-000001; Device Code: 86C36D0C-8F15-59CA-A81E-B1D889205F71) and the licensed package "Statistica 6.1" (serial number BXXR901E246022FA). Statistical data processing was performed by methods of variation statistics with the calculation of average arithmetic values and errors (M ± m), standard deviation (t) and the significance of differences (p-value, the differences were considered statistically significant at $p < 0.05$).

RESULTS

At the beginning of experimental study, rats of both groups were active, the motor activity of the joints were not limited, the general state were not disturbed. In 1-3 days after injection of adjuvant subplantarly in animals of the experimental group the increased aggression, irritability, alertness, increased frequency and depth of respiration and also a significant decrease in motor activity of the left limb and local inflammatory reaction of the injured paw (increased its volume, edema, skin hyperemia, pain sensitivity of the joint) were revealed (Fig. 1).

Table I. WBC count in rats with JAA on the 28th day of the experiment

Types of WBCs	Groups of experimental rats		Types of WBCs	Groups of experimental rats	
	Group I, n = 10	Group II, n = 10		Group I, n = 10	Group II, n = 10
WBCs x 10^9	11.3 ± 0.47	14.27 ± 0.57	Eosinophils, %	1.7 ± 0.30	2.28 ± 0.36
	p <0.001			p >0.05	
Banded neutrophils, %	0.8 ± 0.29	2.4 ± 0.27	Lymphocytes, %	75.3 ± 2.18	63.8 ± 2.23
	p <0.001			p <0.001	
Segmented neutrophils, %	16.6 ± 2.08	25.18 ± 1.94	Monocytes, %	5.60 ± 1.13	5.63 ± 0.59
	p <0.01			p >0.05	
Basophils, %	0.48 ± 0.13	-	Plasma cells,%	0.1 ± 0.05	-

Note: p – the significance of the difference between types of WBCs of the control group and the group of rats with adjuvant arthritis.

Table II. Indicators of caries intensity in experimental rats

Groups of experimental animals	Number of carious teeth	Number of carious cavities
The control group, n = 10	2.2 ± 0.61	2.3 ± 0.65
Experimental group, n=10	4.3 ± 0.30	4.5 ± 0.31
p	p <0.001	p <0.001

Note: p – the significance of the difference between indicators of caries intensity in the control and experimental groups of rats

Table III. Indicators of the depth of tooth carious lesions in experimental animals (in scores)

Groups of experimental animals	Superficial caries	Middle caries	Deep caries
The control group, n = 10	2.1 ± 0.61	0.3 ± 0.20	0
Experimental group, n=10	3.0 ± 0.26	2.1 ± 0.25	0.2 ± 0.20
p	p >0.05	p <0.001	p >0.05

Note: p – the significance of the difference between indicators of the depth in the control and experimental groups of rats

On the 14th day, the progression of JAA and the development of generalized reaction with lesions of both extremities (Fig. 2) were observed: edema, redness and pain in the joints of the intact limb, increased inflammation of the injured rat's paw, ulcers on the skin of its paw, phalanges and knee joint. The peak of the inflammatory process on this day of observation was accompanied by a significant decrease in the overall activity of animals, their mobility and a decrease in food and water intake.

General therapy of adjuvant arthritis from the 14th day of the experiment contributed to the gradual reduction of acute inflammatory phenomena in the joints, which was manifested in the reduction of edema and pain when flexing the inflamed joints. There was a certain tendency to normalize motor activity and emotional state of animals. Treatment of JAA in rats from the 14th to the 28th day of the experiment helped to increase general and motor activity, reduce the severity of the inflammatory process in the hindlimbs: reduce redness, swelling, pain when flexing the joints. There was a certain tendency to normalize motor activity of animals, but was not the full disappearance of inflammatory phenomena.

Due to the frequent transition of acute autoimmune inflammation to chronic, the study of the most obvious indicators of homeostasis – white blood cell (WBC) count in experimental animals were evaluated, the results of which are shown in Table I.

When analyzing the results, it was found that the average statistical parameters of WBC count in intact rats were corresponded to normal values, while in the experimental group – to the changes, that characterized chronic autoimmune process. This was indicated by a significant increase in WBCs, the percentage of banded and segmented neutrophils with a moderate shift to the left and a similar decrease of lymphocytes with a statistically significant difference compare to the control group of rats (p <0.05), as well as a significant increase of eosinophils and the appearance of basophils in the peripheral blood.

Observation of experimental group animals before the end of the basic therapy of adjuvant arthritis (58th day) revealed some improvement in the general condition and local inflammatory reaction in rats, but not their complete normalization, which confirmed the development of chronic autoimmune process, despite the performed treatment course. It should be noted that the death of rats both the control group and with simulated JAA throughout the experiment was not observed.

At the next stage of the experimental study, when comparing the condition of the hard tooth tissues of in-

tact and experimental animals, the carious lesions were revealed in 60% of dental-jaw blocks of rats in group I and in 100% – in group II.

When analyzing the nature of the carious lesion of the teeth (Table II) a low level of caries intensity and almost the same average statistical indicators of the number of carious teeth and cavities in rats of the control group were revealed. At the same time, in animals with adjuvant arthritis, these values were higher in twice then in the control group with a high level of significance of the difference in values ($p < 0.001$). We give an example of a high level of intensity of tooth damage by the carious process in rat number 7 on the 58th day of the experiment after subplantar injection of Freud's adjuvant (Fig. 3).

To assess the dental caries' findings of lesions in experimental animals, it is important to determine the depth of the carious process, the results of which are shown in Table III.

It can be seen from the data given above that in rats with JAA superficial caries was diagnosed by 1.5 times more often than in the control group ($p > 0.05$), and middle caries by 7 times ($p < 0.001$). Deepening of the carious process in rats with adjuvant arthritis was indicated by revealed carious cavities reaching the pulp chamber, while in intact animals there were no deep carious lesions of the teeth.

DISCUSSION

Taking into account the influence of JRA on the clinical course of dental carious lesions, established by the authors in single works [9], the study of this issue was carried out on an experimental model of the disease described by A.M. Bendele [8]. The model, induced by Freund's adjutant according to scientists [10] is the closest to JRA in children.

Inducing of the experimental model of JAA in rats by a single subplantar injection of adjutant into the right hind paw contributed to the development of autoimmune disease: acute local and subsequent generalized joint inflammation with a peak of inflammatory autoimmune process on the 14th day, which confirmed the adequacy of experimental JRA model [11]. The performed basic therapy of JAA in rats did not lead to the elimination of pathological immune inflammation, on the contrary, contributed to the transition from acute to chronic autoimmune process, that agrees with the data literature about clinical manifestations of JRA in childhood [12]. This was evidenced by a significant decrease of protective factors level in the blood of experimental animals: neutrophilic leukocytosis with a moderate shift to the left, lymphocytopenia, a tendency to increase eosinophils and the presence of basophils in the WBC count.

Experimental confirmations of the negative influence of autoimmune disease on the course of dental caries in children, have been described in clinical studies of the authors [13], were: 100% damage of the teeth by carious process of animals with JAA; significantly higher indicators of the number of affected teeth and their cavities compared to intact rats ($p < 0.001$); detection of carious cavities mainly of medium depth and the presence of single deep cavities in experimental animals in contrast to control rats, in which only superficial and sometimes middle caries were diagnosed.

CONCLUSIONS

Thus, a single injection of Freund's adjuvant subcutaneously in right hind paw of experimental rats leads to the manifestation of acute autoimmune inflammation of the joints (juvenile adjuvant arthritis) and the development of local and generalized reaction of animals' organism.

In the process of basic treatment of induced JAA the changes in peripheral blood count (neutrophilic leukocytosis with a shift to the left, the decrease in the percentage of lymphocytes and increase in eosinophils and the presence of basophils), that confirm the transition from acute to chronic autoimmune inflammatory process in experimental animals.

In rats with experimental JAA model, 100% of carious tooth lesions were revealed. Peculiarities of carious tooth lesions in rats with JAA were: significantly higher indicators of caries intensity (number of affected carious teeth and cavities) than in intact animals ($p < 0.001$); the increase in the number of carious cavities of medium depth ($p < 0.001$) and the appearance of deep caries cavities. Thus, in intact rats mainly superficial and middle carious lesions were revealed, but in animals with JAA – middle and deep carious lesions. The negative influence of chronic adjuvant arthritis in experimental animals on the condition of dental hard tissues was confirmed.

The obtained results of the study allow to recommend the use of JAA model in experimental rats for testing of prevention methods of carious tooth lesions in children with JRA.

REFERENCES

1. Casamassimo PS, Flaitz CM, Hammersmith K et al. Recognizing the Relationship Between Disorders in the Oral Cavity and Systemic Disease. Pediatr Clin North Am. 2018;65(5):1007-1032. doi: 10.1016/j.pcl.2018.05.009.
2. Dhinsa K, Saha S, Tripathi AM et al. Assessment of Oral Health-related Quality of Life in Patients Suffering from Systemic Diseases. Int J Clin Pediatr Dent. 2021;14(6):779-783. doi: 10.5005/jp-journals-10005-2079.
3. Kobus A, Kierklo A, Sielicka D, Szajda SD. Juvenile idiopathic arthritis and oral health. Postępy Higieny i Medycyny Doświadczalne. 2016;70:410-419. doi: 10.5604/17322693.1201119 .

4. Skeie MS, Gil EG, Cetrelli L et al. Oral health in children and adolescents with juvenile idiopathic arthritis – a systematic review and meta-analysis. BMC Oral Health. 2019;19(1):285. doi: 10.1186/s12903-019-0965-4.
5. Janicka-Szczepaniak M, Orczyk K, Szymbor K et al. Is it possible to predict a risk of osteoporosis in patients with juvenile idiopathic arthritis? A study of serum levels of markers of bone turnover. Acta Biochimica Polonica. 2018;65(2):297-302. doi: 10.18388/abp.2017_2561.
6. Povsheniuk AV, Kulygina VM, Kapytsia VM et al. Morphological changes in tongue tissues in experimental animals with simulating rheumatoid arthritis. World of Medicine and Biology. 2021; 3(77): 237-242. doi: 10.26724/2079-8334-2021-3-77-237-242.
7. Choudhary N, Bhatt LK, Prabhavalkar KS. Experimental animal models for rheumatoid arthritis. Immunopharmacol Immunotoxicol. 2018;40(3):193-200. doi: 10.1080/08923973.2018.1434793.
8. Bendele AM. Animal models of rheumathoid arthritis. J. Musculoskel Neuron Interact. 2001;1(4):377-385.
9. Skakodub AA, Mamedov AA, Admakin OI et al. Treatment of Fissure Caries of Children with Severe Rheumatic Diseases with Difficulty in Opening the Mouth. Contemp Clin Dent. 2022;13(3):274-279. doi: 10.4103/ccd.ccd_172_20.
10. Orhan CE, Önal A, Uyanıkgil Y, Ülker S. Antihyperalgesic and antiallodynic effect of sirolimus in rat model of adjuvant arthritis. Eur J Pharmacol. 2013;705(1-3):35-41. doi: 10.1016/j.ejphar.2013.02.013.
11. Alavala S, Nalban N, Sangaraju R et al. Anti-inflammatory effect of stevioside abates Freund's complete adjuvant (FCA)-induced adjuvant arthritis in rats. Inflammopharmacology. 2020;28(6):1579-1597. doi: 10.1007/s10787-020-00736-0.
12. Rinkunaite I, Simoliunas E, Alksne M et al. Anti-inflammatory effect of different curcumin preparations on adjuvant-induced arthritis in rats. BMC Complement Med Ther. 2021;21(1):39. doi: 10.1186/s12906-021-03207-3.
13. Pylypiuk OYu, Kulyhina VM, Shinkaruk-Dykovytska Met al. Efektyvnist likuvannia kariiesu zubiv u ditei prepubertatnoho i pubertatnoho viku z yuvenilnym revmatoidnym artrytom [The effectiveness of dental caries treatment in children of prepubertal and pubertal age with juvenile rheumatoid arthritis]. Reports of VNMU. 2023;27(1):63-68. doi: 10.31393/reports-vnmedical-2023-27(1)-12. (in Ukrainian)

The work is a fragment of the research project "Modern trends and latest technologies in the diagnosis and treatment of odontopathology, diseases of periodontal tissues and oral mucosa" (state registration number 0118U005471).

ORCID and contributionship:
Valentyna M. Kulygina: 0000-0001-7846-4441X [A]
Olha Yu. Pylypiuk: 0000-0002-8904-1639 [D]
Iurii V. Turchyn: 0009-0002-7801-2741 [C]
Nataliia G. Gadzhula: 0000-0003-0016-2264 [F]
Anastasiia V. Povsheniuk: 0000-0002-6799-9627 [E]
Mariia M. Shinkaruk-Dykovytska: 0000-0002-0747-4247 [B]
Lina O. Kovalchuk: 0000-0002-1757-6212 [E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olha Yu. Pylypiuk
National Pirogov Memorial Medical University
56 Pyrohov St., 21018 Vinnytsia, Ukraine
tel: +380987500237
e-mail: Olgapylypiuk87@gmail.com

Received: 05.05.2023
Accepted: 09.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

PECULIARITIES OF PARAMETERS OF AEROBIC AND ANAEROBIC PRODUCTIVITY DEPENDING ON THE COMPONENTS OF BODY WEIGHT IN YOUNG MALES FROM THE MOUNTAINOUS DISTRICTS OF ZAKARPATTIA

DOI: 10.36740/WLek202311108

Olena A. Dulo[1], Yurii M. Furman[2], Nataliia M. Hema-Bahyna[1], Petro P. Horvat[1], Tamara B. Kutek[2]
[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]ZHYTOMYR IVAN FRANKO STATE UNIVERSITY, ZHYTOMYR, UKRAINE

ABSTRACT

The aim: To establish differences in the aerobic and anaerobic capacity in young males with different components of body weight who live in the mountainous districts of Zakarpattia region.

Materials and methods: 124 young men aged 17-21 were examined to determine the aerobic and anaerobic capabilities of the body, taking into account the components of body weight.

Results: The level of aerobic productivity, which reflects physical health, depends on the component composition of the body. The highest level of aerobic capacity in terms of the $VO_{2\ max\ rel.}$ is found in young males who have normal body weight with a high and a very high relative content of skeletal muscle, a low relative content of fat, and a normal level of visceral fat. As a consequence, their physical health exceeds "safe health level", namely 42 $ml \cdot min^{-1} \cdot kg^{-1}$, and corresponds to "average" according to Ya.P. Piarnat's criteria.

Conclusions: A high relative fat content negatively affects the functional capabilities of the body of young males in both aerobic and anaerobic modes of energy supply. With the growth in the relative content of skeletal muscles, the increase of the aerobic capacity of the body, as well as the growth of the capacity of alactic and lactic energy supply processes is observed. None of the examined males had "good" or "excellent" parameters of aerobic processes.

KEY WORDS: body mass, fat, skeletal muscles, post-pubertal age

Wiad Lek. 2023;76(11):2389-2394

INTRODUCTION

Physical health forms because of the body's ability to adapt to the external environment, while maintaining normal functional parameters of all physiological systems [1, 2]. Aerobic and anaerobic productivity of the body are integral indicators of physical health. Assessment of physical health by indicators of aerobic and anaerobic productivity makes it possible to characterize it both qualitatively and quantitatively [3-6]. Also, it should be pay attention the set of various morphological factors that determine the somatotype (in particular, the component composition of body weight), affect both functional capabilities of the organism and the susceptibility to certain diseases [7-9]. Therefore, determining the relationship between a person's ability to perform physical work in aerobic and anaerobic modes of energy supply of the body depending on the constitutional characteristics of body composition, is expedient as it allows to individualize the ways of prevention of certain diseases, and to choose effective treatment tactics that are relevant and socially significant [10-13].

THE AIM

The aim was to establish differences in the aerobic and anaerobic capacity in young males with different components of body weight who live in the mountainous districts of Zakarpattia region.

MATERIALS AND METHODS

The task was solved by using a variety of modern diagnostic methods to examine124 young males in the post-puberty period of ontogenesis, aged 17-21, residents of Zakarpattia. The power and capacity of aerobic (VO_{2max}), the power of anaerobic alactic ($WAnT_{10}$), anaerobic lactic ($WAnT_{30}$), and the capacity of anaerobic lactic processes (PPO) of the body's energy supply

were evaluated by bicycle ergometry test; the relative content of fat and skeletal muscles was determined by the bioimpedance method; methods of mathematical statistics using the programs Microsoft Office 2007, Microsoft Excel Stadia 6.1 / prof and Statistica, were used to determine Student's t-test to assess the reliability of the difference in indicators.

RESULTS

When studying the components of body weight in young males from mountainous districts, we identified three groups depending on the fat content and three groups depending on the muscle content. The number of males with a normal relative fat content (8.0 - 19.9%) was the largest – 101 individuals (81.5%). We did not observe any males with a high fat component (>24,9%). The number of males with normal and high relative content of skeletal muscles was the largest – 76 (61.3%) and 39 individuals (31.4%), respectively. There were no males with a low (< 33.3%) relative content of skeletal muscles among those studied (Table I).

The value of the absolute indicator $VO_{2\ max}$ in males with a high relative content of the fat component is higher than the value in males with a normal and low relative fat content ($p>0.05$). At the same time, the average $VO_{2\ max}$ value of males with high relative fat content is 1.32 times significantly lower than the average value in males with low and normal relative fat content ($p<0.05$), and does not reach "safe health level". The average value $VO_{2\ max\cdot rel.}$ exceeds "safe health level", i.e. 42 ml·min^{-1}·kg^{-1}, in young males from mountainous districts with a low relative fat content (44,6±2,1 ml·min^{-1}·kg$^{-1)}$, whereas the average $VO_{2\ max\cdot rel.}$ is 41,6±1,7 ml·min^{-1}·kg^{-1} and does not reach "safe health level" in young males with a normal relative fat content. $WAnT_{10\ rel.}$ was significantly higher in young males from mountainous districts with a low and normal relative content of the fat component compared to individuals with a high relative content of this component (by 31.4% and by 27.3%, correspondingly). Peculiarities of the power of anaerobic lactic processes in representatives of mountainous districts with different component composition of body weight were revealed when determining the $WAnT_{30\ rel.}$ indicator. The lowest average values of $WAnT_{30}$ were registered in males from mountainous districts with a high relative content of the fat component, which is 22.3% lower compared to young males with a low and 20.8% lower compared to young males with a normal relative content of this component (Table II).

As can be seen from Table II, the average values of the absolute capacity of anaerobic lactic energy supply processes of the body according to PPO are the highest in young males with a low and high relative content of the fat component, compared to males with a normal relative fat content ($p>0.05$). Young males from mountainous districts with a high relative content of this component have a significantly lower indicator $PPO_{rel.}$ by 26.4% compared to the indicator of young males with a low and by 10.1% compared to young males with a normal relative content of the fat component ($p<0.05$).

The values of the absolute $VO_{2\ max.}$ index in young males from mountainous districts with different relative content of skeletal muscles do not reliably differ from each other. The average value of $VO_{2\ max.rel.}$ in young males from mountainous districts with normal relative content of skeletal muscles is 38,9±1,1 ml·min^{-1}·kg^{-1}, which is significantly below "safe health level" and corresponds to "below average" level of aerobic productivity ($p<0.05$). In young males with high and very high relative content of skeletal muscles, the indicator $VO_{2\ max.rel\cdot}$ is significantly above the "safe health level", which corresponds to the "average" level of aerobic productivity and is 42,4±2,0 ml·min^{-1}·kg^{-1} and 43,1±0,97 ml·min^{-1}·kg^{-1}, respectively. The absolute value of WAnT10 in young makes from mountainous districts was probably higher

Table I. Distribution of males from the mountain districts of Zakarpattia by component composition of body weight, n=124

Relative fat content (%)							
< 8,0 (-) low		**8,0 – 19,9 (0) normal**		**19,9 – 24,9 (+) high**		**>24,9 (++) very high**	
number of persons	%	number of persons	%	number of persons	%	number of persons	%
7	5,6	101	81,5	16	12,9	-	-
Relative content of skeletal muscles (%)							
< 33,3 (-) low		**33,3 – 39,3 (0) normal**		**39,4 – 44,0 (+) high**		**> 44,0 (++) very high**	
number of persons	%	number of persons	%	number of persons	%	number of persons	%
-	-	76	61,3	39	31,4	9	7,3

Table II. Average values of indicators of aerobic and anaerobic productivity of the body (M±m) of males from the mountain districts of Zakarpattia, depending on the relative fat content, n=124

Indicators	Aerobic productivity		Anaerobic productivity					
	maximum oxygen consumption		power of alactic energy supply processes		power of lactic energy supply processes		capacity of lactic energy supply processes	
Relative fat content (%)	$VO_{2\ max}$ ml·min^{-1}	$VO_{2max\ rel.}$ ml·min^{-1}·kg^{-1}	$WAnT_{10}$, kgm·min^{-1}	$WAnT_{10\ rel}$•, kgm·min^{-1}·kg^{-1}	$WAnT_{30.}$ kgm·min^{-1}	$WAnT_{30\ rel}$•, kgm·min^{-1}·kg^{-1}	PPO, kgm·min^{-1}	$PPO_{rel.}$, kgm·min^{-1}·kg^{-1}
< 8,0 (-) low (n=7)	3098,7± 79,6	44,6 ± 2,1	4683,7 ± 59,6	67,4 ± 2,2	4456 ± 84,3	64,1 ± 3,9	2173,6 ± 51,9	31,4 ± 1,8
8,0 – 19,9 normal (n= 101)	2996,6± 70,3	41,6 ± 1,7	4701,3 ± 76,2	65,3 ± 1,83	4526,0 ± 87,2	62,9 ± 3,8	1898,2 ± 49,8	25,7± 2,2*
19,9 –24,9 (+) high (n=16)	3164,8 ± 68,7	33,7 ± 0,93•*	4821,7 ± 78,6	51,3 ± 1,72•*	4682,1 ± 90,3	49,8 ± 2,7•*	2208,6 ± 60,4	23,1 ± 1,4•*

Note: the probability of a difference in mean values ($p<0.05$):
* - relatively low fat content;
• - relatively normal fat content;

Table III. Average values of indicators of aerobic and anaerobic body productivity (M±m) of males from the mountain districts of Zakarpattia depending on the relative content of skeletal muscles, n=124

Indicators	Aerobic productivity		Anaerobic productivity					
	maximum oxygen consumption		power of alactic energy supply processes		power of lactic energy supply processes		capacity of lactic energy supply processes	
Relative skeletal muscle content (%)	$VO_{2\ max}$ ml·min^{-1}	$VO_{2\ max\ rel.}$ ml·min^{-1}·kg^{-1}	$WAnT_{10}$, kgm·min^{-1}	$WAnT_{10\ rel}$•, kgm·min^{-1}·kg^{-1}	$WAnT_{30.}$ kgm·min^{-1}	$WAnT_{30\ rel}$•, kgm·min^{-1}·kg^{-1}	PPO, kgm·min^{-1}	$PPO_{rel.}$, kgm·min^{-1}·kg^{-1}
33,3 – 39,3 (0) normal (n=76)	2896,5 ± 56,1	38,9 ± 1,1♦•	4658,4 ± 96,3	59,7 ± 2,9 ♦•	4438,9 ± 98,7	56,9 ± 3,6	1966,7 ± 54,2	25,5 ± 1,3
39,4 – 44,0 (+) high (n=39)	3086,4 ± 77,2	42,4 ± 2,0	4703,1 ± 88,2	63,4 ± 3,8	4503,2 ± 76,2	60,1 ± 3,8	2102,3 ± 46,3	27,8 ± 1,2
> 44,0 (++) very high (n=9)	3197,4 ± 59,3	43,1 ± 0,97	4869,8 ± 78,7	65,3 ± 3,2	4572,3 ± 90,6	60,9 ± 4,7	2123,4 ± 51,6	28,7 ± 2,1

Note: the probability of a difference in mean values ($p<0.05$):
* - relatively normal skeletal muscles content;
• - relatively high skeletal muscles content;
♦ - relatively very high skeletal muscles content.

in individuals with a very high relative content of the muscle component compared to individuals with a normal and high relative content of this component of the body. The relative value of $WAnT_{10}$ in young males from mountainous districts with a very high and high content of the muscle component reliably outweighs this indicator by 9.4% and 6.2%, respectively, compared to persons who had a normal relative muscle content. Different anaerobic productivity of young males from mountainous districts, depending on the component composition of the body, was also revealed when determining $WAnT_{30\ rel.}$ Thus, the lowest average values of the relative $WAnT_{30}$ indicator were recorded in young males from mountainous districts with a normal relative content of skeletal muscles compared to individuals with a high and very high relative content of the muscle component. At the same time, regardless of the relative content of the muscle component, indicators of the PPO i PPOrel. of the body's energy supply in absolute and relative values do not differ between themselves ($p>0.05$) (Table III).

Among the young males representing the mountainous districts of the Zakarpattia region, those with a low fat content, or high and very high muscle content had an "average" level of aerobic productivity in terms of the $VO_{2\ max}$•$_{rel.}$ This level of aerobic productivity provided them with a "safe health level" according to H.L. Apanasenko [18]. The level of aerobic performance was

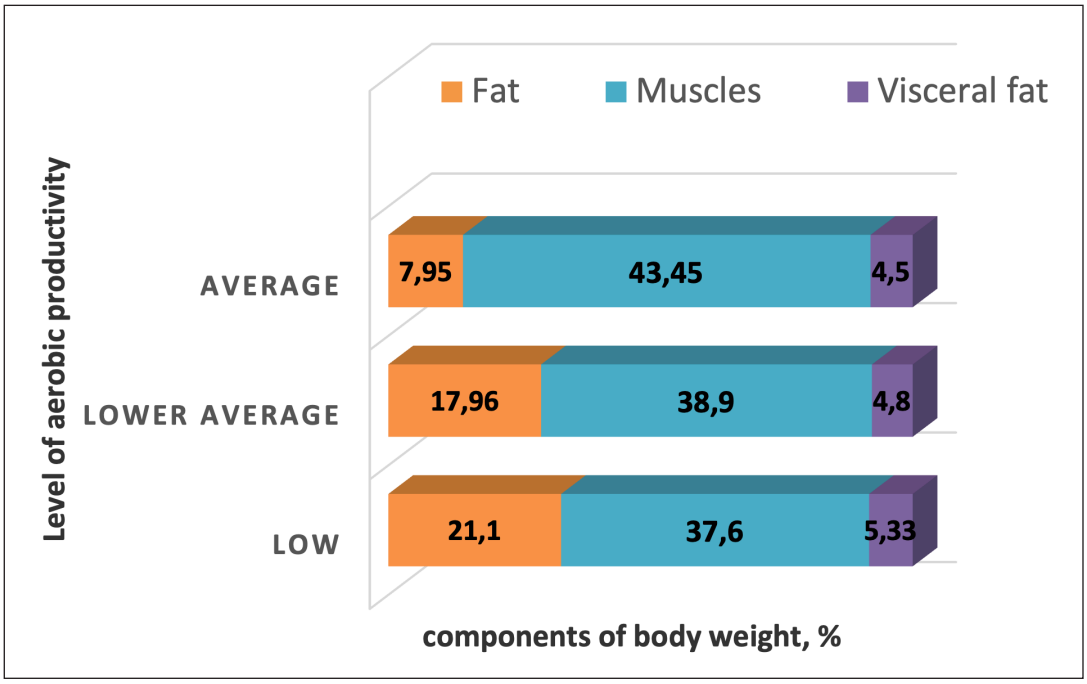


Fig. 1. Graphic representation of the dependence of the level of aerobic productivity of males from the mountain districts of Zakarpattia on the ratio of the component composition of body weight (n=124)

"below average" and "low" in young males with normal and high content of the fat component and with the normal content of the muscle component. Such levels of aerobic performance do not guarantee a "safe health level" according to H.L. Apanasenko, Fig. 1.

DISCUSSION

Studying the level of physical health in post-puberty individuals is extremely important as a young organism of an adult is almost completely formed, and it should prepare for a quality and longer life. Research of physiological indicators that form physical health makes it possible to correctly assess the physical health status in healthy population and allows for a more detailed assessment of the body's adaptive capabilities, as well as to provide a prognostic assessment of the probability of the occurrence of pathological abnormalities on the part of the cardiovascular system and decrease in tolerance to physical exertion.

Thus, Zhang M. and his co-authors in their research of the physical fitness of students at Chinese universities, draw attention to the potential hidden risk for the health of students who, have an excessive fat component with a normal body mass index. The authors also prove that the lower level of physical fitness of the students was partly due to the lower mass of skeletal muscles. That is, they confirm the negative influence of a high content of the fat and a low content of the skeletal muscles on the level of physical performance of young people in the post-pubertal period of ontogenesis [19].

Pigłowska M., investigating the relationship between body mass components, metabolic indicators and function of the endothelial among physically active healthy males, prove that the components of fat and muscle mass are important predictors of the metabolic profile. Maintaining regular high levels of physical activity and metabolic health throughout young and middle adulthood may have beneficial effects on body mass composition parameters and, as a result, may prevent age-related decline in lean body mass and endothelial dysfunction [20].

Our findings are confirmed by the studies of Anwar S. et al., who determined the correlation of the percentage of fat and muscle components of body mass with aerobic and anaerobic performance in 48 male student footballers. According to their findings, the relative content of body fat and skeletal muscle mass is correlated with indicators of aerobic and anaerobic capacity [21].

Researchers Yokota T. confirm the importance of the level of aerobic productivity in relation to the life expectancy of patients with metabolic syndrome. Low aerobic performance is a strong and independent predictor of all-cause mortality in male patients with metabolic syndrome. The use of means to improve the aerobic capacity of the body's energy supply processes and the energy metabolism of skeletal muscles in males with metabolic syndrome leads to a positive prognosis for this category of individuals [22].

Mucha D.K. evaluating the aerobic capacity of males and females depending on the type of posture, came to the conclusion that the research results should find wide practical applications in conducting a comprehensive assessment of body posture and physical per-

formance as a determinant of health preservation [23].

Studying the level of physical health of young males from the mountainous districts of the Zakarpattia region, we understand that the somatotype, component composition of the body, and the functional capabilities of the body are formed in this category of individuals in conditions of relative hypoxia. Szymczak R.K. et al. researching the effects of a long stay at a very high altitude (over 3500 m) on the physiology of male climbers, note that in conditions of hypoxia, the anaerobic productivity of the body decreases, the maximum respiratory volume increases, and the relative content of the fat component of the body weight decreases. At the same time, the lack of oxygen does not affect the level of maximum aerobic power, the indicators of maximum oxygen absorption, hemoglobin and hematocrit levels [24]. Therefore, the study of tests of aerobic and anaerobic capacity in healthy males and females post-puberty aged allows to develop individual and population medical forecasts, form groups of people with an increased risk of pathological processes, and implement medical and social rehabilitation programs.

CONCLUSIONS

A high relative fat content negatively affects the functional capabilities of the body of young males in both aerobic and anaerobic modes of energy supply. With the growth in the relative content of skeletal muscles, the increase of the aerobic capacity of the body, as well as the growth of the capacity of alactic and lactic energy supply processes is observed. None of the examined males had "good" or "excellent" parameters of aerobic processes.

REFERENCES

1. Shakhlina LYA-H, Kohan BH, Tereshchenko TO et al. Sportyvna medytsyna [Sports medicine]. Kyyiv: Natsional'nyy universytet fizychnoho vykhovannya i sportu Ukrayiny. 2019, p.424. (In Ukrainian).
2. Kostyukevych V, Mel'nychuk A, Chkhan' A. Analiz metodiv doslidzhennya problemy shchodo formuvannya modeley zdorovoho sposobu zhyttya students'koyi molodi [Analysis of methods of research on the problem of forming healthy lifestyle models of student youth]. Aktual'ni problemy fizychnoho vykhovannya ta metodyky sportyvnoho trenuvannya. 2021; 1:31-48. (In Ukrainian).
3. Kenney WL, Wilmore JH, Costill DL. Physiology of Sport and Exercise. Human Kinetics. 2021, p. 611.
4. Nesterova S, Sulyma A, Boyko M. Assessment of the level of physical health of the youth with different somatotypes for the ability to adapt to physical activity. Physical education, sports and health culture in modern society. 2019;2(46):35-40.
5. Furman YuM, Miroshnichenko VM, Brezdeniuk OYu et al. Otsinka aerobnoyi ta anaerobnoyi produktyvnosti orhanizmu molodi 17-19 rokiv Podil's'koho rehionu [An estimation of aerobic and anaerobic productivity of an organism of youth aged 17-19 years old of Podilsk region]. Pedagogics, Psychology, Medical-Biological Problems of Physical Training and Sports. 2018; 22(3):136-141. (in Ukrainian).
6. Dulo O, Furman Y, Hema-Bahyna N. Gender and Somatotypological Peculiarities of Indicators of Aerobic and Anaerobic Productivity of Energy Supply of the Body in the Post-Pubertal Period of Ontogenesis in the Residents of the Zakarpattia Region. Wiad Lek. 2022;75(10):2359-2365.
7. Furman YM, Miroshnichenko VM, Bohuslavska VYu et al. Modeling of functional preparedness of women 25-35 years of different somatotypes. Pedagogy of Physical Culture and Sports. 2022; 26(2):118–125. doi:10.15561/26649837.2022.0206.
8. Gaul CA, Docherty D, Cicchini R. Differences in anaerobic performance between boys and men. Int. J. Obes Relat. Metab. Disord. 1995;16(7):451-5. doi: 10.1055/s-2007-973036.
9. Ryan-Stewart H, Faulkner J, Jobson S. The influence of somatotype on anaerobic performance. PLoS ONE. 2018; 13(5):e0197761. doi:10.1371/journal.pone.0197761.
10. Miroshnichenko VM, Furman YuM, Brezdeniuk OYu et al. Correlation of maximum oxygen consumption with component composition of the body, body mass of men with different somatotypes aged 25-35. Pedagogy of Physical Culture and Sports. 2020;24(6):290-297. doi: 10.15561/26649837.2020.0603.
11. Dulo O, Furman Yu, Maltseva O et al. Physical Health of Females from the Lowland Districts of Zakarpattia According to the Metabolic Level of Aerobic and Anaerobic Energy Supply Depending on the Component Body Composition. Wiad Lek. 2023; 76(3):568-574.
12. Kosma M, Ellis R, Cardinal B et al. Psychosocial Predictors of Physical Activity and Health-Related Quality of Life Among Adults with Physical Disabilities: An Integrative Framework. Disability and Health Journal. 2009;2(2):104-9. doi: 10.1016/j.dhjo.2008.10.062.
13. Petukhov AB, Nikityuk DB, Sergeyev VN. Meditsinskaya antropologiya: analiz i perspektivy razvitiya v klinicheskoy praktike [Medical anthropology: analysis and development prospects in clinical practice]. M.: Medpraktika. 2015, p.525. (In Russian).
14. Pyarnat YAP. Vozrastno-polovyye standarty (10-50 let) aerobnoy sposobnosti cheloveka [Age-sex standards (10-50 years) of human aerobic capacity]: avtoref. dis. dokt. med. nauk: 03.00.13. M. 1983, p.44. (In Russian).
15. Furman YuM et al. Funktsional'na pidhotovlenist' [Functional readiness]. Promising models of physical culture and health technologies in physical education of students of higher educational institutions. Kyiv: Olympic literature. 2013, pp. 24-42. (In Ukrainian).

16. Shögy A, Cherebetin G. Minutentest auf dem Fanradergometer zur Bestimmung der Annaeroben Kapazität. J. Appl. Physiol. 1974;33(2):171-6. doi: 10.1007/BF00449517.
17. Dovgiy YuI. Impedansometriya yak metod monitorynhu komponentnoho skladu masy tila studentiv [Impedancemetry as a method of monitoring the component composition of students' body mass]. Prospects, problems and existing achievements of the development of physical culture and sports in Ukraine IV All-Ukrainian Internet Conference «Color of Science», January 29, 2021, pp.299-302. (In Ukrainian).
18. Apanasenko GL, Popova LA, Magliovaniy AV. Sanolohiya [Sanology]. Kyiv-Lviv: PP Kvart. 2011, p.303. (In Ukrainian).
19. Zhang M, Schumann M, Huang T et al. Normal weight obesity and physical fitness in Chinese university students: an overlooked association. BMC Public Health. 2018;18(1):1334. doi: 10.1186/s12889-018-6238-3.
20. Pigłowska M, Kostka T, Drygas W et al. Body composition, nutritional status, and endothelial function in physically active men without metabolic syndrome - a 25 year cohort study. Lipids Health Dis. 2016;15:84. doi: 10.1186/s12944-016-0249-9.
21. Anwar S, Noohu MM. Correlation of Percentage Body Fat and Muscle Mass with Anaerobic an Aerobic Performance in Collegiate Soccer Players. Indian J Physiol Pharmacol. 2016;60(2):137-144.
22. Yokota T, Kinugawa S, Hirabayashi K et al. Pioglitazone improves whole-body aerobic capacity and skeletal muscle energy metabolism in patients with metabolic syndrome. Diabetes Investig. 2017;8(4):535-541. doi: 10.1111/jdi.12606.
23. Mucha DK, Pałka T, Skalska-Izdebska R et al. Aerobic Capacity in Relation to Selected Elements of Body Posture. Int J Environ Res Public Health. 2023;20(2):903. doi: 10.3390/ijerph20020903.
24. Szymczak RK, Grzywacz T, Ziemann E et al. Prolonged Sojourn at Very High Altitude Decreases Sea-Level Anaerobic Performance, Anaerobic Threshold, and Fat Mass. Front Physiol. 2021;12:743535. doi: 10.3389/fphys.2021.743535.

ORCID and contributionship:
Olena A. Dulo: 0000-0003-0473-5605 [A, B, D, F]
Yurii M. Furman: 0000-0002-5206-7712 [C, E, F]
Nataliia M. Hema-Bahyna: 0000-0001-5735-7041 [A, E]
Petro P. Horvat: 0000-0002-3972-0115 [A, C]
Tamara B. Kutek: 0000-0001-9520-4708 [A, E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olena A. Dulo
Uzhhorod National University
3 Narodna Square, 88000 Uzhhorod, Ukraine
e-mail: olena.dulo@uzhnu.edu.ua

Received: 22.05.2023
Accepted: 19.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

EFFECT OF NON-ALCOHOLIC FATTY LIVER DISEASE ON THE COURSE OF DIABETIC POLYNEUROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

DOI: 10.36740/WLek202311109

Andriy Ya. Sabovchyk
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To study the peculiarities of diabetic polyneuropathy in patients with type 2 diabetes mellitus and concomitant NAFLD.

Materials and methods: We examined 75 patients with type 2 diabetes mellitus, including 31 (41.3%) women and 44 (58.7%) men. The main group included 35 patients with NAFLD (46.7%), and the control group included 40 patients without NAFLD (53.3%). The severity of polyneuropathy was assessed using the Toronto clinical neuropathy score. The presence of neuropathic pain syndrome in patients allowed us to divide patients into groups with painful or painless forms of diabetic polyneuropathy. The electroneuromyographic examination was used to study nerve conduction parameters, namely peroneal motor nerve conduction velocity (PMNCV), sensory nerve action potential (SNAP), and sensory nerve conduction velocity (SNCV).

Results: The proportion of patients who did not have diabetic polyneuropathy in the NAFLD group was 12.5%, and in the group without NAFLD - 87.2%. The frequency of diabetic polyneuropathy was higher in the main group, namely: mild, moderate, and severe polyneuropathy was 80%, 56% and 59.3%, respectively, compared to the control group - 20%, 44%, 40.7% (p=0.02). The painful form of DPN was more common in patients of the main group than in the control group, respectively 69.8% and 30.2% (p=0.01). The degree of liver fibrosis did not affect the course of DPN. The study of nerve conduction by PMNCV, SNAP, and SNCV parameters showed that PMNCV was higher in the NAFLD group, and SNAP and SNCV - in the control group, but without statistical significance (p>0.05).

Conclusions: In patients with type 2 diabetes mellitus, the presence of NAFLD affects the severity of diabetic polyneuropathy and increases the risk of painful DPN. The degree of liver fibrosis did not show an effect on the development of diabetic polyneuropathy. ENMG parameters did not demonstrate a statistically significant difference in the study groups.

KEY WORDS: polyneuropathy, non-alcoholic fatty liver disease, diabetes mellitus, electroneuromyography

Wiad Lek. 2023;76(11):2395-2400

INTRODUCTION

Diabetic neuropathy is a clinical and neurophysiological diagnosis that occurs in 45-54% of patients with diabetes mellitus[1].

Focal or diffuse damage to the peripheral somatic and autonomic nervous system is one of the leading complications of diabetes mellitus and leads to other clinically important disorders [2].

The prevalence and severity of this complication increase proportionally with the duration of diabetes mellitus.

The most common form of diabetic neuropathy found in these patients is distal symmetric polyneuropathy or diabetic polyneuropathy proper (DPN), which spreads like socks and gloves and primarily affects the distal arms and legs. Other diffuse diabetic neuropathies are autonomic forms, which include cardiac autonomic neuropathy, gastrointestinal dysfunction, cystopathy, and impotence.

The risk factors for the development of diabetic neuropathy are the duration of the disease, the level of glycosylated hemoglobin, obesity, dyslipidemia, hypertension, and smoking, which are also risk factors for cardiovascular complications [3, 4].

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, affecting nearly 25% of the world's population [5]. The prevalence of NAFLD among patients with type 2 diabetes mellitus is higher than in the general population and can reach 40-70% [6].

It has been shown that metabolic disorders, including dyslipidemia, insulin resistance, and inflammation, have an impact on the pathogenetic components of NAFLD [7].

NAFLD in patients with type 2 diabetes mellitus has been shown to increase the risk of cardiovascular complications and also contributes to the development of microvascular disorders, especially nephropathy and retinopathy [8].

NAFLD, dysmetabolic cardiovascular disorders, and DPN are interrelated conditions and share the same risk factors, increase the incidence of complications, and significantly affect the patient's quality of life. Judging by the fact that patients with NAFLD have an increase in microvascular complications, it is assumed that patients with diabetes mellitus who additionally have NAFLD are at increased risk of developing diabetic polyneuropathy [9].

Given that NAFLD is a consequence of metabolic disorders and has an impact on their course, increasing the risk of complications, and given the data from previous studies on the high affinity of diabetes mellitus with NAFLD and DPN, we tried to assess the relationship between NAFLD and DPN in patients with type 2 diabetes mellitus.

THE AIM

To study the features of diabetic polyneuropathy in patients with type 2 diabetes mellitus in combination with NAFLD.

MATERIALS AND METHODS

From September 2022 to April 2023, we examined 75 patients with type 2 diabetes mellitus on the basis of the neurological and endocrinological departments of the A. Novak ZOCL of the Health Care Facilities of the Health Care District, including 31 (41.3%) women and 44 (58.7%) men. The average age of the patients was 53.4±7.5 years. The correctness of the distribution of patients was checked using the Q-Q chart and the Shapiro-Wilk test. According to the Q-Q graph and the Shapiro-Wilk test ($p>0.05$), a normal distribution of patients was established, so parametric methods of statistical analysis were used in the future. All patients (n=75) were divided into two groups. The main group consisted of 35 patients with NAFLD (46.7%), and the control group included 40 patients without NAFLD (53.3%).

The average age of the patients in the main group was 52.0±6.9 years, and in the control group - 54.7±8.02. Body mass index in patients of the main group was 32.6±3.83, and in the control group - 28.1±3.98. The average duration of diabetes mellitus in patients of the main group was 8.09±5.1 years with an average level of glycosylated hemoglobin of 8.82±1.51%; the control group was 9.38±5.79 and 9.6±2.34%, respectively (Table I).

The need for additional insulin injection to standard treatment in the main group was 27 patients (62.9%), and without the need for insulin therapy in the main group were 13 patients (37.1%). In the control group, 27 patients (67.5%) required insulin therapy, and 13 patients (32.5%) did not require insulin therapy in the control group (Table I).

All patients were familiarized with the purpose and conditions of the study and signed informed consent to the diagnostic and manipulative procedures before the study, and the methodology of their conduct was in accordance with the requirements of the Helsinki Declaration of Human Rights of 1975 and its revised version of 1983 and in accordance with the European Convention on Human Rights, as well as biomedicine and Ukrainian legislation.

The inclusion criteria for the study were age 18-70 years and the presence of type 2 diabetes mellitus. Exclusion criteria for inclusion in the study were alcohol and other toxic substance abuse, autoimmune and oncological processes, as well as chronic infections, including viral hepatitis, in the history.

All patients underwent general clinical, anthropometric, laboratory and instrumental examinations. The study of anthropometric data included measurement of height and body weight. In accordance with the obtained indicators, the body mass index (BMI) was calculated according to the formula $BMI=m/h^2$ (kg/m^2), where *m* is body weight in kilograms, and *h* is body height in meters. According to the WHO recommendations, BMI less than 18.5 was defined as underweight, 18.5-24.9 as normal body weight, 25.0-29.9 as overweight, 30.0-34.9 as grade 1 obesity, 35.0-39.9 as grade 2 obesity, and more than 40.0 as grade 3 obesity.

All patients underwent laboratory general clinical and biochemical studies, which indicate the functional state of the hematopoietic system, liver, kidneys, fat and carbohydrate metabolism.

The degree of liver damage was determined using the online calculator Fibrosis 4 calculator, which calculated FIB-4, an indicator that takes into account the patient's age, ALT and AST activity and platelet count. FIB-4 <1.3 indicates minor or no fibrosis, and >2.67 indicates advanced fibrosis or cirrhosis.

Ultrasound examination of the abdominal cavity was performed according to the generally accepted method.

All patients underwent a neurological examination for diabetic polyneuropathy and the presence of polyneuropathy and its severity were assessed using the Toronto clinical neuropathy score. This scale was used to study 15 subjective and objective indicators of polyneuropathy with a maximum score of 19. The number of points was used to determine the degree of polyneuropathy, where 0-5 is the absence of polyneuropathy, 6-8 is mild polyneuropathy, 9-11 is moderate polyneuropathy, and≥ 12 is severe polyneuropathy.

Table I. Characteristics of the study groups

Clinical and demographic indicators at baseline research	Patients with NAFLD	Patients without NAFLD	Differences between groups at the beginning of the study
Gender, n (%)			
Female	12 (34,3)	19 (47,5)	χ^2=0,85, p=0,36
Male	23 (65,7)	21 (52,5)	
Age, m	52,0±6,9	54,7±8,02	U=517, p=0.052
BMI, kg/m^2	32,6±3,83	28,1±3,98	t=-5.06, p=0.00001
Length of service, years	8,09±5,1	9,38±5,79	U=622, p=0.41
HbA1c, %.	8,82±1,51	9,6±2,34	t=1.73, p=0.088
Need for insulin therapy, n (%)			
No	13 (37,1)	13 (32,5)	χ^2=0,03, p=0,86
Yes.	22 (62,9)	27 (67,5)	

Table II. Distribution of patients by pain syndrome depending on the presence of NAFLD (p=0.01)

Pain syndrome, n (%)	Patients with NAFLD	Patients without NAFLD	Total
No.	13 (40,6)	19 (59,4)	32
Yes.	30 (69,8)	13 (30,2)	43
Total	43 (57,3)	32 (42,7)	75

Table III. ENMG results in patients with and without NAFLD

Indicators of ENMG	Patients with NAFLD	Patients without NAFLD	Differences between groups
PMNCV, m/c	40.9± 9.97	39.6± 10.0	t=-0.52; p=0.6
SNAP, mV	1.84± 2.48	3.96± 6.48	t=1.75; p=0.087
SNCV, m/c	18.8± 19.1	22.1± 27.3	t=0.58; p=0.56

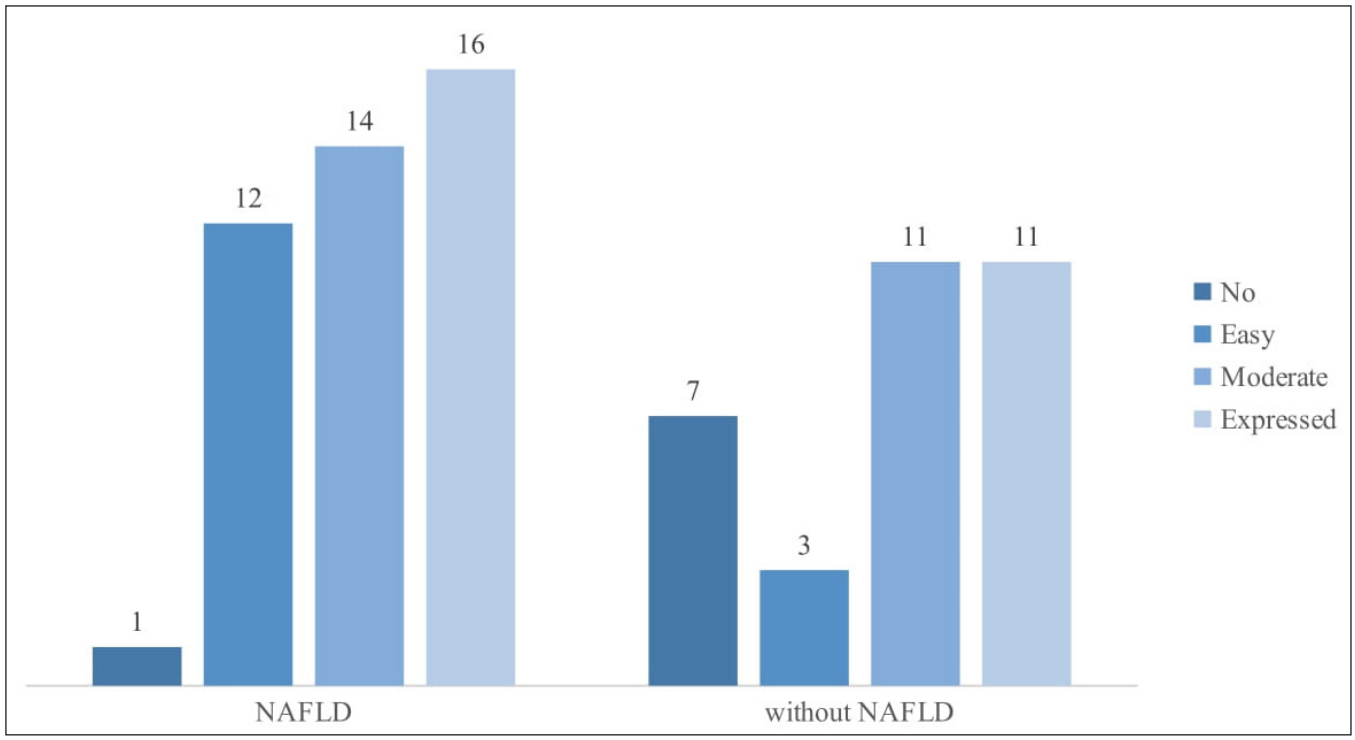


Fig. 1. Distribution of patients by the severity of polyneuropathy depending on the presence of NAFLD (p=0.02)

The electroneuromyographic examination was performed using the Neuro-MVP-Micro apparatus, NEUROSOFT, where the main indicators of nerve conduction in the determination of polyneuritis disorders were determined, namely: peroneal motor nerve conduction velocity (PMNCV), sensory nerve action potential (SNAP), sensory nerve conduction velocity (SNCV).

Data analysis and processing were performed using the computer program Jamovi Version 2.3.26.0 for IOS using parametric and nonparametric methods of evaluating the results. Clinical and demographic parameters were compared between patient groups using the criterion χ^2 for nominal characteristics. For relative and ordinal variables, we first studied the compliance with the normality of the distribution and used the Student's test in the Welch modification in case of compliance or the Mann-Whitney test in case of deviation of the distribution of the variable from the normal. When comparing 3 or more groups, one-factor analysis of variance or the Kruskal-Wallis test was used, depending on the normality of the distribution.

RESULTS

Comparing the groups of patients by painful or painless form of polyneuropathy, it was found that in the NAFLD group, pain syndrome was observed in 69.8% of patients, and in the group without NAFLD - in 30.2%. When assessing the frequency of pain in patients with and without NAFLD using frequency analysis, a statistically significant difference (p=0.01) was found between the presence of pain in these patients, namely the dominance of the painful form in patients with NAFLD – table II.

Our study also compared the effect of fibrosis on the severity of polyneuropathy. According to the results of the study, it was found that the degree of fibrosis (FIB-4) does not affect the severity of polyneuropathy ($\chi^2 = 3.85$, $p = 0.27$).

The influence of NAFLD on the severity of polyneuropathy was established. Thus, according to our data, in the main study group (NAFLD), the proportion of patients who did not have diabetic polyneuropathy was 12.5% (1), and in the control group (without NAFLD) 87.5% (7). The proportion of patients in the main group with mild, moderate and severe polyneuropathy was 80% (12), 56% (14), and 59.3% (16), respectively, compared with the control group - 20% (3), 44% (11), 40.7% (11). According to the χ^2 criterion, there is a statistically significant difference in the comparison groups, indicating that the severity of diabetic polyneuropathy depends on the presence of NAFLD in patients ($\chi^2 = 9.78$, $p = 0.02$) – Fig.1.

According to the data of electroneuromyography (ENMG), in patients with NAFLD, the conduction velocity of the motor fibers of the peroneal nerve (PMNCV) was higher than in the control group and amounted to 40.9± 9.97 in the main group and 39.6± 10.0 in the control group. In contrast to the previous data, the amplitude (SNAP) and conduction velocity (SNCV) of the sensory fibers of the sural nerve were 1.84± 2.48 in the main group and 3.96± 6.48 in the control group, and for SNCP 18.8± 19.1 and 22.1± 27.3, respectively; without signs of statistical significance for all three indicators ($p>0.05$) – table III.

DISCUSSION

We have obtained results indicating that in patients with type 2 diabetes mellitus with concomitant NAFLD, the manifestations of diabetic polyneuropathy are exacerbated.

According to the literature review, there are a limited number of studies that have examined the relationship between diabetic polyneuropathy and NAFLD in patients with type 2 diabetes. Those studies that have been conducted have contradictory results [10].

Mantovani et al. studied the effect of NAFLD on DPN in patients with type 1 diabetes. They used the Michigan Neuropathy Screening Instrument method and the vibration sensitivity threshold to assess diabetic polyneuropathy. A positive relationship between NAFLD and DPN was shown [11].

In our study, there were patients exclusively with type 2 diabetes mellitus, and the severity of polyneuropathy was assessed using the Toronto clinical neuropathy score. According to the study, in patients with concomitant NAFLD, the proportion of patients with statistical significance was higher than in the control group, and vice versa, the proportion of patients with no signs of polyneuropathy was higher in the group without NAFLD (p=0.02).

The study by Williams et al. found that patients with type 2 diabetes with concomitant liver fibrosis due to NAFLD had a higher threshold of vibration sensitivity [12]. Lombardi et al. and Kim et al. showed that neither fibrosis nor steatohepatosis correlated with diabetic polyneuropathy in outpatients with type 2 diabetes [13, 14]. In another similar study, Kim K. et al. found that there was no significant association between the study groups on the NAFLD liver fat score, but NAFLD fibrosis index and FIB-4, which indicate the degree of fibrosis, were higher in patients with DPN compared to patients without DPN [9]. Lv et al. showed a negative correlation between DPN and NAFLD in patients with type 2 diabetes mellitus [15].

Our study also evaluated the impact of fibrosis (FIB-4) on the severity of polyneuropathy. According to our data, there is no relationship between these parameters.

As for the pain syndrome in DPN, we found the influence of NAFLD on the development of the painful form of diabetic polyneuropathy. The proportion of patients with painful DPN was statistically significantly higher in the main group compared with the control group.

Comparing the ENMG data between these groups of patients, we found that PMNCV was higher in the main group, and SNAP and SNCV in the control group, but without statistical significance for all parameters.

Further research is needed to better understand the impact of NAFLD on the progression of DPN in patients with type 2 diabetes mellitus and to study risk factors that may exacerbate this effect.

CONCLUSIONS

NAFLD affects the severity of diabetic polyneuropathy in patients with type 2 diabetes mellitus. The presence of concomitant NAFLD in patients with type 2 diabetes mellitus increases the risk of painful DPN. The degree of liver fibrosis does not affect the course of diabetic polyneuropathy. There was no statistically significant difference in ENMG indices among the study groups.

REFERENCES

1. Feldman EL, Callaghan BC, Pop-Busui R Zet al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5(1):42. doi: 10.1038/s41572-019-0097-9.
2. Callaghan BC, Price RS, Chen KS, Feldman EL. The Importance of Rare Subtypes in Diagnosis and Treatment of Peripheral Neuropathy: A Review. JAMA Neurol. 2015;72(12):1510-8. doi: 10.1001/jamaneurol.2015.2347.
3. Papanas N, Ziegler D. Risk Factors and Comorbidities in Diabetic Neuropathy: An Update 2015. Rev Diabet Stud. 2015;12(1-2):48-62. doi: 10.1900/RDS.2015.12.48.
4. Callaghan BC, Gao L, Li Y et al. Diabetes and obesity are the main metabolic drivers of peripheral neuropathy. Ann Clin Transl Neurol. 2018;5(4):397-405. doi: 10.1002/acn3.531.
5. Marjot T, Moolla A, Cobbold JF et al. Nonalcoholic Fatty Liver Disease in Adults: Current Concepts in Etiology, Outcomes, and Management. Endocr Rev. 2020;41(1):bnz009. doi: 10.1210/endrev/bnz009.
6. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease, or cirrhosis. Nat Rev Gastroenterol Hepatol. 2013;10(6):330-44. doi: 10.1038/nrgastro.2013.41.
7. Kitade H, ChenG, Ni Y, Ota T. Nonalcoholic Fatty Liver Disease and Insulin Resistance: New Insights and Potential New Treatments. Nutrients. 2017; 9(4):387. 2017;9(4):387. doi: 10.3390/nu9040387.
8. Han E, Kim MK, Jang BK, Kim HS. Albuminuria Is Associated with Steatosis Burden in Patients with Type 2 Diabetes Mellitus and Nonalcoholic Fatty Liver Disease. Diabetes Metab J. 2021;45(5):698-707. doi: 10.4093/dmj.2020.0118.
9. Kim K, Oh TJ, Cho HC et al. Liver fibrosis indices are related to diabetic peripheral neuropathy in individuals with type 2 diabetes. Sci Rep. 2021;11(1):24372. doi: 10.1038/s41598-021-03870-z.
10. Huang J, Li R, Liu N et al. Liver fibrosis is independently associated with diabetic peripheral neuropathy in type 2 diabetes mellitus. J Diabetes Investig. 2021;12(11):2019-2027. doi: 10.1111/jdi.13562.
11. Mantovani A, Rigolon R, Mingolla L et al. Nonalcoholic fatty liver disease is associated with an increased prevalence of distal symmetric polyneuropathy in adult patients with type 1 diabetes. J Diabetes Complications. 2017;31(6):1021-1026. doi: 10.1016/j.jdiacomp.2017.01.024.
12. Williams KH, Burns K, Constantino M et al. An association of large-fiber peripheral nerve dysfunction with non-invasive measures of liver fibrosis secondary to non-alcoholic fatty liver disease in diabetes. J Diabetes Complications. 2015;29(8):1240-7. doi: 10.1016/j.jdiacomp.2015.06.015.
13. Lombardi R, Airaghi L, Targher G et al. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. Liver Int. 2020;40(2):347-354. doi: 10.1111/liv.14274.
14. Kim BY, Jung CH, Mok JO et al. Prevalences of diabetic retinopathy and nephropathy are lower in Korean type 2 diabetic patients with non-alcoholic fatty liver disease. J Diabetes Investig. 2014;5(2):170-5. doi: 10.1111/jdi.12139.
15. Lv WS, Sun RX, Gao YY et al. Nonalcoholic fatty liver disease and microvascular complications in type 2 diabetes. World J Gastroenterol. 2013;19(20):3134-42. doi: 10.3748/wjg.v19.i20.3134.

The scientific research was carried out within the scientific topic of the Department of Neurology, Neurosurgery, and Psychiatry "The latest methods of studying the central and peripheral nervous system", state registration number 616.8-047.37.

ORCID and contributionship:
Andriy Ya. Sabovchyk: 0000-0002-5607-5883 [A-F]

Conflict of interest:
The Author declare no conflict of interest

CORRESPONDING AUTHOR

Andriy Ya. Sabovchyk
Uzhhorod National University
3 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380507607719
e-mail: andriy.sabovchyk@uzhnu.edu.ua

Received: 20.05.2023
Accepted: 21.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

HYPERACTIVE BLADDER SYNDROME SECONDARY TO BAROTRAUMA AND CHRONIC STRESS

DOI: 10.36740/WLek202311110

Stepan S. Filip, Rudolf M. Slyvka, Anton I. Batchynsky
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To improve the results of treatment of hyperactive bladder syndrome in men of working age on the background of barotrauma and stress, as a consequence of combat trauma.

Materials and methods: An analysis of the questionnaire and the results of the clinical examination of 32 patients, injured servicemen and people who were injured in combat zones was carried out. The drug solifenacin succinate was used in the treatment complex, which is a specific antagonist of M3 subtype cholinergic receptors. Its influence allows you to achieve relaxation of the bladder detrusor and reduce the contractility of hyperactive bladder.

Results: The main criterion for the effectiveness of the treatment was a decrease in the number of urgent cases, the frequency of urination and manifestations of nocturia by 50% or more, which was considered a positive effect. At the same time, the positive effect was differentiated as follows : an improvement of these parameters by 75% or more from the initial value which is a good result; reduction of symptoms in the range of 50-75% is satisfactory; less than 50% is an unsatisfactory result. A positive effect from the treatment after 8 weeks was observed in 88% of patients, of which 52% had a good result and 36% had a satisfactory result.

Conclusions: The proposed complex of treatment of hyperactive bladder syndrome as a result of combat trauma against the background of barotrauma with neurological consequences and chronic stress allows to achieve a pronounced clinical effect in the vast majority of male patients of working age. And the diagnostic complex allows you to emphasize aspects of clinical vigilance, both for doctors of a specialized branch and of doctors of a general direction.

KEY WORDS: hyperactive bladder, urinary incontinence, nocturia

Wiad Lek. 2023;76(11):2401-2405

INTRODUCTION

The bladder is a muscular sac-shaped organ that stores urine produced by the kidneys. Most people realize that the bladder is filled up when it is half full. In order to empty your bladder, you must be able to relax. This usually means being in a socially comfortable environment, such as a toilet or private space. The brain will then send a signal to the bladder to start squeezing out urine [1-5].

Hyperactive bladder syndrome, or as the people say: «urinary incontinence» is really uncomfortable. You don't have to be a baby to suffer from an unexpected need to go to the toilet when nothing seems to indicate it, or to have difficulties in holding back urination. Hyperactive bladder syndrome is a symptom complex in which there is a strong urge to urinate, with or without signs of urinary incontinence, usually accompanied by frequent urination and nocturia, while there are no pathological changes in the bladder itself and there are no abnormalities in the biochemical and microscopic composition of urine [1,3,6-8]. The results of modern epidemiological studies have shown that the prevalence of overactive bladder symptoms is astounding. The overall prevalence is 30%, at the age of 45-52 it reaches 60%, at the age of 75 an hyperactive bladder occurs in every third person, the distribution by gender is almost the same, but women predominate and only 5% of patients seek medical help. In addition, urgent urination in combination with urinary incontinence is, according to patients, the most unpleasant [3,4,9,10]. They have the greatest negative impact on the quality of life, often causing neuropsychiatric disorders, and in severe cases, they cause social problems at the level of communication with relatives and work colleagues, force them to change their usual lifestyle, leave their favorite job and spend most of their time at home close to the toilet [5,9-12]. The diagnosis of overactive bladder syndrome is not as simple as it may seem. After all, it is important to know whether other pathologies are behind this condition. Identifying the causes of urinary incontinence requires the involvement of various specialists - both a family doctor and doctors of a special-

ized branch. To find out what the reason is, you need to: collect a complete medical history; analyze what medications the patient has already taken; conduct a physical and laboratory examination; if possible, assess the presence of residual urine; ask the patient to keep a voiding diary for three days. First of all, the reason lies in excessive activity or instability of the bladder detrusor, which normally provides controlled urination [7,13-16]. In today's conditions, the number of diagnosed hyperactivity bladder syndrome has increased significantly in men of working age, as a result of combat trauma against the background of barotrauma with neurological consequences and chronic stress. Therefore, the principles of clinical vigilance, both for doctors of a specialized branch and doctors of a general direction, in relation to this syndrome, need accentuation, diagnostic and treatment approaches need clarification.

THE AIM

To improve the results of treatment of hyperactive bladder syndrome in men of working age against the background of barotrauma and stress, as a consequence of combat trauma.

MATERIALS AND METHODS

In the work, an analysis of the questionnaire and the results of the clinical examination of 32 patients, injured servicemen and persons who were injured in combat zones was carried out. The subjects of the study were male patients of the neurological (16), therapeutic (7), surgical (9) departments who were receiving inpatient treatment at the Uzhhorod City Multidisciplinary Clinical Hospital during 2022-2023. The age of the patients ranged from 33 to 53 years. An individual questionnaire, data on disease history and life, data on objective status, data on ultrasound examination of kidneys and bladder, general laboratory indicators of blood and urine, and biochemical indicators of blood were used for the examination of patients.

Hyperactive bladder syndrome includes a number of the following complaints: imperative urges (complaints about sudden irresistible, hard-to-control urges to urinate); increased frequency of urination (more than eight urinations per day); nocturia (urinating at night); Urgent urinary incontinence (due to an imperative urge). Hyperactive bladder is a diagnosis of exclusion and is established in the absence of any inflammatory, metabolic, hormonal or other probable diseases of the lower urinary tract that can cause such symptoms. The diagnosis of this syndrome is based on the following algorithm: survey, questionnaire of patients with the aim of identifying signs of stress and imperative urinary incontinence, patients filling out a urination diary; examination (exclusion of hormone-dependent tumors, genital infections); determination of the amount of residual urine and the volume of the urinary bladder; laboratory studies (urine and blood tests, biochemical analysis; instrumental methods (complex urodynamic diagnostics, ultrasound, cystoscopy). Other diseases of the urogenital tract can also be manifested by hyperactive bladder syndrome, so it is necessary to carry out differential diagnosis with such pathologies as bladder cancer, stones bladder, urinary tract infections, neurogenic bladder [14,17-19].

The dominant symptoms in the observation were the frequency of urination - complaints of patients about too frequent urges during the day and the frequency of urination at night in particular, sometimes in combination with signs of urinary incontinence. At the same time, it is the severity of nocturia that has the greatest negative impact on the quality of life compared to the severity of urination disorders that occur in the first half of the day. Nocturia negatively affects the ability to rest and sleep, somatic health, contributes to a decrease in immunity, the appearance of dizziness, endocrine and metabolic disorders, depression, increases the risk of developing cardiovascular diseases (angina, arrhythmia, myocardial infarction).

At the current stage, there are the following approaches to the therapy of this disease: behavioral methods: visiting the toilet at regular intervals and training the bladder; antimuscarinic and antispasmodic agents (decreasing the contractile activity of the bladder, increasing its functional capacity); surgical interventions aimed at increasing the capacity of the bladder; installation of a neurostimulator. In the treatment of overactive bladder, a general principle is used: from less invasive therapy to more invasive.

These results, combined with data on the role of neurotransmitters (serotonin, g-aminobutyric acid, norepinephrine) in the regulation of the act of urination, allowed us to propose the following therapeutic and preventive complex of hyperactive bladder syndrome. The first link, preventive, lasting up to three months, includes: consumption of an adequate amount of water; reducing coffee consumption; trips to the toilet «on schedule»; perform Kegel exercises to train the muscles of the pelvic floor; normalize body weight, especially if the body mass index exceeds 30; treat concomitant conditions that worsen the situation (constipation, chronic cough); use hygienic means and lay life routes through places where there are toilets for the purpose of psycho-emotional stabilization. The second link, medicinal, lasting up to three months, included the use of the Ukrainian drug «Nigisem» (the active ingredient is solifenacin succinate),which is a competitive specific

antagonist of M3 subtype cholinergic receptors and does not affect other subtypes. Because the bladder is innervated by parasympathetic cholinergic nerves, and acetylcholine contracts the smooth muscles of the detrusor by affecting muscarinic receptors, represented mainly by the M3 subtype. The effectiveness of «Nigisem» - causes the relaxation of the bladder detrusor and reduces the contractility of the overactive bladder. As a result, the frequency of spontaneous contractions decreases, the capacity of the bladder increases, and the spontaneous urge to urinate decreases. Method of use: the usual dose is 5 mg, once a day, if necessary, it can be increased to 10 mg. Its bioavailability is 90%, and the effect is noted already in the first week of treatment, stabilization of the effect occurs in 8-10 weeks, the severity of the effect lasts up to 12 months.

RESULTS

Comparing the state and variability of dysuric symptoms before, during and after the proposed course of treatment, most patients showed improvement in the main indicators of functional disorders of the lower urinary tract. Data were obtained on a significant decrease in frequent urination, nocturia and episodes of urgent urination. This is proved by a decrease in the intensity of sensory symptoms of overactive bladder syndrome. Starting from week 2 of the treatment course, patients were interviewed about the effectiveness and tolerability of the treatment. An appropriate questionnaire was used, in which the intensity of symptoms was expressed in points that characterized the effectiveness of treatment and quality of life. Thus, the quality of life score increased by 17 points after 2 weeks, by 18 points after 4 weeks, by 14 points after 6 weeks, and by 19 points after 8 weeks.

The main criterion for the effectiveness of the treatment was a decrease in the number of urgent urges, the frequency of urination and manifestations of nocturia by 50% or more, which was considered a positive effect. At the same time, the positive effect was differentiated as follows: an improvement of these parameters by 75% or more from the initial value is a good result; reduction of symptoms in the range of 50-75% is satisfactory; less than 50% is an unsatisfactory result. A positive effect from the treatment after 8 weeks was observed in 88% of patients, of which 52% had a good result and 36% had a satisfactory result. This is confirmed by the intensity of reduction of «sensory» symptoms of overactive bladder. Thus, the number of episodes of frequent urination decreased by 41%, nocturia - by almost three times (61%), and the number of urgent urges - by almost four (73%). In 8 (25%) patients, frequent urination remained, but their frequency decreased significantly. In addition, the dynamics of the average effective volume of the bladder was evaluated. The functional volume of the urinary bladder in patients varied from 75 to 135 ml, and against the background of the therapy, after 8 weeks, it increased by 59.13%.

Judging by the daily urine output profile, the average effective bladder volume became larger, indicating a restructuring of its reservoir function. Both before and after treatment, the patients excreted different amounts of urine from urination to urination. However, the number of urinations with a volume of up to 100 ml after the course of treatment decreased from 75% to 19%, and at the same time, the number of urinations in the range of 200-300 ml and 300-400 ml increased by 28% and 20%, respectively.

DISCUSSION

The hyperactive bladder syndrome consists of the following components: imperative urges – a complaint of sudden, uncontrollable, violent urges to urinate; frequent urination - more than 8 urinations per day; nocturia - urination at night; Urgent urinary incontinence - urinary incontinence due to an imperative urge [2,3,4,20,21].

It is necessary to remember that the symptom of urgency can be found in other diseases of the urinary bladder - interstitial cystitis and pain syndrome of the urinary bladder. Urinary incontinence, as well as urgency, frequent daytime urination and nocturia, are among the symptoms of bladder storage dysfunction. Therefore, the following types of urinary incontinence are distinguished: stress urinary incontinence - observation of the involuntary release of urine from the urethra during physical exertion, sneezing or coughing; Urinary incontinence – observation of involuntary urine discharge from the urethra synchronously with the feeling of a sudden imperative urge to urinate, which is difficult to restrain; extraurethral urinary incontinence - observation of urine discharge not through the urethra, but through the fistula (hypospadia of various degrees).

Recently, the assessment of the role of the functional component in the development of urination disorders against the background of chronic stress component and post-traumatic neurological disorders, the main cause of which is considered to be detrusor hyperactivity and a decrease in the tone of the bladder and pelvic floor muscles, has recently become especially relevant. Without posing a direct threat to health, hyperactive bladder syndrome has an extremely negative effect on the psychological state and it significantly reduces the quality of life of patients of this group. The ineffectiveness of preventive measures and late seeking

of medical help, especially of people of working age, requires pharmacological influence [6,7,8, 14,17]. Since hyperstimulation of muscarinic receptors is the cause of detrusor hyperactivity, the main drug treatment for overactive bladder syndrome is anticholinergic drugs. M-cholinoblockers belong to the drugs of the first line. They are antagonists of muscarinic receptors located on smooth detrusor myocytes, which are involved in the regulation of detrusor tone by parasympathetic nerve fibers. Studies show that the main effect of anticholinergic drugs is manifested during the phase of urine accumulation, when there is no activity of parasympathetic fibers, which suppresses urgent urges and improves the storage capacity of the bladder [9,20,21].

CONCLUSIONS

The proposed complex of treatment of hyperactive bladder syndrome as a result of combat trauma against the background of barotrauma with neurological consequences and chronic stress allows to achieve a pronounced clinical effect in the vast majority of male patients of working age. It allows effective elimination of the main objective and subjective symptoms of the disease, which is manifested in a decrease in the frequency of frequent urination by 41%, nocturia by 61%, and episodes of urgency by 73%. And the diagnostic complex allows you to emphasize aspects of clinical vigilance, both for doctors of a specialized branch and doctors of a general direction.

REFERENCES

1. Wrobel A. Overactive bladder syndrome pharmacotherapy: future treatment options. Prz Menopauzalny. 2015; 14(4): 211-217. doi:10.5114/pm.2015.56403.
2. Milsom I, Stewart W, Thuroff J. The prevalence of overactive bladder. Amer. J. Manag. Care. 2000;6(11):S565-73.
3. Yoshida M et al. Management detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. Urology. 2004;63(3(1)):17-23. doi: 10.1016/j.urology.2003.11.003.
4. Willis-Gra MG. Evaluation and management of overactive bladder: strategies for optimizing care. Res RepUrol. 2016; 8: 113-122. doi: 10.2147/RRU.S93636.
5. Epstein BJ, Gums JG, Molina E.. Newer Agents for the Management of Overactive Bladder. Am Fam Physician. 2006;74(12):2061-2068.
6. Nuotio M et al. Urgency, urge incontinence and voiding symptoms in men and women aged 70 years and over. BJU Int. 2002;89(4):350-5. doi: 10.1046/j.1464-4096.2001.01684.x.
7. Ercan O. Comparison of solifenacin and fesoterodine in treatment of overactive bladder SaudiMed J. 2015; 36(10): 1181-1185. doi: 10.15537/smj.2015.10.12016.
8. Steers WD. Pathophysiology of Overactive Bladder and Urge Urinary Incontinence. RevUrol. 2002; 4(4): S7-S18.
9. Nalliah S. Comparison of efficacy and tolerability of pharmacological treatment for the overactive bladder in women: A network meta-analysis. Aust Fam Physician. 2017;46(3):139-144.
10. Tseng L-H et al. The prevalence of urinary incontinence and associated risk factors in Taiwanese women with lower urinary tract symptoms. Chang Gung Med J. 2006;29(6):596-602.
11. Nambiar AK, Bosch R, Cruz F et al. EAU Guidelineson Assessment and Nonsurgical Management of UrinaryIncontinence. EurUrol. 2018;73(4): 596–609.
12. Abrams P, Cardozo L, Wagg A, Wein A. Incontinence: 6th Edition 2017. Bristol UK: International Continence Society. 2016. https://www.ics.org/publications/ici_6/Incontinence_6th_Edition_2017_eBook_v2.pdf [date access 21.06.2023]
13. Lightner DJ, Gomelsky A, Souter L, Vasavada SP.. Diagnosis and Treatment of Overactive Bladder (Non Neurogenic) in Adults: AUA/SUFU Guideline Amendment. J Urol. 2019;202(3):558-563. doi: 10.1097/JU.0000000000000309.
14. Dekhtiar YM, Kostyev FI, Rudenko OV, Kuznietsov DO. Urodynamic patterns of the idiopathic overactive bladder. Science Journal of Clinical Medicine. 2017; 6(5): 74–79. doi: 10.11648/j.sjcm.20170605.12.
15. Isulihem A, Corcos J. Evaluation, treatment and surveillance of neurogenic detrusor overactivity in spinal cordinjury patients. Neuroimmunol. Neuro inflammation. 2019;6:13-26.
16. Drake MJ. Fundamentals of terminology in lower urinary tract function. Neurology and Urodynamics. 2018;37:13-19.
17. Drake MJ, Apostolidis A, Cocci A et al. Neurogenic lower urinary tract dysfunction: clinical management recommendations of the Neurologic Incontinence Committee of the fifth International Consultationon Incontinence 2013. Neurourology and Urodynamics. 2016;35: 657-665.
18. Jayadevappa R, Chhatre S, Neman DK et al. Association between overactive bladder treatment and falls among older adults. Neurourol Urodyn. 2018;37(8):2688-2694. doi: 10.1002/nau.23719.
19. Lightner DJ et al.Overactive Bladder (OAB) in adults: an AUA/SUFU Guideline. J Urol. 2019; 202: 558.
20. Truzzi JC, Come CM, Bezerra CA et al. Overactive bladder – 18 years – Part I. Int. Braz. J. Urol. 2016; 42:188-198.
21. Joint reporton terminology for surgical procedure stotreat stress urinary in continencein women (AUGS – IUGA joint publication). Female Pelvic Med Reconstr Surg. 2020;26(3):162-172. doi: 10.1097/SPV.0000000000000831.

The work was carried out in the framework of research work 0119U102046 «Complex treatment of patients with polytrauma on the background of endocrine disorders».

ORCID and contributionship:
Stepan S. Filip: 0000-0002-6549-3892 [A,B,F]
Rudolf M. Slivka: 0000-0002-0187-2711 [B-D]
Anton I.Batchynsky: 0000-0001-76-42-1889 [E,F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Stepan S. Filip
Uzhhorod National University
71 Minayska st, 81000 Uzhhorod, Ukraine
e-mail: filip.uz@i.ua

Received: 25.06.2023
Accepted: 28.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

HISTOLOGICAL AND MORPHOLOGICAL CHANGES IN THE LYMPHOID STRUCTURES OF THE GASTRIC MUCOUS MEMBRANE IN WHITE RATS WITH THE ADMINISTRATION OF SODIUM GLUTAMATE

DOI: 10.36740/WLek202311111

Mykhailo Yu. Kochmar, Yuliia V. Holosh, Nelli V. Bedey, Ivan I. Pushkash, Lyubov Yu. Pushkash
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To determine the histological and morphological changes of the lymphoid structures of the stomach in male rats under the influence of oral sodium glutamate at the rate of 15 mg/kg of body weight.

Materials and methods: The scientific experiment was performed on 20 white non-linear male rats of reproductive age (4-5 months). The experimental animals were divided into two groups (10 rats in each group), which were orally received monosodium glutamate at a dose of 15 mg/kg body weight every day. We studied the effect of 2 and 4 weekly administration of monosodium glutamate at a dose of 15 mg/kg body weight, respectively, in the I and II groups of experimental animals (depending on the week of their decapitation). Rats of the control groups (n=10) were injected with a placebo for 2 and 4 weeks, namely 0.5 ml of dechlorinated tap water at room temperature. Intact control animals were also divided into two groups, 5 rats each, depending on the week of decapitation: respectively, III group – decapitation on the 2nd week of the experiment; IV group - decapitation on the 4th week of the experiment. After the experiments were completed, animals were decapitated under light ether anesthesia. According to the purpose of the study, pieces of rat stomach measuring 1.0 x 1.0 cm were taken from the front wall of the bottom of the stomach near the great curvature, cardiac and portal parts of the organ. Histological preparations were examined using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out according to the method of S. B. Stefanov, using grids No. 3/16. For electron microscopic examination, pieces of the stomach wall of rats were fixed in a 2.5% solution of glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2-7.4) with subsequent fixation in a 2.0% solution of osmium tetroxide. After dehydration in alcohols and acetone, the material was embedded in eponaraldite. Sections were made on an LKB-8800-III ultramicrotome and studied using a JEM - 100-V microscope. To study the structural components of the lymphoid formations of the mucous membrane of different parts of the stomach of rats, semi-thin sections were made for the purpose of sharpening the blocks, which were stained with methylene blue.

Results: The analysis of the obtained data of the conducted experiment indicates that the administration of monosodium glutamate in a dose of 15 mg/kg of body weight to rats already after 14 days leads to an increase in the density and size of the lymphoid structures of the GMM. The number of immunocompetent cells between the fundus of the gastric glands and the muscle plate increases in the diffuse lymphoid tissue of the gastrointestinal tract of rats in all its parts, both in the I and II groups of experimental animals. These changes are most pronounced in the cardiac and portal parts of the stomach. In both groups of experimental animals, the migration of interepithelial lymphocytes, macrophages, plasma cells, and tissue basophils to the surface epithelium increases. In both groups of experimental animals (and the II group of rats), lymphoid nodules and lymphoid pre-nodules of the gastric mucous membrane (GMM) are located between the bottom of the gastric glands and the muscular plate of the GMM**.** A gradual increase of medium lymphocytes in the GMM was established both in animals of I and II groups, while large lymphocytes increased in almost the same amount in experimental animals of both groups. Similar changes occur in the characteristics of the number of plasma cells, macrophages and tissue basophils in the lymphoid pre-nodules of GMM.

Conclusions: Administering monosodium glutamate to rats at a dose of 15 mg/kg of body weight for 2 weeks leads to an increase in the density and size of lymphoid structures of the mucous membrane in all parts of the stomach with a predominant increase in the number of immunocompetent cells between the bottom of the gastric glands and the muscle plate. At the same time, more pronounced changes were found in the number of small lymphocytes, which tend to decrease by the 2nd week of the experiment, and vice versa – their density increases by the 4th week of monosodium glutamate administration.

KEY WORDS: monosodium glutamate, stomach, lymphoid structures

Wiad Lek. 2023;76(11):2406-2412

INTRODUCTION

Mucosa Associated Lymphoid Tissue or MALT are dispersed aggregates of non-encapsulated organized lymphoid tissue in the mucosa that are associated with local immune reactions on the surface of the mucosa. There are three main types of MALT - this is lymphoid tissue associated with the intestine (GALT – Gut Associated Lymphoid Tissue), lymphoid tissue associated

with the bronchi (BALT – bronchus associated lymphoid tissue) and lymphoid tissue associated with nasal part of the pharynx (NALT – nasal associated lymphoid tissue). MALT determines the effectiveness of the immune defense system of the mucous membranes of internal organs [1]. The study of lymphoid formations of the mucous membranes, including the stomach, is an extremely urgent task of both clinical and experimental research, which is due to the fact that in recent years the pollution of the external environment by toxic and harmful substances, in particular, antigens, and as well as products and components of food products that enter a person's stomach with food.

Various experimental and clinical studies are conducted to determine the impact of food additives on health. The most common of them is monosodium glutamate. The food supplement, which has been known as a "taste enhancer" for more than 100 years, is the fifth type of taste in Japan – "umami", after sour, bitter, salty and sweet. This additive was first discovered by the Japanese scientist Professor Kikunae Ikeda and introduced into mass production as one of the most common food additives after salt and pepper [2]. Studies have shown that even minimal doses of monosodium glutamate (0.6 and 1.6 mg/g of body weight for two weeks or 100-500 mg/kg of body weight for three weeks) can cause harmful effects on the body of humans and laboratory animals. animals, in particular rodents [3]. It was established that monosodium glutamate induced pronounced changes in the organs of the digestive system. Experimental feeding of monosodium glutamate to rats for 1-9 months at a dose of 2 mg/g of body weight caused characteristic histological changes in the pancreas, which were characterized by a decrease in the number of β-cells, hemorrhages, and signs of fibrosis [4].

Therefore, information about the negative impact of monosodium glutamate on the structural organization of organs, in particular the nervous, digestive, immune and other systems, is increasingly appearing in the professional literature [2]. The study of the peculiarities of histological and morphological changes in the lymphoid structures of the stomach against the background of sodium glutamate administration in the experiment can supplement knowledge about the effect of this food additive and the structural organization of the body's defense system on its administration.

THE AIM

The aim is to determine the histological and morphological changes of the lymphoid structures of the stomach in male rats under the influence of oral sodium glutamate at the rate of 15 mg/kg of body weight.

MATERIALS AND METHODS

The scientific experiment was performed on 20 white non-linear male rats of reproductive age (4-5 months) weighing 220-280 g, which were divided into two groups of 10 rats each. Experimental animals were kept in vivarium conditions in compliance with all regulations, namely the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine No. 3447–IV "On the Protection of Animals from Cruel Treatment", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

The experimental animals were divided into two groups (10 rats in each group), which were orally received monosodium glutamate at a dose of 15 mg/kg body weight every day. Monosodium glutamate was dissolved in 0.5 ml of dechlorinated tap water at room temperature. We studied the effect of 2 and 4 weekly administration of monosodium glutamate at a dose of 15 mg/kg body weight, respectively, in the I and II groups of experimental animals (depending on the week of their decapitation).

Rats of the control groups (n=10) were injected with a placebo for 2 and 4 weeks, namely 0.5 ml of dechlorinated tap water at room temperature. Intact control animals were also divided into two groups, 5 rats each, depending on the week of decapitation: respectively, III group – decapitation on the 2nd week of the experiment; IV group - decapitation on the 4th week of the experiment.

All experimental animals were kept in the conditions of the vivarium of the Lviv National Medical University named after Danylo Halytskyi. Control and experimental animals were kept in separate boxes in the vivarium.

After the experiments were completed (after administration of monosodium glutamate on weeks 2 and 4 of the experiment), animals were decapitated under light ether anesthesia. Animals were weighed before decapitation. The skin and soft tissues of the abdomen were dissected, the abdominal cavity was cut, and the stomach of control and experimental animals was sampled. According to the purpose of the study, pieces of rat stomach measuring 1.0 x 1.0 cm were taken from the front wall of the bottom of the stomach near the great curvature, cardiac and portal parts of the organ. The obtained pieces of stomach preparations were fixed for 2 weeks in a 10.0% solution of neutral formalin, after which they were embedded in paraffin blocks. Histological preparations were examined using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out

according to the method of S. B. Stefanov, using grids No. 3/16. At the same time, the density (number) of cellular elements was determined in a large grid square on an area of 625 μm in diffuse lymphoid tissue, lymphoid pre-nodules, and lymphoid nodules of the gastric mucous membrane (GMM). In lymphoid nodules, lymphoid pre-nodules, and diffuse lymphoid tissue, the number of small, medium, and large lymphocytes, plasma cells, macrophages, and tissue basophils was counted in 20 large squares of the 3/16 morphometric grid (the area of one large square is 625 μm2). Subsequently, the average value of cell density in one large square was calculated.

For electron microscopic examination, pieces of the stomach wall of rats were fixed in a 2.5% solution of glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2-7.4) with subsequent fixation in a 2.0% solution of osmium tetroxide. After dehydration in alcohols and acetone, the material was embedded in eponaraldite. Sections were made on an LKB-8800-III ultramicrotome and studied using a JEM - 100-V microscope. To study the structural components of the lymphoid formations of the mucous membrane of different parts of the stomach of rats, semi-thin sections were made for the purpose of sharpening the blocks, which were stained with methylene blue.

The analysis and processing of the results of the examination of patients was carried out by the computer program Statistics 10.0 (StatSoftInc, USA) for Windows, using parametric and non-parametric methods of evaluating the obtained results.

RESULTS

It should be noted that in the control group of rats (III-IV) groups) at different stages of experiment, we did not find any differences in the histological and morphometric evaluation of the lymphoid structures of the GMM. Therefore, in the future, the results of control animals of III and IV groups were combined into a single control group III (n= 10), with which the data of experimental animals of groups I and II at different stages of monosodium glutamate administration were compared.

The analysis of the obtained data of the conducted experiment indicates that the administration of monosodium glutamate in a dose of 15 mg/kg of body weight to rats already after 14 days leads to an increase in the density and size of the lymphoid structures of the GMM. The number of immunocompetent cells between the fundus of the gastric glands and the muscle plate increases in the diffuse lymphoid tissue of the gastrointestinal tract of rats in all its parts, both in the I and II groups of experimental animals. These changes are most pronounced in the cardiac and portal parts of the stomach. In both groups of experimental animals, the migration of interepithelial lymphocytes, macrophages, plasma cells, and tissue basophils to the surface epithelium increases. In both groups of experimental animals (and the II group of rats), lymphoid nodules and lymphoid pre-nodules of the GMM are located between the bottom of the gastric glands and the muscular plate of the GMM.

The cellular composition of lymphoid formations of GMM in rats changes after administration of monosodium glutamate at a dose of 15 mg/kg of body weight. As shown in Table 1, two weeks after the introduction of monosodium glutamate in the lymphoid nodes of the GMM in rats, the number of small lymphocytes in all sections of the stomach significantly decreases compared to similar data in animals of the control group, and after 1 month after the introduction of a food supplement, on the contrary, it is significantly increases both in comparison with the data of the I group and with the indicators of the control rats of the III group.

Average lymphocytes significantly increased in both groups of experimental animals in lymphoid nodules of the SOS in all its parts. A similar trend was followed in the analysis of plasma cells. Large lymphocytes, on the contrary, in the lymphoid nodules of the GMM, on the 2nd week of taking monosodium glutamate, increased significantly in all parts of the stomach, with their gradual decrease until the 4th week of the experiment (rats of the II group) (Table I).

In an electron microscopic study, we established that when monosodium glutamate was administered to rats at a dose of 15 mg/kg of body weight, cytoplasmic outgrowths appeared in lymphocytes, which indicates their active migration. The penetration of lymphocytes through the basement membrane into the epithelial layer of the GMM is also determined, which indicates an increase in the number of interepithelial lymphocytes when sodium glutamate is administered.

The activity of immune reactions upon administration of monosodium glutamate is indicated by an increase in the number of macrophages in all lymphoid formations of in gastric mucous membrane (GMM) of experimental animals of groups I and II. A significant increase in macrophages was established in both groups I and II rats. At the same time, no significant difference was established between the data of the animals of the experimental groups, that is, the number of macrophages and tissue basophils actually remained the same both on the 2nd week and on the 4th week of the experiment. The ultrastructure of macrophages changes significantly from the 2nd week of the introduction of the food supplement, namely, the number of microvilli and pseudopodia on the plasmolemma increases, and the cytoplasm becomes heterogeneous,

Table I. Cellular composition of lymphoid nodules of the mucous membrane of the stomach of rats in the control and experimented groups

Cell type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	9.4±0.21**	12.04±0.16*+	11.16±0.23
	Bottom	10.44±0.28**	14.23±0.21*+	13.08±0.26
	Portal part	11.06±0.25**	16.45±0.18**+	14.02±0.21
Medium lymphocytes	Cardiac part	0.86±0.06	1.14±0.06**+	0.72±0.06
	Bottom	0.95±0.05	1.25±0.08*+	0.80±0.09
	Portal part	1.16±0.09	1.44±0.03*+	0.91±0.08
Large lymphocytes	Cardiac part	0.77±0.05*	0.63±0.07	0.51±0.07
	Bottom	0.75±0.07**	0.61±0.04*	0.42±0.05
	Portal part	0.50±0.06**	0.44±0.08*	0.28±0,07
Plasma cells	Cardiac part	1.61±0.09**	1.85±0.07**	0.80±0.06
	Bottom	1.82±0.10**	1.80±0.07**	0.92±0.10
	Portal part	2.28±0.12*	2.30±0.24*	1.21±0.07
Macrophagocytes	Cardiac part	1,69±0.07**	1.60±0.03**	0.68±0.05
	Bottom	1,40±0.06**	1.44±0.05**	0.60±0.07
	Portal part	2.20±0.08**	2.26±0.21**	0.84±0.11
Tissue basophils	Cardiac part	1.70±0.05**	1.77±0.05**	0.85±0.03
	Bottom	2.50±0.24**	2.54±0.06**	1.21±0.14
	Portal part	1.60±0.07**	1.72±0.05**	0.68±0.07

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – $p<0.05$; ** – $p<0.01$; the difference between the indicators in rats of the I and II groups is significant: + - $p<0.05$.

Table II. Cellular composition of pre-lymphoid nodules of the gastric mucosa of rats in the control and experimental groups

Cells type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	8.12±0.18*	10.06±0.15*+	9.11±0.33
	Bottom	10.37±0.21*	12.80±0.17*+	11.42±0.26
	Portal part	10.70±0.23*	13.55±0.28*+	12.30±0.25
Medium lymphocytes	Cardiac part	0.85±0.07	1.08±0.06*	0.71±0.08
	Bottom	0.97±0.09	1.22±0.08*+	0.88±0.06
	Cardiac part	0.98±0.07	1.25±0.06*+	0.89±0.05
Large lymphocytes	Cardiac part	0.44±0.06	0.43±0.09	0.34±0.10
	Bottom	0.53±0.12	0.50±0.09	0.32±0.04
	Portal part	0.56±0.06	0.54±0.07	0.43 ±0.07
Plasma cells	Cardiac part	1.08±0.13*	1.11±0.06**	0.50±0.12
	Bottom	1.43±0.06**	1.41±0.10**	0.75±0.08
	Portal part	1.68±0.05*	1.73±0.06**	0.94±0.06
Macrophagocytes	Cardiac part	1.58±0.10**	1.54±0.05**	0.64±0.05
	Bottom	1.30±0.05**	1.22±0.07**	0.55±0.09
	Portal part	2.15±0.11**	2.14±0.09**	0.84±0.07
Tissue basophils	Cardiac part	1.60±0.04**	1.76±0.09**	0.86±0.07
	Bottom	2.21±0.14**	2.35±0.13**	1.14±0.12
	Portal part	1.77±0.06**	1.70±0.09**	0.85±0.08

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – $p<0.05$; ** – $p<0.01$; the difference between indicators in rats of the I and II groups is significant: + - $p<0.05$.

Table III. Cellular composition of the diffuse lymphoid tissue of the gastric mucous membrane of rats in the control and experimental groups

Cell type	Parts of the stomach	Groups of animals		
		I group	II group	III group
Small lymphocytes	Cardiac part	3.94±0.26*	4.77±0.16*+	4.30±0.12
	Bottom	3.36±0.18*	4.08±0.24*+	3.70±0.16
	Portal part	4.30±0.21*	5.23±0.16*+	4.86±0.23
Medium lymphocytes	Cardiac part	0.65±0.08	0.84±0.04*+	0.54±0.09
	Bottom	0.77±0.09	1.06±0.05*+	0.65±0.08
	Portal part	1.34±0.06	1.50±0.08*	1.18±0.06
Large lymphocytes	Cardiac part	0.26±0.06*	0.25±0.04*	0.14±0.08
	Bottom	0.37±0.04	0.37±0.09	0.23±0.12
	Portal part	0.42±0.05	0.47±0.07*	0.27±0.06
Plasma cells	Cardiac part	1.11±0.09**	1.15±0.10**	0.52±0.08
	Bottom	1.15±0.06**	1.35±0.12**	0.66±0.07
	Portal part	1.30±0.05**	1.57±0.04**	0.72±0.05
Macrophagocytes	Cardiac part	0.44±0.10**	0.49±0.08**	0.21±0.04
	Bottom	0.60±0.08**	0.77±0.03**	0.22±0.07
	Portal part	1.05±0.08**	0.97±0.09**	0.37±0.06
Tissue basophils	Cardiac part	118±0.08**	1.26±0.09**	0.55±0.04
	Bottom	2.15±0.10**	2.21±0.08**	0.88±0.14
	Portal part	1.65±0.05**	1.69±0.07**	0.62±0.12

Note: the difference between the parameters of the rats of the experimental groups (I and II groups) and the control group (III group) is significant: * – $p<0.05$; ** – $p<0.01$; the difference between indicators in rats of the I and II groups is significant: + - $p<0.05$.

containing many lysosomes, phagosomes, and pinocytotic vesicles. Macrophagocytes have various shapes and the cytoplasm contains many specific granules.

Tissue basophils are also significantly increased in animals of both experimental groups in all departments of lymphoid nodules of GMM. They migrate in the direction of the surface epithelium of the GMM.

In the 2nd week of the experiment, the number of small lymphocytes decreases in the lymphoid pre-nodules in comparison with such data in rats of the control group, while on the 4th week of administration of monosodium glutamate, on the contrary, a significant increase in them was found in all departments of the GMM (Table II).

A gradual increase of medium lymphocytes in the GMM was established both in animals of I and II groups, while large lymphocytes increased in almost the same amount in experimental animals of both groups. Similar changes occur in the characteristics of the number of plasma cells, macrophages and tissue basophils in the lymphoid pre-nodules of GMM.

The dynamics of changes in the density of cellular elements in the diffuse lymphoid tissue of GMM in rats is similar to the changes in lymphoid nodules and lymphoid pre-nodules (Table III).

On the 2nd week of sodium glutamate administration, the number of small lymphocytes in all parts of the stomach in rats also decreases in the diffuse lymphoid tissue of the GMM. But after a 4-week introduction of the food supplement, its density begins to gradually increase, just as in the lymph nodes and pre-nodules of GMM. Average lymphocytes in the diffuse lymphoid tissue of GMM gradually increase with minimal changes in animals of the I group and maximum deviations from the data of the control group in the experimental animals of the II group. Large lymphocytes, plasma cells, macrophages, and tissue basophils also increased at all stages of the experiment (both on the 2nd and 4th week of sodium glutamate administration). At the same time, we found no significant difference between the obtained indicators in animals of the I and II groups.

So, against the background of sodium glutamate administration at a dose of 15 mg/kg of body weight, changes in their cellular composition occur in lymphoid nodes and pre-nodules, as well as in diffuse lymphoid tissue of GMM compared to rats of the control group. As early as on the 2nd week of the experiment, the number of small lymphocytes in these lymphoid structures of the GMM in rats increases compared to the indicators in control animals, and 4 weeks after the administration of monosodium glutamate, the density of small lymphocytes increases even more in all lymphoid structures of the GMM.

DISCUSSION

The Various experimental and clinical studies are being conducted on the effects of monosodium glutamate on the organs and systems of the body. The results of a clinical study conducted by Boutry C. et al. with the involvement of volunteers who took monosodium glutamate in a dose of 2 g within 7 days indicate an increase in the volume of the portal cavity of the stomach in comparison with such data of the control group. The authors attribute the obtained result to an increase in gastric secretion, while the rate of gastric emptying remained unchanged [5].

The results of Xu JZ et al (2022) showed that administration of 30 mg/kg monosodium glutamate had no significant effect on serum C-reactive protein, trimethylamine N-oxide, angiotensin II, intestinal interleukin (IL)-1β, IL-6, factor tumor necrosis-α, secretory IgA, and fecal albumin in mice, but promotes intestinal inflammation and changes in intestinal flora. Moreover, the administration of 1500 mg/kg of monosodium glutamate increased the risk of cardiovascular diseases and damaged the intestinal structure and disturbed the composition of the intestinal microflora. The authors also established that with an increase in the dose of monosodium glutamate, inflammatory changes in the intestines of mice increase, while intestinal immunity sharply decreases [6].

The results of our research also indicate the reaction of the immunocompetent tissue of the digestive tract to the administration of monosodium glutamate to rats at a dose of 15 mg/kg of body weight. The activity of immune processes when sodium glutamate is administered is indicated by an increase in the number of macrophages in all lymphoid formations of GMM in an experiment in rats. Changes in all cell types of elements of lymphoid nodules, pre-nodules and diffuse lymphoid tissue of GMM were also found, which indicates the reaction of the body, including the local immunity of the stomach, to sodium glutamate intake. This is manifested by an increase in the density of cellular elements of the lymphoid tissue of the GMM and indicates a protective reaction of the animal body to the action of a negative factor, namely, the food additive – monosodium glutamate.

CONCLUSIONS

Administering monosodium glutamate to rats at a dose of 15 mg/kg of body weight for 2 weeks leads to an increase in the density and size of lymphoid structures of the mucous membrane in all parts of the stomach with a predominant increase in the number of immunocompetent cells between the bottom of the gastric glands and the muscle plate. At the same time, more pronounced changes were found in the number of small lymphocytes, which tend to decrease by the 2nd week of the experiment, and vice versa – their density increases by the 4th week of monosodium glutamate administration.

REFERENCES

1. Elmore SA. Enhanced Histopathology of Mucosa-Associated Lymphoid Tissue. Toxicol Pathol. 2006; 34 (5): 687–696. doi: 10.1080/01926230600939989.
2. Vashcheniuk MI. Glutamat natriju: mechanizmi vplivu na rizni organi ta systemi (Ohlad literature). [Sodium glutamate: mechanisms of influence on different organs and body systems (Literature rewiew)]. Art of Medicine. 2023; 1 (25): 101-105. doi: 10.21802/artm.2023.1.25.101. (in Ukrainian)
3. Rutska AV, Getsko NV, Krynytska IY. Toksychnyy vplyv hlutamatu natriyu na zhyvyy orhanizm (Ohlyad literatury) [Toxic impact of monosodium glutamate on a living organism (Literature review)]. Medychna ta klinichna khimiia. 2017; 1: 119-127. doi: 10.11603/mcch.2410-681X.2017.v0.i1.7685. (in Ukrainian)
4. Kinash OV, Chupryna OB, Donets IM et al. Mechanizm diji glutamate natriju na organi ta sistemy. [Mechanisms of monosodium glutamate impact on organs and systems]. Actualni pitanja suchasnoji medicine. 2021; 4 (21): 178-183. doi: 10.31718/2077-1096.21.4.178 (in Ukrainian)
5. Boutry C, Matsumoto H, Airinei G et al. Monosodium glutamate raises antral distension and plasma amino acid after a standard meal in humans. Am J Physiol Gastrointest Liver Physiol. 2011; 300 (1): 137-145. doi: 10.1152/ajpgi.00299.2010.
6. Xu JZ, Tang M, Liu Y et al. Safety assessment of monosodium glutamate based on intestinal function and flora ina mice. Food Science and Human Wellness. 2022; 11(1): 155-164. doi:10.1016/j.fshw.2021.07.016.

The study was performed within the framework of the scientific topics "Morphological characteristics of internal organs and vascular bed in normal ontogeny and patterns of their restructuring in obesity and the effect of physical factors on the body" (state registration number 0119U102059) researched by the Department of Anatomy and Histology.

ORCID and contributionship:
Mykhailo Yu. Kochmar: 0000-0002-0219-0552 [A, F]
Yuliia V. Holosh: 0000-0001-8516-0545 [B-D]
Nelli V. Bedey: 0000-0002-8885-0258 [B]
Ivan I. Pushkash: 0000-0003-4651-4608 [B]
Lyubov Yu. Pushkash: 0000-0003-4651-4608 [B]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mykhailo Yu. Kochmar
Uzhhorod National University
1 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380509135074
e-mail: kaf-anatomy@uzhnu.edu.ua

Received: 12.05.2023
Accepted: 18.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

RESPIRATORY MICROBIOME AND ITS RELATIONSHIP WITH INFLAMMATORY MARKERS

DOI: 10.36740/WLek202311112

Olesya M. Horlenko, Iryna Yu. Pikina, Lyubomyra B. Prylypko, Gabriella B. Kossey, Maria A. Derbak, Adrian I.Tomey, Volodymyr Yu. Mashyka
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: This study aims to investigate and analyze the microbiome of the nasopharyngeal zone in acute respiratory infections (ARI) and their relationship with inflammatory markers.

Materials and methods: Examination of 112 children (10-14 years old) with acute respiratory infections (ARI) of the upper respiratory tract was carried out. The control group consisted of 25 healthy children identical in age and examination parameters.

Results: When analyzing the microflora of the nasopharynx of patients, 29,0% of strains were gram-positive bacteria and 71,0% were gram-negative bacteria (*Escherichia coli* representing 37,0%). Biochemical examination of the biomaterial revealed the presence of sucrase (n=69), maltase (n=87), lactorepoxidase (n-89) and alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86). Regarding the markers of inflammatory response, the following dynamics was noted: increase in the level of IgM (3,91 ± 1,79 g/l, p<0,01) by 2,2 times, elevation of Ig G level by 10 times (145, 91 ± 53,04 g/l, p< 0,01), slightly higher than the reference values IgE level. In addition, increased IL-1, IL-4, IL-6, γ-IFN, TNF-α, Neopterin levels were detected. The level of Thyroid stimulating hormone (TSH) was significantly different compared to the control group (0,62 ± 0,57 vs. 1,98 ± 0,30 mIU/ml, p< 0,01), but within the reference values.

Conclusions: Predominance of Gram-negative bacteria in the nasopharyngeal microflora of patients along with elevated inflammatory markers and lactoperoxydase enzyme predominance was detected in the study.

KEY WORDS: acute respiratory infections, nasopharyngeal microflora, inflammatory markers, correlation, children

Wiad Lek. 2023;76(11):2413-2419

INTRODUCTION

Pathogenetic features of acute respiratory infections (ARI) are related to the clinical presentation caused by the respiratory microbiome of the upper respiratory tract. ARI, mainly of the upper respiratory tract, are especially common in preschool-age children [1].

In healthy children, acute inflammation of the mucous membrane and lymphoid structures of the oropharynx is usually a self-limiting disease, except for episodes caused by GAS. Due to an insufficiently developed immune system, children primarily suffer from ARI and are prone to the development of complications, including bronchitis, pneumonia, sinusitis, otitis. Each year, up to 12 cases of ARI can occur in a child, and the frequency of complications reaches 30% and leads to cases where the use of antibiotics is considered [2,3].

Colonization of the nasopharyngeal zone is the first stage in the development of pathology. The next stage of primary colonization is the transmission of infection in the environment. Nasopharyngeal carriage of microorganisms can play a leading role in the development and spread of respiratory infections, and the so-called «healthy» carriage under the influence of various pathological influences can transform into an infectious process [4].

Active reproduction of microorganisms occurs during acute respiratory viral infections. Under the influence of infectious factors and other factors that suppress immunity, the bacterial process develops [5,6].

THE AIM

To Investigate and analyze the microbiome of the nasopharyngeal zone in ARI and their relationship with inflammatory markers

MATERIALS AND METHODS

Examination of 112 sick children (10-14 years old) with acute respiratory infections (ARI) of the upper respi-

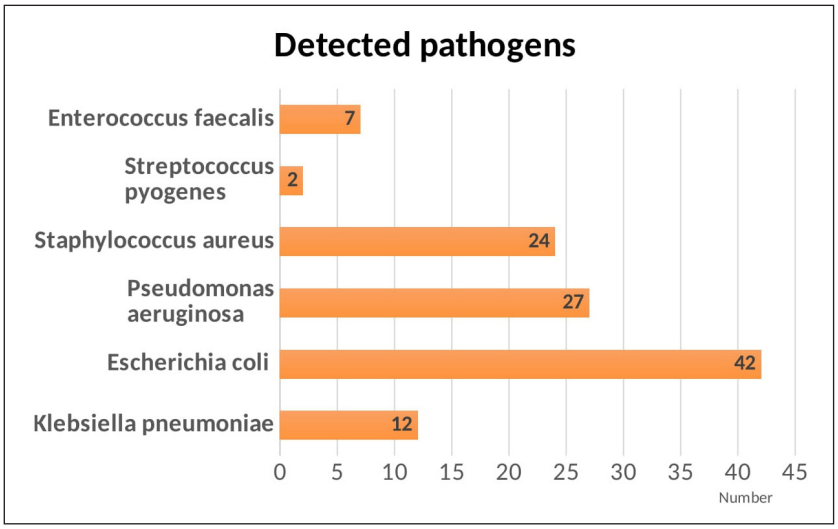


Fig. 1. Characteristics of detected pathogens in children (absolute values)

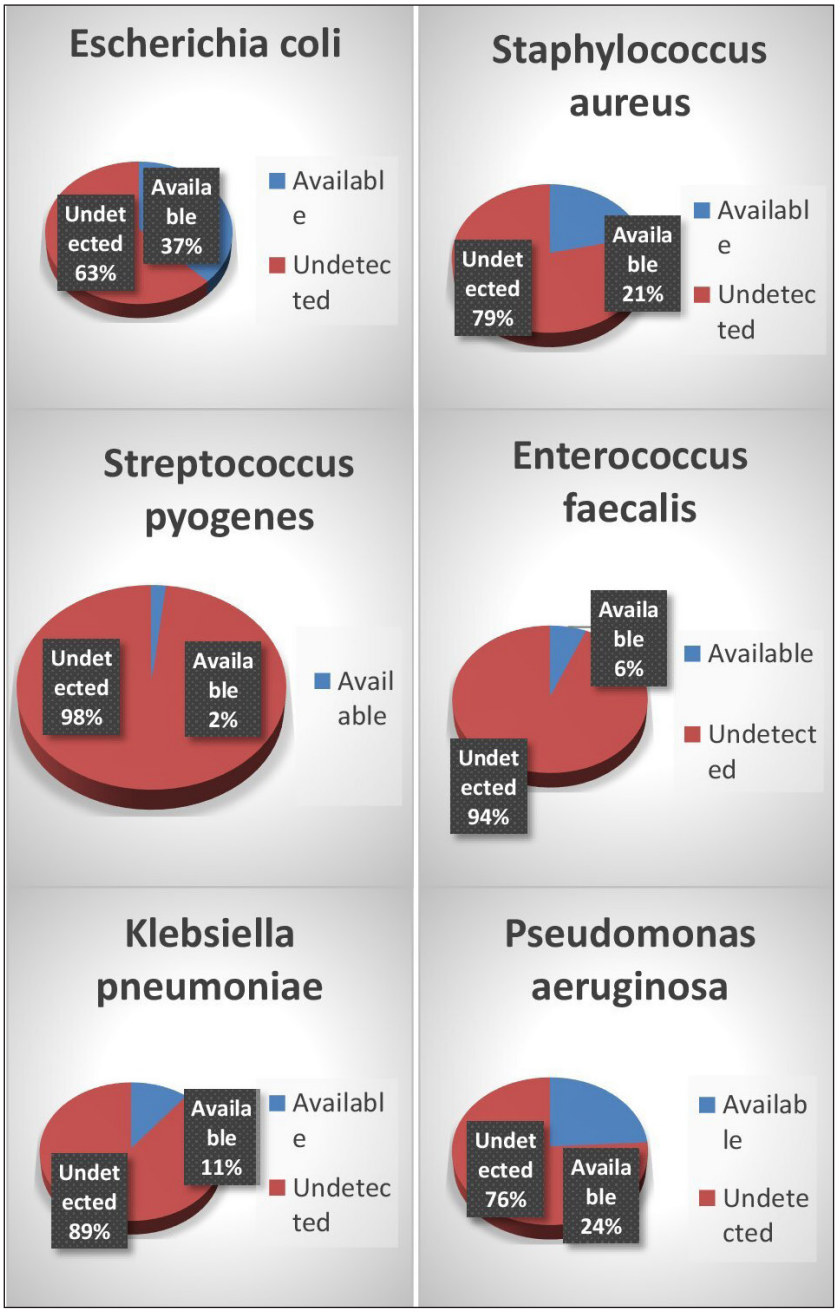


Fig. 2. The structure of representatives of the microflora of the nasopharynx in children with ARI: A) Percentage of *Escherichia coli* strains in the microflora of nasopharynx in children with ARI; B) Percentage of *Staphylococcus aureus* strains in the microflora of nasopharynx in children with ARI; C) Percentage of *Streptococcus pyogenes* strains in the microflora of nasopharynx in children with ARI; D) Percentage of *Enterococcus faecalis* strains in the microflora of nasopharynx in children with ARI; E) Percentage of *Klebsiella pneumoniae* strains in the microflora of nasopharynx in children with ARI; F) Percentage of *Pseudomonas aeruginosa* strains in the microflora of nasopharynx in children with ARI

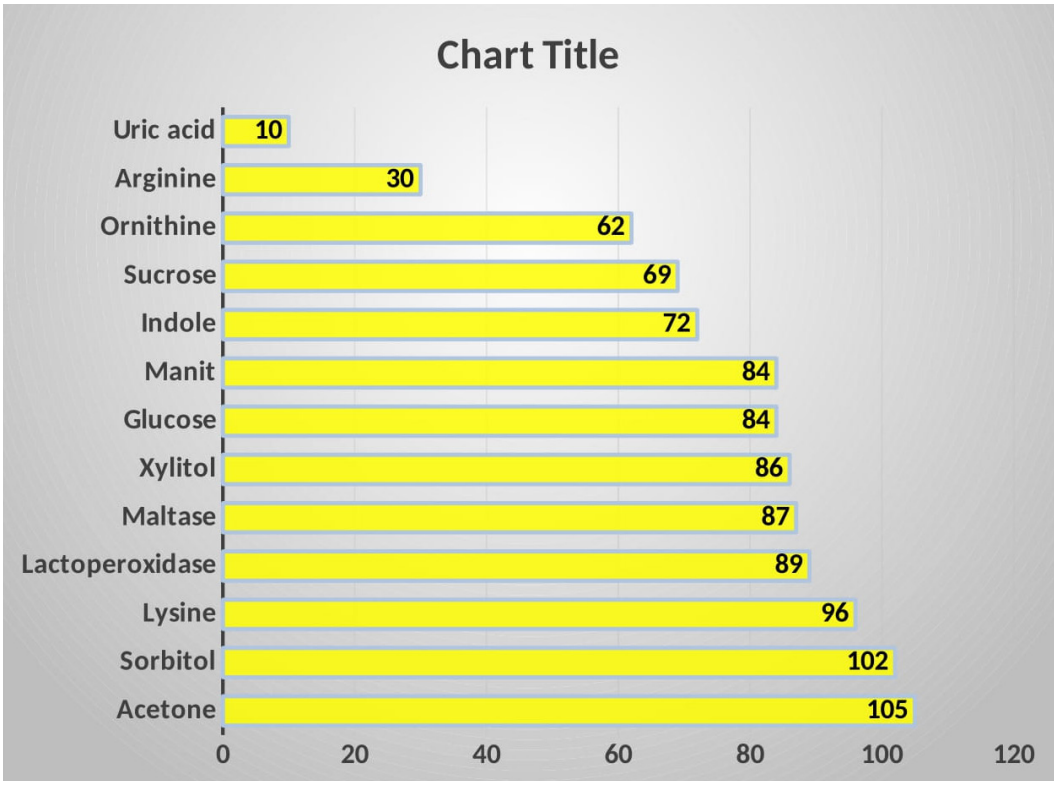


Fig. 3. Biochemical components of the biomaterial in children with ARI

ratory tract who were being treated at the Uzhgorod Clinical City Hospital was carried out. The control group consisted of 25 healthy children identical in age and examination parameters.

A study of the immunological and hormonal status was conducted to identify the levels of markers of the inflammatory response. Microbiological research included the selection of pathogens of the pathological process, identification by morphological, cultural and biochemical properties based on the analysis of nasopharyngeal washes and the establishment of correlational relationships between them.

RESULTS

When analyzing the microflora of the nasopharynx of patients with acute upper respiratory tract infections, 114 strains of opportunistic microorganisms were isolated, of which 33 strains (29,0%) were gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71,0%) were gram-negative bacteria (*Klebsiela pneumoninae, Pseudomonas aeruginosa, Escherichia coli*) (fig. 1, 2).

According to the research data, there is a predominance of 81 strains of gram-negative bacteria (71.0%), in particular, *Klebsiela pneumoninae* (11%), *Pseudomonas aeruginosa* (24%), *Escherichia coli* (37.0%) and 33 gram-positive bacteria (29.0%) - *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), *Enterococcus faecalis* (6.0%). According to groups, the leading pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The composition of the microflora of the nasopharynx of healthy children depends on many factors, both on the age category and on the hormonal status and pathology [4].

During the biochemical examination of the biomaterial, the following components were observed (fig. 3)

The design of biomaterial research included the detection of a group of enzymes such as sucrase (n=69), maltase (n=87) and lactorepoxidase (n-89). Lactoperoxidase, which is a heme-containing glycoprotein, was shown to have the highest level in our research. Its function is to use H_2O_2 for the synthesis of hypothiocyanate (OSCN–), which has the ability to suppress the replication of bacteria, fungi, viruses, parasites, neutralization of intestinal pathogens in infants. The enzyme maltase (α-glucosidase) splits maltose disaccharide to form glucose. Sucrase breaks down both sucrose and maltose [4]. The obtained level of glucose in children (n=84) indicates the functionality of the system of destructuring and formation of monosaccharides. Amino acids Lysine (n-96), ornithine (n=62), arginine (n=30) were also found in the biomaterial.

The presence of alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86) indicates the possibility of detoxification by the organism. Factors of the intoxication plan were also found: acetone (n=105), which indicates excessive replication of bacteria in the oral cavity. Iden-

Table I. Immunogram of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
Ig M (0,31-1,79, g/l)	3,91 ± 1,79	1,05 ± 0,09	< 0,01
Ig G (6,98-15,49, g/l)	145, 91 ± 53,04	10,39 ± 0,79	< 0,01
Ig E (till 120 IU/ml)	144,68 ± 61,09	41,71 ± 3,18	< 0,01

Table II. Markers of the inflammatory response of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
IL-1 (0-11, pg/ml)	30,37 ± 28,19	2,08 ± 0,49	< 0,01
IL-2 (0-10, pg/ml)	9,43 ± 5,80	0,41 ± 0,05	< 0,01
IL-4 (< 0,5 ng/ml)	5,98 ± 3,19	0,37 ± 0,14	< 0,01
IL-6 (0-10, pg/ml)	16,99 ± 6,89	3,29 ± 0,66	< 0,01
IL-10 (0-20, pg/ml)	15,55 ± 11,88	2,13 ± 0,31	< 0,01
γ-IFN (< 15, pg/ml)	54,16 ± 7,35	5,65 ± 0,85	< 0,01
TNF-α (< 6, pg/ml)	156,70 ± 20,70	3,43 ± 0,47	< 0,01
Neopterin (< 10, nmol/l)	90,42 ± 54,33	6,34 ± 1,14	< 0,01
Cortisol (110-692, nmol/l)	540,32 ± 132,17	251,46 ± 118,82	< 0,01

tification of indole in 72 cases indicates effects in the regulation of various aspects of bacterial physiology and the level of virulence. Tryptophan is an indole derivative and precursor of the neurotransmitter serotonin and can cause vomiting and angiospasm in the patient [5].

Respiratory diseases of the upper respiratory tract (URI), like all inflammatory diseases, are accompanied by an adequate response of the child's organism. We investigated the inflammatory response of the child's organism by studying the following markers (Table I).

As we can see from Table I, there is an increase in the level of IgM (3,91 ± 1,79 g/l, p<0,01) by 2,2 times, which indicates an increase in their synthesis after the pathogen enters the body for bactericidal activation of human blood serum.

Let's consider the next stage of the humoral link of immunogenesis - the synthesis of IgG molecules. According to our data, Ig G level is increased 10 times (145, 91 ± 53,04 g/l, p< 0,01). According to scientific data, immunoglobulins of class G (IgG) are the main class of antibodies and are synthesized in response to a secondary infectious factor. In the process of implementation and formation of the immune response, the synthesis of IgM is switched to IgG. We observe the predominance of IgG synthesis.

The level of IgE is slightly higher than the reference values, but 3,5 times higher than the level in children of the control group (144,68 ± 61,09 versus 41,71 ± 3,18 IU/ml, p<0,01). According to literature data, it is believed that the main role of IgE is the protection of mucous membranes due to the induction of a local inflammatory reaction. This causes an inflammatory reaction [6].

In the vast majority of cases, cytokines are close-acting mediators of local interactions between cells in foci of certain processes in tissues, even pairs of cells. Depending on the known parameters of irradiation, the effects of cytokines are classified into autocrine effects (on the cell itself that secreted cytokines) and paracrine effects (on cells located nearby). There are also endocrine or remote effects, they are also called systemic,

Table III. Hormonal status of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
Free Triiodothyronine (1,2 - 2,8, nmol/l)	1,38 ± 0,57	1,32 ± 0,12	0,60
Free thyroxine (12,5 - 21,0, pmol/l)	14,59 ± 3,03	13,72 ± 0,51	0,15
Thyroid stimulating hormone (0,4 - 4,0, mIU/ml)	0,62 ± 0,57	1,98 ± 0,30	< 0,01
Thyroid peroxidase antibody (< 0,9, IU/ml)	7,26 ± 4,71	5,52 ± 0,21	0,07

Table IV. Correlation relationships of nasopharyngeal microflora and inflammatory response markers

Laboratory indicators		Correlation coefficient (r)	Statistical significance (p)
Escherichia coli	Free T4	0,19	0,05
	TNF-α	0,20	0,04
Staphylococcus aureus	Lactoperoxidase	-0,20	0,04
	Acetone	-0,21	0,03
Pseudomonas aeruginosa	Free T3	0,20	0,04
	Free T4	-0,28	0,003
	TNF-α	-0,20	0,04
Streptococcus pyogenes	γ-IFN	0,32	0,001
Klebsiella pneumoniae	Free T4	0,20	0,04
Enterococcus faecalis	Cortisole	0,26	0,007

since in this case the cytokine reaches the target cell, circulating in the blood. But endocrine effects were found only for four cytokines (TNF-α, IL-1, IL-6, M-CSF) and not in healthy organisms, only in severe systemic pathology such as septic shock [6,7].

According to the data of Table II, there is significant increase in the level of the following cytokines with a significant predominance in comparison with the data of the control group of children: IL-1 (30,37 ± 28,19 vs. 2,08 ± 0,49 pg/ml, p< 0,01), IL-2 (9,43 ± 5,80 vs. 0,41 ± 0.05 pg/ml, p< 0,01), IL-4 (5,98 ± 3,19 vs. 0,37 ± 0,14ng/ml, p< 0,01), IL-6 (16,99 ± 6,89 vs. 3,29 ± 0,66 pg/ml, p< 0,01), IL-10 (15,55 ± 11,88 vs. 2,13 ± 0,31 pg/ml, p< 0,01), γ-IFN (54,16 ± 7,35 vs. 5,65 ± 0,85pg/ml, p< 0,01), TNF-α (156,70 ± 20,70 vs. 3,43 ± 0,47pg/ml, p< 0,01), Neopterin (90,42 ± 54,33 vs. 6,34 ± 1,14nmol/l, p< 0,01), Cortisol (540,32 ± 132,17 vs. 2 51,46 ± 118,82 nmol/l, p< 0,01).

The level of IL-1 increased 2,7 times, IL-4 11,8 times, IL-6 1,7 times, γ-IFN – 3,6 times, TNF-α – 26 times, Neopterin – 9 times. IL-2 and Cortisol levels are within reference values.

The formation and biological activity of cytokines are interconnected and mutually regulated in response to a stimulus. They form the so-called cytokine cascade, which corresponds to the inflammatory response of the child's body and, as a result, the clinical presentation is formed. Regulators of natural resistance - interferons α and β, interleukins 1, 6 and 12, TNF-α, chemokines (IL 8, MCP-1, RANTES, etc.) are the main activators and regulators of nonspecific reactions of the body to protect it from colonization by carriers of foreign genetic information [6].

The investigated parameters of the hormonal status of children with ARI are considered in Table III.

According to the data in Table III, changes are observed only in the Thyroid peroxidase antibody (TPO) indicator (7,26 ± 4,71 IU/ml), but they are unreliable in comparison with the data of the control group p=0,07. Indicators of Free triiodothyronine (T3) and Free thyroxine (T4) do not show differences with the control group (0,60 and 0,15 respectively). The level of Thyroid stimulating hormone (TSH) is significantly different compared to the control group (0,62 ± 0,57 vs. 1,98 ± 0,30 mIU/ml, p< 0,01), but within the reference values. Changes in thyroid status correspond to scientific research on their involvement in the inflammatory process [8].

On the basis of the data obtained from the study of inflammatory markers and indicators of the nasopharyngeal microflora in children with ARI, a correlation analysis of relationships was carried out (Table IV).

According to Table IV, correlations between the fre-

quency of detection of microorganisms and indicators of the inflammatory response of the child's organism are the following. The values of *Escherichia coli* infection have reliable positive relationships with Free T4 (r =0,19, p=0,05), TNF-α (r =0,20, p=0,04). The value of *Staphylococcus aureus* has a negative correlation with the levels of the *enzyme Lactoperoxidase* (r =-0,20, p=0,04) and Acetone (r =-0,21, p=0,03). The microorganism Pseudomonas aeruginosa presents negative correlations with Free T4 (r =-0,28, p=0,003), TNF-α (r =-0,20, p=0,04) and positive with Free T3 (r =0,20, p=0,04). The microorganism *Streptococcus pyogenes* has the highest correlations in our study with γ-IFN (r = 0,32, p = 0,001). Positive correlations of Klebsiella pneumoniae with Free T4 (r =0,20, p=0,04), *Enterococcus faecalis* with Cortisol (r =0,26, p=0,007) are observed.

DISCUSSION

Given the high prevalence of acute respiratory diseases in childhood, there is a need for new developments to identify pathogenetic factors for the treatment and prevention of this pathological process. The respiratory microbiome plays a leading role in diseases[1,2]. Deviations of the microflora and carriage causes the clinical presentation of acute respiratory diseases. Disturbances of the balance and the presence of pathogenic microorganisms cause an inflammatory reaction in the mucous membranes of the respiratory tract. Changes in the enzymatic, detoxification and characterological features of the microbial landscape present the inflammatory response of the child's organism with the presence of increased levels of their markers, in particular Il-1,4,6, γ-IFN, TNF-α [4,5].. Correlation relationships with thyroid hormones, TNF-α in the detection of *Escherichia coli* are observed. *Staphylococcus aureus* causes a violation of enzymatic properties in the oral cavity, in particular Lactoperoxidase and signs of intoxication of the organism. Respiratory pathology is the most common problem in clinical pediatrics and its relevance in childhood is related to the prevalence, predicted severe course and complications and requires further treatment and solving the etiopathogenetic plan for understanding the disease and its prevention.

CONCLUSIONS

1. 114 strains of opportunistic microorganisms were identified, of which 33 strains (29,0%) were Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71,0%) were Gram-negative bacteria (*Klebsiela pneumoninae, Pseudomonas aeruginosa, Escherichia coli*). The leading pathogens were *Escherichia coli* (37,0%) and *Staphylococcus aureus* (21,0%).
2. The biomaterial included the detection of enzyme groups such as sucrase (n=69), maltase (n=87), lactorepoxidase (n-89). Lactoperoxidase showed the highest level in our research.
3. Sucrase breaks down both sucrose and maltose. The obtained level of glucose in the analysis of children (n=84) testifies to the functionality of the system of destructuring and formation of monosaccharides. The presence of alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86) indicates the possibility of detoxification of the child's body. Factors of the intoxication plan were also found: acetone (n=105), which indicates excessive replication of bacteria in the oral cavity. Identification of indole in 72 cases indicates effects in the regulation of various aspects of bacterial physiology and the level of virulence. Tryptophan is an indole derivative and precursor of the neurotransmitter serotonin and may cause vomiting and angiospasm in the patient.
4. The level of IL-1 is increased 2,7 times, IL-4 11,8 times, IL-6 1,7 times, γ-IFN – 3,6 times, TNF-α – 26 times, Neopterin – 9 times. IL-2 and Cortisol levels are within reference values.
5. According to the data of the immunogram, changes are observed only in the indicator of Thyroid peroxidase antibody (TPO) levels (7,26 ± 4,71 IU/ml), but they are unreliable in comparison with the data of the control group p=0,07.
6. The most frequent detection of *Escherichia coli* microorganisms has a reliable positive relationship with Free T4 (r = 0,19, p = 0,05), TNF-α (r = 0,20, p = 0,04) and *Staphylococcus aureus*, which has a negative correlation with Lactoperoxidase enzyme (r =-0,20, p=0,04) and Acetone levels (r =-0,21, p=0,03).

REFERENCES

1. Havrylenko AO, Smiian OI, Moshchych OP et al. Klinichni osoblyvosti ta kharakter perebihu hostroho bronkhitu v ditei doshkilnoho viku u poiednanni iz syndromom eutyreoidnoi patolohii ta bez noho 1 Modern pediatrics. [Clinical features and nature of the course of acute bronchitis in preschool children in combination with the syndrome of euthyroid pathology and without it]. Suchasna Pediatriya. Ukrayina. 2021;8(120):47-54. doi: 10.15574/SP.2021.120.47. (in Ukrainian)
2. Fesenko MIe, Fastovets MM, Ziuzina LS et al. Hostri respiratorni rekurentni infektsii u ditei. [Acute respiratory recurrent infections in children]. Visnyk Ukrainskoi. Medychnoi stomatolohichnoi akademii. 2019:19,4(68):34-38. http://repository.pdmu.edu.ua/handle/123456789/17879 [date access 20.05.2023] (in Ukrainian)

3. Lutsenko OI, Rudyshyn SD, Borovyk TS. Medyko-sotsialnyi monitorinh zakhvoriuvanosti u ditei na hostryi bronkhit i pnevmoniiu (na prykladi mikroiolohichnoi laboratorii mistia Hlukhova, Sumska oblast. [Medical and social monitoring of the incidence of acute bronchitis and pneumonia in children (on the example of the microbiological laboratory of the city of Glukhova, Sumy region]. Visnyk Ukrainskoi medychnoi stomatolohichnoi akademii. Aktualni problemy suchasnoi medytsyny. 2021; 21,3(75):74-82.
4. Minukhin VV, Kovalenko NI, Tkachenko VL et al. Normalna mikroflora nosohlotky yak rezervuar polirezystentnykh shtamiv zbudnykiv infektsii verkhnikh dykhalnykh shliakhiv. [Normal microflora of the nasopharynx as a reservoir of polyresistant strains of causative agents of upper respiratory tract infections]. Annals of Mechnikov Institute. 2015;2:191-199.
5. Koliada KD, Fomenko RS, Dzyza AV, Lupyr AV. Mikrobiom nosohlotky ta yoho rol u patohenezi khvorob. [The microbiome of the nasopharynx and its role in the pathogenesis of diseases]. Abstracts book ΛΌΓΟΣ. 2021. doi: 10.36074/logos-26.11.2021.v3.33.
6. Rezaei N. Pediatric Immunology. A Case-Based Collection with MCQs. Springer. 2019;2: 863.
7. Katilov OV, Dmytriiev DV, Dmytriieva KYu. Klinichna pulmonolohiia dytiachoho viku. [Clinical pulmoomology of childhood]. Medytsyna. 2020, p.320.
8. De Luca R, Davis PJ, Lin Hu-Yu. et al. Thyroid Hormones Interaction With Immune Response, Inflammation and Non-thyroidal Ilness Syndrome. Front Cell Dev Biol. 2021. doi:10.3389/fcell.2020.614030.

ORCID and contributionship:
Olesya M. Horlenko: 0000-0002-2210-5503 [A,D,F]
Iryna Yu. Pikina: 0000-0003-1565-8174 [B,E]
Lyubomyra B. Prylypko: 0000-0002-4131-5450 [C,F]
Gabriella B. Kossey: 0000-0003-0811-4929 [D,F]
Mariya A. Derbak: 0000-0003-4791-4080 [B]
Adrian I. Tomey: 0000-0002-5095-8937 [E]
Volodymyr Yu. Mashyka: 0000-0002-0236-9958 [B]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olesya M. Horlenko
Uzhhorod National University
46 Pidhirna st., 88000 Uzhhorod, Ukraine
tel: +380505269658
e-mail: ohorlenko@gmail.com

Received: 21.05.2023
Accepted: 20.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

IMMUNE-INFLAMMATORY-ENDOCRINE REGULATION DISORDERS IN CHILDREN WITH CORONAVIRUS INFECTION

DOI: 10.36740/WLek202311113

Olesya M. Horlenko, Khrystyna A. Hechko, Lyubomyra B. Prylypko, Olga Blaga, Fedir V. Horlenko, Oleksandr O. Kutsyn, Bohdan M. Halay
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To study the of homeostasis links desorder and indicators imbalance in children with Coronavirus infection.

Materials and methods: A clinical and laboratory study was conducted in children (50 persons) with Coronavirus infection. The children were used outpatient treatment.

Results: It was found that Direct bilirubin was increased in 3 times (10.55±7.67 vs. 3.63±0.49 μmol/l, p<0.01), Alanine aminotransferase – in 1.7 times (37,02±20.53 vs. 21.90±1.82 IU/l, p<0.01). An levels increassng of Ig G – in 12.3 times, Ig E – in 4.6 times, Ig M – in 3.4 times was observed. The CRP level was increased in 3.1 times (8.76 ± 2.16 vs. 2.54 ± 0.53 mg/l, p< 0.01), C-peptide (4.65±1.67 vs. 1.23±0.08 ng/ml, p<0.01) – in 3.8 times. Negative correlations of T3 with Procalcitonin (r=-0.30) and Creatinine (r=-34) were revealed. T4 values are correlated with Total cholesterol (r=-0.65) and Creatinine (r=0.29). Leptin was presented positive correlations with Alanine aminotransferase (r=0.48) and with C-peptide (r=0.39).

Conclusions: There was an increase in the Ig G levels in 12.3 times, Ig E – in 4.6 times, and the Ig M level - in 3.4 times. The Thyroid stimulating hormone level was significantly lower (in 4.7 times). An increase in the C-reactive protein levels (in 3.1 times) and C-peptide (in 3.8 times) was observed. It should be noted that the strongest negative correlation between T4 and Total cholesterol (r=-0.65) and the highest positive correlation between Leptin and Alanine aminotransferase (r=0.48) and C-peptide (r=0. 39).

KEY WORDS: Coronavirus infection, acute respiratory distress syndrome, pathogenesis, inflammatory immune response, children

Wiad Lek. 2023;76(11):2420-2428

INTRODUCTION

In December 2019, an outbreak of a new infectious disease occurred in the city of Wuhan, Hubei Province, China. This is already the seventh coronavirus. On March 11, 2020, the World Health Organization (WHO) classified the outbreak as a pandemic. A number of studies have examined the symptoms and characteristics of adults with COVID-19. A smaller number of these studies cover the morbidity of children with COVID-19 [1]. To contain infection and develop effective systems to treat viral infections in an outbreak scenario, we must understand the nature of infection and the immune system's response about a new virus, and assess the similarities and differences of the new virus from viruses that have caused outbreaks in the past. Scientists focus on studying the reaction of the immune system against SARS-CoV-2 in comparison with cases of other CoVs (SARS and MERS) [2,3]. SARS-CoV-2 is a new infectious agent that has entered human civilization. As a rule, emerging and re-emerging viral infections belong to the RNA virus family, because these viruses have a high mutation rate, which allows them to adapt significantly to the environment with rapid evolution. We have a little and not inough about SARS-CoV-2 in children currently [4]. Also, the systemic landscape of immune responses in patients with COVID-19 is unclear. The mechanisms of development of the response reaction of the innate and adaptive immune system of the macroorganism to infection with the SARS-CoV-2 virus are described. Because the clinical features and immunopathogenesis of SARS-CoV-2 and SARS-CoV and MERS-CoV are somewhat similar, the knowledge gained from SARS-CoV and MERS-CoV has important implications for understanding [2,5]. Identification of characteristic clinical signs, understanding of the adaptive response of the child's organism to an infectious factor in the near future will be aimed at preventing the development and treatment of both acute respiratory distress syndrome and chronic conditions caused by damage to other target organs in the case of COVID-19 [3,6,7] .

Table I. Biochemical parameters in children

Laboratory indicators	Main group (n = 50) M ± m	Control group (n = 28) M ± m	Statistical significance (p)
Total protein (64-83, g/l)	67,31 ± 7,49	74,50 ± 3,39	< 0,01
Albumin (35-53, g/l)	45,38 ± 5,84	44,53 ± 2,32	0,46
Total bilirubin (<21,0, μmol/l)	18,37 ± 10,26	15,19 ± 0,84	0,11
Direct bilirubin (0-5,1, μmol/l)	10,55 ± 7,67	3,63 ± 0,49	< 0,01
Alanine aminotransferase (< 40,0, IU/l)	37,02 ± 20,53	21,90 ± 1,82	< 0,01, 1,7
Alkaline phosphatase (42-128, IU/l)	141,59 ± 99,44	52,02 ± 3,38	< 0,01 2,7
Creatinine (33-110, μmol/l	93,82 ± 34,63	61,21 ± 5,54	< 0,01 1,5
Urea (3,3-7,7, mmol/l)	6,84 ± 3,36	3,81 ± 0,32	< 0,01 1,8
Total cholesterol (3,1-5,0, mmol/l)	5,01 ± 1,71	4,31 ± 0,24	0,04 1,2
Glucose (3,3-6,1, mmol/l)	5,69 ± 2,37	4,75 ± 0,04	0,04 1,2
Potassium (3,5-5,1, mmol/l)	4,77 ± 0,47	4,21 ± 0,17	< 0,01 1,1
Sodium (135-155, mmol/l)	137,58 ± 4,91	143,71 ± 2,28	< 0,01
Chlorine (95-110, mmol/l)	102,47 ± 4,19	101,97 ± 2,82	0,58

THE AIM

To study the homeostasis links diorders and the indicators imbalance in children with Coronavirus infection

MATERIALS AND METHODS

Clinical and laboratory research was carried out in children with confirmed Coronavirus infection. The main group consisted of 50 children aged 14-16yy. (26 girls, 24 boys). The control group included healthy children (n=28), identical by the studied parameters. The children were used outpatient treatment of the City Multidisciplinary Clinical Hospital, Uzhhorod. Laboratory tests were performed on the 5th day of the disease and included biochemical and immunological examination, indicators of the pituitary-thyroid panel, markers of inflammatory-endocrine regulation.

RESULTS

The most common laboratory abnormalities associated with the new CoV include hypoalbuminemia, lymphopenia, decreased neutrophils, elevated C-reactive protein (CRP) and lactate dehydrogenase (LDH), and decreased CD8 counts; according to the researchers. The viral load of SARS-CoV-2 detected through the respiratory tract of patients was found to be positively related with the severity of lung disease. Albumin, lymphocytes, LDH, neutrophils, and CRP are highly correlated with acute lung injury. Age, viral load, lung injury score, blood biochemical parameters, albumin, CRP, LDH, lymphocytes (%) and neutrophils (%) can be disease severity predictors [8,9].

The obtained results are presented in the Table I.

There are no significant differences in the levels of albumin, total bilirubin, and chlorine in comparison with the results obtained in children of the control group (p=0.11-0.58), according to Table I. There is a significant predominance of Total protein in children of the control group in comparison with patients (74.50 ± 3.39 to 67.31 ± 7.49 g/l, p< 0.01) and Sodium level - (143.71 ± 2, 28 to 137.58 ± 4.91 mmol/l, p< 0.01). It should be noted that, the values were increased in sick children in all parameters, according to other indicators.

Table II. Immunogram of children with Coronavirus

Laboratory indicators	Main group (n = 50) M ± m	Control group (n = 28) M ± m	Statistical significance (p)
Ig M (0,31-1,79, g/l)	4,11 ± 1,74	1,20 ± 0,06	< 0,01 3,4
Ig G (6,98-15,49, g/l)	151,07 ± 39,77	12,29 ± 0,07	< 0,01 12,3
Ig E (till 120 IU/ml)	163,47 ± 43,29	35,60 ± 1,07	< 0,01 4,6

Table III. Results of the study of the homeostasis pituitary-thyroid link

Laboratory indicators	Main group (n = 50) M ± m	Control group (n = 28) M ± m	Statistical significance (p)
Thyroid stimulating hormone (0,4 - 4,0, mIU/ml)	0,40 ± 0,08	1,87 ± 0,46	< 0,01
Free triiodothyronine (1,2 - 2,8, nmol/l)	1,30 ± 0,24	1,39 ± 0,08	0,09
Free thyroxine (12,5 - 21,0, pmol/l)	15,25 ± 1,99	15,32 ± 0,49	0,86
Thyroid peroxidase antibody (< 35, IU/ml)	4,84 ± 3,56	5,69 ± 0,11	0,21

Table IV. Parameters of the levels of inflammatory endocrine regulation markers

Laboratory indicators	Main group (n = 50) M ± m	Control group (n = 28) M ± m	Statistical significance (p)
Ferritin (7-140, ng/ml)	79,32 ± 34,07	77,07 ± 10,40	0,73
C-reactive protein (<3, mg/l)	8,76 ± 2,16	2,54 ± 0,53	< 0,01
Procalcitonin (0-11, pg/ml)	9,27 ± 2,96	1,61 ± 0,23	< 0,01
Adiponectin (5-37, ng/ml)	27,92 ± 8,52	7,73 ± 0,86	< 0,01
Leptin (2,05-11,09, ng/ml)	10,44 ± 2,77	6,97 ± 0,32	< 0,01
C-peptide (0,81-3,85, ng/ml)	4,65 ± 1,67	1,23 ± 0,08	< 0,01

Table V. The correlations relationship of the pituitary-thyroid panel parameters

Laboratory indicators		Correlation coefficient (r)	Statistical significance (p)
Free triiodothyronine	Procalcitonin	-0,30	0,04
	Creatinine	-0,34	0,01
Free thyroxine	Total cholesterol	-0,65	< 0,01
	Creatinine	0,29	0,04
Thyroid stimulating hormone	Ig E	0,32	0,03
Thyroid peroxidase antibody	Albumin	0,28	0,05

Table VI. Correlationship of system of immune-inflammatory-endocrine regulation

Direct Bilirubin was increased in 3 times (10.55 ± 7.67 vs. 3.63 ± 0.49 μmol/l, p< 0.01) and exceeded the limits of reference values; Alanine aminotransferase - in 1.7 times (37, 02 ± 20.53 vs. 21.90 ± 1.82 IU/l, p< 0.01), Alkaline phosphatase – in 2.7 times (141.59 ± 99.44 vs. 52.02 ± 3.38 IU/ l, p< 0.01), Creatinine – in 1.5 times (93.82 ± 34.63 to 61.21 ± 5.54 μmol/l, p< 0.01), Urea – in 1.8 times (6.84 ± 3.36 vs. 3.81 ± 0.32 mmol/l, p< 0.01),

Laboratory indicators		Correlation coefficient (r)	Statistical significance (p)
Leptin	C-peptide	0,39	0,005
	Alanine aminotransferase	0,48	< 0,01
Adiponectin	Potassium	0,31	0,03
C-reactive protein	Direct bilirubin	0,38	0,007
	Albumin	-0,31	0,03
	Ferritin	-0,33	0,02
Procalcitonin	Free triiodothyronine	-0,30	0,04
Ig E	Thyroid stimulating hormone	0,32	0,03
	Albumin	-0,34	0,02
	Ig M	0,32	0,02
Ig G	Sodium	-0,33	0,02
	Total protein	-0,29	0,04

Total cholesterol, Glucose and Potassium – in 1.1-1,2 times within the reference values. That is, inflammatory markers are mainly increased compared to the control group against the background of a decrease in the level of total protein. Corresponding data were obtained by scientists too when have considered the laboratory examination of children with Coronavirus infection [8,9]

The immunogram of children with Coronavirus are presented in Table II.

Pathological increases in all Immunoglobulins beyond the reference values were observed in children with Coronavirus, according to the data in the table II. The highest comparative characteristic was the level of Ig G – in 12.3 times (151.07 ± 39.77 to 12.29 ± 0.07 g/l, p< 0.01). The level of Ig E with lower values of increase was observed – in 4.6 times (163.47 ± 43.29 to 35.60 ± 1.07 IU/ml, p< 0.01) and the level of Ig M – in 3.4 times (4.11 ± 1.74 to 1.20 ± 0.06 g/l, p< 0.01).

Consider the obtained results of the study of the homeostasis pituitary-thyroid link, which are presented in Table III.

As we can see by the data in Table III, all indicators are within the reference values and most have an unreliable difference, except of Thyroid stimulating hormone level, which is significantly lower (in 4.7 times) in patients (0.40 ± 0.08 to 1.87 ± 0.46 mIU/ml, p< 0.01).

The levels of inflammatory endocrine regulation markers are presented in table IV.

All obtained data are significantly different in patients and children of the control group (p<0.01), except for Ferritin values (p=0.73). An increase beyond the reference values is observed in the levels of C-reactive protein (8.76 ± 2.16 vs. 2.54 ± 0.53 mg/l, p< 0.01), the comparative ratio is 3.1 times and C-peptide (4.65 ± 1.67 to 1.23 ± 0.08 ng/ml, p< 0.01), which is increased in patients in 3.8 times.

Also, reliable correlation relationships between the studied parameters were found.

The correlations of the parameters of the pituitary-thyroid panel are shown in Table V.

The negative correlationships of T3 with Procalcitonin (r=-0.30) and Creatinine (r=-34) are observed, according to Table V. T4 values correlated with Total cholesterol (r=-0.65) and Creatinine (r=0.29). TSH presents positive correlationship with Ig E (r=0.32) and TPA level with Albumin (r=0.28). It should be noted about the highest negative correlation between T4 and Total cholesterol. As reported in viral infections, especially in immunocompromised patients, there is a two-way relationship between the Immune system and Thyroid hormones in both physiological and pathophysiological conditions. The Immune system and the Endocrine system interact with each other. Specifically involved in the relationship between inflammation and THS-related diseases, they can be considered not only as biomarkers but also as potential therapeutic targets [10].

The revealed correlations of indicators of immune-inflammatory-endocrine regulation are presented in Table VI.

According to the table VI, the most correlationship were found in the values of IgE with Thyroid Stimulating hormone(r=0.32), Ig M(r=-0.32) and Albumin(r=-0.34) and CRP with Direct bilirubin(r=0.38), Albumin(r=-0.31), Ferritin(r=-0.33). Leptin indicator presents positive relationships with inflammatory markers, in particular C-peptide (r=0.39) and Alanine Aminotransferase (r=0.48). C-peptide, formed as a result of proinsulin proteolysis, is not only an Insulin Chaperone in β-cells, but also a signaling molecule that regulates many physiological and biochemical processes through specific C-peptide receptors. The regulatory effects of

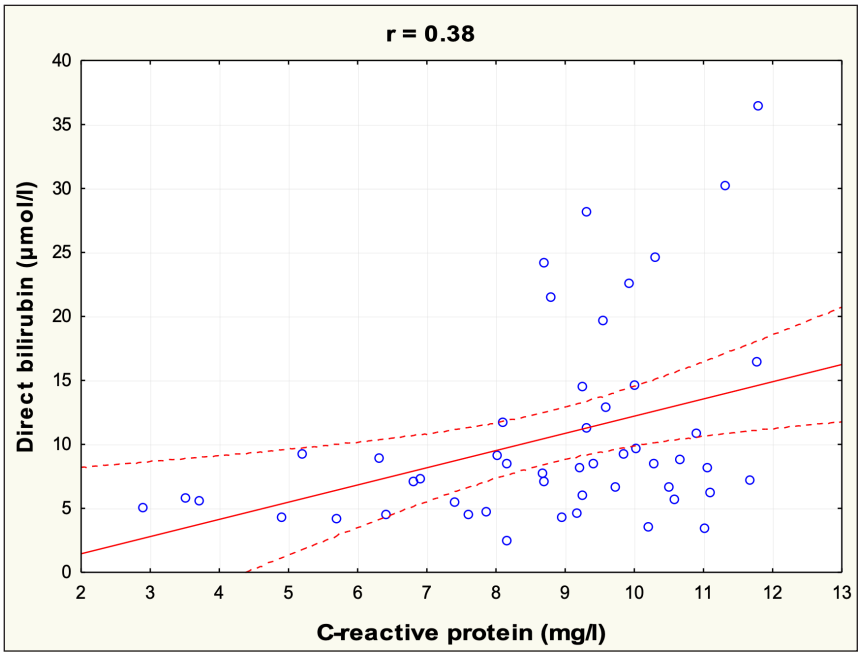


Fig. 1. Correlationship between CRP and Direct Bilirubin

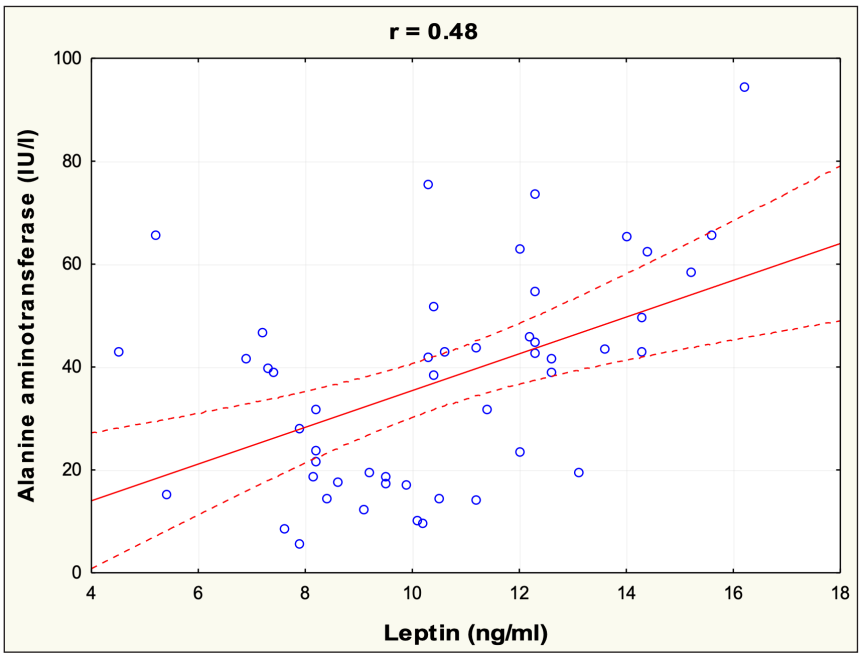


Fig. 2. Correlationship between Leptin and Alanine Aminotransferase

C-peptide are tissue-specific and largely depend on the physiological state of the organism, the concentration of C-peptide and its ability to form complexes. It was established that C-peptide is involved in the regulation of the synthesis and secretion of adipokines, which indicates its role in the control of energy homeostasis [11,12]. In addition to inflammatory diseases, it has been proven that some infectious diseases can be associated with the development of obesity. Mechanisms may include reprogramming of the host's metabolism, exchange of microbiota components, and adaptations of the host's immune and metabolic systems in the presence of chronic viral infection, which causes changes in cytokine and interferon levels that may play a role in the development of obesity. On the other hand, obesity has been found to be an important risk factor for the severity of some viral infections, such as severe acute respiratory syndrome, Coronavirus 2 (SARS-CoV-2). And leptin has also been suggested as a possible link [13-15].

The Procalcitonin (PCT) indicator correlates with the Free Triiodothyronine values (r=-0.30). Because of the low sensitivity of PCT for all outcomes, normal PCT levels should not be used to guide treatment decisions in patients with COVID-19. PCT is mainly used as a biomarker of bacterial infections. The results of the present study show a novel use of PCT in a specific group of

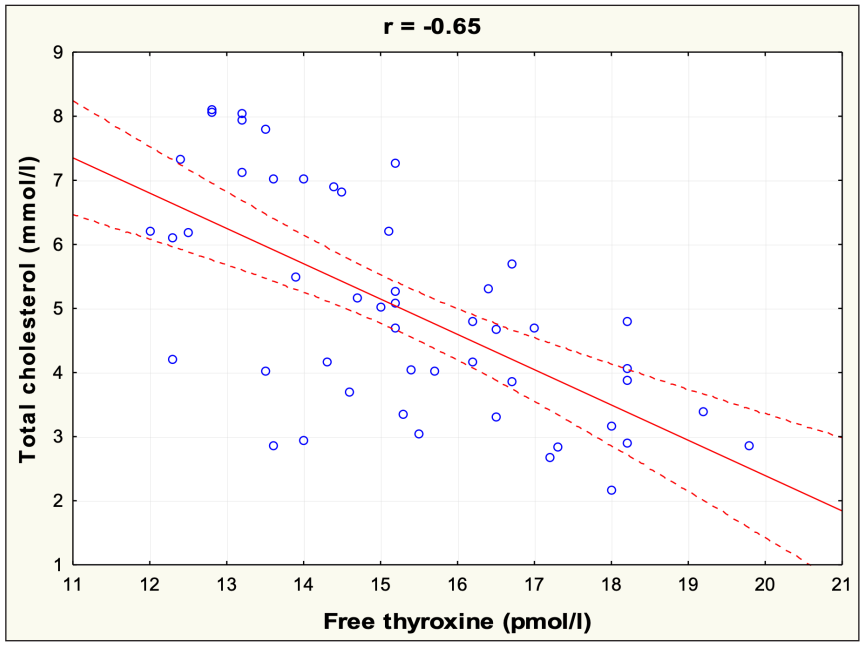


Fig. 3. Corelationship between Free T4 and Total Cholesterol

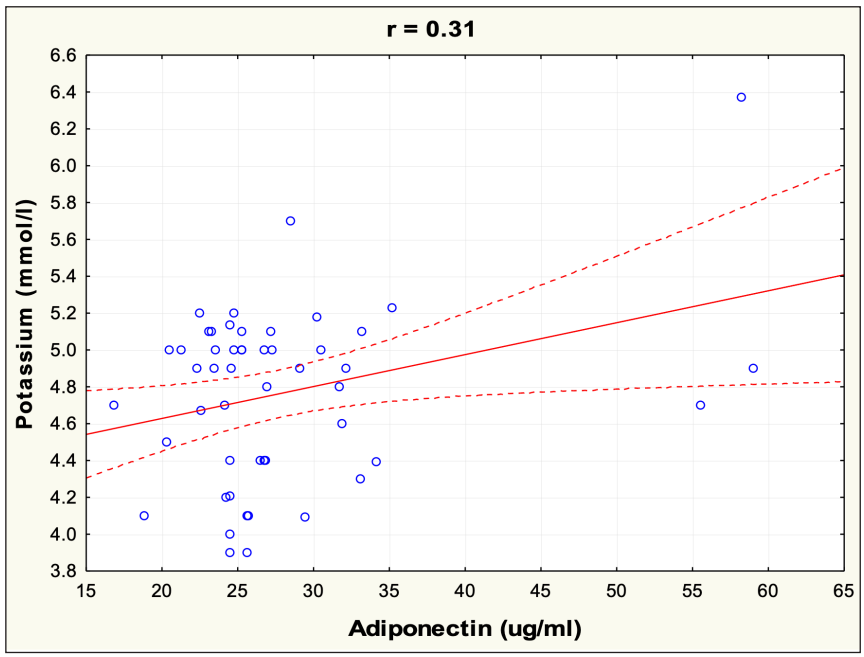


Fig. 4. Corelationship between Adiponectin and Potassium levels

patients with COVID-19 as a marker of disease severity. Even after adjusting for bacterial co-infections and the inflammatory markers CRP and d-dimer, elevated PCT levels remained associated with severe COVID-19 infection. Elevated PCT in severe COVID-19 infections can be explained by looking at the pathways of PCT synthesis, which is regulated by various cytokines such as Interleukin-6 and TNF-α. As hyperinflammation has been shown to be an important factor in the progression of COVID-19 infections, dysregulation of the immune response may also trigger the production of PCT[16]

The most representative correlogram of relationship, in particular the correlation between CRP and Direct Bilirubin is illustrated on Fig. 1.

There is growing evidence that Bilirubin, which is formed during the physiological breakdown of heme, has a powerful anti-inflammatory effect. Evidence suggests that Bilirubin can suppress inflammatory responses by preventing Leukocyte migration to target tissues through disruption of vascular cell adhesion molecule-1 (VCAM-1)-dependent cell signaling. Because VCAM-1 is an important mediator of tissue damage, in vitro experimental studies have shown significantly less tissue damage and reduced infiltration of inflammatory cells into tissues. The scientists' conclusions suggest that Bilirubin functions as an endogenous regulator of inflammatory reactions[17].

The correlogram of the relationship between Leptin and Alanine Transierase is represented on Fig.2.

In addition to the metabolic effect, the range of functional capabilities of the Leptin hormone includes the regulation of inflammatory processes, the effect on the Immune system and the synthesis of Thyroid hormones that regulate metabolism. Therefore, it is reasonable to interact with the inflammatory marker - ALT, the level of which increases due to the pathological destruction of cells and the release of the intracellular enzyme into the bloodstream [14,18]. The pronounced inflammatory process in the child's body was confirmed by the data of our survey and its correlation analysis the following correlogram of the relationship between Free T4 and Total Cholesterol is shown on Fig. 3.

The level of Cholesterol depends on the level of hormones, therefore, when diagnosing Hypothyroidism, it is so important to test Cholesterol and its fractions (HDL and LDL). Thyroxine is produced by the Thyroid gland, which transmute into the active form of Triiodothyronine and have affects on the level of Cholesterol in our organism. This means that the impaired action of both hormones will affect the level of Cholesterol in the same way [10]. In our research, a strong negative relationship between these parameters is observed.

Scientific information is provided by the correlogram about correlations between Adiponectin and Potassium levels and are illustrated on Fig. 4.

Consider the relationship between serum Potassium level - Adiponectin level and the risk of Metabolic syndrome development accordingly. The prevalence of Metabolic syndrome was 51.7% in participants with Hypokalemia and 37.7% in those who were Normokalemic. The level of Potassium in the blood serum significantly decreased with an increase in the number of components of the Metabolic syndrome. Low serum Potassium is significantly associated with the prevalence of Metabolic syndrome in middle-aged and elderly Chinese. That is, according to scientists, there are relationships between the level of potassium and the adipose tissue, hormones Adiponectin and Leptin, which is also proven in our research [19,20].

DISCUSSION

COVID-19 is either rare in children or undiagnosed, because this age group have asymptomatic course of disease. The proportion of children with COVID-19 with elevated inflammatory markers was reported as low. Severe cases of COVID-19 are associated with elevated PCT levels [16]. When patient have infected with COVID-19, the vast majority of homeostasis links were violated.

Activation of virus-specific B-cells leads to their differentiation into plasma cells, which successively produce specific IgM and IgG class antibodies. During the development of COVID-19, it is observed a gradual increase in the concentration of SARS-CoV-2-binding antibodies of IgM and IgG class in serum blood from the 7th to the 20th day of the disease. It has been demonstrated that SARS-CoV-2-specific antibodies of the IgM class disappear at the end of the 12th week from the moment of the onset of the disease, and the IgG class remains for a long period of time, determining the level of protection against re-infection[21]. There is a study that replacement therapy with C-peptide prevents the development of inflammation in the endothelial cells of vessels, and an excess of C-peptide, on the contrary, reveals its pro-inflammatory properties. It was established that C-peptide is involved in the regulation of the synthesis and secretion of Adipokines, which indicates its role in the control of energy homeostasis[11,12]. Clinically, the immune response induced by SARS-CoV-2 infection is biphasic. During the incubation and mild stages, a specific adaptive Immune response is required to eliminate the virus and prevent disease progression to severe stages. To develop an endogenous protective Immune response during the incubation and non-severe stages, the child must have good general health and an appropriate genetic background (eg, HLA) that cases specific antiviral immunity. However, when the protective Immune response is impaired, the virus will spread and massive destruction of affected tissues will occur, especially in organs that have high ACE2 expression, such as the intestine and kidney. Damaged cells induce innate inflammation in the lung, which is largely mediated by proinflammatory macrophages and granulocytes. [3,22]. Initiation of the development of the Inflammatory response of the child's organism, violation of the immune-inflammatory-endocrine regulation system, metabolic processes, requires the constant attention of doctors to identify markers of the pathological process and develop therapeutic treatment schemes.

CONCLUSIONS

1. The level of Direct bilirubin was increased in 3 times (10.55 ± 7.67 vs. 3.63 ± 0.49 μmol/l, p< 0.01) and exceeded the limits of reference values, Alanine aminotransferase - in 1.7 times (37.02 ± 20.53 vs. 21.90 ± 1.82 IU/l, p< 0.01), Alkaline phosphatase – in 2.7 times (141.59 ± 99.44 vs. 52.02 ± 3, 38 IU/l, p<0.01), Creatinine – in 1.5 times (93.82 ± 34.63 to 61.21 ± 5.54 μmol/l, p<0.01), Urea - in 1 .8 times (6.84 ± 3.36 vs. 3.81 ± 0.32 mmol/l, p< 0.01), Total cholesterol, Glucose and Potassium – in 1.1-1.2 times, but within the reference values . That is, inflammatory markers are mainly increased compared to the control group

against the background of a decrease in the level of Total Protein.

2. An increase in Ig G levels was observed – in 12.3 times (151.07 ± 39.77 to 12.29 ± 0.07 g/l, p< 0.01). With lower values of increase, the level of Ig E was observed – in 4.6 times (163.47 ± 43.29 to 35.60 ± 1.07 IU/ml, p< 0.01) and the level of Ig M - 3.4 times (4.11 ± 1.74 to 1.20 ± 0.06 g/l, p< 0.01).
3. All indicators of the Thyroid panel were within the reference values and most had an unreliable difference, except for the level of Thyroid Stimulating hormone, which is significantly lower (by 4.7 times) in patients (0.40 ± 0.08 to 1.87 ± 0 .46 mIU/ml, p< 0.01).
4. An increase beyond reference values was identified in the levels of CRP (8.76 ± 2.16 vs. 2.54 ± 0.53 mg/l, p< 0.01), the comparative ratio is 3.1 times and C -peptide (4.65 ± 1.67 to 1.23 ± 0.08 ng/ml, p< 0.01), which is increased in patients in 3.8 times.
5. Negative correlations of T3 with Procalcitonin (r=-0.30) and Creatinine levels (r=-34) were revealed. T4 values correlate with Total Cholesterol (r=-0.65) and Creatinine levels (r=0.29). TSH presents positive correlations with Ig E levels (r=0.32), Leptin with Alanine aminotransferase (r=0.48) and with C-peptide levels (r=0.39), TPA with Albumin indicator levels (r=0, 28). It should be noted that the highest negative correlationship of T4 with Total cholesterol levels and the highest positive correlation of Leptin with Alanine Aminotransferase levels were releaved

REFERENCES

1. Jonas F. Ludvigsson. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults Acta Pediatrica Nurturing the child. 2020;109(6): 1088-1095. doi:10.1111/apa.15270.
2. Abaturov AE, Agafonova EA, Krivusha EI at al. Pathogenesis of COVID-19. Child`s health. 2021;15(2): 133–144. doi:10.22141/2224-0551.15.2.2020.200598.
3. Vafaeinezhad A, Reza Atashzar M, Baharlou R. The Immune Responses against Coronavirus Infections: Friend or Foe? Int Arch Allergy Immunol. 2021; 182(9): 863–876. doi:10.1159/000516038.
4. Cao Q, Chen Yi-Ch, Chen Ch-L at al. SARS-CoV-2 infection in children: Transmission dynamics and clinical characteristics. J Formos Med Assoc. 2020; 119(3):670-673. doi: 10.1016/j.jfma.2020.02.009.
5. Chen Y, Guo Y, Pan Y at al. Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Commun. 2020;525(1):135-140. doi: 10.1016/j.bbrc.2020.02.071.
6. Wei-jie Guan, Zheng-yi Ni, Yu Hu at al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020; 382:1708-1720. doi: 10.1056/NEJMoa2002032.
7. Guo YR, Cao QD, Hong ZS at al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res. 2020;7(1):11. doi:10.1186/s40779-020-00240-0.
8. Jin Y, Yang H, Ji W at al. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses. 2020;12(4):372. doi:10.3390/v12040372.
9. Thevarajan I, Nguyen THO, Koutsakos M at al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med . 2020; 26:453–455. doi:10.1038/s41591-020-0819-2.
10. De Luca R, Davis PJ, Lin HY at al. Thyroid Hormones Interaction With Immune Response, Inflammation and Non-thyroidal Illness Syndrome. Front Cell Dev Biol. 2021;8:614030. doi: 10.3389/fcell.2020.614030.
11. Shpakov AO. Mechanisms of action and therapeutic potential of proinsulin C-peptide. J Evol Biochem Phys. 2017;53:180–190. doi:10.1134/S0022093017030024.
12. Lewandowski KC, Biesiada L, Grzesiak M at al. C-Peptide and leptin system in dichorionic, small and appropriate for gestational age twins—possible link to metabolic programming?. Nutr. Diabetes. 2020;10(1):29. doi:10.1038/s41387-020-00131-2.
13. Jameson DV, Nikhil VD. Viral Infections and Obesity Curr Obes Rep. 2017; 6(1):28-37. doi: 10.1007/s13679-017-0251-1.
14. Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T at al. Role of Leptin in Inflammation and Vice Versa International Journal of Molecular Sciencesст 1-24. 2020;21(16):5887. doi: 10.3390/ijms21165887.
15. Rebello CJ, Kirwan JP, Greenway FL. Obesity, the most common comorbidity in SARS-CoV-2: Is leptin the link? Int J Obes (Lond). 2020;44(9):1810-1817. doi: 10.1038/s41366-020-0640-5.
16. Tong-Minh K, van der Does Yu, Engelen S at al. High procalcitonin levels associated with increased intensive care unit admission and mortality in patients with a COVID-19 infection in the emergency department. BMC Infect Dis. 2022;22(1):165. doi:10.1186/s12879-022-07144-5.
17. Vogel ME, Zucker SD. Bilirubin acts as an endogenous regulator of inflammation by disrupting adhesion molecule-mediated leukocyte migration. Inflamm Cell Signal. 2016;3(1):1178. doi: 10.14800/ics.1178.
18. Shi Yu, Wang Yi, Shao Ch at al. COVID-19 infection: the perspectives on immune responses. Nature. Cell Death & Differentiation. 2020;27(5):1451-1454. doi:10.1038/s41418-020-0530-3.

19. Sun K, Su T, Li M at al .Serum potassium level is associated with metabolic syndrome: A population-based study. Clinical Nutrition. 2014;33(3): 521-527. doi:10.1016/j.clnu.2013.07.010.
20. Lee Yo-S, Lee S-Ye. The Relationship between Dietary Sodium-to-Potassium Ratio and Metabolic Syndrome in Korean Adults: Using Data from the Korean National Health and Nutrition Examination Survey 2013-2015. Korean Journal of Health Promotion. 2020;33(3): 521-527. doi:10.1016/j.clnu.2013.07.010.
21. Thevarajan I, Nguyen TH, Koutsakos M et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. Nat Med. 2020;26(4):453-455. doi: 10.1038/s41591-020-0819-2.
22. Rothe C, Schunk M, Sothmann P at all.Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. The New England journal of medicine. 2020; 382(10):970-971. doi: 10.1056/NEJMc2001468.

ORCID and contributionship:
Olesya M. Horlenko: 0000-0002-2210-5503 [A,D,F]
Khrystyna A. Hechko: 0000-0003-4989-7659 [B,D]
Lyubomyra B. Prylypko: 0000-0002-4131-5450 [A,C]
Olga S. Blaga: 0000-0002-5627-1403 [B,D]
Fedir V. Horlenko: 0000-0002-0496-2069 [D,E]
Oleksandr O. Kutsyn: 0000-0001-7902-4598 [D,E]
Bohdan M. Halay: 0000-0002-7566-4982 [B,E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olesya M. Horlenko
Uzhhorod National University
46 Pidhirna st., 88000 Uzhhorod, Ukraine
tel: +38055269658
e-mail: ohorlenko@gmail.com

Received: 10.05.2023
Accepted: 22.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

S-AMLODIPINE AS A MODERN EFFECTIVE ANTIHYPERTENSIVE AND AN ANTIANGINAL AGENT

DOI: 10.36740/WLek202311114

Ivan V. Chopei, Ksenia I. Chubirko, Mykhailo V. Bychko, Mykhaylo M. Hechko, Antonina V. Kedyk, Ivan I. Kogutych
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: Study of the clinical and hemodynamic effects of S-amlodipine in patients with arterial hypertension associated with coronary artery disease, in individuals with preserved LV systolic function.

Materials and methods: The study includes 51 patients with arterial hypertension associated with coronary artery disease, who were treated with S-amlodipine.

Results: This study shows the high clinical effectiveness of the use of S-amlodipine in patients with arterial hypertension associated with coronary artery disease. We reveal that treatment of hypertensive patients with coronary artery disease with S-amlodipine leads to improvement of LV diastolic dysfunction, bringing it closer to normal values.

Conclusions: Clinical effectiveness was associated with positive changes in hemodynamics, and was expressed in the normalization of the left ventricle diastolic function parameters, about which indirectly indicates decreasing of end-diastolic pressure.

KEY WORDS: S-amlodipine, arterial hypertension, hypertrophy, angina pectoris, threshold physical load, central hemodynamics, diastolic dysfunction

Wiad Lek. 2023;76(11):2429-2434

INTRODUCTION

Progress in basic sciences have made possible to study the mechanisms of coronary blood circulation at the molecular and cellular level. The study of ion channels, in particular those that ensure the penetration of calcium ions into the cell, made it possible to create a fundamentally new drugs group – calcium channel blockers (CCB) [1]. The first clinically used drug was verapamil (1962). Later, in 1967, Fleckenstein announced the discovery of a fundamentally new drug group – CCB (he separated them from b-adrenoblockers). Since then, in different periods, the attitude towards CCB has changed [1].

In recent years, CCB has again found its wide use, especially after cardiosurgical interventions (coronary artery bypass grafting, stenting, balloon angioplasty) [2-4]. One of the reasons for the growing popularity of CCB is metabolic neutrality and improvement of diastolic function of the left ventricle (LV) in hypertensive patients and patients with coronary disease.

Data from multicenter studies indicate the special effectiveness of dihydropyridine CCB group for reducing the risk of strokes [1,5].

Such a representative of the dihydropyridine series of the III generation as amlodipine (known as «norvask», «normodipine», «Amlo») is particularly popular among CCBs [2,3,5,6]. It is characterized by high expected efficiency (60-80% bioavailability) and stability of plasma concentration (24-36 hours), which means that is unnecessary to create retarded forms [1-3,5].

The antihypertensive effectiveness of amlodipine therapy in mild and moderate hypertension is similar to that of other basic antihypertensive drugs, sometimes even better and can reach 60-70%. On the background of therapy, there is an improvement in the daily profile of blood pressure (BP); with long-term use (>4-6 months) develops regression of LV hypertrophy. The reduction of cerebral strokes risk in people with hypertension is one of the important effects of the drug, confirmed in a number of large-scale studies,. Amlodipine combines well with all groups of drugs. In coronary artery disease (CAD), amlodipine is used in patients with stable and vasospastic angina. In patients with CAD, the drug has a coronary dilating effect, which increases the blood supply to the myocardium and reduces the total peripheral vascular resistance. In addition, amlodipine has an antiatherosclerotic effect, which was convincingly confirmed in the PREVENT study (2000) [1,2]. The clinical effects of this drug in CAD include a reduction in the number and duration of

anginal episodes and episodes of painless myocardial ischemia; at the same time, the antianginal effect is more pronounced compared to a number of other CCBs (CAPE-II study, 2002) [1].

The most common side effect of amlodipine in clinical practice is peripheral edema. There are present data that this complication may occur in more than 8% of patients receiving amlodipine [5,7]. The solution to this issue was the use of the levorotatory isomer of S-amlodipine. It is known that optical isomerism is characteristic of almost all molecules in the body. Most proteins consist of levorotatory amino acids. It is believed that drugs based on levorotatory molecules are safer and more effective when used. Most of the drugs used are a mixture of dextrorotatory and levorotatory isomers (R+S). At the same time, the active substance is the levorotatory S-isomer [7-10].

Taking into account the available literature data, we conducted our own research.

THE AIM

Study of the clinical and hemodynamic effects of S-amlodipine in patients with arterial hypertension associated with coronary artery disease, in individuals with preserved LV systolic function.

MATERIALS AND METHODS

The study includes 51 patients with arterial hypertension associated with coronary artery disease, who were treated with S-amlodipine. Among them: 27 patients - with first stage hypertension, 24 - with second stage hypertension. It was noted angina pectoris II functional class (FC) in 25 patients, and angina pectoris III FC in 26 patients; there were 25 (49%) women and 26 (51%) men aged from 34 to 73 years (on average 64.1±4.2 years). Exclusion criteria were: acute coronary syndromes, symptomatic hypertension, history of myocardial infarction, stenting or coronary bypass surgery, diabetes mellitus. As a baseline, all patients received standard therapy with enalapril 20 mg per day, bisoprolol 2,5 or 5 mg per day, rosuvastatin 10 mg per day, and acetylsalicylic acid 100 mg per day. Doses of drugs did not change during two months before inclusion in the study.

Clinical effectiveness was assessed by lowering BP to target levels and reducing the number of angina attacks by 30% or more (positive antianginal effect). A positive ergometric effect was considered as an increase in power (W) of the threshold load by one step (25 W). Hemodynamic indicators were studied by echocardiography. LV hypertrophy was ascertained according to the recommendations of A. Canau et al. [10]. To analyze the structural and functional state of the heart, the following indicators were studied: the anterior-posterior size of the left ventricle, left ventricle parameters like end-systolic dimension (ESD), end-diastolic dimension (EDD), interventricular septum (IVS) and posterior wall thickness, were calculated end-systolic volume (ESV), end-diastolic volume (EDV), ejection fraction (EF), myocardial mass index of left ventricle (MMILV). Myocardial mass was calculated according to the formula of R.B. Devereux 1995. MMILV was calculated as the ratio of MMLV to body area (S), which was determined according to the Dubois table. There were distinguished three types of LV geometry: normal geometry – MMILV<125 g/m^2, relative wall thickness (RVS) <0.45; eccentric LV hypertrophy: MMILV >125 g/m^2, RVS <0.45; concentric LV hypertrophy: MMI LV >125 g/m^2, RVS >0.45. The control group consisted of 20 healthy people.

Diastolic heart function was studied by Doppler echocardiography. The following parameters were determined: isovolumic relaxation time (IVRT), maximum speed of early diastolic filling (E), deceleration time of early diastolic filling (DT), maximum speed of late diastolic filling (A) and ratio E/A.

After stabilization of hemodynamic indicators (with enalapril and bisoprolol), treatment with S-amlodipine was prescribed for ten weeks. In the absence of a decrease in blood pressure to the target levels of systolic and diastolic blood pressure, the dose of the drug was increased from 2.5 mg to 5 mg. The studied indicators were determined before the appointment of S-amlodipine and after 10 weeks of taking the drug.

RESULTS

The average systolic blood pressure (SBP) and diastolic blood pressure (DBP) in patients from general population before the start of treatment was 162/100 mm Hg. In group patients for which S-amlodipine in dose 2.5 mg was prescribed (n=30) after 10 weeks of treatment BP decreased to 128/83 mm Hg. The decreasing of SBP and DBP was considered statistically significant (p <0.005). The mean SBP and DBP in the group treated with S-amlodipine in dose 5 mg (n=21) decreased to 126/ 81 mm Hg (p <0.005).

In general group, treatment of patients for 10 weeks led to decrease in the number of angina attacks (the need for nitroglycerin (NG) decreased more than twice per week), to increase the power of the threshold load (W), to decrease the threshold demand of oxygen to myocardium (TDO) without significantly affecting on the contractility of the myocardium (see Table I).

The criteria for terminating the test with dosed physical activity were also not significantly different. We also did not notice any side effects from S-amlodipine in

Table I. Clinical and instrumental indicators of the effectiveness of treatment patients with AH and CAD by S-amlodipine (M±m)

Stages of research	Indicators of treatment effectiveness				
	Number NG tablets in a week	Power (W)	Threshold demand of oxygen (TDO) at rest, conditional units	TL (threshold load), conditional units	EF, %
Hypertensive patients with coronary artery disease before treatment	28,7±2,3	81,9±3,0	87,1±6,2	177±5,8	59,2±2,4
Hypertensive patients with coronary artery disease after treatment	12,9±25	123,7±2,8	88,0±2,9	149±4,5	58,6±3,7
P	<0,01	<0,01	>0,05	>0,05	>0,05

Table II. Indicators of the effectiveness of treatment of patients with arterial hypertension and concomitant coronary artery disease depending on the severity of coronary artery disease

Hypertensive patients with coronary artery disease	Number patients (n=51)	Threshold load power, W (Вт)		P
		Before treatment	After treatment	
Angina pectoris II FC	25	100,2±4,1	149,0±3,8	<0,01
Angina pectoris III FC	26	49,4±2,3	74,5±2,8	<0,05

Table III. Clinical and instrumental parameters of the effectiveness of patients treatment with S-amlodipine depending on the type of LV hypertrophy (M±m)

Indicators of treatment effectiveness	Patients with concentric type of LV hypertrophy, n=25			Patients with eccentric type of LV hypertrophy, n=22		
	Before treatment	After treatment	P	Before treatment	After treatment	P
Number of nitroglycerine tablets in a week	26,7±1,5	11,9±2,0	<0,01	29,4±3,1	15,7±1,8	<0,01
Power (W)	79,7±2,0	134±4,1	<0,01	82,1±3,6	119±3,1	<0,01
Threshold demand of oxygen (TDO) at rest, conditional units	87,0±2,1	86,8±1,5	>0,05	87,3±2,2	88,9±1,5	>0,05
Ejection fraction (EF), %	59,0±1,4	58,8±1,8	>0,05	58,9±1,5	58,7±1,9	>0,05

Table IV. Indicators of central and intracardiac hemodynamics in patients with hypertension associated with CAD under the influenceof S-amlodipine treatment (M±m)

Hemodynamic indicators	Patients with hypertension associated with coronary artery disease		
	Before treatment	After treatment	P
Heart rate, bpm	71,9±2,5	72,1±3,1	>0,05
BP, mmHg	161,2±5,0	127,4±3,8	<0,02
Threshold demand of oxygen (TDO) at rest, conditional units	90,1±3,7	88,1±2,9	>0,05
EDV, cm^3	145,7±3,4	141,8±3,7	>0,05
ESV, cm^3	74,8±3,1	71,2±2,8	>0,05
EF, %	59,2±2,4	59,1±3,0	>0,05

patients with arterial hypertension in combination with CAD. Blood pressure decreased to target levels in 87% of patients.

The initial functional condition of myocardium, that is, the functional class of angina pectoris, is important for the effectiveness of treatment. Table II shows data on the effectiveness of course treatment of patients with S-amlodipine depending on the severity of the disease (presence of concomitant CAD).

Treatment with S-amlodipine significantly increases the level of threshold load power (W) both in patients with hypertension and II FC of angina as in patients with III FC.

LV hypertrophy was noted in the majority of patients with hypertension and coronary heart disease (in 47 of 51 patients). Only four patients with hypertension combined with coronary artery disease did not have LV hypertrophy. This phenomenon can be explained by the increased inotropic properties of the myocardium and the relatively short-term history of hypertension in these patients.

Table III presents the results of the study of the clinical effectiveness of S-amlodipine in patients with AH and CAD, depending on the type of LV hypertrophy.

The frequency of concentric and eccentric types of LV hypertrophy was approximately the same (25 versus 22

patients). According to literature data, in patients with hypertension, this ratio is 4:1 [11,12]. The addition of ischemic heart disease to hypertension leads to increase in the frequency of eccentric LV hypertrophy, as well as to increase the risk of developing LV dilatation - an increase in EDV (LV dilatation index) [11,12].

The analysis of Table III shows the positive dynamics of indicators. Irrespective of the type of LV hypertrophy, in both groups, the clinical efficacy of S-amlodipine was high, which was expressed in a decrease of using of nitroglycerine per week and an increase in the level of threshold exercise power (W). The criteria for terminating the test with dosed physical activity were not significantly different.

Performance of higher threshold loads by patients with AH associated with CAD, after treatment with amlodipine, occured on the background of minor changes in the parameters of central hemodynamics (see Table IV).

We note a positive trend of LV volumetric indicators (EDV, ESV) on the background of practically unchanged EF and threshold demand of oxygen. The pronounced antihypertensive effect was observed on the background of unchanged heart rate (without activation of the sympatho-adrenal system).

It is impossible to explain the high clinical effectiveness of amlodipine in patients with AH and CAD, based on the data of changes in the specified hemodynamic parameters.

The study of the hemodynamic structure of diastole made it possible to establish in the examined patients hypertrophic type of diastolic dysfunction (in 27 persons) and «pseudonormal» type (in 24 persons).

In patients with a hypertrophic type of diastolic dysfunction was found a lengthening of IVRT (82.3±1.7 relative to 68.3±1.3 ms, P<0.05) and decreasing of late diastolic filling speed (A; 64.2±1.5 relative to 43,0±1.6 cm/sec, P<0.05). At the same time, the speed of early diastolic filling (E; 60.2±0.9 relative to 70.5±0.9 cm/sec, P<0.05) and the E/A ratio (0.96±0.6 relative to 1,65±0.04 conditional units, P<0.05) increased. Unreliable increasing in DT was noted (190.0±8.2 relative to 181±9.7 ms, P>0.05).

With the «pseudo-normal» type of filling of the left ventricle, the following changes were noted: IVRT increased (62.4±2.5 relative to 66.7±1.8 ms, P<0.05) and DT increased (171.4±8.2 relative to 182,4±10.7 ms, P>0.05) and the E indicator decreased (78.7±1.9 relative to 71.2±1.8 cm/sec, P<0.05). The E/A ratio approached normal values (1.59±0.05 relative to 1.65±0.04 conventional units, P>0.05). This orients us to increase in end-diastolic pressure in the left parts of the heart (left atrial and LV).

During treatment with S-amlodipine for 10 weeks, a decrease in systolic and diastolic blood pressure was noted. Average daily SBP decreased by 36.5±2.20 mm Hg, or by 20.1±0.8% from the initial level (P<0.05), DBP – by 26.2±0.86 mm Hg , or by 18.1±0.8% (P<0.05). At the same time, heart rate did not increase.

S-amlodipine therapy in patients with hypertrophic type of diastolic dysfunction significantly reduced IVRT (82.3±1.7 to 69.0±1.9 ms, P<0.05), not significantly – DT (from 190.0±8.2 to 175.4±8.9 ms, P>0.05) and late diastolic filling speed (A; from 64.2±1.5 to 59.3±1.6 cm/sec, P>0.05). Under the influence of amlodipine treatment, the speed of early diastolic filling significantly increased (E; from 60.2±0.9 to 71.4±1.2 cm/sec, P<0.05), and as a result E/A value also increased (from 0.96±0.06 to 1.28±0.04 conventional units, P<0.05). All this indicates hemodynamic unloading of the LV due to a reduction of preload, first of all, – decreasing in pressure in the left heart.

The following hemodynamic effects of S-amlodipine were noted in patients with the «pseudonormal» type of diastolic dysfunction: rasing of IVRT (from 62.4±2.5 to 84.5±1.7 ms, P<0.05), lowering of E (from 78.7±1.9 to 67.2±1.6 cm/sec, P<0.05) and E/A ratio (from 1.59±0.05 to 1.24±0.06 conventional units, P<0.05). There was revealed a tendency to increase the speed of late diastolic filling (A; from 50.1±1.5 to 56.4±2.7 cm/sec.; P>0.05) and DT (from 171.4±8.2 to 182,0±8.1 ms, P>0.05). These changes were considered as positive, i.e. hemodynamic indicators approached the hypertrophic type of diastolic dysfunction.

Summarizing the above, we reveal that treatment of hypertensive patients with coronary heart disease with S-amlodipine leads to improvement of LV diastolic dysfunction, bringing it closer to normal values.

DISCUSSION

The literature describes, mainly, data relating to the improvement of LV systolic function in patients with AH and CAD under the influence of amlodipine therapy. In our study we found the superiority in the mechanism of therapeutic action of S-amlodipine in hypertensive patients, evaluating systolic function by volumetric indicators, and diastolic function by transmitral blood flow [1-3,8,11,12].

We note the safety of treatment with S-amlodipine, which indicates the rare frequency of side effects. Similar conclusions are given in the literature.

Thus, the SESA study [13] was conducted to estimate the effectiveness and tolerability of S-amlodipine (2.5/5 mg) - pure levorotatory amlodipine in the treatment of patients with AH. The study showed that S-amlodipine in doses of 2.5/5 mg is an effective drug for the treatment of hypertension, well tolerated, and can also be an ideal replacement therapy for patients with peripheral edema when using racemic amlodipine.

Despite the fact that the study was published back in 2002, and the recommendations for the treatment of

hypertension have been revised four times since then, these data were not reflected in relation to S-amlodipine.

There is an opinion that the presence of two isomers provides the therapeutic properties of amlodipine, which have brought the drug to the forefront in the treatment and prevention of major cardiovascular events.

Analyzing the data of the latest epoch-making large-scale multicenter studies and taking into account the results of our study, it is possible to assert with high probability the positive effects of the use of the levorotatory isomer of amlodipine (S-amlodipine) [7-10,14].

But the use of monotherapy, as a rule, cannot control all pathogenetic mechanisms of BP increasing: activity of the sympathetic nervous system, renin-angiotensin-aldosteron system, volume-dependent and other secondary mechanisms. Today, necessity of use combined antihypertensive drugs is obvious, especially in the treatment of high-risk patients.

The most effective combination of hypotensive drugs, based on the modern research base (ACCOMPLISH, ADVANSE, HYYET, ASCOT, ONTARGET), is ACE inhibitors + CCB, angiotensin II receptor antagonists + CCB, ACE inhibitor + thiazide diuretic or angiotensin II receptor antagonists (ARA) + thiazide diuretic.

Among modern combinations of antihypertensive drugs, the combination of CCB and ACE inhibitors receives special attention. This combination, according to the data of two large-scale international studies ASCOT and ACCOMPLISH, allows to further reduce the risk of major cardiovascular events.

Given that CCBs (including levorotatory isomers) are almost ideal drugs for combined therapy, which can be used in combination with ACE inhibitors, ARA, diuretics, ready-made combined drugs, which include amlodipine, appeared on the pharmacological market [14-16]. These combined drugs have proven themselves well in the treatment of arterial hypertension with diabetes, chronic kidney diseases, coronary heart disease (angina pectoris, vasospastic angina). Therapy with these combined medicines is highly effective, convenient for patients (one tablet with two active substances), safe and relatively unexpensive.

CONCLUSIONS

1. S-amlodipine is characterized by high clinical efficacy and safety, which makes it the drug of choice in the treatment of low-renin arterial hypertension in combination with coronary heart disease.
2. The high clinical effectiveness of S-amlodipine is due to positive changes in hemodynamics, which are expressed in the normalization of indicators of LV diastolic function. These changes are represented in a decrease of preload, and also indirectly indicate about the reduce of LV end-diastolic pressure.
3. The use of combined drugs therapy of amlodipine and ACE inhibitors or amlodipine and ARA in patients with hypertension and CAD significantly reduces the risk of cardiovascular complications.

REFERENCES

1. Amosova KM. Porivnialna otsinka antyanhinalnoi efektyvnosti ta perenosymosti livoobertaiuchoho izomeru amlodypinu i oryhinalnoho amlodypinu u khvorykh na esentsialnu arterialnu hipertenziiu. [Comparative assessment of the antianginal efficacy and tolerability of the levorotatory isomer of amlodipine and the original amlodipine in patients with essential arterial hypertension]. Spetsializovanyy medychnyy portal. 2015. https://health-ua.com/article/15521-porvnyalna-otcnka-antigpertenzivno-efektivnost-ta-perenosimost-lvoobertayuc [date access 20.05.2023] (In Ukrainian).
2. Bychko MV. Antahonisty kaltsiiu v likuvanni khvorykh na arterialnu hipertenziiu asotsiovanu iz ishemichnoiu khvoroboiu sertsia [Calcium antagonists in the treatment of patients with arterial hypertension associated with coronary heart disease]. Uzhhorod: Lira. 2019, p. 248. https://dspace.uzhnu.edu.ua/jspui/handle/lib/47306 [date access 20.05.2023] (In Ukrainian).
3. Sen S, Demir M, Yigit Z, Üresin AY. Efficacy and safety of S-amlodipine 2.5 and 5 mg/d in hypertensive patients who were treatment-naive or previously received antihypertensive monotherapy. J Cardiovasc Pharmacol Ther. 2018;23(4):318-328. doi: 10.1177/1074248418769054.
4. Dalal J, Mohan JC, Iyengar SS et al. S-Amlodipine: an isomer with difference-time to shift from racemic amlodipine. Int J Hypertens. 2018; 2918:8681792. doi: 10.1155/2018/8681792.
5. Lutai MI, Lysenko HF. Efektyvnist preparatu amlodypinu (stamlo) u patsiientiv z ishemichnoiu khvoroboiu sertsia v poiednanni z arterialnoiu hipertenziieiu [The effectiveness of the drug amlodipine (stamlo) in patients with coronary heart disease in combination with arterial hypertension]. Ukr. kardiol. zhurn. 2001; 2: 87-99. (In Ukrainian).
6. Chen Q, Huang Q, Kang Y et al. Efficacy and tolerability of initial high vs low doses of S-(-)-amlodipine in hypertension. The Journal of Clinical Hypertension. 2017; 19(10): 973–982. doi: 10.1111/jch.13022.
7. Sica DA. Pharmacotherapy review: calcium channel blockers. J Clin Hypertens. 2006; 8(1):53-56. doi: 10.1111/j.1524-6175.2005.04140.x.
8. Netiazhenko VZ., Bychko MV. Klinichna efektyvnist amlodypina u khvorykh arterialnoiu hipertenziieiu v poiednanni z ishemichnoiu khvoroboiu sertsia [Clinical effectiveness of amlodipine in patients with arterial hypertension in combination with coronary heart disease]. Naukovyi visnyk UzhNU. Seriia „Medytsyna". 2003; 21: 126–130. (In Ukrainian).

9. Maksimova MA, Lukina YV, and Martsevich SY. The role of S-amlodipine in arterial hypertension therapy with combination of calcium channel blockers and beta-blockers. Ration Pharmacother Cardiol. 2013; 9: 236–240. doi:10.20996/1819-6446-2013-9-3-236-240.
10. Canau A, Devereux RB, Roman MJ. Patterns of left ventricular hypertrophy in essential hypertension. J. Amer. Coll. Cardiology. 1992; 12:1550-1558. doi:10.1016/0735-1097(92)90617-v.
11. Mohan JC, Iyengar SS, Hiremath J et al. "S-Amlodipine: An Isomer with Difference – Time to Shift from Racemic Amlodipine". International Journal of Hypertension. 2018. doi: 10.1155/2018/8681792.
12. Netiazhenko VZ, Bychko MV. Sposib otsinky efektyvnosti likuvannia khvorykh na ishemichnu khvorobu sertsia z stenokardiieiu napruzhennia ta arterialnoiu hipertenziieiu amlodypinom [The method of evaluating the effectiveness of treatment of patients with coronary heart disease with angina pectoris and arterial hypertension with amlodipine] Patent na korysnu model № 30491 vid 25.02.2008p, vlasnyk patentu Natsionalnyi medychnyi universytet im.O.O.Bohomoltsia. https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=96367 [date access 20.05.2023] (In Ukrainian).
13. Liu F, Qiu M, Zhai SD. Tolerability and effectiveness of (S)-amlodipine compared with racemic amlodipine in hypertension: a systematic review and meta-analysis. Curr Ther Res Clin Exp. 2010; 71(1):1-29. doi: 10.1016/j.curtheres.2010.02.005.
14. Park CG, Ahn TH, Cho EJ et al. Comparison of the Efficacy and Safety of Fixed-dose S-Amlodipine/Telmisartan and Telmisartan in Hypertensive Patients Inadequately Controlled with Telmisartan: A Randomized, Double-blind, Multicenter Study. Clinical Therapeutics. 2016; 38 (10): 2185–2194. doi:10.1016/j.clinthera.2016.09.006.
15. Bychko MV, Bychka YaM. Sposib otsinky efektyvnosti likuvannia benidypinom khvorykh na ishemichnu khvorobu sertsia z stenokardiieiu ta arterialnoiu hipertenziieiu z porushenniam diastolichnoi funktsiieiu livoho shlunochka po «hipertrofichnomu» typu [The method of evaluating the effectiveness of treatment with benidipine in patients with coronary heart disease with angina pectoris and arterial hypertension with impaired diastolic function of the left ventricle of the "hypertrophic" type]. Patent na korysnu model №59603 vid 25.05.2011r, vlasnyk patentu UzhNU. https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=159007 [date access 20.05.2023] (In Ukrainian).
16. Kim DK, Ahn JH, Lee KH, et al. Ambulatory blood pressure response to S-amlodipine in Korean adult patients with uncontrolled essential hypertension: A prospective, observational study. J Clin Hypertens. 2022;24(3):350–357. doi: 10.1111/jch.14442.

ORCID and contributionship:
Ivan V. Chopei: 0000-0003-4626-0855 [A]
Ksenia I. Chubirko: 0000-0002-4379-0538 [E]
Mykhailo V. Bychko: 0009-0003-7880-6581 [A, B]
Mykhaylo M. Hechko: 0000-0003-2793-5044 [C]
Antonina V. Kedyk: 0000-0002-7021-9644 [B, D]
Ivan I. Kogutych: 0000-0002-3064-8292 [F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Antonina V. Kedyk
Uzhhorod National University
15A Timiryazeva st, 88000 Uzhhorod, Ukraine
tel: +380968844247
e-mail: kedyk.tonja@gmail.com.

Received: 22.05.2023
Accepted: 28.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

THE STATE OF MOTIVATION TO STUDY AND PROFESSIONAL DEVELOPMENT AMONG MEDICAL STUDENTS OF THE MEDICAL FACULTY OF THE STATE INSTITUTION "UZHHOROD NATIONAL UNIVERSITY"

DOI: 10.36740/WLek202311115

Angelika O. Keretsman
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To determine the level of motivation for learning and professional development among 6th-year students of the medical faculty.

Materials and methods: Included a comparative analysis of contemporary scientific psychological-pedagogical works on this issue and questionnaire surveys. The research design involved the use of psychological diagnostic testing methods by A.O. Rean, V.O. Yakunin, and K. Zamfir in the modification by A.O. Rean.

Results: When studying the levels of motivation for acquiring knowledge in students from the two investigated groups, differences were found in the interpretation and perception of motivation content. For the first group, the main motivation is the possibility of becoming a highly qualified specialist, while for the second group, it is to ensure the success of their future profession. However, both groups of students have high levels of internal motivation. The main reason for the difference in attitudes toward the learning process is the different motivational complexes in students, which determine a different hierarchy of motives in the studied groups in achieving the goal of becoming a doctor, albeit through different means (due to a greater influence of internal negative motivation, especially in the second group).

Conclusions: The main motivation for students of the first group to study is the opportunity to become a highly qualified specialist, while for students of the second group, it is to ensure the success of their future professional activity, primarily through high levels of internal motivation. The effectiveness of the educational process and the future development as a doctor are determined by the type of their motivational complex. Taking this into account can serve as the main reserve of motivation for students and be useful in developing measures to attract and retain medical personnel.

KEY WORDS: motivation, learning, professional development, personnel

Wiad Lek. 2023;76(11):2435-2441

INTRODUCTION

The modern system of higher medical education is developing and functioning in new political and socio-economic conditions that determine not only the ways and directions of its development, but also put forward new requirements for students and create new challenges in the process of their educational activities [1, 2]. Because of this, the requirements for the training of future doctors have undergone structural changes. In particular, in 2022, higher medical institutions of Ukraine developed new editions of educational programmes with an updated list of competencies, programme learning outcomes and educational components in accordance with the standard of higher education in the specialty 222 Medicine, approved by the order of the Ministry of Education and Science of Ukraine dated 08.11.2021 No. 1197 [3]. The need for such changes is also due to the implementation of medical reform in Ukraine, the impact of the COVID-19 pandemic and the consequences of the ongoing war. At the same time, higher medical education as a leading channel for transferring scientific knowledge to future doctors is currently under strong pressure from society, which is putting forward increasing demands for its improvement, improving the quality of knowledge, ensuring the provision of quality healthcare in line with the real needs of the population in modern conditions, etc.

That is why recently, more and more scientists have emphasised the need to restructure higher medical education in the new conditions of mixed (full-time and distance) learning, in particular for medical students [4]. According to some scholars, the main approach to solving the problems with ensuring learning conditions in modern conditions is to develop an adaptive system of individualisation and personalisation of professional

training of future medical professionals, which requires a combination of the traditional, informative learning paradigm and an innovative, competence-based system [5].

However, these learning conditions, which have arisen in Ukraine as a result of the spread of coronavirus infection and are forced by the military aggression of a neighbouring state, also determine the need to develop new approaches to the organisation and provision of the learning process itself. After all, although learning management systems and their use in a distance format allow for an individual approach to teaching students, they do not take into account the personal component of the student (the desire to learn and develop).

Thus, in the European educational space, the key concepts of higher education according to the Bologna system of education, which is also used by medical students, are competences and learning outcomes in the form of acquired theoretical knowledge, practical skills and abilities that can be quantified and measured by obtaining the appropriate number of points for the acquired knowledge [6]. The acquisition of such knowledge and skills can be exclusively technical, when a student receives the minimum points only to pass the exam and close the subject, and professional development is defined as obtaining a diploma (and does not guarantee becoming a doctor).

Therefore, one of the key tasks of modern higher medical education is not only to train medical specialists with a high level of professionalism, but also to ensure the comprehensive development of students' personalities, in particular medical students who are capable of continuous self-improvement, especially in view of the new paradigm in medicine "continuous lifelong learning".

Improving the training of students in a higher medical education institution is due to many factors, among which one of the most important is the motivation of their educational and cognitive activities. It is motivation that is one of the leading factors in the successful learning of a young person, and hence their professional development in the future.

In view of the above, in the process of training a future doctor, the priority should be given to the personality of a medical student who, being in constant professional and personal development, should strive for continuous professional training and have the opportunity and desire to receive it.

The formation of students' positive motivation to learn a future profession, and thus to the educational and cognitive process, is of great importance in this process. The formation of motivation raises the issue of searching and selecting priority motives and the optimal structure of the student's motivational sphere [7].

Therefore, despite the existence in modern science of a significant number of psychological and pedagogical works devoted to the problem of forming the motivation of students' educational and cognitive activity, this issue is one of the most relevant, especially for future doctors. Given the medical staffing crisis and in light of the martial law in Ukraine, it is extremely important to study the state of motivation to study and professional development of medical students.

THE AIM

To determine the state of motivation to study and professional development of 6th year students of the Medical Faculty of the Uzhhorod National University in order to develop measures to improve the learning process of medical students and their practical use in overcoming the problems of the medical staffing crisis.

MATERIALS AND METHODS

To achieve this goal, a comparative analysis of modern scientific psychological and pedagogical works was carried out and a survey of medical students in their final year of study was conducted. The study involved 146 6th year medical students of the Medical Faculty of the Uzhhorod National University who studied at the Department of Social Medicine and Hygiene during the academic year 2022-2023. Medical students were distributed almost equally by gender ($p<0.5$), the age of respondents ranged from 21-26 years.

The study design involved the use of psychological diagnostic testing methods: to determine the general motives of learning activities in the modification of A. A. Rean and V. O. Yakunin and K. Zamfir in the modification of A. O. Rean to determine the level of professional development [8]. Data processing was carried out using STATISTICA 6.0, the difference in results was considered significant at $p<0.05$.

RESULTS

Motivation to study is a complex and multilevel process, the study of which is an important element in optimising the training of future highly qualified doctors. When conducting the survey, we divided the 6th year students into 2 groups: Group 1 - students who studied after school and Group 2 - students who studied after graduation (from previous special groups of the Medical Faculty).

Thus, the respondents were asked to choose the 5 most important general motives for learning from the

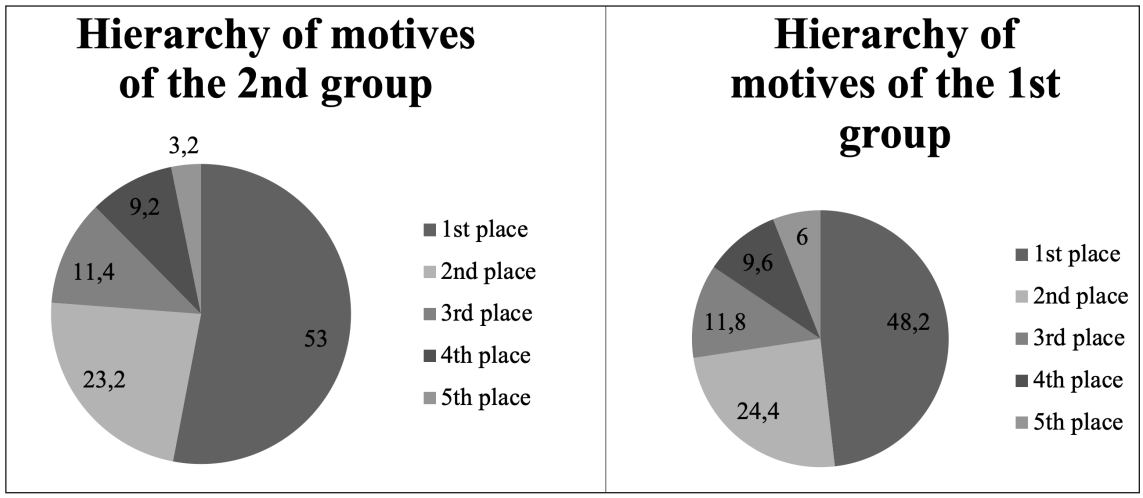


Fig. 1. Distribution of motives for learning according to the answers of respondents to the questionnaire by A. A. Rean and V. A. Yakunin (n=146). A) Hierarchy of motives of the 2nd group; B) Hierarchy of motives of the 1st group

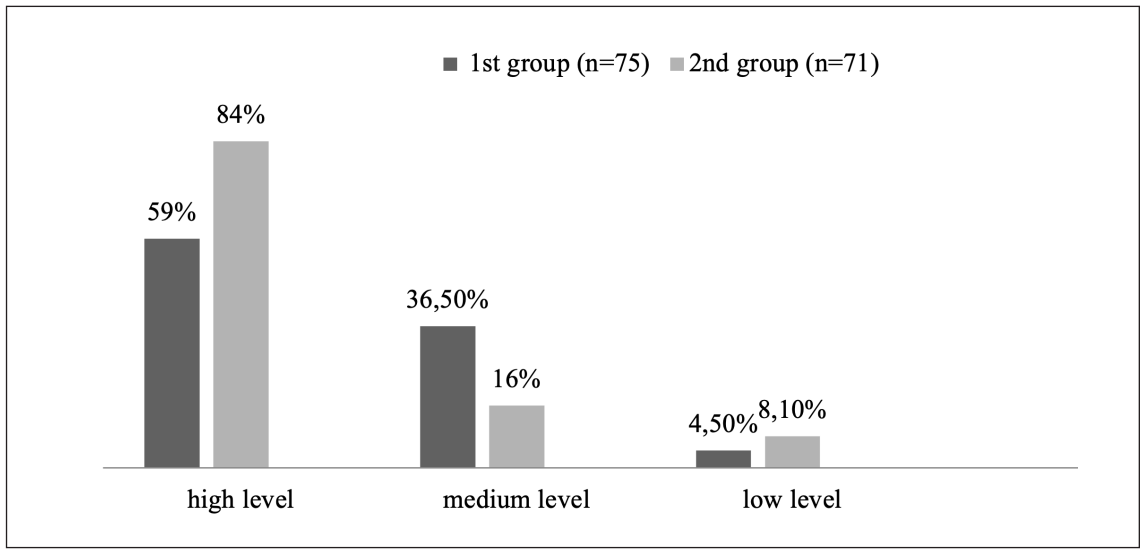


Fig. 2. Distribution of students by levels of motivation according to the methodology of A. O. Rean and V. O. Yakunin (n=146).

16 options presented. After analysing and processing the results of the questionnaire, the leading motives that characterise the essence of the learning motivation of 6th year medical students as the object of study were identified. The hierarchy of motives in the two selected groups was different. Thus, medical students from group 1 identified becoming a highly qualified specialist as the main goal in their studies, while respondents from group 2 chose ensuring the success of their future professional activities as the first and main motive. The further hierarchy of motives of the respondents from the first group was as follows: the second was to acquire in-depth knowledge in medicine, the third was to study well, pass exams with good and excellent marks, the fourth was to get intellectual satisfaction from studying, and the fifth was to ensure the success of future professional activities.

In the second group of respondents, the hierarchy of general motivations for studying looked somewhat different: obtaining a diploma ranked second, followed by studying the subjects of the academic cycle in third place, seeking approval from parents and others in fourth place, and avoiding judgment and punishment for poor performance in fifth place (Fig. 1).

The analysis of the responses allowed us to compare the frequency with which students indicated the motive that motivates them to study. Thus, for students from Group 1, the most important motives were the reasons for professional development rather than the acquisition of knowledge, as well as for the students from Group 2. The acquisition of deep knowledge among students of group 1 as a motive for learning is only the second step in the hierarchy of motives, while in group 2 it is only the third.

Table I. The type of motivational complexes in the two groups of students under study

№	Type of motivation (in points on a 5-point scale)		
	IM	EP	EN
1.	4	5	3
2.	2	3	5

The opportunity to get high marks at exams and intellectual satisfaction from learning, which are present in the hierarchy of motives of group 1, actually contribute to the motivation of students in this group to the least extent and do not motivate students in group 2 at all. At the same time, it should be noted that the majority of respondents from Group 2, who already have previous college experience, have a better understanding of the prospects for their future profession, but do not believe that their success directly depends on the knowledge they acquire. In most cases, students from this group already have some experience working in hospitals or combine their studies with non-medical work. They are older, so they are well aware of their responsibility for the future, especially to themselves. As a result, they are better able to adapt to changing learning environments, preferring practical experience to acquired theoretical knowledge. Also, the majority of them (78%) are residents of rural areas of Transkarpattia region, where the peculiarities of living create an additional external factor influencing the formation of the hierarchy of motives for learning and motivation for professional activity.

There is a distinction between extrinsic and intrinsic motivation for any type of activity, including study and professional development. The intrinsic type of motivation is indicated if the activity itself is important to the individual. If the motivation is based on the desire to satisfy other needs (external to the content of the activity itself), we should speak of extrinsic motivation. In turn, extrinsic motives are differentiated into positive and negative ones. Extrinsic positive motives are undoubtedly more effective and more desirable from all points of view than extrinsic negative ones [9, 10].

In order to determine the features and level of intrinsic (personal) motivation in students of the two groups, we used another method of psychodiagnostic testing in the modification A. O. Rean and V. O. Yakunin, which involved choosing from the proposed 20 statements of assessments of their own judgements in the form of statements "correct", "not completely correct", "not completely incorrect" or "wrong" (Fig. 2).

Based on the interpretation of the survey data, it can be argued that in both groups of students surveyed, intrinsic motivation is at medium and high levels, with a higher level for students from group 2. At the same time, 8.1% of students from this group and 4.5% from the first group of students said that they were studying at the university at the request of their parents or for other external reasons and had not yet decided on their future profession, and were not sure that their future would be related to medicine. This situation is extremely regrettable, as it indicates a potential loss of medical personnel potential in our region.

The vast majority of students from group 1 (86%) are highly motivated to study and acquire practical skills. The level of high motivation among these students is significantly ($p<0.05$) lower (59%) than among students from group 2.

There is no problem of motivation to study at foreign higher medical universities at all. The complex process of enrolment, other approaches to studying (independent choice of time for certain disciplines, additional incentives for studying in the form of free choice of elective disciplines, etc.) and a more conscious approach to higher education [11] explains this.

For a more in-depth disclosure of the reasons for such a difference in the state of motivation of the two groups of medical students under study, we used the methodology of K. Zamfir in the modification of A.A. Rean, which is based on the concept of intrinsic and extrinsic motivation for professional activity. On the basis of the results obtained, the motivational type of students from the first and second groups was determined, respectively, based on the ratio of three types of motivation: intrinsic (IM), extrinsic positive (EP) and extrinsic negative (EN). When interpreting the data, not only the type of motivational complex was taken into account, but also the degree of predominance of different types of motivation among themselves.

Thus, according to our data, students from group 1 have the optimal type of motivational complex, i.e. intrinsic positive motivation prevails over intrinsic and negative motivation. On the contrary, students from group 2 have a suboptimal type of motivational complex, when intrinsic positive motivation prevails over intrinsic motivation, but is inferior to intrinsic negative motivation (Table I).

Based on the above, it should be noted that the reason for the more "light-hearted" attitude to learning among students in Group 2 may be the influence of a wider range of internal negative motivations (such as

completing studies at any cost, obtaining a profession for greater profit, obtaining a medical degree to pander to the interests of others, etc.)

A more complete description of the structure of internal negative motivation in students, in particular from group 2, was obtained by analyzing the answers to an anonymous questionnaire that we created, which provided a choice of 15 answers regarding negative factors of motivation for the educational process. The main negative motivations that do not contribute to the interest of our students in studying and becoming a doctor were: lack of confidence in the future (89%), lack of decent salary (72%), lack of social benefits (especially housing), particularly in rural areas (56%), insufficient practical experience in applying the acquired skills during training (in particular, up to the 6th year - 78%) and inability to obtain knowledge on a flexible schedule (own choice of disciplines and time of their study - 62%).

Based on the above, when developing measures to increase motivation to study, the management of the university and the teaching staff of the medical faculty in the process of organising the educational process in modern unfavourable conditions should take into account the above features of motivation to study of medical students, including graduates.

DISCUSSION

One of the most challenging tasks of today is to ensure access to trained healthcare workers, who are the main guarantor of effective healthcare services and improved health indicators. Therefore, most WHO strategic documents state that the effectiveness of healthcare services is primarily determined by the presence of a sufficient number of experienced and motivated healthcare workers in the right place and at the right time. In view of this, improving the quality of medical staff training is perhaps the most important aspect in ensuring the proper quality of medical services and building the human resources capacity of the healthcare system, including in a particular region [12].

Medical education, obtained with considerable effort over a considerable number of years of study at a medical university, is a key factor in ensuring quality medical services through an appropriate level of professionalism. In other words, professional competence directly depends on the education and experience that a medical graduate implants in his or her practice. This, at the same time, requires significant efforts from the future specialist - from the efforts made during training to the efforts to provide quality healthcare services. In turn, efforts without appropriate motivation are meaningless.

One of the types of extrinsic motivation is systematic control. If we transfer this judgement to the learning process, strict control of student's knowledge can act as an extrinsic motivation, but is not able to ensure high quality of learning [11]. This can be evidenced, for example, by the failure of 6th year students to pass the KROK-2 licensing exam or by graduation from the medical faculty with low scores in most disciplines. Thus, the process of proper control of knowledge, without taking into account the motivational components of its implementation by the student, is not always a criterion for achieving quality knowledge, but rather may be a manifestation of meeting the student's immediate needs (to complete their studies and receive a diploma even with satisfactory grades).

The study of various aspects of motivational activity as a factor of efficiency in student learning has received a lot of attention in recent years and remains one of the most pressing scientific tasks in the context of reforming the medical industry. The analysis of modern scientific psychological and pedagogical research on this topic shows a wide range of studies of this problem: from the study of motives for entering a higher education institution and the dynamics of changes in motives in different courses to the conditions for students to have positive motivation for learning and cognitive activity [13-15].

The main characteristic of the motivational sphere is the hierarchy of motives, which allows to identify the personal meaning of activity for any person of any profession. The formation of optimal positive internal motives in students should be manifested in the form of a true persistent desire to obtain medical education, which ultimately is the key to the formation of high professional fulfilment of the student.

That is why, in our opinion, the study of the motivational sphere of medical students' personality should become a priority in the search for new forms and methods of improving the teaching of medical disciplines in the conditions of mixed adaptive learning and become a promising direction in the development of measures to overcome the problems of the personnel crisis at the regional level.

CONCLUSIONS

1. It has been determined that one of the main reasons for the high motivation to acquire knowledge among students from group 1 is the opportunity to become a highly qualified specialist, and among students from group 2 - to ensure the success of future professional activities.
2. The internal component of motivation to the educational process in the vast majority of 6th year students of the Medical Faculty of the Uzhhorod

National University is at the average and the number of students from previous special groups is higher, and it is more pronounced in students from previous special groups.
3. It has been proved that students of group 1 have the optimal type of motivational complex, unlike respondents from group 2, which directly determines the effectiveness of the educational process and future training as a doctor from the point of view of the respondents.
4. Reserves for motivating students to study and professional development are diverse and should be taken into account when organizing the educational process at the School of Medicine of the Medical Faculty of the Uzhhorod National University.
5. The results of the study can be used in the process of developing measures to attract and retain medical staff in the Transcarpathian region on the basis of sound organisational and persistent management efforts not only by the university management and teaching staff, but also require a balanced policy of local authorities and those involved in the formation of personnel policy, in particular, taking into account the characteristics of the Transcarpathian region, which will give confidence in the future to graduates of the Medical Faculty.

REFERENCES

1. Zakon Ukrainy «Pro vyshchu osvitu» [Law of Ukraine «On Higher Education»]. http://zakon4.rada.gov.ua/laws/show/1556-18. [date access 08.05.2023] (In Ukrainian).
2. Osadcha K, Osadchiy V, Kruglyk The Role of Information and Communication Technologies during Epidemics: Attempted Analysis. Ukrainian Journal of Educational Studies and Information Technology. 2020;8(1):62-82. doi: 10.32919/uesit.2020.01.06.
3. Standart vyshchoi osvity druhoho (mahisterskoho) rivnia, haluz znan 22 Okhorona zdorovia, spetsialnist 222 Medytsyna [Higher Education Standard for the Second (Master's) Level, Field of Knowledge 22 Health Care, Specialty 222 Medicine]. Approved and implemented by the Order of the Ministry of Education and Science of Ukraine dated November 8, 2021, No. 1197. (In Ukrainian).
4. Sisoeva SO, Osadcha KP. Condition, technologies and prospects of distance learning in the higher education of Ukraine. Information Technologies and Learning Tools. 2019;70(2):271-284. doi: 10.33407/itlt.v70i2.2907.
5. Osadcha KP, Osadchiy VV, Spirin OM, Kruglyk VS. Kontseptualni zasady rozrobky adaptyvnoi systemy indyvidualizatsii TA personalizatsii profesiinoi pidhotovky maibutnikh fakhivtsiv v umovakh zmishanoho navchannia [Conceptual Principles of Developing an Adaptive System for Individualization and Personalization of Professional Training of Future Specialists in the Conditions of Blended Learning]. Pedahohika formuvannya tvorchoyi osobystosti u vyshchiy i zahal'noosvitniy shkolakh. 2021;74(3). doi: 10.32840/1992-5786.2021.74-3.12. (In Ukrainian).
6. Bolonskyi protses - strukturna reforma vyshchoi osvity na yevropeiskomu prostori [Bologna Process - Structural Reform of Higher Education in the European Space]. https://euroosvita.net/?category=8&id=233. [date access 08.05.2023] (In Ukrainian).
7. Hylyun OV. Osvitni motyvatsii studentskoi molodi [Educational Motivation of Students. Facets]. Sotsiologia. 2012;1(81):102-104. https://www.libr.dp.ua/text/grani_2012_1_27.pdf. [date access 08.05.2023] (In Ukrainian).
8. Agranovich NV, Knyshova SA. Yzuchenye motyvatsyy uchebnoi diatelnosty studentov medytsynskykh vuzov y ee rol v formyrovanyy hotovnosty k budushchei professyy [Study of Motivation for Learning Activity of Medical University Students and Its Role in the Formation of Readiness for the Future Profession]. Sovremennyye problemy nauki i obrazovaniya. 2015;2(2):551. (In Russinian).
9. Artyushina MV et al. Psykholohiia diialnosti ta navchalnyi menedzhment: navch. posib. [Psychology of Activity and Educational Management: Textbook]. Kyiv: KNEU. 2007, p.366. (In Ukrainian).
10. Meretsky VM. Rol samostiinoi roboty studentiv v orhanizatsii navchalnoho protsesu [The Role of Independent Work of Students in the Organization of the Educational Process]. Medical Education. 2015;3:113-115. doi: 10.11603/me.v0i3.4989. (In Ukrainian).
11. Pro zatverdzhennia Poriadku provedennia monitorynhu yakosti osvity [On the Approval of the Procedure for Monitoring the Quality of Education]. https://euroosvita.net/index.php/?category=17&id=1664. [date access 08.05.2023] (In Ukrainian).
12. Hlobalnaia stratehyia razvytyia kadrovыkh resursov zdravookhranenyia: trudovie resursi 2030 h. Global Strategy for Human Resources for Health: Workforce 2030 - World Health Organization. Geneva. 2015. https://apps.who.int/iris/handle/10665/253394. [date access 08.05.2023] (In Russinian). Yegorova YV, Ignatovich OM, Korobchenko VV. Psykhodiahnostychne zabezpechennia proforiientatsii v systemi pedahohichnoi osvity : posibnyk [Psychodiagnostics of Career Guidance in the System of Pedagogical Education: Manual]. Kirovohrad: Imex-LTD. 2014. https://lib.iitta.gov.ua/7685/ [date access 08.05.2023] (In Ukrainian).
13. Fira DB. Symuliatsiine navchannia studentiv yak odyn iz perspektyvnykh metodiv formuvannia ta vdoskonalennia profesiinykh khirurhichnykh navychok u maibutnikh likariv [Simulation Learning of Students as One of the Promising Methods for Forming and Improving Professional Surgical Skills in Future Doctors]. Medical Education. 2017;4:58-60. doi: 10.11603/me.2414-5998.2017.4.8209. (In Ukrainian).
14. Pryadko NO. Motyvatsiia dosiahnennia yak determinanta uspikhu v profesiinii diialnosti osobystosti [Achievement Motivation as a Determinant of Success in Professional Activities of an Individual]. Visnyk Natsional'noho universytetu «Chernihivs'kyy kolehium» imeni T. H. Shevchenka. 2015;127(20):177-180. http://visnyk.chnpu.edu.ua/?wpfb_dl=1574. [date access 08.05.2023] (In Ukrainian).

ORCID and contributionship:
Angelika O. Keretsman: 0000-0002-8902-2227 [A-F]

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR

Angelika O. Keretsman
Uzhhorod National University
1 Narodna Square, 88000 Uzhhorod, Ukraine
tel: +380506309227
e-mail: angelika.keretsman@uzhnu.edu.ua

Received: 10.05.2023
Accepted: 21.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

IMMUNITY CHANGES IN PATIENTS WITH ACUTE MAXILLOFACIAL ODONTOGENIC INFECTIONS DURING TREATMENT STAGES: AN ANALYSIS

DOI: 10.36740/WLek202311116

Oksana V. Klitynska[1], Roksolana Yu. Kruchak[2], Viacheslav R. Gurando[1], Volodimyr V. Shetelya[1], Nataliya V. Layoch[1], Iurii O. Mochalov[1]

[1]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

[2]DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

The aim: Evaluate the expediency of using different methods of treatment for patients with acute purulent-odontogenic inflammatory processes in both the main and control groups. This assessment will be based on various indicators of non-specific immunity.

Materials and methods: This study involved the evaluation of the humoral component of nonspecific immunity in 114 patients. We assessed changes in total protein and its fractions, C-reactive protein (CRP), lysozyme, and immunoglobulins (A, M, and G) during three distinct time intervals: 1-3 days, 5-7 days, and 8-14 days after treatment initiation. Statistical analysis was conducted using Statistica 10.0 (StatSoft, Inc., USA) and Microsoft Office Excel 2010.

Results: At different postoperative follow-up periods, a significant improvement in humoral nonspecific immunity indicators ($p>0.05$) was observed when comparing patients treated with and without platelet-rich plasma. This improvement is expected to enhance reparative processes and expedite recovery.

Conclusions: The incorporation of platelet-rich plasma, immunocorrective, and adaptogenic therapy into the comprehensive treatment of acute purulent odontogenic inflammatory processes in the maxillofacial region not only leads to pronounced and enduring positive outcomes but also results in substantial improvements, including the potential normalization of key humoral and cellular factors associated with innate immunity.

KEY WORDS: Infectious and inflammatory processes of the maxillofacial area, treatment, platelet-rich plasma, immunity indicators

Wiad Lek. 2023;76(11):2442-2447

INTRODUCTION

Purulent inflammatory processes occupy a significant position in the realm of surgical dentistry due to various factors, including host organism changes and infectious agent properties [1-3].

Improving diagnostic and treatment methodologies for patients afflicted with odontogenic inflammatory diseases in the maxillofacial and neck regions remains a critical concern in practical healthcare. This is driven by their high incidence, severe symptoms, and frequent complications, which can disrupt the dentoalveolar system and lead to aesthetic facial defects, including deformities stemming from postoperative scars, thus directly affecting patient well-being.

Numerous methods and strategies for treating purulent wounds have been explored, yet none are universally effective [4-6]. Contemporary standards for managing patients with purulent inflammatory diseases in the maxillofacial and neck regions include surgical interventions to drain purulent foci, antimicrobial therapy, detoxification, anti-inflammatory measures, and homeostasis correction [4, 7]. Nonetheless, the prevalence of these pathologies continues to rise. This increase can be attributed to factors such as the growing antibiotic resistance of microorganisms, their virulence, and adaptability [8, 9].

Platelet-rich plasma (PRP), a blood concentrate containing 3-5 times the normal platelet count, has emerged as a valuable tool in maxillofacial surgery and surgical dentistry [10-12]. Upon activation, platelets release specific biological factors that stimulate mesenchymal progenitor cell migration and proliferation, foster neoangiogenesis, and promote tissue regeneration in both hard and soft tissues [10-14]. This study aims to provide an overview of PRP's success and discuss the technical preparation and biological foundations of its clinical use.

THE AIM

The aim of this work was to evaluate the expediency of using different methods of treatment for patients with acute purulent-odontogenic inflammatory processes

Table I. Indicators of the Humoral Component of Innate (Non-specific) Immunity in the Treatment of Acute Purulent Odontogenic Inflammatory Processes in the Maxillofacial Area at Different Postoperative Time Points.

Indicators of humoral activity. Factors of innate immunity	Average statistical norm	1-3 days		5-7 days		8-14 days	
		The main group (n=60)	Control group (n=54)	The main group (n=60)	Control group (n=54)	The main group (n=60)	Control group (n=54)
Total protein, g/l	74,00±8,10	62,12±2,12	58,85±2,32	67,21±2,14	62,13±2,18	73,60±4,20°	68,25±4,22
α1-globulins, %.	4,20±0,70	3,30±0,82	3,21±0,84	3,85±0,83	3,42±0,82	4,12±0,85	3,72±0,86
α2-globulins, %.	8,70±1,30	7,20±1,56	7,12±1,62	7,94±1,60	7,36±1,63	8,45±1,60	8,12±1,64
β-globulins, %.	9,90±1,90	13,00±2,63	13,25±2,68	10,28±2,60	12,15±2,69	10,00±2,63	11,24±2,65
γ-globulins, %.	15,50±2,10	27,30±3,24*	27,90±3,30*	19,24±3,18	24,40±3,31**	16,18±3,32°	21,25±3,30
Albumin, %.	61,70±2,30	49,10±2,21*	48,13±2,48*	57,28±2,22°	53,06±2,44**	61,40±2,22°°	55,20±2,48°
C-reactive protein, mg/l	5,00±0,50	27,15±6,25*	27,93±6,32*	10,18±6,21°	18,56±6,30**,°°	7,16±2,70°	12,48±3,42**,°
Lysozyme titer, μg/ml	3,74±0,03	1,16±0,15*	1,09±0,17*	3,04±0,17*,°°,▲	1,25±0,20*	3,47±0,19°°,▲	2,00±0,21*,°°
IgA, g/l	2,54±0,62	2,90±0,39	2,90±0,40	2,67±0,40	2,80±0,44	2,50±0,41	2,65±0,45
IgM, g/l	1,47±0,43	1,70±0,29	1,75±0,31	1,53±0,28	1,69±0,33	1,48±0,30	1,54±0,32
IgG, g/l	12,10±2,35	12,98±1,89	13,07±1,92	12,80±1,90	12,94±1,92	12,10±1,92	12,46±1,94

Notes:

*p<0.01; **p<0.05 - Indicates a significant difference in values compared to the established average statistical norm;

°p1 <0.05; °°p1 <0.01 - Represents a significant difference in values when compared to the data recorded during the initial postoperative period (days 1-3).

▲p2 <0.01 - Demonstrates a significant difference in values compared to the control group.

in both the main and control groups. This assessment will be based on various indicators of non-specific immunity.

MATERIALS AND METHODS

The study involved 114 patients with infectious and inflammatory processes of the maxillofacial area. Of these, 60 patients were treated using the developed treatment and prevention complex (referred to as the main group), while the remaining 54 patients underwent treatment according to standard protocols for surgical patient management.

The etiology of clinical and laboratory changes was determined through the examination of specific indicators of non-specific immunity. These obtained values were subsequently compared to established reference values. The dynamics of the humoral component of non-specific immunity were evaluated through alterations in total protein and its fractions, C-reactive protein (CRP), lysozyme, as well as immunoglobulins A, M, and G within the patients' blood.

Assessments were conducted at three distinct time points: 1-3 days, 5-7 days, and 8-14 days postoperatively.

Statistical analysis was carried out using Statistica 10.0 (StatSoft, Inc., USA) and Microsoft Office Excel 2010. For normally distributed samples, descriptive statistics were employed, with quantitative characteristics being represented as mean (M) ± standard deviation (SD).

RESULTS

The examination of the immune status of patients afflicted with acute purulent odontogenic processes in the maxillofacial region during varying postoperative intervals relied on laboratory parameters.

The assessment of non-specific immunity dynamics encompassed the investigation of cellular components, including neutrophils evaluated through tests measuring spontaneous nitroblue tetrazolium (NST sp.) and stimulated nitroblue tetrazolium (NST stim.) reduction, non-enzymatic lysosomal cationic proteins (lysosomal cationic test - LCT), phagocytic index (FI), phagocytic count (PC), phagocytosis completion index (PCI), and natural killer cells expressing CD16+ and CD56 (NK cells). Humoral factors were assessed by evaluating protein levels and its fractions, C-reactive protein, tetralysocyme (TL), as well as immunoglobulins of classes A, M, and G in blood serum. Neutrophil functionality was assessed by determining the overall oxygen-dependent bactericidal activity of peripheral blood neutrophils using either spontaneous (NST sp.) or stimulated (NST stim.) tests.

The results of the study regarding the humoral component of innate (non-specific) immunity in the treatment of acute purulent odontogenic inflammatory processes in the maxillofacial area at various postoperative time points are presented in Table I.

On day 1-3 of the postoperative period, a decrease in the content of total protein in the blood by 16.05% in the main group and by 20.47% in the control group

Table II. Indicators of the cellular link of innate (nonspecific) immunity in the treatment of acute purulent odontogenic processes at different times of the postoperative period.

Indicators. Cellular factors of innate immunity	Average statistical norm	1-3 days		5-7 days		8-14 days	
		The main group (n=60)	Control group (n=54)	Main group (n=60)	Control group (n=54)	Main group (n=60)	Control group (n=54)
NST spons.test, %	9,34±0,40	17,48±1,08 *	17,52±1,09 *	10,21±0,25 °,▲	15,10±0,86 *	9,40±1,12 °,▲▲	13,25±0,87 *,°
NST stim.test, %	62,00±9,40	27,70±5,20 *	27,68±5,31 *	58,41±5,21 °,▲▲	33,12±5,30 **	61,00±5,40 °,▲▲	44,10±5,35 °°
Lysosomal cation test, %.	84,10±2,50	73,80±2,78 **	73,49±2,83 **	79,20±2,71°°	75,14±2,68	83,90±2,74	80,13±2,72
Phagocytic indicator, %.	56,20±4,62	30,60±2,73 *	20,87±2,79 *,▲▲	48,70±2,74 ▲	26,53±2,82 *	55,18±2,73 ▲	32,80±2,80 *
Phagocytic number, abs.	12,80±1,40	9,90±1,96	8,34±1,90	11,90±2,05	9,09±1,93	12,52±2,10	10,25±1,90
Phagocytosis completion rate, %.	39,00±2,80	30,73±3,06 **	26,21±2,44 *	38,70±2,91 ▲▲	29,22±2,46 *	39,00±2,44 °°	32,75±2,83
NK cells $CD16^+$, $CD56^+$	15,60±2,65	23,30±3,47 **	24,20±3,05 **	20,60±3,06	24,00±3,10 **	16,30±3,12	20,85±3,18

Notes:
*p<0.01; **p<0.05 - significant difference in values relative to the average statistical norm.
°p1 <0.01; °°p1 <0.05 - significant difference in values compared to the data on days 1-3 of the postoperative period.
▲p2 <0.01; ▲▲ p2 <0.05 - significant difference in values compared to the control group.

compared to the normal values (p>0.05) was established; the fraction of α-globulins in the blood of patients in the main group was 18.60% and in the control group - 20.00% lower than the average (p>0.05). In the main group, the fraction of α-globulins in the blood was 1.7% higher than in the control group (p1 >0.05). The concentration of β-globulins in the blood of patients increased relative to the norm: by 31.31% in the main group and by 33.84% in the control group (p>0.05); in patients of the main group, the content of β-globulins in the blood exceeded the same indicator in patients of the control group by 1.92% (p1 >0.05). In all patients, a significant increase in the content of γ-globulins in the blood was observed in relation to the normative data: by 74.19% in the main group and by 80.00% in the control group (p<0.01). The concentration of γ-globulins in the blood of patients in the main group was 2.15% lower than in patients in the control group (p1 >0.05). There was a decrease in the concentration of albumin in the blood: by 20.42% in the main group and by 22.00% in the control group compared to the average (p<0.01). However, in patients of the main group, the albumin content in the blood did not differ significantly from the data in the control group (p_1 >0.05).

The concentration of C-reactive protein (CRP) in the blood of the subjects remained elevated, surpassing normative data (27.15±6.25 mg/l and 27.93±6.32 mg/l vs. 5.00±0.50 mg/l, respectively, p<0.01). Simultaneously, the lysozyme titer in the blood decreased significantly, showing a reduction of 69.00% in the main group and 70.86% in the control group compared to the average values (p<0.01). In patients from the main group, there were increases in blood concentrations of IgA by 14.17%, IgM by 15.65%, and IgG by 7.27%, although these changes were not statistically significant (p>0.05). In contrast, patients in the control group exhibited elevated levels of IgA by 17.7%, IgM by 19.05%, and IgG by 8.02%, with no statistically significant differences between the groups (p1 >0.05).

On days 5-7 of the postoperative period, total protein content in the blood increased by 8.19% in the main group and by 5.57% in the control group compared to the data from days 1-3 of the postoperative period (p2 >0.05). The α-globulin fractions in the blood increased on average by 12.38% in the main group and by 4.45% in the control group (p2 >0.05). Conversely, the concentration of β-globulins decreased in both groups on day 5-7 of the postoperative period, showing a reduction of 20.92% in the main group and 8.30% in the control group (p2 >0.05). A notable decrease in γ-globulin levels in the blood of subjects was observed, with a decrease of 29.52% in the main group and 12.54% in the control group. It is noteworthy that in the control group, the concentration of γ-globulins in the blood was 57.42% higher than the average (p>0.05). The concentration of albumin in the blood of the study population increased

significantly on day 5-7 of the postoperative period, rising by 16.66% in the main group (p1 <0.05) and by 10.24% in the control group (p1 <0.05) compared to the data from days 1-3 after treatment. Importantly, in patients in the control group, the albumin content in the blood remained significantly lower than normative values (p<0.05). In patients from the main group, the concentration of albumin in the blood was 7.37% higher than in patients from the control group (p1 <0.05).

In the main group, on day 5-7 of the postoperative period, there was a significant decrease in C-reactive protein content in the blood by 62.50% (p1 <0.05), compared to 33.55% in the control group (p1 <0.01), relative to the data from day 1-3 of the postoperative period. However, in the control group, the analyzed CRP index, with a value of 18.56±6.30 mg/l, remained significantly higher than the average statistical norm (p<0.05).

The lysozyme titer in the blood of patients in the main group increased, reaching a value of 3.04±0.17 µg/ml, which was significantly higher than that on days 1-3 of the postoperative period (p1 <0.01) and markedly higher than the value (1.25±0.20 µg/ml) in patients from the control group (p2 <0.01). There was a decrease in the content of IgA, IgM, and IgG in the blood, although these changes were not statistically significant (p1 >0.05), indicating a reduction in the inflammatory response.

On day 8-14 of the postoperative period, in patients with acute purulent odontogenic inflammatory processes in the main group, blood protein content increased (p1 <0.05), as did albumin and lysozyme titer (p1 <0.01). This was accompanied by a decrease in the concentrations of γ-globulin and C-reactive protein (p1 <0.05) compared to the data from days 1-3 of the postoperative period. All other analyzed parameters remained within reference values (p>0.05).

In the control group of patients, the concentration of C-reactive protein (CRP) in the blood remained elevated (p1 <0.01), while the lysozyme titer and albumin levels remained lower (p1 <0.01 and p1 <0.05, respectively) compared to the data from days 1-3 of the postoperative period. It is worth noting that the concentration of CRP in the blood was also higher (p < 0.05), and the lysozyme titer in the blood was lower (p < 0.01) compared to normative values

The results of indicators of the cellular link of innate (non-specific) immunity in the treatment of acute purulent odontogenic processes at different times of the postoperative period are presented in Table II.

During the initial period of the postoperative phase (days 1-3), there was an increase in neutrophil surface triggering (NSTsp.) to 17.48±1.08% in the main group and 17.52±1.09% in the control group, relative to the average statistical norm (p<0.01). Concurrently, during this research period, the neutrophil surface triggering stimulation (NSTstim.) decreased to 27.70±5.20% in the main group and 27.68±5.31% in the control group, relative to normative values (p<0.01).

Thus, in patients of the main group during days 1-3 of the postoperative period, lysosomal cationic protein content decreased to 73.80±2.78%, and in patients of the control group to 73.49±2.83%, compared to the average statistical norm (p<0.05); in patients of the main group the phagocytic index was 1.8 times higher (30.60±2.73% vs. 56.20±4.62%, p<0.01), the phagocytic number was 1.3 times higher (9.90±1.96 abs. vs. 12.80±1.40 abs., p>0.05), and the phagocytosis completion rate (PCR) was 1.3 times lower (30.73±3.06% vs. 39.00±2.80%, p<0.01) compared to the average statistical norm. In patients of the control group, there was a decrease in the phagocytic index by 2.7 times, phagocytic number by 1.5 times, and PAE by 1.5 times (p<0.01).

The cytotoxic activity of NK cells occurs in the absence of sensitized lymphocytes, characteristic of cellular immunity reactions. In patients of the main group, the content of NK cells CD16+ , CD56+ was 1.5 times higher (23.38±3.47%), and in patients of the control group, it was 1.6 times higher (24.20±3.05%) compared to average values (15.60±2.65%) during days 1-3 of the postoperative period (p<0.01). It should be noted that a significant difference between the groups was only observed in the phagocytic index (p2 <0.05) during this analyzed research period.

On the 5th-7th day of the postoperative period, in the main group, we observed a significant decrease in NST spons. (p1 <0.01) and NK cells CD16+ , CD56+ (p1 >0.05), alongside an increase in NST stim. (p1 <0.05), lysosomal cation test (p1 <0.05), phagocytic index, phagocytic number, and phagocytosis completion index (p1 >0.05) relative to data from the first 1-3 days post-treatment. Conversely, in the control group on the 5th-7th day postoperatively, the dynamics of the studied parameters did not significantly differ from the data observed during the first 1-3 days post-treatment (p1 >0.05). Notably, NST sp. and NK cells CD16+ , CD56+ values were significantly higher (p>0.01), while NST stim. (p<0.05), phagocytic index (p<0.01), and PFD (phagocytosis completion index) (p<0.05) were below normative values.

As a result of the study, it was demonstrated that on the 5th-7th day of the postoperative period, patients in the main group exhibited significantly lower NST spons. values (p2 <0.01) and higher NST stim., phagocytic index, and phagocytosis completion index values (p2 <0.05) compared to patients in the control group.

In the control group, on the 8th – 14th day of the postoperative period, NST sth. values were significantly higher than both the average data and the values from the first 1-3 days of the postoperative period (p1 <0.01), while the phagocytic index values were below normative values (p1 <0.01). In contrast, in the main group on the 8th-14th day of the postoperative period, NST stm. (p2 <0.05) and phagocytic index (p2 <0.01) were higher, and NST sp. (p2 <0.05) values were lower than those in the control group

DISCUSSION

The treatment of patients with acute purulent odontogenic processes of the maxillofacial area is complex, comprehensive and timely. According to many authors, existing treatment methods are characterized by trauma, a long recovery period and frequent complications, which is associated with increased antibiotic resistance of microorganisms, their virulence and variability [8, 9].

The results of recovery are significantly improved with the use of platelet-enriched plasma at the treatment stages, the main characteristics of which are the presence of a large number of platelets in it, which during activation change their shape and secrete specific biological factors that induce migration and proliferation of mesenchymal progenitor cells, stimulate neoangiogenesis and regeneration in both hard and soft tissues [10-12].

According to many authors [13, 14] plasma enriched with platelets is widely used in maxillofacial surgery and surgical stomatology.

The effectiveness of recovery in the postoperative period is determined by the improvement of the patient's physical condition, which is confirmed by immunological parameters.

According to the results obtained by us, in the patients of the main group who received platelet-enriched plasma, the indicators of humoral (non-specific) immunity approached the norm for 1-3 days, in particular, the content of total protein, α-globulins, β-globulins, γ-globulins in the blood and albumins. However, the content of CRP and IgA, IgM, IgG in the blood remained high.

On the 5th-7th day of the postoperative period, the treatment led to positive changes in the indicators of the humoral arm of nonspecific immunity, most notably in patients with acute purulent odontogenic inflammatory processes, who were treated with our proposed pharmacotherapy, that the decrease in the content of C-reactive protein and IgA, IgM, IgG in the blood (p>0.05) was confirmed, which indicates a decrease in the inflammatory reaction.

On the 8th-14th day of the postoperative period in the main group, the application of our proposed treatment and prophylactic model resulted in all cellular immunity indicators returning to values within the range of the average statistical norm (p>0.05). Additionally, NST spons. values were lower, NST stim. (p2 <0.01), and the indicator of phagocytosis completion (p2 <0.05) were higher than those observed during the first 1-3 days of the postoperative period.

The presence of an infectious and inflammatory process led to an increase in the intrinsic (spontaneous) enzymatic activity of neutrophilic granulocytes, indicating their antigenic overload, while simultaneously reducing the coefficient of neutrophil chemiluminescence stimulation, confirming a decrease in the reserve potential of phagocytic cells.

CONCLUSIONS

In conclusion, our study of the key components of non-specific immunity in the treatment of acute purulent odontogenic inflammatory processes in the maxillofacial region revealed significant alterations in both humoral and cellular factors, involving both decreases and dangerous increases in many of the parameters studied. Conventional standard treatment, following traditional protocols, failed to produce significant and sustained improvements in non-specific immunity factors. However, the inclusion of platelet-rich plasma, immunocorrective therapy, and adaptogenic therapy in the comprehensive treatment of acute purulent odontogenic inflammatory processes in the maxillofacial region not only resulted in the most pronounced and sustained positive outcomes but also led to significant improvements and even normalization of key humoral and cellular factors of innate immunity.

REFERENCES

1. Olsen I, van Winkelhoff Acute focal infections of dental origin. A J.Periodontol 2000. 2014;65 (1):178-89. doi: 10.1111/prd.12018.
2. Malanchuk V, Sidoryako A, Vardzhapetian S Modern treatment methods of phlegmon in the maxillo-facial area and neck. Georgian Med News. 2019;(294):57-61.
3. Taub D, Yampolsky A, Diecidue R, Gold L. Controversies in the Management of Oral and Maxillofacial Infections. Oral Maxillofac Surg Clin North Am. 2017;29 (4):465-473. doi: 10.1016/j.coms.2017.06.004.
4. Greenstein G, Greenstein B Clinical management of acute orofacial infections. Compend Contin Educ Dent. 2015;36 (2):96-103; quiz 104,114.

5. Klitynska OV, Layosh NV, Zorivchak TI et al. Local anesthesia in childrens dental receptions. Problems of clinical pediatrics. 2021; 4 (54): 77–80. doi: 10.24144/1998-6475.2021.54.77–80.
6. Layosh NV. Evaluation of the effectiveness of local anesthesia on the lower jaw in adolescents at an outpatient dental appointment. Journal of dentistry.2022; 4(121): 74. doi: 10.35220/2078-8916-2022-46-4.13.
7. Avetikov DS, Lokes KP, Ivanytska OS et al. Fatures of incinal anesthesia in thetreatment of acute periodontitis ofthe frontal teeth of maxilla in adolescent children. Ukrainian dental almanac. 2022;4:25–29.
8. Mochalov I, Kryvtsova M, Chobey A, Kulynych M. Identification of Pathogenic Microflora and Its Sensitivity to Antibiotics in Cases of the Odontogenic Purulent Periostitis and Abscesses in the Oral Cavity. Prague Med Rep. 2023;124(1):16-32. doi: 10.14712/23362936.2023.2.
9. Biron A, Halperin D, Sichel JY, Eliashar R. Deep neck abscess of dental origin draining through the external ear canal. Otolaryngol Head Neck Surg. 2005;133(1):166-7. doi: 10.1016/j.otohns.2004.09.101.
10. Krammer TL, Kollars M, Kyrle PA et al. Plasma levels of platelet-enriched microRNAs change during antiplatelet therapy in healthy subjects. Front Pharmacol. 2022; 13:1078722. doi: 10.3389/fphar.2022.1078722.
11. Fetisov SO, Nikityuk DB, Klochkova SV et al. Regenerative Potential of Spinal Ganglion Neurons During Topical Administration of Platelet-Enriched Blood Plasma Into the Skin Wound. Bull Exp Biol Med. 2018;165(6):808-812. doi: 10.1007/s10517-018-4270-z.
12. Miller ED, Song F, Smith JD et al. Plasma-based biomaterials for the treatment of cutaneous radiation injury. Wound Repair Regen. 2019;27(2):139-149. doi: 10.1111/wrr.12691.
13. Pohranychna K, Ohonovskyi R, Pasternak Yu et al. Effectiveness of arthrocentesis in the treatment of internal temporomandibular joint disorders after mandibular condylar fractures. International Journal of Medical Dentistry. 2022; 26(1):152-7.
14. Kruchak RY, Ilnytskyy YM. Modern ideas about the etiology of infectious-inflammatory processes of the maxillofacial area. Analytic review of the literature. East European scientific Journal. 2019; 9(49):4-8.

ORCID and contributionship:
Oksana V. Klitynska: 0000-0001-9969-2833 [A]
Roksolana Yu. Kruchak: 0000-0002-9235-1662 [E]
Viacheslav R. Gurando: 0000-0001-6303-3799 [F]
Volodimyr V. Shetelya: 0000-0001-6058-9708 [B]
Nataliya Vasilyvna Layoch: 0000-0003-4741-1731 [C]
Iurii O. Mochalov: 0000-0002-5654-1725 [D]

Conflict of interest:
The Authors declare no conflicts of interest

CORRESPONDING AUTHOR

Oksana V. Klitynska
Uzhhorod National University
3,Narodna Square, 88000 Uzhhorod, Ukraine
e-mail: klitinskaoksana@i.ua

Received: 19.05.2023
Accepted: 29.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

CLINICAL AND EPIDEMIOLOGICAL FEATURES OF MENINGOCOCCAL INFECTION AND ITS EARLY DIAGNOSIS IN RESIDENTS OF THE TRANSCARPATHIAN REGION

DOI: 10.36740/WLek202311117

Anton I. Kohutych[1], Andriana A. Halamba[1], Halyna M. Koval[1], Vlasta V. Vysochanska[1], Marian S. Malchytskyi[2]
[1]STATE UNIVERSITY UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2]REGIONAL CLINICAL INFECTIOUS DISEASES HOSPITAL, UZHHOROD, UKRAINE

ABSTRACT

The aim: To identify clinical and epidemiological features of meningococcal infection on the initial day of a patient's medical consultation, as well as the efficacy of laboratory examinations.

Materials and methods: A retrospective analysis of 76 patients' histories diagnosed with meningococcal disease was carried out.

Results: Children were more susceptible to meningococcal disease ($p < 0.001$). The majority of children were of preschool age, with the minority being adolescents and children under the age of one year. Among children disease incidence did not depend on gender. Among adults, the majority were women ($p=0.002$). All patients had a family history of a disease, close relatives tested positive for meningococcal diseases ($p=0.039$). The main symptom discovered during the primary examination on the day of admission to the hospital was a hemorrhagic rash ($p<0.001$). Most cases were of moderate severity ($p<0.001$) and cases of children having meningococcemia ($p<0.001$). A typical rash was found in 40% of patients with generalized meningococcal disease. A complete blood count showed leukocytosis in 47.8% of all cases. The most effective method of confirming the diagnosis was a thick blood smear and microscopic examination of cerebrospinal fluid ($p<0.001$).

Conclusions: Patients in the Transcarpathian region mainly develop an atypical form of meningococcal disease. Only half of all patients diagnosed with meningococcemia had a classical hemorrhagic rash. Generalized forms of meningococcal disease may proceed with normal or subfebrile temperature and without severe leukocytosis. We doubt the use of bacteriological methods of laboratory diagnosis due to their low effectiveness. The most sensitive method of laboratory diagnosis is a microscopic examination of blood smear, and cerebrospinal fluid.

KEY WORDS: meningococcal disease, epidemiology, thick blood smear

Wiad Lek. 2023;76(11):2448-2454

INTRODUCTION

Meningococcal disease (MD) is an anthroponotic infection that is transmitted via droplets and has a wide spectrum of symptoms (from asymptomatic nasopharyngeal carriage to generalized forms with high mortality) [1]. The causative agent of MD is *Neisseria meningitidis*. Clinical forms of MS may wary from asymptomatic course to a fulminant form that leads to multiple organ failure and death within hours or days [2]. The overall mortality rate of generalized meningococcal infection (GMI) can be as high as 10%, and in some cases - 40-80% [3]. Despite low incidence rates in economically developed countries over the past decade, MD has consistently been the leading infectious cause of death in young children [4].

According to the International Health Regulations 2005, MD is included in the list of infectious diseases regulated by these regulations that constitute an exceptional national or regional problem [5]. Ukraine and the Transcarpathian region are not an exception. In Ukraine, 896 cases were registered in 2018-2022, of which 706 (78.8%) were children, with an intensive incidence rate ranging from 0.218 to 3.26 per 100,000 population for all patients and from 0.91 to 3.02 for children. In the Transcarpathian region, 205 patients were detected during this period, including 160 (78.0%) children, which is almost a quarter of all patients in our country. The intensive incidence rate per 100,000 population was 4-11 times higher than the national rate, ranging from 0.96 to 6.69, and for children it was 3-9 times higher, ranging from 3.1 to 22.3.

Meningococcal disease is the leading cause of bacterial meningitis and septicemia worldwide and

is associated with high mortality and serious lifelong complications in survivors [6]. Treatment requires antibiotics. The sooner treatment is started, the better it is likely to be. If MD is suspected, treatment should begin in at the pre-hospital phase [7].

THE AIM

The aim of the study is to identify the clinical and epidemiological particularities of meningococcal disease that may be found during primary medical examination, in order to suspect the diagnosis and begin an early antibiotic therapy.

MATERIALS AND METHODS

To determine the clinical, epidemiological and laboratory characteristics of the course of MD in the Transcarpathian region, 76 patients' histories were analyzed. All patients were admitted to the Regional Clinical Infectious Diseases Hospital in 2018-2020 years with a diagnosis of MD, including: 21 adults aged 19 to 43 years and 55 children aged from 3 months to 18 years. There were 34 males and 42 females. Family history of meningococcal disease, contact with a patient with meningococcal disease, main complaints, clinical manifestations and laboratory tests on the first day of admission were recorded. The diagnosis of MD was confirmed following the discovery of gram-negative diplococci in blood and cerebrospinal fluid smears, or the identification of the causative agent *N. meningitidis* based on bacteriological investigations of nasopharyngeal swabs, blood, and cerebrospinal fluid, as well as their combination. The survey was conducted at the permission of the Bioethics Commission of the Faculty of Medicine of the Uzhhorod National University (Minutes No. 4 dated February 2, 2022).

Statistical processing was performed in Jamovi 2.2.5. The Kruskal-Wallis test was employed to calculate the significance of individual variables in diagnosing and measuring the severity of the condition, and then a pairwise comparison of variables using the Dwass-Steel-Critchlow-Fligner test. The normality of the distribution of quantitative data was assessed using the Shapiro-Wilk test. The results were represented as M±SD and Me (Q1; Q3). A critical level of reliability of α=0.05 was employed.

RESULTS

Among patients diagnosed with MD, children were the dominant group with 55 cases (74.4%) compared to other age groups, with a p-value of <0.001. This includes: Nine individuals were under one year of age (16.4%), with slightly more patients aged between 1 and 3 years - a total of 13 individuals (23.9%). A majority of the patients were preschool children - 17 individuals (30.9%), which is significantly higher than the group comprised of individuals under one year and primary school age, with a total of 11 individuals (20.0%). The smallest percentage of patients were adolescents - 5 individuals (9.1%), which is significantly lower than all groups except for children under one year. There were no variations in gender composition, with 28 boys (50.9%) and 27 girls (49.1%).

The mean age of adult patients diagnosed with MD was (29.9±6.6) years, with a significant preponderance of female cases (14 individuals (66.7% (p=0.002)).

It is noteworthy that a total of 60.5% of individuals diagnosed with MD were found to be part of family foci (p<0.001), particularly among adults at 71.4% (p<0.001) and to a slightly lesser extent among children at 56.4% (p=0.039). Meanwhile, patients diagnosed with MD were likely less frequently identified in organized groups, with a decrease of 44.7% (p=0.042), particularly among adults, with a reduction of 23.8% (p<0.001). Such association was not observed during childhood (52.7% and 47.3%).

The primary motive for conducting patient examinations for the detection of MD was typically the appearance of a rash, either on its own or along with other symptoms of the disease (51.3% (p=0.039)), the presence of contact with a patient who has MD or with other related symptoms is reported at a rate of 26.3%. In other cases, 22.4% of patients underwent disease detection examinations for reasons unrelated to their disease. In children (61.8%, p<0.001), a rash was frequently the primary reason for examination, either alone or accompanied by other clinical manifestations. Conversely, in adults, contact with an infected individual was the main reason for examination, either alone or with other manifestations(47.6%, p=0.015).

The diagnoses of MD for which patients received treatment at the Regional Clinical Infectious Diseases Hospital can be found in Table I.

The table displayed above indicates that adults were primarily affected by meningococcal nasopharyngitis and meningococcemia. It is not likely that there was a discernible variation in the rate of detection between the generalized and localized forms of the disease. In childhood, there was a significantly higher frequency of meningococcemia diagnosis than adulthood (p<0.001). The prevalence of generalized meningococcal infections was nearly nine times higher than that of localized meningococcal infections, with statistical significance (p<0.001). In general, the prevalence of generalized

Table I. Forms of meningococcal disease in the examined patients

	Forms of meningococcal disease Absolute number	Adults (n=21)		Children (n=55)		Total (n=76)	
		%	Absolute number	%	Absolute number	%	
Localised forms	Asymptomatic carrier of meningococcal disease	1	4.8	3	5.5	4	5.3
	Meningococcal nasopharyngitis	9	42.9[*, 1, 2]	3	5.5	12	15.8
	Total	10	47.6[3]	6	10.9	16	21.1
Generalised forms	Meningococcemia	8	38.1[*]	45	81.8[**, 4]	53	69.7[***]
	Mixed form of meningococcal disease	3	14.3	4	7.3	7	9.2
	Total	11	52.4 [5, 6]	49	89.1 [7]	60	79.8 [8]

Notes: [*] – probable difference from asymptomatic carrier of meningococcal disease in adults p<0.001;
[**] – probable difference from asymptomatic carrier of meningococcal disease and meningococcal nasopharyngitis in childhood p<0.001;
[***] – probable difference from asymptomatic carrier of meningococcal disease and meningococcal nasopharyngitis of generalised forms in all subjects p<0.001;
[1] – probable difference of meningococcal nasopharyngitis in adults from meningococcal nasopharyngitis in children p<0.001;
[2] – probable difference of meningococcal nasopharyngitis in adults from meningococcal nasopharyngitis of all examined p=0.008;
[3] – probable difference between localised forms of meningococcal disease in adults and localised forms of meningococcal disease in children p=0.004;
[4] – probable difference between localised forms of meningococcal disease in adults and localised forms of meningococcal disease of all examined p=0.015;
[5] – probable difference between generalised forms of meningococcal disease in adults and generalised forms of meningococcal disease in children p<0.001;
[6] – probable difference between generalised forms of meningococcal disease in adults and generalised forms of meningococcal disease of all examined p=0.015;
[7] – probable difference between localised forms of meningococcal disease and generalised forms of meningococcal disease in children p<0.001;
[8] – probable difference between localised forms of meningococcal disease and generalised forms of meningococcal infection of all examined p<0.001.

Table II. Severity of meningococcal disease in patients of the Regional Clinical Hospital

Degrees of severity of the disease	Adults (n 21)		Children (n 55)		Total (n 76)	
	Absolute number	%	Absolute number	%	Absolute number	%
Asymptomatic course	1	4.8	2	3.6	3	3.9
Mild course	7	33.3 [3, 5]	4	7,3	11	14.5 [8]
Moderate degree of severity	10	47.6 [1, 2]	43	78.2 [4, 6]	53	69.7 [7]
Severe course	3	14.3	6	10.9	9	11.8

Notes:
[1] – difference between the moderate severity and asymptomatic course in adults p=0.0016;
[2] – difference between moderate severity and severe severity in adults P=0.0218;
[3] – difference between moderate and asymptomatic course in adults p=0.018;
[4] – difference between moderate severity and asymptomatic course, mild severity and severe course in children p<0.001;
[5] – difference between the mild severity of the disease in adults and children p= 0.0041;
[6] – difference between the moderate severity of the disease in adults and children p=0.0094;
[7] – the difference between moderate severity and asymptomatic course, mild severity and severe course in all subjects p<0.001;
[8] – difference between mild severity and asymptomatic course in all subjects p=0.0238.

forms of the disease was nearly quadruple that of localized forms amongst patients at the Regional Clinical Infectious Diseases Hospital (p < 0.001).

The degree of MD in patients who received treatment at the Regional Clinical Infectious Diseases Hospital is displayed in Table II.

Patients with moderate severity were significantly more likely to be identified among those diagnosed with MD, particularly during childhood.

In each instance, localized forms of meningococcal infection were bacteriologically confirmed via the detection of N. meningitidis in nasopharyngeal swabs. Generalized forms of MD were confirmed through bacterioscopic detection of gram-negative diplococci in blood and cerebrospinal fluid, and bacteriological detection of N. meningitidis in blood, cerebrospinal fluid and nasopharyngeal swabs. The efficacy of bacterioscopic and bacteriological examination in individuals with generalized meningococcal infection is illustrated in Table III.

The microscopic blood examination is the most effective means of confirming a diagnosis of gener-

Table III. Efficacy of microscopic and bacteriological examination in generalised forms of meningococcal disease

Research method	Generalised forms of meningococcal infection					
	Meningococcemia (n=53)		Mixed form of meningococcal disease (n=7)		Total (n=60)	
	Absolute number	%	Absolute number	%	Absolute number	%
Bacterioscopic blood test	49	92.3[1,2]	6	85.7[4]	55	91.6[6,7]
Bacteriological blood test	3	5,7	1	14.3	4	6.8
Bacterioscopic test of CSF	0	0	4	57.1	4	6,6
Bacteriological test of CSF	0	0	5	71.4[5]	5	8,3
Bacteriological test of nasopharyngeal swabs	24	43.4[3]	4	57.1	28	46.7

Notes:

[1] – difference between bacterioscopic and bacteriological blood tests in patients with meningococcemia $p<0.001$;

[2] – difference between bacterioscopic test of blood and bacteriological test of nasopharyngeal swabs in patients with meningococcemia $p<0.001$;

[3] – difference between the bacteriological test of nasopharyngeal swabs and bacteriological test of blood in patients with meningococcemia $p<0.001$;

[4] – difference between bacterioscopic and bacteriological blood tests in patients with generalised forms of meningococcal infection $p=0.008$;

[5] – difference between bacteriological tests of blood and CSF in patients with generalised forms of meningococcal infection $p<0.031$;

[6] – difference between bacterioscopic and bacteriological blood tests in all subjects $p<0.001$;

[7] – the difference between bacterioscopic tests of blood and bacteriological tests of nasopharyngeal swabs in all subjects $p<0.001$.

Table IV. Complete blood count in patients with different forms of meningococcal disease on the day of admission

Laboratory indicator	Forms of meningococcal disease			
	Asymptomatic carrier of meningococcal disease	Meningococcal nasopharyngitis	Meningococcemia	Mixed form of meningococcal disease
WBC ($*10^9$/L) *	6,78±2,04	7,719(6,29;9,41)	8,15(6,99; 11,2)	20,3±6,32[1,2,3]
LYM ($*10^9$/L)	3,38±1,55	2,11±1,13	2,5(1,29; 3,46)	1,1 (0,825; 1,56)
MID ($*10^9$/L)	0,295±0,154	0,22(0,165; 0,27)	0,39(0,21; 0,63)	0,16 (0,075; 0,235)
GRA ($*10^9$/L) *	2,35±1,65	5,66 (3,85; 6,59)	5,12(3,84; 7,49)	18,8±6,8[1,2,3]
RBC ($*10^{12}$/L)	4,86±0,215	4,43±0,429	4,78(4,42; 5,09)	4,45±0,732
HGB (g/L)	134±15,8	135±16,1	129 (120; 134)	117±56,7
PLT ($*10^9$/L)	290±82,4	271±78,5	285(229; 393)	217±84,7
GRA/LYM (%) *	0,895±0,689	2,31(2,01;4,34)	1,95(1,18; 3,43)	19,6±14,9[1,2,3]
MID/GRA(%) *	0,111(0,0965;0,236)	0,0428±0,0387	0,0634(0,0406; 0,123)	0,00661 (0,00291; 0,0147)[3]
MID/LYM (%)	0,102±0,0703	0,225(0,0685;	0,155(0,103; 0,246)	0,174 (0,0509; 0,222)
PLT/LYM (%)	94,5±37,9	120(109;202)	166(85,8; 183)	203±129

Notes:

* Statistically significant indicators (Kruskal-Wallis) * P≤0,001

[1]- difference between WBC counts in patients with generalised forms of meningococcal disease and asymptomatic carrier of meningococcal disease $p=0.041$;

[2] – difference between WBC counts in patients with generalised forms of meningococcal disease and meningococcal nasopharyngitis $p=0.013$;

[3] – difference between the WBC counts in patients with generalised forms of meningococcal disease and meningococcemia $p=0.003$;

[4] – difference between the GRA counts in patients with generalised forms of meningococcal disease and asymptomatic carrier of meningococcal disease $p=0.041$;

[5] – difference between the GRA counts in patients with generalised forms of meningococcal disease and meningococcal nasopharyngitis $p=0.01$;

[6] – difference between the GRA counts in patients with generalised forms of meningococcal disease and meningococcemia $p<0.001$;

[7] – difference between the GRA/LYM ratio in patients with generalised forms of meningococcal disease and asymptomatic carrier of meningococcal disease $p=0.041$;

[8] – difference between the GRA/LYM ratio in patients with generalised forms of meningococcal disease and meningococcal nasopharyngitis $p=0.035$;

[9] – difference between the GRA/LYM ratio in patients with generalised forms of meningococcal disease and meningococcemia $p=0.001$;

[10] – difference between the MID/GRA ratio in patients with generalised forms of meningococcal disease and meningococcemia $p=0.01$.

alized meningococcal infection. In this examination, a specific diplococcus was found most frequently in individuals with MD and in over half of meningococcal disease cases. Bacteriological testing of nasopharyngeal swabs was positive in almost half of the patients. The bacteriological blood test was determined to have the

lowest efficacy, as it validated the disease diagnosis in only one out of 15-17 patients. Bacteriological examination of cerebrospinal fluid and nasopharyngeal swabs proved more efficient, yielding a confirmatory diagnosis in nearly fifty percent of the patients. Bacteriological analysis of cerebrospinal fluid and nasopharyngeal swabs has been demonstrated to be a superior method, verifying approximately half of the patients.

When analyzing reasons for referral for a specific diagnosis of MD upon admission, we categorized all patients into the subsequent groups: patients with complaints of rash, either alone or in combination with other symptoms of the disease; patients who have been in contact with a MD patient without clinical manifestations or complaints and with their presence; patients who were not in contact with MD patients, did not exhibit a rash, but rather presented with complaints and clinical manifestations of the disease. The study found that a rash alone or in combination with other complaints and clinical manifestations, contact with a patient with MD alone or in combination with other symptoms, was the reason for provisional diagnosis of MD and referral for specific diagnosis in 45 patients (59.2%, $p<0.001$). For 20 (26.3%) patients, the reason was contact with a patient with MD or with other clinical manifestations, and for 11 (14.5%) patients, it was other complaints and manifestations. It is noteworthy that 31.6% (24 patients) were found to have a hemorrhagic rash consistent with meningococcemia, while 27.6% (21 patients) had presented with an atypical rash. Rash was not present as a pathognomonic sign of the disease in 16 patients (30.9%) with generalized forms of MD. Meningeal symptoms with a positive indication were found in 7 (9,2%) patients. Amongst other complaints, 46 (60.5%) individuals experienced fever, 17 (22.3%) reported a runny nose, 15 (19.7%) complained of a sore throat, and 14 (18.4%) experienced vomiting.

Body temperature, as an indicators of intoxication, in patients with MD ranged widely upon admission - from 36.1 ^{0}C to 40.2 ^{0}C and was in asymptomatic carriage of MD the temperature was (36.6±0.05) ^{0}C and all patients had a normal temperature; at meningococcal nasopharyngitis 7 patients had normal temperature and 5 had subfebrile, with an average temperature reading of (36.9±0.42) ^{0}C; at meningococcemia - (37.6±1.04) ^{0}C, a normal temperature was recorded in 20 patients, subfebrile - 11, febrile - 15, high - 6, excessive - 1; at mixed forms of MD - (38.0±1.01) ^{0}C, a normal temperature - 1 person, subfebrile - 3, febrile - 1, excessive - 1.

Thus, out of the 72 patients diagnosed with meningococcal nasopharyngitis, meningococcemia, and mixed forms of meningococcal infection, 28 (38.9%) had a normal temperature, 19 (26.4%) had a subfebrile temperature, 16 (22.2%) had a febrile temperature, 7 (9.8%) had a high temperature, and 2 (2.7%) had an excessively high temperature. In nearly two-thirds of cases (65.3%), MD manifested in patients with a normal or subfebrile temperature.

A typical rash, indicative of generalized meningococcal infection, was observed in 24 patients (40.0%), while 19 (31.6%) had an atypical rash, and 17 (28.4%) did not have any rash.

The complete blood count (CBC) results are detailed in the following table IV.

The complete blood count analysis showed that solely the leukocyte and granulocyte counts, along with the granulocyte-linked ratios (GRA1/LYM1, MID1/GRA1), had statistical significance ($p\leq0.001$). In a pairwise comparison, significant differences were observed in only the indicators of patients with mixed forms of MD when compared to all other examined groups. An increase in the number of leukocytes in the analysis of the entire examined group was found in 37 people (48.7%), a normal number - in 34 people (44.7%) without a statistically significant difference, and in 5 people (6.6% ($p<0.001$) the number of leukocytes was reduced. An elevation of leukocytes was noted in 37 individuals (48.7%) of the analyzed group, while 34 people (44.7%) demonstrated typical leukocyte counts without any significant statistical variation. Conversely, leukocyte levels were lowered in 5 participants (6.6%) ($p<0.001$). The latter were diagnosed with moderate meningococcemia and all of them belonged to the childhood age group.

DISCUSSION

Antibiotics constitute the primary treatment for meningococcal infection. Early use is crucial for successful recovery and a positive disease outlook. [1] It is particularly important in severe forms [8]. Our study identified the clinical and epidemiological features of meningococcal infection in residents of the Transcarpathian region, which enable early suspicion of meningococcal infection and prescribe proper treatment on the initial day of treatment. The crucial epidemiological feature is age, with particular emphasis on infants up to one year old [1, 2]. The peculiarity of our region lies in the fact that the majority of ill children are of pre-school age. We did not find any elderly individuals [7]. Another significant epidemiological indicator we identified was interaction with an ill individual, primarily in the family.

The vast majority of patients were diagnosed with moderate meningococcemia. The primary clinical manifestation is a characteristic stellate hemorrhagic rash accompanied by a range of general intoxication

symptoms [3]. During the initial examination, a characteristic hemorrhagic rash was identified in only a third of the patients in our study, while a different type of rash was observed in a quarter of the patients. Thus, a rash, characteristic and uncharacteristic in combination with other manifestations of the disease, was detected in more than half of patients with meningococcemia. Almost a quarter of the patients had been in contact with a patient with meningococcal infection during the clarification of the epidemiological history. A combination of clinical signs, including the presence of a rash, and epidemiological data on contact with a patient suffering from meningococcal infection, were present in the vast majority of hospitalized patients with a confirmed diagnosis of generalized meningococcal infections. This confirms the significance of considering epidemiological data.

One manifestation of intoxication is changes in body temperature. Over a third of patients with clinical signs of meningococcal infection at the Regional Clinical Infectious Diseases Hospital had a normal temperature. Only one-third of patients presented with febrile temperature, indicating a level of intoxication. The identification of an elevated quantity of leukocytes and granulocytes on a complete blood count serves as a vital indication of generalized infectious disease. A satisfactory leukocyte and granulocyte count were found in over half of the patients with meningococcal infection. Only in mixed forms of meningococcal infection was a significant rise in the number of the latter observed.

The diagnosis of meningococcal infection can be confirmed with reliability when the presence of Neisseria meningitidis is detected in the blood, nasopharyngeal mucosa, or CSF [3]. Bacteriological examination of nasopharyngeal swabs proved most efficient in localized forms, though markedly less so in cases of meningococcemia and mixed meningococcal infection. Nonetheless, it enabled us to reliably confirm the etiology of the diagnosis in almost half of all the patients we examined. Bacteriological examination of the blood samples from patients with meningococcemia was unsuccessful in our research. Bacteriological examination of the cerebrospinal fluid was more effective in the identification of the causative agent. Our findings are consistent with the literature [7]. In cases of generalized forms of disease, the most efficient method was the microscopic examination of blood and CSF to identify the presence of gram-negative diplococci. This approach enabled us to identify the causative agent in almost all cases.

CONCLUSIONS

1. Meningococcal infection is prevalent in children, with 74.4% of all cases occurring in this age group, particularly among preschoolers. Adolescents and infants under one year of age may be less affected.
2. The incidence of meningococcal infection in childhood does not exhibit any significant dependence on gender. However, during adulthood, women are 66.7% more likely to be affected by the infection compared to men.
3. Meningococcal infection patients are predominantly found in family foci, 74.6% in adults and 56.4% in children.
4. The most common sign of meningococcal infection was a skin rash, either alone or accompanied by other symptoms of the disease, notably in children (61.8%).
5. In most patients diagnosed with meningococcal infection, clinical symptoms of rash were combined with epidemiological evidence of contact with a patient or carrier of meningococcal infection.
6. Generalised forms of meningococcal infection are dominant during childhood, with a prevalence rate of 89.1%.
7. The condition generally exhibits moderate severity (69.7%), with a higher prevalence in children (78.2%).
8. The most efficient methods for confirming the diagnosis of meningococcal infection are: for localised forms, bacteriological examination of nasopharyngeal swabs; and for generalised forms, microscopy of blood smears and cerebrospinal fluid (92.3%, 85.7%).
9. Bacteriological examination of blood and cerebrospinal fluid is not a reliable method to confirm the diagnosis of generalised meningococcal infection.
10. In the majority of cases, meningococcal infection presents itself in an atypical manner. This means that it occurs with either a normal temperature (28.9%) or subfebrile temperature (26.4%); less than half of patients (43.6%) exhibit the characteristic haemorrhagic rash associated with meningococcemia, and only 47.5% show an increase in leukocyte count.

REFERENCES

1. Meninhokokova infektsiya. [Meningococcal infection]. Tsentr hromadskoho zdorovia Ukrainy. https://www.phc.org.ua/kontrol-zakhvoryuvan/inshi-infekciyni-zakhvoryuvannya/krapelni-infekcii/meningokokova-infekciya [date access 15.06.2023]
2. Schaffer De Roo S, Torres RG, Fu LY. Meningococcal disease and vaccination in college students. Hum Vaccin Immunother. 2021;17(11):4675–4688. doi: 10.1080/21645515.2021.1973881.

3. Ahmed-Abakur EH. Meningococcal meningitis: Etiology, diagnosis, epidemiology and treatment. Am J Med Sci. 2014;4(6):266–271. doi:10.5923/j.ajmms.20140406.13.
4. Pollard AJ. Meningococcal Disease. Ulster Med J. 2018 ;87(2):81–82.
5. Mizhnarodni pravyla okhorony zdorov"ya. [International health regulations second edition]. WHO. 2005. https://apps.who.int/iris/bitstream/handle/10665/43883/9789663836911-ukr.pdf?sequence=33&isAllowed=y [date access 15.06.2023]
6. Parikh SR, Campbell H, Bettinger JA et al. The everchanging epidemiology of meningococcal disease worldwide and the potential for prevention through vaccination. J.Infec. 2020;81(4):483–498. doi: 10.1016/j.jinf.2020.05.079.
7. Barer MR, Irving W, Swann A, Perera N. Medychna mikrobiolohiia. Posibnyk z mikrobnykh infektsii: patohenez, imunitet, laboratorna diahnostyka ta kontrol [Medical microbiology. A guide to microbial infections: pathogenesis, immunity, laboratory diagnosis, and control: 19th Edition]. Kyiv: VSV Medytsyna. 2020, p. 448. (in Ukrainian).
8. Usachova OV, Pakholchuk TM. Suchasnyi stan problemy meninhokokovoi infektsii: diahnostyka i startova terapiia. [The current state of meningococcal infection: diagnosis and initial therapy]. Zdorovia Ukrainy. Pediatriia 2019;1(48):29-30. (in Ukrainian).

The article was written within the framework of the research topic of the Department of Microbiology, Virology, and Epidemiology, as part of the infectious diseases course at the State University "UzhNU" "Health and Recreation. Peculiarities of the clinical and epidemiological course of infections and parasitoses typical for the Transcarpathian region" State registration number 0117002833. Theme code 02070832. UDC index: 614.4. Subject code 76.33.

ORCID and contributionship:
Anton I. Kohutych: 0000-0003-3838-9106 [A, D, F]
Andriana A. Halamba: 0000-0003-0175-9943 [A, C, D]
Galina M. Koval: 0000-0002-0623-2326 [E, F]
Vlasta V. Vysochanska: 0000-0003-0998-554X [C, E]
Marian S. Malchitsky: 0000-0002-6102-6146 [B]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Anton I. Kohutych
Uzhhorod National University
1 sq. Narodna, 88000 Uzhhorod, Ukraine
tel:+380503722960
e-mail: a.kohutych@uzhnu.edu.ua

Received: 18.06.2023
Accepted: 29.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

INFLUENCE OF PROBIOTICS ON THE MESOTHELIN LEVEL IN WOMEN WITH ENDOMETRIOSIS ASSOCIATED WITH INFERTILITY IN COMPLEX PREPARATION FOR ASSISTED REPRODUCTIVE TECHNOLOGIES

DOI: 10.36740/WLek202311118

Oksana V. Bakun, Halyna D. Koval, Yevheniia A. Dudka, Iryna A. Oshchepkova, Khrystyna Y. Makoviichuk
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim: To study the determination of Mesothelin level in women with endometriosis associated with infertility and estimate influence of probiotic on endometriosis according of Mesothelin level in complex preparation before assisted reproductive technologies.

Materials and methods: In this study, we conducted a retrospective analysis of the medical records of 40 infertile women who underwent assisted reproductive technologies while also using the probiotic "Femina Probiz." We divided the participants into two groups. The control group comprised 11 women who had tubal infertility due to a previous inflammatory condition but were otherwise found to be in good health through comprehensive clinical and laboratory assessments. These women, aged between 21 and 42 with an average age of 29.75 years, did not use the probiotic "Femina Probiz." The main group consisted of 29 women diagnosed with external genital endometriosis who were undergoing assisted reproductive technologies. Women in the main group received the probiotic "Femina Probiz" from Unic Biotech Ltd, India. They took one tablet twice a day for one month as part of their overall treatment before undergoing assisted reproductive technologies. We measured the Mesothelin levels before and after this preparation phase. This study was conducted at Bukovinian State Medical University and Centre of Reproductive Medicine. It's worth noting that the primary infertility incidence was significantly higher in the main group of patients.

Results: In the main group, we observed that the Mesothelin level was 0.73±0.01, which was significantly higher than the post-preparation level (0.59±0.01). In contrast, the control group had a Mesothelin level of 0.49±0.01. Interestingly, we noted that the Mesothelin level in patients increased approximately twofold before preparation compared to those who had undergone preparation. This suggests that the use of the probiotic led to a sharp reduction in the elevated Mesothelin levels.

Consequently, the significant decrease in Mesothelin levels after using the probiotic indicates its effectiveness and potential utility in the preparation phase of assisted reproductive technologies programs.

Conclusions: The elevated Mesothelin levels indicate a strong association between the pathogenesis of endometriosis and inflammation, as well as damage to the peritoneum.

The incorporation of a probiotic as part of a comprehensive preparation regimen prior to assisted reproductive technologies notably enhances the overall health of patients and leads to a reduction in Mesothelin levels. Based on our findings, we highly recommend the inclusion of this probiotic preparation in clinical practice.

KEY WORDS: endometriosis, assisted reproductive technologies, infertility, mesothelin

Wiad Lek. 2023;76(11):2455-2459

INTRODUCTION

Endometriosis is a chronic inflammatory condition characterized by the growth of tissue similar to the uterine lining outside the uterus, commonly in areas like the peritoneum, ovaries, and cervix. Clinical symptoms often include progressive dysmenorrhea, chronic pelvic pain, deep dyspareunia, and infertility, all of which significantly impact a patient's quality of life [1]. It is estimated that approximately 10% of women in their reproductive years are affected by endometriosis [2].

While the exact cause and development of endometriosis remain unclear, the retrograde menstruation theory, proposed by Sampson in 1921, is widely accepted. Other hypotheses, such as coelomic metaplasia and vascular/lymphatic metastasis [3], have also been proposed but cannot fully explain all forms of the condition. Additionally, factors such as the immune system, hormones, genetics, and the environment are believed to play roles in the pathogenesis of endometriosis [4].

Considering the involvement of natural killer (NK) cells in endometriosis due to reduced toxicity, one potential treatment approach is the activation of these cells. In animal models, intraperitoneal injections of Lactobacillus gasseri OLL2809, a probiotic that stimulates IL-12 production, led to the activation of NK cells and a reduction in ectopic endometriotic lesions. A

randomized, double-blind, placebo-controlled study also suggested that this probiotic could alleviate endometriosis-related pain [5].

Furthermore, blocking inhibitory receptors like KIR2DL1, LILRB1/2, and NKG2A with monoclonal antibodies may offer therapeutic benefits in endometriosis [6]. Reducing TGF-β levels is another potential treatment option [7]. Additionally, stimulating NK cells through intraperitoneal injections of IL-2 has been shown to decrease endometriosis lesion sizes in animal models [8], though evidence in humans is lacking. The Bacillus Calmette–Guérin (BCG) tuberculosis vaccine, known to recruit NK cells, enhance cytotoxicity, and reduce ectopic endometrial lesions in animal models, has also been considered as a potential treatment [9].

Mesothelin (MLN) is a surface molecule found on mesothelial cells and has been observed in various cancers, including mesothelioma, ovarian cancer, pancreatic cancer, and squamous cell carcinoma [10]. It may play a role in peritoneal implantation and metastasis, particularly through interactions with CA125 [11]. MLN, when combined with CA125, demonstrated higher sensitivity in identifying ovarian cancer patients compared to using either marker alone. Notably, elevated MLN levels were found in the urine of early-stage ovarian cancer cases, outperforming serum measurements [12]. However, sensitivity decreased when distinguishing early-stage cases from healthy controls. Subsequent research showed sensitivity at a specific threshold in another cohort of prediagnostic sera.

THE AIM

The objective of this study is to investigate the measurement of Mesothelin levels in women experiencing infertility due to endometriosis and assess the impact of a probiotic on endometriosis in relation to Mesothelin levels during the comprehensive preparation phase before assisted reproductive technologies.

MATERIALS AND METHODS

We conducted a study involving 40 women, who were categorized into two distinct groups. The control group comprised 11 women with tubal infertility resulting from a previous inflammatory condition. These individuals, following an extensive clinical and laboratory examination, were found to have no other detectable diseases and were considered to be in a state of good health. Their ages ranged from 21 to 42 years, with an average age of 29.75 years. Importantly, they did not receive the probiotic "Femina Probiz."

The main group consisted of 29 women diagnosed with external genital endometriosis, all of whom were undergoing assisted reproductive technologies. Patients in the main group were administered the probiotic "Femina Probiz," manufactured by Unic Biotech Ltd, India. They were instructed to take one tablet twice daily, each containing 10×109 Lactobacillus, as part of a one-month comprehensive preparation (treatment) regimen before undergoing assisted reproductive technologies. Mesothelin levels were assessed both before and after this preparation phase.

This study was conducted at Bukovinian State Medical University and the Centre of Reproductive Medicine.

The measurement of Mesothelin levels was carried out utilizing the Human Msln (Mesothelin) ELISA Kit, employing sandwich enzyme-linked immunosorbent assay technology. Initially, a capture antibody was pre-coated onto 96-well plates, and subsequently, biotin-conjugated antibodies were used for detection. Standard test samples and biotin-conjugated detection antibodies were sequentially added to the wells, followed by a wash step with a wash buffer. HRP-Streptavidin was introduced, and any unbound conjugates were removed with wash buffer. The enzymatic reaction catalyzed by HRP was visualized using TMB substrates, resulting in the formation of a blue-colored product that changed to yellow upon the addition of an acidic stop solution. The density of yellow coloration was directly proportional to the quantity of the target sample captured on the plate.

Statistical analysis was performed utilizing the STATISTICA-10 software package (StatSoft, Inc., USA), and the significance of differences ($p < 0.05$) was assessed using Student's t-criterion.

RESULTS

The mean age of women in control group (who did not take probiotic "Femina probiz"- 29,75±7,09 years) and in main group (who took probiotic "Femina probiz") 30,65±2,04 ($p>0,05$)

Women main and control group has been examined and determined Mesothelin level. The Mesothelin level in blood serum in women before preparation to assisted reproductive technologies is shown in Table I.

We determined the Mesothelin level in blood serum before and after preparation

The Mesothelin level in blood serum in women before and after preparation to assisted reproductive technologies is shown in Table II.

Comparison the Mesothelin level in blood serum and peritoneal fluid in women before preparation to assisted reproductive technologies is shown in Table III.

The decrease in Mesothelin levels observed in the control group can be attributed to the fact that these patients were essentially in good health. Upon examining the data presented in Table I, we can discern two distinct

Table I. The Mesothelin level in blood serum in women before preparation to assisted reproductive technologies (M±m)

Groups of women under studies	n	Mesothelin level in blood serum, ng/ml	p
Endometriosis	25	0,73±0,01 ng/ml	<0,05
Control	11	0,49±0,01 ng/ml	<0,05

Table II. The Mesothelin level in blood serum in women before and after preparation to assisted reproductive technologies (M±m)

Group	Mesothelin level in blood serum, ng/ml		p
	Before preparation (treatment)	After preparation (treatment)	
Endometriosis	0,73±0,0 ng/ml	0,59±0,01 ng/ml	<0,05
Control	0,49±0,01 ng/ml	-	-
p	<0,05	-	

Table III. Comparison the Mesothelin level in blood serum and peritoneal fluid in women before preparation to assisted reproductive technologies (M±m)

Mesothelin level (ng/ml)	n	Group of women under studies (main group) with endometriosis	p
blood serum	25	0,73±0,01 ng/ml	<0,05
peritoneal fluid	29	0,55±0,01 ng/ml	<0,05

groups: the main group, comprising women with endometriosis, who received our proposed preparation for assisted reproductive technologies, including probiotics, and the control group, who underwent preparation for assisted reproductive technologies without the addition of probiotics. Within the main group, the Mesothelin level measured at 0.73±0.01 was notably higher than the post-preparation level of 0.59±0.01. In contrast, the control group had a Mesothelin level of 0.49±0.01. It is worth noting that the Mesothelin level increased approximately twofold in patients before the preparation phase compared to patients after the preparation, respectively. Consequently, the utilization of the probiotic resulted in a sharp reduction in the elevated Mesothelin levels, underscoring its effectiveness and its potential application in assisted reproductive technology preparation programs.

DISCUSSION

In this study, our primary objective was to assess the serum mesothelin levels in patients diagnosed with endometrial cancer, specifically [13] examining variations across different histopathological subtypes. Our overarching aim was to investigate the potential utility of serum mesothelin as a diagnostic marker for endometrial cancer. Several prior studies have explored the use of serum mesothelin, a 40-kDa glycoprotein originating from mesothelial cells, as a diagnostic or screening tool for mesothelioma [11, 12]. A recent meta-analysis reported summary estimates of 64% sensitivity and 89% specificity for this test, which is commercially available as an ELISA kit. It's important to note that most of these studies have utilized tissue bank samples and focused on specific diagnostic subgroups rather than consecutive patient series typically encountered in clinical practice. Among the largest studies conducted on mesothelin testing using pleural fluid, sensitivities of 67% and 71% were reported, with specificities of 98% and 89% for diagnosing mesothelioma. Mesothelin, a 40-kDa protein, is typically expressed in mesothelial cells found in the pleural cavity, peritoneal cavity, and peritoneum [13, 14]. Additionally, mesothelin has a strong affinity for cancer antigen 125 (CA125) through N-linked glycans [15]. Several studies have indicated that the co-expression of mesothelin and CA125 is associated with aggressive tumor characteristics and poor prognosis in various cancers, including ovarian carcinomas [16-19]. However, only a limited number of studies have explored the correlation between mesothelin and CA125 expression in endometrial cancers [20-28]. Hence, our study aimed to investigate this relationship and its potential implications for clinicopathological features. Our findings suggest that Mesothelin levels could serve as a non-invasive marker in women with endometrial cancer.

CONCLUSIONS

Based on the findings of this study, we have reached the following conclusions: The utilization of a quantitative ELISA to detect mesothelin in both serum and peritoneal fluid revealed elevated levels in 80% of patients diagnosed with endometriosis. These outcomes suggest that serum mesothelin has the potential to serve as a biomarker for this condition. Nevertheless, further investigations involving larger patient cohorts are necessary to comprehensively evaluate the sensitivity and specificity of this assay.

REFERENCES

1. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med. 2020;382(13):1244–56. doi: 10.1056/NEJMra1810764.
2. Shafrir AL, Farland LV, Shah DK et al. Risk for and consequences of endometriosis: A critical epidemiologic review.Best Pract Res Clin Obstet Gynaecol. 2018;51:1–15. doi:10.1016/j.bpobgyn.2018.06.001.
3. Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: Pathogenesis and treatment.Nat Rev Endocrinol. 2014; 10(5):261–75. doi:10.1038/nrendo.2013.255.
4. Giudice LC, Kao LC. Endometriosis Lancet. 2004;364(9447):1789–99. doi:10.1016/s0140-6736(04)17403-5.
5. Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstetrics Gynecol. 1927;14(4):422–69. doi:10.1016/S0002-9378(15)30003-X.
6. Itoh H, Uchida M, Sashihara T et al. Lactobacillus gasseri OII2809 is effective especially on the menstrual pain and dysmenorrhea in endometriosis patients: Randomized, double-blind, placebo-controlled study. Cytotechnology. 2011; 63(2):153–61. doi:10.1007/s10616-010-9326-5.
7. Hoogstad-van Evert J, Paap R, Nap A, van der Molen R. The promises of natural killer cell therapy in endometriosis. Int J Mol Sci. 2022; 23(10):5539. doi: 10.3390/ijms23105539.
8. Velasco I, Quereda F, Bermejo R et al. Intraperitoneal recombinant interleukin-2 activates leukocytes in rat endometriosis. J Reprod Immunol. 2007;74(1-2):124–32. doi: 10.1016/j.jri.2006.12.001.
9. Maksym RB, Hoffmann-Młodzianowska M, Skibińska M et al. Immunology and immunotherapy of endometriosis. J Clin Med. 2021;10(24):5879. doi: 10.3390/jcm10245879.
10. Luo L, Shi H-Z, Liang Q-L at al. Diagnostic value of soluble mesothelin-related peptides for malignant mesothelioma: a meta-analysis. Respir Med. 2010; 104(1): 149–156. doi: 10.1016/j.rmed.2009.05.017.
11. Davies HE, Sadler RS, Bielsa S et al. Clinical Impact and reliability of pleural fluid mesothelin in undiagnosed pleural effusions. Am J Respir Crit Care Med. 2009; 180(5): 437–444. doi: 10.1164/rccm.200811-17290C.
12. Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer. 1992;50(3):373–381. doi: 10.1002/ijc.2910500308.
13. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. Proc Natl Acad Sci U S A. 1996;93(1):136–140. doi:10.1073/pnas.93.1.136.
14. Gubbels JA, Belisle J, Onda M et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. Mol Cancer. 2006; 5(1):50. doi:10.1186/1476-4598-5-50.
15. Li YR, Xian RR, Ziober A et al. Mesothelin expression is associated with poor outcomes in breast cancer.Breast Cancer Res Treat. 2014;147(3):675–684. doi:10.1007/s10549-014-3077-5.
16. Shiraishi T, Shinto E, Mochizuki S et al. Mesothelin expression has prognostic value in stage II/III colorectal cancer.Virchows Arch. 2019;474(3):297–307. doi:10.1007/s00428-018-02514-4.
17. Nahm CB, Turchini J, Jamieson N et al. Biomarker panel predicts survival after resection in pancreatic ductal adenocarcinoma: a multi-institutional cohort study.Eur J Surg Oncol. 2019;45(2):218–224. doi:10.1016/j.ejso.2018.10.050.
18. Einama T, Kamachi H, Nishihara H et al. Co-expression of mesothelin and CA125 correlates with unfavorable patient outcome in pancreatic ductal adenocarcinoma. Pancreas. 2011;40(8):1276–1282. doi:10.1097/MPA.0b013e318221bed8.
19. Rump A, Morikawa Y, Tanaka M et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. J Biol Chem. 2004;279(10):9190–9198. doi:10.1074/jbc.M312372200.
20. Einama T, Homma S, Kamachi H et al. Luminal membrane expression of mesothelin is a prominent poor prognostic factor for gastric cancer.Br J Cancer. 2012;107(1):137–142. doi:10.1038/bjc.2012.235.
21. Einama T, Kamachi H, Nishihara H et al. Importance of luminal membrane mesothelin expression in intraductal papillary mucinous neoplasms.Oncol Lett. 2015;9(4):1583–1589. doi:10.3892/ol.2015.2969.
22. Yildiz Y, Kabadayi G, Yigit S et al. High expression of mesothelin in advanced serous ovarian cancer is associated with poor prognosis. J BUON. 2019;24(4):1549–1554.
23. Inoue S, Tsunoda T, Riku M et al. Diffuse mesothelin expression leads to worse prognosis through enhanced cellular proliferation in colorectal cancer. Oncol Lett. 2020;19(3):1741–1750. doi:10.3892/ol.2020.11290.
24. Felder M, Kapur A, Gonzalez-Bosquet J et al. MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress. Mol Cancer. 2014;13(1):129. doi:10.1186/1476-4598-13-129.
25. Dainty LA, Risinger JI, Morrison C et al. Overexpression of folate binding protein and mesothelin are associated with uterine serouscarcinoma. GynecolOncol. 2007;105(3):563–570. doi:10.1016/j.ygyno.2006.10.063.
26. Ginath S, Menczer J, Fintsi Y et al. Tissue and serum CA125 expression in endometrial cancer. Int J Gynecol Cancer. 2002;12(4):372–375. doi:10.1046/j.1525-1438.2002.01007.x.
27. Metindir J, Dilek GB, Pak I. Staining characterization by immunohistochemistry of tumor cancer antigen in patients with endometrial cancer. Eur J Gynaecol Oncol. 2008;29(5):489–492.

28. Kakimoto S, Miyamoto M, Einama T et al. Co-expression of mesothelin and CA125 is associated with the poor prognosis of endometrial serous carcinoma and mixed carcinomas including serous carcinoma. Pathol Oncol Res. 2020;26(4):2299–2306. doi:10.1007/s12253-020-00823-1.

Ethical approval for this study was obtained from the Medical Ethics Committee of the Bukovinian State Medical University, Chernivtsi, Ukraine (approval ID: No.9.02.2023). Consent to participate: All the persons involved in the study, including researchers and patients, gave their written informed consents for participation.

ORCID and contributionship:
Oksana V. Bakun: 0000-0002-4742-2265 [B,D]
Halyna D. Koval: 0000-0002-7120-9253 [F]
Yevheniia A. Dudka: 0000-0002-8301-500X [A,C]
Iryna A. Oshchepkova: 0009-0004-4591-6730 [C]
Khrystyna Y. Makoviichuk: 0000-0002-9143-9574 [E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Oksana V. Bakun
Bukovinian State Medical University
129 Golovna St, 58000 Chernivtsi, Ukraine
e-mail: kupchanko06@gmail.com

Received: 05.05.2023
Accepted: 24.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

POLYMORPHISM OF ACE AND AT2R1 GENES AS A GENETIC BACKGROUND FOR DIFFERENT TYPES OF ENCEPHALOPATHIES

DOI: 10.36740/WLek202311119

Khrystyna Duve[1], Shkrobot Svitlana[1], Olena Tkachenko[2]
[1]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY OF THE MINISTRY OF HEALTH OF UKRAINE, TERNOPIL, UKRAINE
[2]THE SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE

ABSTRACT

The aim: To study the prevalence of ACE I/D and AT2R1 A1166C gene polymorphisms in patients with CTE, SVD, AIE, and PIE and to assess the influence of the presence of a particular genotype of the studied genes on the occurrence and/or progression of encephalopathies.

Materials and methods: A total of 96 patients with encephalopathies of various genesis (chronic traumatic encephalopathy (CTE) n=26; chronic alcohol-induced encephalopathy (AIE) n=26; microvascular ischemic disease of the brain (or cerebral small vessel disease, (SVD)) n=18; post-infectious encephalopathy (PIE) n=26) were involved in the study. The molecular genetic study was performed in the molecular genetics laboratory of the State Institution «Reference Center for Molecular Diagnostics of the Ministry of Health of Ukraine», Kyiv. Statistical processing of the results was performed using the STATISTICA 10.0 program.

Results: In patients with various types of encephalopathies, probable changes in the frequency distribution of genotypes of polymorphic variants I/D of the ACE gene were established (11.11% vs. 33.33% - carriers of the I/I genotype, 27.78% vs. 50.00% - carriers of the I/D genotype and 61.11% vs. 16.67% – carriers of the D/D genotype) and A1166C of the AT2R1 gene (22.22% vs. 66.67% – carriers of the A/A genotype, 50.00% vs. 25.00% – carriers A/C genotype, 27.78% versus 8.33% – carriers of the C/C genotype) compared to individuals of the control group only in patients with SVD. The presence of the D allele and the D/D genotype of the ACE gene is associated with a statistically significant increase in the risk of SVD development and progression (respectively, 4.2 times (95% CI (1.39-12.72)) and 7.9 (95% CI (1.31-47.05)) times). A similar trend was established for the carrier of the C allele of the A1166C polymorphic variant of the AT2R1 gene in patients with SVD: a 4.3-fold increase in the risk of development and progression (95% CI (1.30-13.86). In addition, there is a probable dependence between carrier genotype A/C of the AT2R1 gene and increased risk of PIE and AIE by 4.8 and 5.7 times, respectively.

Conclusions: Therefore, results suggest the reasonability to include the I/D of the ACE gene polymorphism investigation in the genetic panel of encephalopathies.

KEY WORDS: encephalopathies, ACE I/D polymorphism, AT2R1 A1166C polymorphisms

Wiad Lek. 2023;76(11):2460-2468

INTRODUCTION

Encephalopathies of various genesis remain the most relevant and socially significant issue in neurological pathology. The marked interest nowadays is focused on this brain disease due to the steady increase in its morbidity rate, the development of marked neuropsychiatric disorders, the negative impact on the quality of life, and the early disability of patients, which determines the medical and social importance of performing the early diagnosis, adequate treatment and prevention [1]. It has been scientifically proven that the brain is the first organ to suffer from hypoxia and toxins in the case of systemic blood circulation disorders, viral intoxications, and brain injuries. As a result, clinically it may manifest with the development of encephalopathy, which can develop at any age of a person [2].

Microvascular ischemic disease of the brain (or cerebral small vessel disease, (SVD) is a type of encephalopathy caused by chronic cerebrovascular insufficiency and/or repeated episodes of acute cerebral blood circulation disorders. It is characterized by neurological, neuropsychological, and/or mental disorders resulting from multifocal or diffuse damage to the brain [3]. According to T. S. Mishchenko and co-authors, atherosclerosis and arterial hypertension are the main causes of this syndrome [4]. According to statistical data, in Ukraine, 6.1% of the population suffers from SVD [5].

Recent global data shows a concerning rise in chronic alcohol-induced encephalopathy (AIE) spreading worldwide and continuously affecting more countries [6-8]. Ukraine ranks among the top ten countries in terms of annual alcohol consumption, with an average

of 13.9 liters. In addition, according to the results of WHO research, several causes that lead to the death of a person and are in one way or another related to alcohol abuse have been identified, including injuries and accidents - 29.60%, as well as cardiovascular diseases - 14, 00% [9]. The World Health Organization (WHO) reports that every year, approximately 50-60 million people worldwide suffer from traumatic brain and skull injuries. In Ukraine, official data shows that from 2014 to 2019, around 200,000 individuals were hospitalized due to brain injuries. The recent invasion has caused a critical increase in traumatic brain injuries among both military personnel and civilians [10, 11]. Furthermore, traumatic brain and skull injuries are now recognized as chronic diseases with long-term consequences, such as an increased risk of progressive neurodegeneration and chronic traumatic encephalopathy (CTE) [12, 13].

Many cases of encephalopathy are caused by infections, even if the pathogen does not directly affect the central nervous system. This means that more than 19 million people develop sepsis each year, and 70% of them may experience sepsis-associated encephalopathy according to Robba C. and co-authors [14]. Some strains of the influenza virus are considered neurotropic/neurovirulent because they can enter the CNS. Ludlow M. and co-authors explain that encephalopathy is the most common non-respiratory complication of influenza, typically occurring a week after the first symptoms of the virus [15]. Encephalopathy (also known as HIV-associated dementia or AIDS-dementia complex) is the most commonly observed nervous system lesion in HIV-infected individuals, with 60-90% of AIDS patients experiencing it. This is according to the nosological structure of nervous system lesions in HIV-infected persons, as reported in reference [16].

It is crucial to comprehend the genetic foundation of encephalopathies due to their high prevalence. This understanding plays a crucial role in assessing the risk of their development and progression. Allelic polymorphism, the variation of the genome resulting from point changes in genes or different amounts of tandem repeats, determines an individual's resistance or predisposition to certain diseases, including encephalopathy [17]. Several scientific studies have been published that have identified probable associations between certain types of encephalopathies and candidate genetic factors. The study of the polymorphism of the angiotensin-I converting enzyme (ACE) gene, which is responsible for the synthesis of angiotensin I-converting enzyme and plays a vital role in the regulation of blood pressure, indicates its role in the mechanisms of occurrence of certain encephalopathies. Polymorphism in the 16th intron of the ACE gene is associated with the presence (insertion or I allele) and absence (deletion or D allele) of 287 bp. areas.

Angiotensin II type 1 receptor (AT2R1) is involved in the implementation of the main physiological and pathophysiological functions of angiotensin. Many studies have shown that the activation of the renin-angiotensin system directly or indirectly leads to the activation of angiogenesis processes [18]. The AT2R1 gene is located on the long arm of chromosome 3 at the 3Q24 locus. More than twenty polymorphic variants of the AT2R1 gene are known, and the most studied is the A1166C mutation (replacement of adenine for cytosine at position 1166) in the 3' region. Considering the synergistic effect of the ACE and AT2R1 genes and their influence on the maintenance of homeostatic processes, it is advisable to investigate the polymorphic variants of these genes in patients with all types of encephalopathies under investigation.

THE AIM

Thus, the aim of the study is to study the prevalence of ACE I/D and AT2R1 A1166C gene polymorphisms in patients with CTE, SVD, AIE, and PIE and to assess the influence of the presence of a particular genotype of the studied genes on the occurrence and/or progression of encephalopathies.

MATERIALS AND METHODS

We conducted a study on 96 patients with encephalopathies of different origins who were receiving treatment at the neurological departments of the "Ternopil Regional Clinical Psychoneurological Hospital" in Ternopil, Ukraine from 2021 to 2022. The patients were classified according to the cause of the encephalopathy**.** The distribution of encephalopathy types was: chronic traumatic encephalopathy (CTE) n=26, chronic alcohol-induced encephalopathy (AIE) n=26, microvascular ischemic disease of the brain (or cerebral small vessel disease, (SVD)) n=18, and post-infectious encephalopathy (PIE) n=26. The control group comprised 12 individuals who were representative in terms of age and gender.

Currently, there is no universal classification for encephalopathies that considers their severity, origin, and clinical presentation. Therefore, the verification of different types of encephalopathies was conducted based on criteria proposed by various authors [19-21]. The progression of each subtype of encephalopathy is determined by multiple factors, including the underlying cause of the condition, its impact on the rate of brain tissue damage and clinical presentation, as well as

the influence of concurrent illnesses. Encephalopathy encompasses a variety of subtypes, each characterized by a distinct set of neurological symptoms that are contingent upon the severity and progression of the condition. Such symptoms may include behavioral disturbances, apathy, altered memory and attention, cognitive decline leading to eventual dementia, as well as moderate neurological deficits such as extrapyramidal and pyramidal insufficiency.

Patient inclusion criteria were the following: age from 18 to 75 years; compliance with diagnosis criteria; and availability of the patient's informed consent. Exclusion criteria: the presence of oncopathology; concomitant pathology in the stage of decompensation; use of psychoactive substances; the presence of other diseases that could cause psychoneurological disorders, behavioral and mental disorders.

The performed study is a single-moment clinical study of the "case-control" type. The study protocol included screening of patients to determine compliance with inclusion and exclusion criteria, carrying out laboratory determinations, genetic research, and statistical analysis of the obtained data. All patients were informed about the purpose of the clinical study and gave written informed consent for their participation in it. Confidentiality about the patient's identity and state of health was preserved. The patient's informed consent form, examination card, and all stages of the research were approved by the bioethics commission of the Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine.

MOLECULAR GENETIC STUDY OF THE POLYMORPHIC VARIANT I/D OF THE ACE GENE AND A1166C OF THE AT2R1 GENE

Its first stage was isolating DNA from whole peripheral blood on a paper blank using the commercial kit "Quick-DNA Miniprep Plus Kit" (Zymo Research, USA) according to the instructions. Molecular and genetic differentiation of the studied gene variants was carried out by allele-specific PCR or PDRF PCR (restriction fragment length polymorphism) by standard operational protocols developed in the molecular genetics laboratory of the SI "RCMD of Public Health Ministry of Ukraine"

Electrophoretic separation was carried out in the System for horizontal electrophoresis multi Sub Midi (Cleaver Scientific, Great Britain). The size of amplified and restriction fragments was estimated by comparison with the molecular weight marker GeneRuler DNA Ladder (Thermo Scientific, USA) in an ethidium bromide-stained 3% agarose gel (Cleaver Scientific, UK). In the visualization process, the formed fragments for each sample were evaluated, and photofixation of the obtained images was carried out. The genotypes of the pieces were determined according to the SOPs approved by the institution by evaluating the molecular weight of the restriction/amplified fragments compared to the molecular weight and corresponding positive control samples (Table I).

STATISTICAL ANALYSIS

The Hardy-Weinberg law was used to assess the correspondence between the genotypes of the selected sample and the general population. Comparison of observed and expected frequencies (Pearson Chi-Square, $\chi 2$) was calculated using Pearson's formula: $p2 + 2pq + q2 = 1$ (Hardy-Weinberg equilibrium), using Pearson's $\chi 2$-square. When obtaining values of the reliability coefficient $p>0.05$, we accepted the «null» hypothesis about the equality of the samples, that is, the correspondence between the selected model and the general population. Comparative analysis of frequency tables was performed using Pearson Chi-Square ($\chi 2$) and Fisher exact p, two-tailed (in those cases when the values of expected frequencies (expected frequencies) of individual indicators did not exceed 5). To assess the influence of the factor (the presence of a particular gene genotype) on the investigated feature (occurrence and progression of the disease), the odds ratio (OR) and its 95% confidence interval (95% CI) were calculated. The influence was considered statistically probable at $p<0.05$ for the odds ratio.

RESULTS

Analysis of the frequency distribution of ACE and AT2R1 gene genotypes according to the Hardy-Weinberg law in patients with the studied types of encephalopathies and assessment of compliance with population balance was carried out in all observation groups and the control group. It was established that the frequency of the genotype responsible for the I/D polymorphism of the ACE gene both in patients with various types of encephalopathies and in the control group did not significantly deviate from Hardy-Weinberg equilibrium ($p>0.05$) (Table II). Analysis of the frequency distribution of the genotype responsible for the A/C polymorphism of the AT2R1 gene showed that the frequency of genotypes for this gene in patients with CTE, SVD, and in the control group also did not deviate significantly from the Hardy-Weinberg equilibrium. At the same time, in patients with AIE and PIE, the frequency distribution of genotypes responsible for the A/C polymorphism of the AT2R1 gene did not correspond to the general population according to the Hardy-Weinberg law.

Table I. Molecular weight of restriction/amplified fragments

Gene and polymorphism, rs	The size of the restriction/amplified fragments and the corresponding genotype
ACE I/D, rs4340	Genotype *II*: 479 bp Genotype *ID*: 479 and 192 bp Genotype *DD*: 192 bp
AT2R1 A1166C, rs5186	Genotype *AA:* 351 bp Genotype *AC*: 351, 238 and 113 bp Genotype *CC:* 238 and 113 bp

Table II. ACE and AT2R1 gene polymorphism according to the Hardy-Weinberg law in patients with different types of encephalopathies.

Genotype		CTE		SVD		AIE		PIE		Control	
		expected	available	expected	available	expected	available	expected	available	expected	available
ACE gene polymorphism											
Homozy-gotes that occur frequently	I/I	5,54	6	1,13	2	12,46	12	4,65	4	4,08	4
Heterozy-gotes	I/D	12,92	12	6,75	5	11,08	12	12,69	14	5,83	6
Homozy-gotes, which are rare	D/D	7,54	8	10,13	11	2,46	2	8,65	8	2,08	2
χ^2, p		χ^2=0,13; p>0,05		χ^2=1,21; p>0,05		χ^2=0,18; p>0,05		χ^2=4,36; p>0,05		χ^2=0,01; p>0,05	
AT2R1 gene polymorphism											
Homozy-gotes that occur frequently	A/A	13,16	13	4,01	4	12,46	10	10,47	8	7,52	8
Heterozy-gotes	A/C	10,67	11	8,97	9	11,08	16	12,06	17	3,96	3
Homozy-gotes, which are rare	C/C	2,16	2	5,01	5	2,46	0	3,47	1	0,52	1
χ^2, p		χ^2=0,02; p>0,05		χ^2=0,01; p>0,05		χ^2=5,14; p<0,05*		χ^2=2,25; p<0,05*		χ^2=0,70; p>0,05	

Note. * – statistically significant result.

The results of the frequency distribution of ACE gene genotypes showed that the I/D genotype predominated in patients with CTE, PIE and the control group (Table III). Genotype I/I was detected least often in patients with the studied types of encephalopathies (except for the AIE group, where genotype D/D was observed least usually).

Comparing the distribution of genotypes of the ACE gene in patients with the studied types of encephalopathies and controls, statistically significant differences were found only in patients with SVD, in whom the distribution of genotype frequencies according to the polymorphic variant of the ACE gene was as follows: 11.11% of people are carriers of the I/I genotype, 27, 78% – I/D genotype and 61.11% – D/D genotype. At the same time, in the group of patients with AIE, the frequency distribution of ACE gene genotypes probably differed from the data of patients with SVD and PIE ($\chi 2 = 20.05$; $p = 0.010$).

The results of the distribution of genotype frequencies according to the A1166C polymorphic variant of the AT2R1 gene showed that the A/C genotype predominated in patients with CTE, AIE, and PIE, while the distribution of genotypes A/C and A/A was even in patients with CTE. It should be noted that the obtained results of the distribution of genotype frequencies in patients with CTE, AIE, and PIE probably did not differ from the data of the control group. Comparing the distribution of genotypes of the AT2R1 gene in patients with SVD and the control group, statistically significant differences were found (22.22% vs. 66.67% - carriers of the A/A genotype, 50.00% vs. 25.00% - carriers of the A/C genotype, 27, 78% versus 8.33% - carriers of the C/C genotype). In addition, the data of patients with

Table III. Polymorphism of ACE and AT2R1 genes in patients with different types of encephalopathies

	CTE (1)		SVD (2)		AIE (3)		PIE (4)		Control	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ACE gene polymorphism										
I/I	6	23,08	2	11,11	12	46,15	4	15,38	4	33,33
I/D	12	46,15	5	27,78	12	46,15	14	53,85	6	50,00
D/D	8	30,77	11	61,11	2	7,69	8	30,77	2	16,67
χ^2 (EP/CG), p	χ^2=0,97; p=0,614		χ^2=6,03; p=0,049*		χ^2=0,97; p=0,614		χ^2=1,90; p=0,386		–	
χ^2, p	χ^2=20,05; p=0,010*, $p_{2-3, 3-4}$<0,05*									
AT2R1 gene polymorphism										
A/A	13	50,00	4	22,22	10	38,46	8	30,77	8	66,67
A/C	11	42,31	9	50,00	16	61,54	17	65,38	3	25,00
C/C	2	7,69	5	27,78	0	0,00	1	3,85	1	8,33
χ^2 (EP/CG), p	χ^2=1,08; p=0,581		χ^2=6,04; p=0,049*		χ^2=5,74; p=0,057		χ^2=5,74; p=0,068		–	
χ^2, p	χ^2=19,30; p=0,013*; p_{2-3}<0,05*									

Note. * – statistically significant result.

Table IV. Frequency of ACE and AT2R1 gene alleles in patients with different types of encephalopathies

Frequency of alleles	CTE		SVD		AIE		PIE		Control	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
ACE gene polymorphism										
Allele I	24	46,15	9	25	36	69,23	22	42,31	14	58,33
Allele D	28	53,85	27	75	16	30,77	30	57,69	10	41,67
p (EP/CG)	p=0,460		p=0,015*		p=0,437		p=0,798		–	
AT2R1 gene polymorphism										
Allele A	37	71,15	17	47,22	36	69,23	33	63,64	19	79,17
Allele C	15	28,85	19	52,78	16	30,77	19	36,54	5	20,83
p (EP/CG)	p=0,580		p=0,017*		p=0,421		p=0,196		–	

Note. * – statistically significant result.

SVD probably differed from those of patients with AE (χ2=19.30; p=0.013).

Analyzing the frequency distribution of alleles of the ACE gene, it was established that among patients with CTE and PIE, the number of carriers of alleles I and D was almost the same, while among patients with SVD, carriers of allele D predominated, and among patients with AIE, carriers of allele I predominated (Table IV). Comparing the frequencies of alleles of the ACE gene among patients with the studied types of encephalopathies, probable differences were established in the SVD group relative to control data (frequency of allele I - 25.00 vs. 58.33%; frequency of allele D - 75.00% vs. 41.67%).

A similar trend was noted regarding the frequency of distribution of alleles A and C according to the polymorphic variant A1166C of the AT2R1 gene, in particular, among patients with the studied types of encephalopathies, probable differences were found in the SVD group compared to control data (frequency of allele A - 47.22% vs. 79.17%; frequency C alleles – 52.78% versus 20.83%) (Table IV). At the same time, the analysis of the frequency distribution of alleles of the AT2R1 gene showed that among patients with SVD, the number of carriers of alleles A and C was equal; while in the group of CTE, AIE, and PIE, carriers of the A allele predominated.

Analyzing the odds ratio and its confidence interval for alleles of the ACE gene in patients with the studied types of encephalopathies, it was established that there is a statistically significant relationship between the carriage of alleles I and D and the occurrence of encephalopathy only in patients with SVD (Table V). Thus, the presence of the D allele increases the risk of encephalopathy in this cohort of patients more than 4 times. A similar trend has been established regarding the presence of the C allele of the AT2R1 gene in patients with SVD.

Table V. Odds ratio (OR) and its confidence interval (95% CI) for ACE and AT2R1 gene alleles in patients with different types of encephalopathies

Allele	CTE		SVD		AIE		PIE	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
ACE								
Allele I	0,61	0,23–1,63	0,24*	0,08–0,72	1,61	0,59–4,38	0,52	0,20–1,40
Allele D	1,63	0,61–4,34	4,20*	1,39–12,72	0,62	0,23–1,70	1,91	0,72–5,09
AT2R1								
Allele A	0,65	0,20–2,06	0,24*	0,07–0,77	0,59	0,18–1,87	0,46	0,15–1,42
Allele C	1,54	0,49–4,88	4,25*	1,30–13,86	1,69	0,54–5,32	2,19	0,70–6,81

Note. * – statistically significant result.

Table VI. Odds ratio (OR) and its confidence interval (95% CI) for ACE genotypes in patients with different types of encephalopathies

Encephalopathy type	ACE gene polymorphism					
	I/I		I/D		D/D	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
CTE	1,20	0,25–5,77	0,86	0,22–3,37	2,22	0,39–12,56
SVD	0,25	0,04–1,67	0,38	0,08–1,78	7,86*	1,31–47,05
AIE	1,71	0,41–7,14	0,86	0,22–3,37	0,42	0,05–3,38
PIE	0,36	0,07–1,81	1,17	0,30–4,59	2,22	0,39–12,56

Note. * – statistically significant result.

Table VII. The odds ratio (OR) and its confidence interval (95% CI) for AT2R1 genotypes in patients with different types of encephalopathies

Encephalopathy type	AT2R1 gene polymorphism					
	A/A		A/C		C/C	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
CTE	0,50	0,12–2,08	2,20	0,48–10,07	0,92	0,07–11,22
SVD	0,14*	0,03–0,73	3,00	0,61–14,86	4,23	0,43–41,88
AIE	0,31	0,07–1,32	4,80*	1,04–22,10	0,14	0,01–3,82
PIE	0,22*	0,05–0,96	5,67*	1,22–26,33	0,44	0,03–7,69

Note. * – statistically significant result.

Analyzing the odds ratio and its confidence interval for ACE gene genotypes in patients with the investigated types of encephalopathies, it was established that there is a statistically significant relationship between carrying the D/D genotype and the occurrence of encephalopathy in patients with SVD (Table VI). Thus, the presence of the D/D genotype increases the risk of encephalopathy in this cohort of patients by almost 8 times.

Analyzing the odds ratio and its confidence interval for the genotypes of the AT2R1 gene in patients with the studied types of encephalopathies, it was established that there is a statistically significant relationship between the carrier of the A/C genotype and the increased risk of encephalopathy in patients with PIE and patients with AIE (Table VII). On the other hand, the protective properties of the A/A genotype in patients with SVD and PIE have been established.

DISCUSSION

Cerebral small vessel disease (SVD) develops against the background of arterial hypertension, atherosclerosis, diabetes, and a number of other diseases and is a slowly progressive disorder of cerebral blood circulation with the development of multifocal or diffuse ischemic damage to the brain [22]. The renin-angiotensin-aldosterone system (RAAS) is central to the regulation of blood pressure and plays an important role in the central nervous system,

as its inhibition can reduce the rate of cognitive decline in patients with SVD and dementia [23]. ACE affects angiotensin metabolism in the RAAS system, and ACE inhibitors suppress microglial activation and preserve dendritic integrity and cognitive function [24]. One of the main pathogenetic links of cerebrovascular pathology is the development of endothelial dysfunction, which is characterized by the development of specific changes in the endothelium, manifested by a decrease in vasodilation, a change in prothrombotic properties, and the development of a proinflammatory state [25, 26].

Individualization of universal protection and damage mechanisms is determined mainly by allelic polymorphism – genome variations that determine the individual characteristics of a person and consist in the presence of point changes in genes or tandem repeats in different quantities. On the one hand, allelic polymorphism determines the resistance of an individual to a certain disease, and in others, it determines the predisposition to the occurrence of pathology, including encephalopathy [17, 27]. The results of our study, which indicate the association of the D allele and the D/D genotype of the ACE gene with a statistically increased risk of SVD development and progression, are confirmed by a number of other studies.

Thus, according to scientific data, middle-aged and older patients with SVD who have an allele encoding a highly active variant (D) of the ACE gene show greater cognitive impairment, while carriers of a low-active allele (I) have an increased risk of dementia [28, 29] . In addition, the angiotensin II type II (Ang II) gene receptor 1 polymorphism plays an important role in the regulation of blood pressure and is associated with a decrease in prefrontal and hippocampal volumes, hippocampal volume and memory loss in the elderly [30, 31].

As a result of chronic alcohol intoxication, oxidative and nitrosative stress develops, which can lead to significant damage to brain neurons, which can be clinically manifested by the development of neurological deficits and a noticeable decrease in cognitive and memory functions [32, 33]. In addition, excessive alcohol consumption is almost always associated with systemic hypertension, as well as endothelial dysfunction and arterial atherosclerosis. Ang II has been shown to cause vasoconstriction by increasing superoxide production via induction of NADPH oxidase (NOX) in the vessel wall [34]. Ang II is known to stimulate superoxide generation through its receptor type 1 (AT1) to activate NOX in the vascular wall [35]. Therefore, the role of polymorphic variants of the AT2R1 gene in increasing the risk of development and progression of SVD and AIE is justified. Bai et al. first showed a direct causative role of Ang II through interaction with the AT1 receptor in the induction of alcoholic oxidative stress/aortic damage, inflammation, cell death and proliferation, as well as their remodeling [36].

It is known that Ang II, in addition to its prohypertensive properties, is able to activate various cells of the immune system, including, for example, macrophages, inducing the production of proinflammatory cytokines, such as IL-6, TNFα, and other proinflammatory cytokines [37]. Accordingly, Ang II was associated with the development of inflammatory lung injury. The results of our study indicate an increased risk of PIE in carriers of the A/C genotype of the AT2R1 gene. Researchers, using the example of SARS-CoV-2, cannot answer affirmatively whether neurological syndromes are a direct effect of the virus [38]. It is possible that these clinical-neurological syndromes are secondary effects caused by viral mechanisms that remain to be elucidated. Viral infection is known to activate inflammatory, prothrombotic, and endothelial pathways that can lead to a systemic inflammatory response syndrome characterized by cytokine hyperproduction that affects brain function [39, 40].

CONCLUSIONS

In patients with various types of encephalopathies, probable changes in the frequency distribution of genotypes of polymorphic variants I/D of the ACE gene were established (11.11% vs. 33.33% - carriers of the I/I genotype, 27.78% vs. 50.00% - carriers of the I/D genotype and 61.11% vs. 16.67% – carriers of the D/D genotype) and A1166C of the AT2R1 gene (22.22% vs. 66.67% – carriers of the A/A genotype, 50.00% vs. 25.00% – carriers A/C genotype, 27.78% versus 8.33% – carriers of the C/C genotype) compared to individuals of the control group only in patients with SVD. The presence of the D allele and the D/D genotype of the ACE gene is associated with a statistically significant increase in the risk of SVD development and progression (respectively, 4.2 times (95% CI (1.39-12.72)) and 7.9 (95% CI (1.31-47.05)) times). A similar trend was established for the carrier of the C allele of the A1166C polymorphic variant of the AT2R1 gene in patients with SVD: a 4.3-fold increase in the risk of development and progression (95% CI (1.30-13.86). In addition, there is a probable dependence between carrier genotype A/C of the AT2R1 gene and increased risk of PIE and AIE by 4.8 and 5.7 times, respectively.

REFERENCES

1. Pashkovska NV. Diabetychni entsefalopatiyi: mekhanizm rozvytku, dyferentsiyovana diahnostyka, likuvannya ta profilaktyka. [Diabetic encephalopathies: mechanism of development, differential diagnosis, treatment and prevention]. Dissertation abstract for obtaining the scientific degree of Doctor of Medical Sciences. 14.01.15 - nervous diseases Kharkiv. 2010, p.32. http://dspace.bsmu.edu.ua:8080/xmlui/handle/123456789/57 [date access 25.04.2023] (in Ukrainian)
2. Ushakova GO, Babets YV, Kyrychenko SV et al. Molekulyarni mekhanizmy rozvytku entsefalopatiyi. [Molecular mechanisms of encephalopathy development: monograph]. Dnipro. 2017, p.203. https://www.biochemistry-dnu.dp.ua/wp-content/downloads/metodichki/ushakova_molekularni-mehanizmy-encephalopatiji.pdf [date access 25.04.2023] (in Ukrainian)
3. Mishchenko TS, Mishchenko VM, Zdesenko IV. Porushennya snu i yoho korektsiya u khvorykh na dystsyrkulyatornu entsefalopatiyu. [Treatment of patients with dyscirculatory encephalopathy against the background of metabolic syndrome]. Ukrayins'kyy psykhonevrolohichnyy visnyk. 2019; 12. doi:10.26565/2312-5675-2019-12-05. (in Ukrainian)
4. Yarkova SV, Revenko AV, Demchenko AV. Klinichna efektyvnist' metabolichnoyi terapiyi v likuvanni khvorykh na khronichnu ishemiyu holovnoho mozku. [Clinical effectiveness of metabolic therapy in the treatment of patients with chronic cerebral ischemia]. Man'kovs'kyy zhurnal nevrolohiyi. 2018: 6(2):39-43. http://dspace.zsmu.edu.ua/handle/123456789/16238 [date access 25.04.2023] (in Ukrainian)
5. Dudnyk S. Sertsevo-sudynni zakhvoryuvannya v Ukrayini: prohnozy — nevtishni. [Cardiovascular diseases in Ukraine: forecasts are disappointing]. Tvoye zdorov'ya. 2015. https://www.vz.kiev.ua/.../sercevo-sudinni-zaxvoryuvannya-v-ukrayini-prognozi-nevt... [date access 25.04.2023] (in Ukrainian)
6. Lysyuk IV, Khristenko OM. Pidlitkovyy alkoholizm: sotsial'ni faktory ryzyku. [Adolescent alcoholism: social risk factors]. Medsestrynstvo. 2017;2:36-38. doi:10.11603/2411-1597.2017.2.8506. (in Ukrainian)
7. Brynzyuk M. Formuvannya zdorovoho sposobu zhyttya pidlitkiv: problemy i perspektyvy. [Formation of a healthy lifestyle of teenagers: problems and prospects Innovative processes in the development of preschool and primary education]. 2018, p.64. http://eprints.zu.edu.ua/id/eprint/26748 [date access 25.04.2023] (in Ukrainian)
8. Townshend JM, Duka T. Patterns of alcohol drinking in a population of young social drinkers: a comparison of questionnaire and diary measures. Alcohol and Alcoholism. 2002; 37(2):187-192. doi: 10.1093/alcalc/37.2.187.
9. Roschupkina TM. Suchasni pohlyady na vyvchennya alkoholizatsiyi naselennya ta yiyi naslidkiv. [Modern views on the study of alcoholism in the population and its consequences]. Medychna psykholohiya. 2016;1:77-84. http://www.mps.kh.ua/archive/pdf/2018/2/17/Modern_views_on_the_study_of_population_alcoholation_and_its_consequences.pdf [date access 25.04.2023] (in Ukrainian)
10. Kramareva OG, Volosovets AO, Maznichenko OYu et al. A clinical case of operative and intensive treatment of an open craniocerebral injury according to the «Management of severe traumatic brain injury» protocol. Ukrayins'kyy medychnyy chasopys. 2023. doi: 10.32471/umj.1680-3051.153.239596.
11. Dubinski D, Kolesnyk V. War in Ukraine: a neurosurgical perspective. Acta Neurochir (Wien). 2022; 164(12):3071-3074. doi: 10.1007/s00701-022-05388-3.
12. Maas AIR, Menon DK, Manley GT et al. InTBIR Participants and Investigators. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. Lancet Neurol. 2022; 21(11):1004-1060. doi: 10.1016/S1474-4422(22)00309-X.
13. Saliy ZV. Khronichna posttravmatychna entsefalopatiya. Pohlyad na problemu. [Chronic post-traumatic encephalopathy. view of the problem]. Visnyk medychnykh i biolohichnykh doslidzhen'. 2020; 3:167–174. doi:10.11603/bmbr.2706-6290.2020.3.11528.
14. Robba C, Crippa IA, Taccone FS. Septic encephalopathy. Curr Neurol Neurosci Rep. 2018; 18(12):82. doi: 10.1007/s11910-018-0895-6.
15. Ludlow M, Kortekaas J, Herden C et al. Neurotropic virus infections as the cause of immediate and delayed neuropathology. Acta Neuropathol. 2016; 131(2):159–184. doi: 10.1007/s00401-015-1511-3.
16. Moskalyuk VD, Boyko YuI, Randyuk YuO et al. Klinichni proyavy vil-asotsiyovanoho urazhennya tsentral'noyi nervovoyi systemy ta vplyv na nykh antyretrovirusnoyi terapiyi. [Clinical manifestations of HIV-associated lesions of the central nervous system and the effect of antiretroviral therapy on them]. Infectious diseases. 2020; 2(100):73-76. doi: 10.11603/1681-2727.2020.2.11288. (in Ukrainian)
17. Stepanova EI, Kolpakov IE, Kondrashova VH et al. Efficiency of endothelial dysfunctioncorrection in children-residents of radioactively contaminated areas using the method of intermittent normobaric hypoxi therapy. 2016(21):322-335. doi:10.33145/2304-8336-2016-21-322-335.
18. Fishchuk LE, Gorovenko NG. Genetic polymorphisms of the renin-angiotensin system in breast cancer patients. Exp Oncol. 2013; 35(2):101-4.
19. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019; 18(5):459-480. doi: 10.1016/S1474-4422(18)30499-X.
20. Erkkinen MG, Berkowitz AL. A Clinical Approach to Diagnosing Encephalopathy. Am J Med. 2019; 132(10):1142-1147. doi: 10.1016/j.amjmed.2019.07.001.
21. Frontera JA, Melmed K, Fang T. Toxic Metabolic Encephalopathy in Hospitalized Patients with COVID-19. Neurocrit Care. 2021; 35(3):693-706. doi: 10.1007/s12028-021-01220-5.

22. Petrenko MS, Grabovetsky SA. Analysis of the relationship between the concentration of iron in the basal nuclei and cognitive impairment in patients with hypertensive and atherosclerotic encephalopathy during the study of changes in the magnetic susceptibility of the subcortical structures of the brain. East European Journal of Neurology. 2018; 3(21):19-25. doi:10.33444/2411-5797.2018.3(21).19-25.
23. Wan C, Zong RY, Chen XS. The new mechanism of cognitive decline induced by hypertension: High homocysteine-mediated aberrant DNA methylation. Frontiers in cardiovascular medicine. 2022; 9:928701. doi:10.3389/fcvm.2022.928701.
24. McHugh J. ACE inhibitors preserve cognitive function. Nat Rev Rheumatol. 2018; 14(11):623. doi: 10.1038/s41584-018-0102-9.
25. Zhou T, Zheng Y, Sun L et al. Microvascular endothelial cells engulf myelin debris and promote macrophage recruitment and fibrosis after neural injury. Nat Neurosci. 2019; 22(3):421-435. doi: 10.1038/s41593-018-0324-9.
26. Zagorodnyi MI, Svintsitskyi IA. Endotelial'na dysfunktsiya pry arterial'niy hipertenziyi: suchasni pohlyady na prychyny y mekhanizmy rozvytku, diahnostyku ta korektsiyu. [Endothelial dysfunction in arterial hypertension: modern views on causes and mechanisms of development, diagnosis and correction]. Praktychnyy likar. 2013; 2:17-27. http://nbuv.gov.ua/UJRN/PraktLik_2013_2_6 [date access 25.04.2023] (in Ukrainian)
27. Li Y, Zhang Z, Deng L et al. Genetic variation in angiotensin converting-enzyme affects the white matter integrity and cognitive function of amnestic mild cognitive impairment patients. J Neurol Sci. 2017; 380:177–81. doi: 10.1016/j.jns.2017.06.026.
28. Zhang Z, Deng L, Bai F et al. ACE I/D polymorphism affects cognitive function and gray-matter volume in amnestic mild cognitive impairment. Behav Brain Res. 2011; 218(1):114–20. doi: 10.1016/j.bbr.2010.11.032.
29. Taylor WD, Benjamin S, McQuoid DR et al. AGTR1 gene variation: association with depression and frontotemporal morphology. Psychiatry Res. 2012; 202(2):104–9. doi: 10.1016/j.pscychresns.2012.03.007.
30. Zannas AS, McQuoid DR, Payne ME et al.Association of gene variants of the renin-angiotensin system with accelerated hippocampal volume loss and cognitive decline in old age. Am J Psychiatry. 2014;171(11):1214–21. doi: 10.1176/appi.ajp.2014.13111543.

ORCID and contributionship:
Khrystyna Duve: 0000-0001-9036-2459 [A-D, F]
Olena Tkachenko: 0000-0001-5486-4996 [A, B, E, F]
Shkrobot Svitlana: 0000-0002-5115-0207 [A, B, E, F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Khrystyna Duve
I. Horbachevsky Ternopil National Medical University
14 Troleybusna St., 46000 Ternopil, Ukraine
e-mail: duve.khrystyna@gmail.com

Received: 30.04.2023
Accepted: 15.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

MANIFASTATIONS OF COMPLICATION IN THE ANEMIC SYNDROME IN NON-HODGKIN LYMPHOMAS OF TRANSCARPATIA

DOI: 10.36740/WLek202311120

Myroslava Yu. Popovych
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To investigate the main indicators characterising clinical and haematological manifestations in patients with NHL complicated by anaemia at the stages of the disease development for optimisation of diagnostics and prognosis of the disease.

Materials and methods: Data from 40 patients were analysed, 40 (100%) of whom were diagnosed with NHL complicated by anaemia. The severity of anaemia was divided into: mild anaemia - haemoglobin 10 - 12 g/dl; moderate anaemia - 8 - 10 g/dl; severe anaemia - 6.5 - 8 g/dl; life-threatening anaemia - below 6.5 g/dl. Patients were divided into 2 groups: the first group included 27 patients with haemoglobin levels of 100-120 g/l; the second group consisted of 13 patients with haemoglobin levels of 80 to 99 g/l. All patients were examined using clinical, laboratory, instrumental and special research methods. Statistical processing of the results was performed using the methods of variation statistics using the Microsoft Excel XP.

Results: The course of lymphoproliferative diseases in which the proliferation of a malignant clone is often complicated by anaemia. The prognostically unfavourable factors were identified. The article discusses possible pathophysiological mechanisms of the identified changes.

Conclusions: The results of the study demonstrate that as the disease progresses and the tumour mass increases, NHL patients tend to develop more severe anaemia. The leading cause of anaemia in patients is the infiltration of the bone marrow by tumour cells and their negative impact on erythropoiesis.

KEY WORDS: non-Hodgkin's lymphoma, anaemia, diagnosis, treatment, Transcarpathian region

Wiad Lek. 2023;76(11):2469-2473

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is an actual problem of modern haematological and oncological practice, as in recent years there has been a constant trend towards their growth [1,2]. NHLs are malignant formations of lymphoid tissue, which are a heterogeneous group of lymphoproliferative diseases that differ in morphological variants and clinical features associated with the molecular biological characteristics of the tumour process and different prognosis. The identification of different NHL variants is based on the ultrastructural, molecular biological, and immunophenotypic characteristics of cells and is most often the main factor in the course of the disease [1,2]. According to the Ministry of Health and the National Academy of Medical Sciences of Ukraine (2020), the prevalence of NHL in the adult population in our country was 22.85 cases per 100,000 adults, and the incidence was 3.25 cases per 100,000 adults [3].

One of the most aggressive variants of NHL is B-cell lymphoma. Its proportion is 35% of all NHL cases, and in 30% of cases it is characterised by a recurrent course [4].

Numerous recent studies have shown that the R-CHOP regimen is currently the gold standard in the treatment of patients with diffuse B-cell non-Hodgkin's lymphoma (DBCL). The development of this regimen has shown progress in prolonging remission in patients with DBCL, but studies to modify this regimen are ongoing [5-7].

Optimisation of treatment is carried out in the direction of changing the number of polychemotherapy (PCT) courses and their duration for people at high risk of toxic complications, among whom the studies primarily include elderly patients. The effectiveness of the newly developed regimens is assessed primarily by the response to the course of chemotherapy, the duration of the relapse-free period, and the frequency of toxic complications, as they worsen the prognosis and may cause the patient's death [2, 8-9].

There is a serious obstacle that limits the effectiveness of the main chemotherapy regimens - their high toxicity, which affects both the tumour tissue and the body as a whole. A decrease in the effectiveness of cytostatic treatment forces the haematologist to

Table I. Clinical and laboratory characteristics of the examined patients (absolute number (%), M±m)

Clinical and laboratory parameters	Patients with NHL (n=40)
Gender.	
men	20 (50,0)
women	20 (50,0)
Age, years	
Range.	24-78
mean value ± std. dev.	57,85±14,29
Median	60
Anthropometric indicators	
Body weight	76,97±8,84
Height	174,47±6,29
Body surface area	1,92±0,12
The stage of the disease	
I	24 (60,0)
II	10 (25,0)
III	3 (7,5)
IV	3 (7,5)
B-symptoms (n=29)	29 (72,5)
Local forms Number of affected lymph node groups	1 g - 7 (17.5) 2 g - 8 (20.0) 3 g - 5 (12.5) 4 g - 7 (17.5) none - 13 (32.5)
Generalised forms	
were	9 (22,5)
was not	31 (77,5)
Involvement of the authorities	
Lungs	7 (17,5)
CNS	1 (4,0)
Bone marrow	0 (0)
Liver	1 (4,0)
Spleen	2 (5,0)
Pericardium	1 (4,0)
Bones	3 (7,5)
Stomach	4 (10,0)
Red blood cells, 10^9	4,01±0,25
Haemoglobin	103,13±9,16
White blood cells, 10^9	8,27±2,62
Lymphocytes, %.	25,18±9,22
Neutrophils, %.	60,50±11,70
Platelets	241,46±84,50
Glucose, mm	4,59±0,87
Urea	7,11±2,22
Creatinine	87,57±15,18
ALT	21,71±4,78
AST	23,25±6,86
LDG	363,65±20,41
Number of cases of haematological toxicity	36 (90,0)
Number of cases of hepatological toxicity	10 (25,0)
Number of cases of nephrological toxicity	9 (22,5)
Absence of any toxicity	4 (10,0)
One type of toxicity During treatment	36 (90,0)
Two types of toxicity	10 (25,0)
Three types of toxicity	3 (7,5)
Response to chemotherapy	
Full	29 (72,5)
Partial	8 (20,0)
None	3 (7,5)

intensify treatment by transferring patients to high-dose polychemotherapy (HDP) and including additional cytostatic [8-9]. Despite the obvious relevance of this problem for haematology, few studies have been devoted to the study of clinical and haematological features of NHL with anaemic syndrome, with conflicting results of observations, insufficient number of controlled studies, lack of evidence base, and unclear data on haematological changes at different stages of the disease, which prompted us to conduct this study.

THE AIM

The aim of the study is to establish the frequency and determine the features of clinical manifestations of haematological and non-haematological toxicity in patients with NHL with anaemic syndrome to assess their prospective use as prognostic markers.

MATERIALS AND METHODS

The subject of the study was the clinical and haematological manifestations of NHL in 40 patients (20 women and 20 men), including 20 patients (8 women and 11 men) living in highland areas and 20 patients (11 women and 9 men) from lowland areas of Transcarpathia. All patients were treated in the haematology department of the Municipal Non-Profit Enterprise "Andriy Novak Transcarpathian Regional Clinical Hospital" of the Transcarpathian Regional Council and were under outpatient care or registered at the outpatient clinic of the same institution.

The diagnosis of NHL was verified based on the results of clinical, laboratory, cytological, histological and immunophenotypic data before specific treatment. The diagnosis was verified in all patients on the basis of the current generally accepted criteria for the classification of tumours of haematopoietic and lymphoid tissues of the World Health Organization (2001 and 2008). In order to conduct the planned research, inclusion criteria were determined. The inclusion criteria for patients were the absence of internal organ diseases and organ failure (cardiac, pulmonary, hepatic and renal), HIV infection and other infectious diseases, and a decrease in leukocytes that required a change in the number and duration of chemotherapy courses, especially for older patients. All patients in the study met the inclusion criteria and underwent the necessary clinical and laboratory examination. The statistical processing of the results was performed using the methods of variation statistics using the computer program Microsoft Excel XP.

RESULTS

Among the examined patients with NHL, we did not find any patients with severe (haemoglobin concentration 6.5 - 8 g/dl) or life-threatening anaemia (haemoglobin concentration below 6.5 g/dl). Mild anaemia (haemoglobin 10 - 12 g/dl) was observed in 27 patients, and moderate anaemia (haemoglobin 8 - 10 g/dl) in 13 patients. We did not find significant differences in the examined patients with NHL depending on gender ($p>0.05$), which, in our opinion, may be due to common pathogenetic mechanisms of anaemic syndrome formation.

Clinical and laboratory characteristics of patients with NHL are presented in Table I.

As can be seen from Table I, patients with NHL had foci of lymphoproliferative process in the lungs, pericardium, bones and stomach.

Clinical signs of toxicity were determined in accordance with clinical and laboratory criteria during the first four courses of radiotherapy. Based on their results, patients were divided into subgroups for statistical analysis based on the presence or complete absence of toxic manifestations and/or the type of toxic complications, as well as their total number.

Clinical manifestations of haematological, hepatological and nephrological toxicity during the first four courses of radiotherapy were analysed in all the patients studied (Table I).

In a comparative analysis of clinical and laboratory data in the examined patients, we found no differences depending on their residence in highland or lowland regions ($p>0.05$).

DISCUSSION

In patients living in the Transcarpathian region, as the disease progresses, a more severe form of anaemia is noted, which is probably due to the infiltration of tumor cells into the bone marrow. Clinical manifestations cannot be associated with patients staying in different altitude areas. The method of choice for treatment of patients with NHL is chemoradiation therapy with the use (according to indications) of targeted drugs. The possibility of individualized selection of concomitant therapy during PCT will reduce the risk of toxic complications and improve the effectiveness of PCT in patients with NHL. Treatment of patients with malignant non-Hodgkin's lymphoma involves receiving a full course of PCT treatment according to the prescribed regimens and completing it within the time frame outlined in the treatment plan. This is the main condition for long-term remission and prevention of early relapses of the disease [10]. Urbanization has caused

significant migration processes and redistribution in the social population structure - increase to 68% the size of the urban population, reflected at the level and structure of oncological incidence. It is determined that the increase in the incidence of cancer is largely due to the peculiarities of the demographic structure of Ukraine - a significant aging of the population [11]. However, the increase in the specific weight of the elderly in the demographic structure, belonging to the groups with higher incidence of NHL, must be taken into account when carrying out measures of prevention, diagnosis and dispensary supervision [11]. Diagnosis and treatment of extranodal NHLs remain one of the most difficult problems of modern oncology, which is due to the probable tendency towards the growth of extranodal NHL in recent years, the difficulty of timely detection, often the late start of therapy and, as as a result, worsening the prognosis for such patients.

CONCLUSIONS

1. The anaemic syndrome in NHL patients living in Transcarpathia was mild (67.5%) or moderately severe (32.5%). As the disease progresses and the tumour mass increases, NHL patients tend to develop more severe anaemia. The leading cause of anaemia is the infiltration of the bone marrow by tumour cells and their negative impact on erythropoiesis.
2. Further analysis of the results obtained is necessary to identify diagnostic risk markers for several types of toxicity in patients, especially taking into account the assessment of concomitant therapy, which is associated with an increased risk of toxic effects during polychemotherapy in patients with NHL.
3. The prospect of further research in this area lies in the possibility of modifying the risk of toxic complications in patients during chemoradiotherapy by means of an individualised choice of concomitant therapy.
4. Clinical and laboratory manifestations in the examined patients with NHL did not depend on their residence in highland or lowland regions.

REFERENCES

1. Greer JP, Arber DA, Glader B et al. Wintrobe's clinical haematology. 13th ed., Philadelphia: Lippincott Williams & Wilkins. 2014, p.2278.
2. Illidge T, Specht L, Yahalom J et al. Modern radiation therapy for nodal non-Hodgkin lymphoma-target definition and dose guidelines from the International Lymphoma Radiation Oncology Group. Int J Radiat Oncol Biol Phys. 2014;89(1):49-58. doi: 10.1016/j.ijrobp.2014.01.006.
3. Kryachok IA, Karamenesht EE, Kuschevoy EV et al. Dosvid zastosuvannia khimioterapii druhoi linii v likuvanni limfom [Experience of using second-line chemotherapy in the treatment of lymphoma]. Onkologiya, 2010;12(1): 61-65. https://www.oncology.kiev.ua/article/1087/opyt-primeneniya-ximioterapii-vtoroj-linii-v-lechenii-limfom [date access 09.05.2023] (In Ukrainian).
4. Michallet AS, Coiffier B. Treatment of patients with diffuse large B-cell lymphoma. Hematologia, 2009;1: 29-40. https://core.ac.uk/download/pdf/268448276.pdf [date access 09.05.2023]
5. Gaidukova SN, Vydyborets SV, Perekhrestenko TP et al. Epidemiolohiia, patohenez, molekuliarna kharakterystyka, klasyfikatsiia ta prohnoz dyfuznoi B-klitynnoi limfomy: novi pohliady na isnuiuchi problemy [Epidemiology, pathogenesis, molecular characterisation, classification and prognosis of diffuse B-cell lymphoma: new perspectives on existing problems]. Haematology. Transfusiology. Eastern Europe. 2020;6(2):217-225. doi: 10.34883/PI.2020.6.2.007. (In Ukrainian).
6. Gaidukova SN, Sivak LA. Perebih nekhodzhkins'koyi limfomy z peryferychnykh V-klityn: prohnostychne znachennya anemiyi [The course of non-Hodgkin's lymphoma from peripheral B-cells: prognostic significance of anaemia]. Gematologiya. Transfuziologiya. Vostochnaya Yevropa. 2019;5(1): 34-37. http://recipe.by/wp-content/uploads/woocommerce_uploads/2019/04/1_2019_Gematologiya.pdf [date access 09.05.2023] (In Ukrainian).
7. Ignatieva EV, Kryukov EV, Chernetsov VA et al. Analiz komorbidnosti u khvorykh na limfoproliferatyvni zakhvoriuvannia. Vplyv suputnoi patolohii na smertnist [Analysis of comorbidity in patients with lymphoproliferative diseases. Influence of comorbidity on mortality]. Gematologiya. Transfuziologiya. Vostochnaya Yevropa. 2020;6(4):436-448. doi: 10.34883/PI.2020.6.4.001. (In Ukrainian).
8. Stiff PJ, Unge JM, Cook JR et al. Autologous Transplantation as Consolidation for Aggressive Non-Hodgkin's Lymphoma. N Engl J Med. 2013; 369(18): 1681–1690. doi: 10.1056/NEJMoa1301077.
9. Sivkovich SO. Taktyka likuvannia khvorykh iz nekhodzhkinskymy limfomamy [Tactics of treatment of patients with non-Hodgkin's lymphomas]. Visnyk naukovykh doslidzhen'. 2004;1:29. https://tdmuv.com/kafedra/journals/investigation/2004_3/all.pdf#page=27 [date access 09.05.2023] (In Ukrainian).
10. Shchepotin IB, Fedorenko ZP, Haysenko AV et al. Porivnialna kharakterystyka stanu urazhennia zloiakisnymy novoutvorenniamy miskoho ta silskoho naselennia Ukrainy [Comparative characteristics of the state of damage by malignant neoplasms of the urban and rural population of Ukraine]. Klinichna onkolohiya. 2011;1(1): 6-8. https://www.clinicaloncology.com.ua/article/278/porivnyalna-xarakteristika-stanu-urazhennya-zloyakisnimi-novoutvorennyami-miskogo-ta-silskogo-naselennya-ukraini [date access 09.05.2023] (In Ukrainian).
11. Balashova OI, Basarab MYu, Jhanjulia AO et al. Nekhodzhkinski limfomy z ekstranodalnoiu lokalizatsiieiu - dyferentsialna diahnostyka ta likuvannia [Non-Hodgkin's lymphomas with extranodal localization - differential diagnosis and treatment]. Naukovyy visnyk Uzhhorods'koho universytetu, seriya «Medytsyna». 2011;3(42): 9-11. (In Ukrainian).

The article is done within the complex research framework for «Prevalence of risk factors, features of the clinic, diagnosis and treatment of comorbid pathologies of internal organs in the inhabitants of Transcarpathia depending on the height of residence above sea level» state registration number 012015U104561.

ORCID and contributionship:
Myroslava Yu. Popovych: 0000-0001-7424-8365 [A-F]

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR

Myroslava Yu. Popovych
Uzhhorod National University
1 Narodna Sq., 88000 Uzhhorod, Ukraine
tel: +380996319061
e-mail: popovych.mira@ukr.net

Received: 11.05.2023
Accepted: 21.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

RECONSTRUCTION OF THE TROCHANTERIC ZONE IN PRIMARY ARTHROPLASTY OF UNSTABLE PERTROCHANTERIC FRACTURES

DOI: 10.36740/WLek202311121

Anatolii I. Kanziuba[1], Petro P. Popyurkanych[1], Vasyl V. Stoyka[1], Maksym A. Kanzyuba[2]
[1] UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE
[2] ST. JOHN PAUL II MAZOVIAN PROVINCIAL HOSPITAL, SIEDLCE, POLAND

ABSTRACT

The aim: To justify the concept and features of acetabular reconstruction during primary endoprosthesis for transcatheter fractures from the standpoint of radiological data, biomechanical calculations and intraoperative observations.

Materials and methods: A retrospective analysis of the use of primary cement arthroplasty for osteoporotic fractures of the trochanteric zone in 52 elderly and senile patients was conducted. Before implantation of the femoral component, fragments of the proximal metaphysis were fixed with cerclage tightening loops which depended on the type of fracture. For fractures 31-A2.1, 31-A2.2, 31-A2.3, reconstruction of the destroyed trochanteric zone and the walls of the bone marrow canal opening was performed using our own methodology. Finite-element modeling with the SolidWorks program was used to investigate the influence of the reconstruction of the trochanteric zone on the distribution of strain on the bone tissue around the implant under osteopenic conditions and load during single-support standing.

Results: Clinical and X-ray results were studied in 39 (74.36%) operated patients within 3 to 33 months. There were no complications associated with reconstruction of the proximal part of the femur and implantation of endoprostheses. Restoration of movements in the hip joint and full loading of the operated limb was allowed the day after surgery, depending on the patients` physical condition. The results of finite-element modeling indicate a significant reduction of the strain on the proximal metaphysis in the zone of predominant destruction of the medial and posterior walls of the bone marrow canal of the trochanteric zone reconstruction and ensuring the stability of the femoral component.

Conclusions: Clinical results and biomechanical calculations confirm the possibility and feasibility of using primary arthroplasty in unstable osteoporotic fractures of the trochanteric zone with the aim of early restoration of the support function of the damaged limb in individuals with limited physical capabilities. Reconstruction of the intertrochanteric area with a ring-shaped autograft contributes to the achievement of primary stability of the femoral component, restoration of the total femoral offset and stabilizing function of muscles around the joints.

KEY WORDS: pertrochanteric fractures, osteopenic fractures, endoprosthesis, hip arthroplasty

Wiad Lek. 2023;76(11):2474-2480

INTRODUCTION

The constant increase in the number of elderly and senile people in the world is accompanied by an increase in cases of osteoporotic fractures in the area of the hip joint [1]. About 45% of them are fractures of the trochanteric zone [2, 3]. Due to the presence of concomitant diseases in patients of this age category, the treatment of trochanteric fractures represents a complex medical, social and economic problem. In China, they are called "the last injury in life", due to the high rate of complications and mortality. Without surgical intervention, the mortality rate reaches 34.6% [4].

Modern technologies of osteosynthesis are a generally recognized standard for the treatment of fractures of the trochanteric zone, because they are efficient and relatively less traumatic [5].

However, with unstable fractures (31-A A2.2, 31-A2.3, 31-A3.3 according to the OA classification) after osteosynthesis, a high level of complications is observed (from 0.5 to 56%) depending on the type of fracture, patients' condition, quality of the reposition and fixation. The most common, about 20%, are migration of fixing structures, complications of osteosynthesis, destruction (cut out effect) of the proximal fragment by the fixator, secondary displacement of fragments, and in 2.8% of cases infectious complications are observed [1, 6]. The possibility of repeated restorative or reconstructive operations is limited due to the comorbid condition of patients, risks of complications and increased mortality [1, 7].

According to research data, primary hemiarthroplasty with unstable pertrochanteric fractures

provides more guarantees of early restoration of function of the damaged limb and, therefore, is used as an alternative to osteosynthesis [2, 8]. However, if osteoporosis and a destroyed proximal metaphysis is present, the implantation of the femoral component requires careful mechanical evaluation to ensure the primary stability of the femoral component and the entire artificial hip joint. The expediency of preliminary restoration of the acetabular area by fixing fragments of the posterior, medial (area of the calcarus and small trochanter) and external surfaces using wire, tension tapes or plates or bone plastic remains under consideration considered [9, 10].

THE AIM

The aim of this study was to justify the concept and features of acetabular reconstruction during primary endoprosthesis for transcatheter fractures from the standpoint of radiological data, biomechanical calculations and intraoperative observations.

MATERIALS AND METHODS

The study is based on the results of a retrospective analysis of the implementation of primary arthroplasty for trochanteric fractures in 52 patients aged 72 to 89 years (mean age – 76.34 ± 3.28 years). Among them were 38 women aged 72 to 88 years (mean age - 74.18 ± 3.46 years) and 14 men aged 73 to 89 years (mean age - 76.4 ± 4.14 years). The periods of hospitalization for patients in the traumatology departments were 1 to 5 days after the injury. The injuries were of low-energy - as a result of falling on the thigh while walking or from a standing position.

As per the radiographic examination data according to the OA classification, 17 of the injured had fractures of the proximal part of the femur belonging to type 31-A1 (31-A1.2, 31-A1.3), 28 had multifragmentary fractures of type 31-A2 (31 -A2.1, 31-A2.2, 31-A2.3), and 7 – type 31-A3 fractures (31-A3.1, 31-A3.3).

For fractures 31-A2.2 and 31-A2.3, spiral computed tomography (SCT) of the hip joints was performed before arthroplasty.

The criteria for selecting patients were age, the presence of an osteopenic condition, the severity of the comorbid condition due to concomitant diseases (including the presence of two or more osteoporotic fractures of different localization for 12 patients), which determined the impossibility of mechanical unloading the damaged limb after surgery. Among the selection criteria in 13 patients, we included excessive body weight according to the body mass index - the average value was 27.31 ± 2.41 (from 26.18 to 32.33).

Arthroplasty was performed 3-9 days after the injury (average time 5.2 ± 1.4 days). In all cases, external-anterior surgical access to the hip joint and cement fixation of the femoral component were used. Endoprostheses with a standard stem were used for hemiarthroplasty in 36 patients, and with an extended stem in 16. In 5 patients, due to the presence of destructive-dystrophic changes in the damaged joint that occurred before the injury, total arthroplasty was performed.

In addition to hemiarthroplasty of the hip joint, 6 patients underwent simultaneous interventions in connection with concomitant injuries during a single anesthetic procedure: osteosynthesis for fractures of the proximal metaphysis of the humerus – 2, distal metaepiphysis of the forearm bones – 3, ulnar condyle – 1.

For all patients, fragments of the proximal metaphysis were fixed with cerclage tightening loops, the nature of which depended on the type of fracture, before the implantation of the femoral component. In case of fractures 31-A2.1, 31-A2.2, 31-A2.3, in order to ensure the primary stability of the stem of the endoprosthesis, as well as to restore the optimal alignment between the proximal part of the femur and the pelvis, before the implantation of the femoral component, the destroyed trochanteric area section of the calcar and bone walls of the entrance to the bone marrow canal were reconstructed. By means of numerical analysis on a mathematical 3D model, the stress-deformed state in the proximal part of the femur around the leg of the endoprosthesis was investigated under the conditions of the presence of an osteoporotic pertrochanteric fracture and in due to the functional load of the limb. According to the modeling conditions, the bone fragments of the trochanteric area are pre-connected with a cerclage wire followed by cement fixation of the implant. In the first option, implantation is performed without restoration of the intertrochanteric area and calcar (Fig. 1b). The second option includes the reconstruction of the intertrochanteric area with a ring-shaped autograft taken from the base of the proximal fragment, which is removed in accordance with the proposed method of surgery (Fig. 1c). The models were built in the SolidWorks program [11]. The stress state analysis was carried out in the ANSYS Workbench [GMN] program. The Mises stress test was chosen to evaluate the stress state.

The impact of the body mass without the investigated limb (single-support standing) and the impact of the muscular-ligamentous apparatus in

Table I. The value of the strained-deformed state (Mises stress) at the control points of the calculation models

Types of calculated models with an implanted femoral component of an endoprosthesis during single-support standing	Value of SDS (Mises stress) at control points (MPa)							
	1	2	3	4	5	6	7	8
	the dissection of the femur in the frontal plane				on the surface of the femur			
Fracture-free model for intact bone tissue	10	2.2	1.9	10	11.2	13.5	16.2	14.3
Model with osteoporotic bone tissue, trans-acetabular fracture, implanted femoral component without inter-acetabular reconstruction	14.7	6	13.5	13.8	8.3	12.8	9	6.9
The model with osteoporotic bone tissue, with a transtrochanteric fracture, with reconstruction of the intertrochanteric area with a ring-shaped graft	6.3	4.9	12.7	13.8	4.2	11.5	8.9	6.7
Changes in the strain level (%)	42.9	18.3	5.3	0	49.4	10.2	1.1	2.9

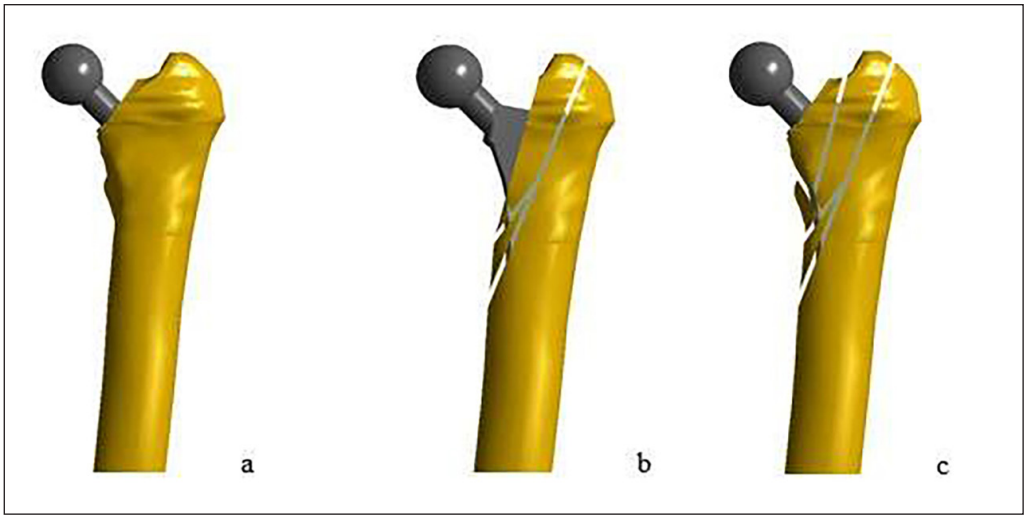


Fig. 1. Calculation models of the left femur: a) model of the femur without fracture, unchanged bone; b) a model of a multifragmentary pertrochanteric fracture, osteoporotic bone, endoprosthesis implantation without restoration of the intertrochanteric area; c) model of a multifragmentary pertrochanteric fracture, osteoporotic bone, implantation of an endoprosthesis with plasty of the intertrochanteric area, Adams's arch and calcar with a ring-shaped graft.

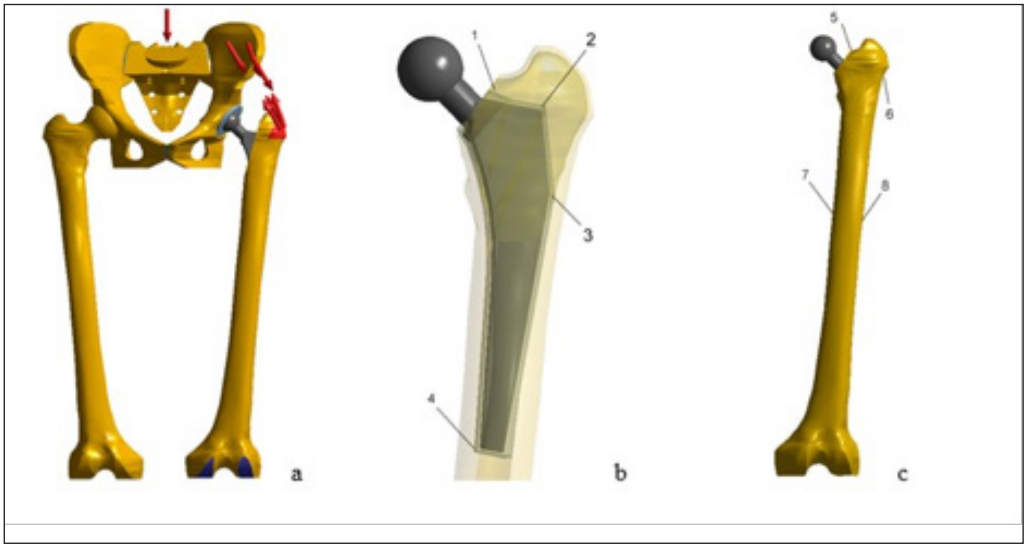


Fig. 2. Calculation model: a) Direction of action of forces and fixation of the model; b) control points in the frontal section of the femur; c) control points on the surface of the femur.

the vertical position of the body were chosen as the load. The total load is reduced to a set of equivalent forces. The points of application of forces, their magnitudes and directions of action are taken from references [12].

The comparative analysis was carried out according to the stress values at the control points - on the inner surface of the bone marrow canal in the places of contact with the lower endoprosthesis (Fig. 2b), as well as on the outer surface of the femur (Fig. 2c).

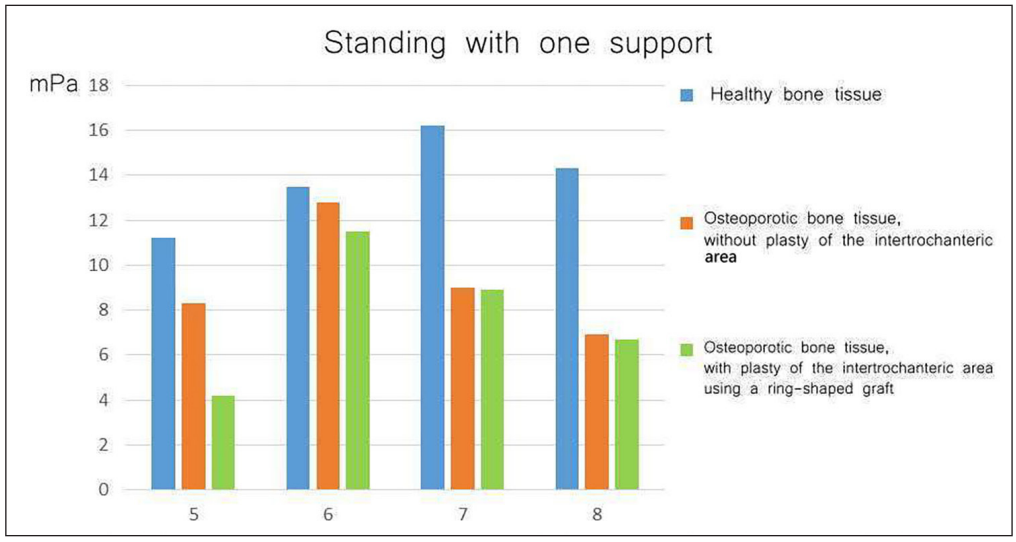


Fig. 3. Mises stress values - the surface of the femur at control points 5, 6, 7, 8.

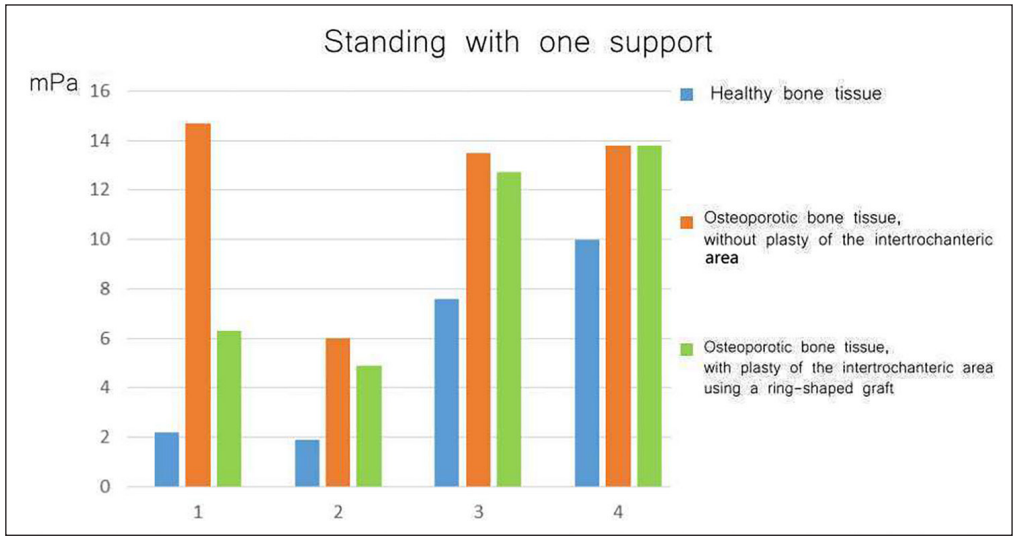


Fig. 4. Mises stress values - frontal section of the femur (control points 1, 2, 3, 4).

RESULTS

We did not observe complications related to reconstruction of the proximal part of the femur and implantation of endoprostheses, as well as infectious complications. Restoration of movements in the hip joint and full loading of the operated limb was allowed, depending on the physical condition of the patient, the day after the operation. The duration of stay in the hospital varied from 6 to 11 days (8.3 ± 1.8 days on average). On the day of discharge, all patients could move with the help of a walker or crutches without limiting the load on the operated limb.

Clinical and radiological examination was performed 3, 6 and 12 months after the day of surgery. A total of 39 patients were studied after arthroplasty for 31-A1 (4), 31-A2 (28) and 31-A3 (7) fractures. Among them, 11 patients were examined between 18 and 33 months. There were no cases of hip dislocations after hemi- and total arthroplasty. The level of mobility depended on the comorbid condition, but all patients noted the capability to apply full load on the operated limb during walking. There were found no signs of instability of the femoral component and destruction of bone tissue around the implants on control radiographs within the observation period. In 4 patients, moderate proximal displacement of fragments of the apex of the greater trochanter was noted, which did not affect the function of support and gait.

In the case of primary arthroplasty for type 31-A2 fractures, we supplement the reconstruction of the trochanteric zone with bone plasty with an annular autograft to restore the medial cortical wall (Adams' arch) and form the upper opening of the bone marrow canal. The graft is taken from the basal part of the proximal fragment that is being removed. It contains 2 to 3 cm of cortical medial wall (Adams' arch). Its size is determined by the X-ray of the contralateral hip joint during surgery planning. The transplant is placed on the edges of the fragments of the trochanteric zone previously fixed with a wire. In this way, we unsure an

established optimal level of immersion of the stem in the bone marrow channel, restoration of the offset and stabilizing function around the joint muscles.

Analysis of the strained-deformed state (SDS) of the model with an unstable osteoporotic fracture of the trochanteric zone and an implanted femoral component of the endoprosthesis shows that the most strained element of the model is the femur. The influence of the mineral density of bone tissue on the nature of the distribution and value of strains on the outer surface of the femur and on the inner surface of the bone-marrow canal around the leg of the implant was established. In the presence of osteoporosis, the strain value on the outer surface of the bone decreases (Fig. 3), but significantly increases on the inner surface around the implant (Fig. 4). Especially in the area of the destroyed proximal metaphysis. The reconstruction of the trochanteric zone, in comparison with the model without reconstruction, does not affect the nature of the SDS in the area of the diaphyseal part of the bone marrow canal. The main result of the reconstruction is a significant decrease in the strained state in the bone tissue of the restored proximal metaphysis, namely in the area of the restored medial and posterior wall of the bone marrow canal (Table I).

In the area of the entrance to the medullary canal along the medial surface, it decreased to 6.3 MPa (compared to 14.7 MPa for the model without reconstruction). On the lateral surface, the stress level decreased by 18.4% and amounted to 4.9 MPa (compared to 6 MPa for the model without reconstruction) (Fig. 4).

DISCUSSION

In the case of fractures at the level of the small trochanter, and in the absence of an osteopenic state, endoprostheses with diaphyseal fixation stems are offered, which significantly reduces the level of stress in the proximal metaphysis of the femur.

In case of unstable osteoporotic pertrochanteric fractures, ensuring the stability of the femoral component is provided by using cement fixation of the leg and reconstruction of the destroyed acetabular area. The argument in favor of hemiarthroplasty with cement fixation is the relatively low traumatic nature of the surgery and a much lower level of complications in the medium-term observation period [14, 15]. The presence of a collar (collared versions) prevents stem subsidence due to being supported by the medial-posterior cortical wall (calcar) [16].

Due to the lack or insufficient fixation of fragments in pertrochanteric fractures, there is a threat of displacement and non-union of fragments, bursitis, pain on the outer surface of the thigh, weakness of the abductors and associated lameness, and the possibility of dislocations are observed [17]. Repositioning and "rigid fixation" of fragments of the trochanteric zone during primary arthroplasty contributes to the restoration of the function of the muscles attached to them and the anatomical and physiological features of this area, since the total strain these muscles put on the trochanteric zone during regular walking is doubled, and when going up the stairs - up to 4 times more than the body weight [9, 18].

Techniques for fixing fragments of the trochanteric zone vary. Trochanteric Buttress Plates are used, which fixate the apex of the greater trochanter and are fixed with screws on the outer surface of the trochanteric zone [19]. However, the most common is fixation with a wire or steel cable, including small and large trochanters [9, 20, 21].

Computer tomography with 3D modeling provides the most accurate assessment of the nature of the destruction of the trochanteric zone and the justified choice of surgical tactics [22, 23].

Viewing the data of Li M. et al., according to the results of CT studies of unstable fractures, when the posterior-medial surface of the proximal metaphysis is destroyed, in 32.2% of cases a single fragment of the intertrochanteric ridge with a small trochanter is formed, in 42.37% they are represented by two separate fragments, and in 25.42% the separated small trochanter was presented as 3 or more fragments [24, 25].

From the standpoint of these studies, we consider fixation of fragments with a cerclage wire in the form of tightening wire loops depending on the type of fracture to be the most reasonable. In our patients with type 31-A2 fractures, we form a tension wire loop in such a way that the apex of the greater trochanter is fixed, the crossing of the wire over the interacetabular ridge and two parallel wraps above and below the lesser trochanter are created, which ensures fixation of the most destroyed posterior-medial wall of the trochanteric zone.

Unstable fractures of type 31-A3 (reverse fractures) are mainly due to destruction of the medial wall of the proximal metaphysis. This is illustrated by the requirements to ensure positive contact of the proximal and distal fragments in the area of Adam's arch - Positive medial cortex support (PVCS) when applying osteosynthesis [26]. In arthroplasty for reverse fractures, we form a tightening wire loop in such a way as to create compression tension between the fragments along the entire fracture surface.

In case of 31-A2 fractures, before the implantation of the endoprosthesis stem, various options are offered for replacing the defect of the Adams' arch and the area of the

calcar. The authors suggest filling the defect of the posterior-medial wall by impaction of bone fragments with or without wire fixation [27]. Compact autografts from the lower part of the removed head and neck are used [28], or a semicircular graft from the removed capitus [29].

Thus, the results of the calculations and gathered data confirm the biomechanical effectiveness of the reconstruction of the trochanteric zone in unstable osteoporotic trans-acetabular fractures by fixing the fragments with a tightening wire loop and plasty of the proximal opening of the entrance to the bone-medullary canal with a ring-shaped autograft with the restoration of the Adams arch and Calcar zone.

CONCLUSIONS

Despite the limited number of operated patients, the obtained clinical results, as well as the results of biomechanical calculations, confirm the possibility and feasibility of using primary arthroplasty in unstable osteoporotic fractures of the trochanteric zone with the aim of early restoration of the support function of the damaged limb in individuals with limited physical capabilities. Reconstruction of the intertrochanteric area with a ring-shaped autograft contributes to the achievement of primary stability of the femoral component, restoration of the total femoral offset and stabilizing function of muscles around the joints.

REFERENCES

1. Liu P, Jin D, Zhang C, Gao Y. Revision surgery due to failed internal fixation of intertrochanteric femoral fracture: current state-of-the-art. BMC Musculoskelet Disord. 2020;21(1):573. doi:10.1186/s12891-020-03593-8.
2. Özdemir E, Okkaoglu MC, Evren AT et al. The Cost and Consequences of Failed Osteosynthesis of Intertrochanteric Femur Fractures: A Matched Cohort Study. Indian J Orthop. 2021;55(3):629-635. doi:10.1007/s43465-020-00322-0.
3. Marks R. Hip fracture epidemiological trends, outcomes, and risk factors, 1970-2009. Int J Gen Med. 2010;3:1-17.
4. Huang G, Zhang M, Qu Z et al. Fixation options for reconstruction of the greater trochanter in unstable intertrochanteric fracture with arthroplasty. Medicine (Baltimore). 2021;100(26):e26395. doi:10.1097/MD.0000000000026395.
5. Ouyang X, Ding Yu, Yu L, Xin F et al. Comparison of the curative effects of PFNA and DHS fixation in treating intertrochanteric fractures in elderly patients. Biomed Research-tokyo. 2017;28(6);2717-2723.
6. Martinho T, Stoffel K. Treatment of Intertrochanteric Femur Fractures with Hip Arthroplasty in Older Patients: A Narrative Review of Indications and Outcomes. Medicina (Kaunas). 2021;57(8):763. doi:10.3390/medicina57080763.
7. Müller F, Galler M, Zellner M et al. Total hip arthroplasty after failed osteosynthesis of proximal femoral fractures: Revision and mortality of 80 patients. J Orthop Surg (Hong Kong). 2017;25(2):2309499017717869. doi:10.1177/2309499017717869.
8. Fahad Ul Islam M, Ahmed Qureshi O. Consequences of treatment of extracapsular fractures of hip with cemented bipolar hemiarthroplasty and dynamic hip screw fixation: A clinical study International Journal of Orthopaedics Sciences 2020;6(2):701-706. doi: 10.22271/ortho.2020.v6.i2k.2123.
9. Huang G, Zhang M, Qu Z et al. Fixation options for reconstruction of the greater trochanter in unstable intertrochanteric fracture with arthroplasty. Medicine (Baltimore). 2021;100(26):e26395. doi:10.1097/MD.0000000000026395.
10. Fichman SG, Mäkinen TJ, Safir O et al. Arthroplasty for unstable pertrochanteric hip fractures may offer a lower re-operation rate as compared to cephalomedullary nailing. Int Orthop. 2016;40(1):15-20. doi:10.1007/s00264-015-2794-7.
11. Paul M. Kurowski Engineering Analysis with SolidWorks Simulation 2012. 2012, p.475
12. Crowninshield RD, Brand RA. A physiologically based criterion of muscle force prediction in locomotion. J Biomechanics. 1981;14(11):793-801. doi: 10.1016/0021-9290(81)90035-x.
13. Vyrva OE, Golovina YaA, Karpinsky MYu et al. The study of the stress-strain state in the "implant-bone" system on the model of the allocomposite endoprosthesis of the proximal femur. Travma. 2020; 21(1). doi:10.22141/1608-1706.1.21.2020.197797.
14. Kelly M, Chen AF, Ryan SP et al. Cemented Femoral Fixation in Total Hip Arthroplasty Reduces the Risk of Periprosthetic Femur Fracture in Patients 65 Years and Older: An Analysis From the American Joint Replacement Registry. J Arthroplasty. 2023;38(7/2):S351-S354. doi:10.1016/j.arth.2023.04.039.
15. Rassir R, Schuiling M, Sierevelt IN et al. What Are the Frequency, Related Mortality, and Factors Associated with Bone Cement Implantation Syndrome in Arthroplasty Surgery?. Clin Orthop Relat Res. 2021;479(4):755-763. doi:10.1097/CORR.0000000000001541.
16. Demey G, Fary C, Lustig S et al. Does a collar improve the immediate stability of uncemented femoral hip stems in total hip arthroplasty? A bilateral comparative cadaver study. J Arthroplasty. 2011;26(8):1549-1555. doi:10.1016/j.arth.2011.03.030.
17. Frankel A, Booth RE Jr, Balderston RA et al. Complications of trochanteric osteotomy. Long-term implications. Clin Orthop Relat Res. 1993;(288):209-213.
18. Pachore JA, Shah VI, Upadhyay S, Patel SB. Primary Arthroplasty for Unstable and Failed Intertrochanteric Fractures: Role of Multi-Planar Trochanteric Wiring Technique. Hip Pelvis. 2023;35(2):108-121. doi:10.5371/hp.2023.35.2.108.
19. Ganjale SB, Gadegone W, Kothadia P. Trochanteric Buttress Plate Combined with Proximal Femoral Nail for Unstable Intertrochanteric Fractures. [Innovative Technique]. Open Journal of Orthopedics. 2018. doi: 10.4236/ojo.2018.86027.

20. Huang C, Wu X. Surgical Selection of Unstable Intertrochanteric Fractures: PFNA Combined with or without Cerclage Cable. Biomed Res Int. 2021;2021:8875370. doi:10.1155/2021/8875370.
21. Wu HF, Chang CH, Wang GJ et al. Biomechanical investigation of dynamic hip screw and wire fixation on an unstable intertrochanteric fracture. Biomed Eng Online. 2019;18(1):49. doi:10.1186/s12938-019-0663-0.
22. Kalia RB, Arora SS, Sarkar B et al. A comprehensive 3D CT based classification of intertrochanteric fracture. J Clin Orthop Trauma. 2022;30:101912. doi:10.1016/j.jcot.2022.101912.
23. Zhang Y, Sun Y, Liao S, Chang S. Three-Dimensional Mapping of Medial Wall in Unstable Pertrochanteric Fractures. Biomed Res Int. 2020;2020:8428407. doi:10.1155/2020/8428407.
24. Li M, Li ZR, Li JT et al. Three-dimensional mapping of intertrochanteric fracture lines. Chin Med J (Engl). 2019;132(21):2524-2533. doi:10.1097/CM9.0000000000000446.
25. Mei J, Pang L, Jiang Z. Strategies for managing the destruction of calcar femorale. BMC Musculoskelet Disord. 2021;22(1):460. doi:10.1186/s12891-021-04324-3.
26. Kozono N, Ikemura S, Yamashita A et al. Direct reduction may need to be considered to avoid postoperative subtype P in patients with an unstable trochanteric fracture: a retrospective study using a multivariate analysis. Arch Orthop Trauma Surg. 2014;134(12):1649-1654. doi:10.1007/s00402-014-2089-2.
27. Sinno K, Sakr M, Girard J, Khatib H. The effectiveness of primary bipolar arthroplasty in treatment of unstable intertrochanteric fractures in elderly patients. N Am J Med Sci. 2010;2(12):561-568. doi:10.4297/najms.2010.2561.
28. Vital PR, Rao S, Rao NRP. Primary cemented bipolar hemiarthroplasty with trochanteric and calcar reconstruction in unstable intertrochanteric fractures in elderly: a prospective study. J Evid Based Med Healthc. 2015;2(40):6586-6586. doi: 10. 18410 /je bmh /2015/ 898.
29. Kadam R, Gupta A, Chhallani A. Calcar reconstruction in bipolar hemiarthroplasty in elderly patients. Journal of Evolution of Medical and Dental Sciences. 2016;5(77):5701-5704. doi: 10.14260/jemds/2016/1286.

ORCID and contributionship:
Anatolii I. Kanziuba 0000-0002-8762-1330 [A-F]
Petro P. Popyurkanych 0000-0002-4809-2640 [A-D]
Vasyl V. Stoyka 0000-0003-2219-2544 [A,B,D,E]
Maksym A. Kanzyuba 0009-0002-9463-5586 [A-E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Petro P. Popyurkanych
Uzhhorod National University
71 Mynaiska st, 88000 Uzhorod, Ukraine
tel: +380666984988
e-mail: popyurkanich.petro@gmail.com

Received: 12.05.2023
Accepted: 27.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

CESAREAN SECTION IN CASES OF STRESS-COMPROMISED PREGNANCY IN WOMEN WITH UNDIFFERENTIATED CONNECTIVE TISSUE DYSPLASIA

DOI: 10.36740/WLek202311122

Volodymyr Maliar, Oksana Korchynska, Vitalii Maliar, Vasyl Maliar
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: Assessment of abdominal delivery by cesarean section in preterm pregnancies in women with undifferentiated connective tissue dysplasia.

Materials and methods: Analyzed were 3,371 cases of cesarean section deliveries in preterm pregnancies complicated by undifferentiated connective tissue dysplasia (UCTD). Based on a scoring assessment of external and visceral UCTD markers, three groups were identified: Group 1 included 466 patients with no signs of UCTD, Group 2 consisted of 798 patients with mild UCTD, and Group 3 comprised 2,107 patients with moderate to severe UCTD. The severity of connective tissue dysplasia manifestations was assessed based on external and internal signs of connective tissue dysplasia, as well as gynecological and obstetric history, indications for abdominal delivery in preterm pregnancies, and maternal and perinatal outcomes of the deliveries.

Results: It has been established that in 71.4% of patients with stress-compromised pregnancies resulting in preterm birth and delivered by cesarean section, the most common indications were: inability of the uterine scar in 23.8%, breech presentation of the fetus in 19.1%, and detachment of the normally placed placenta in 4.9%. An unfavorable factor was moderate to severe connective tissue dysplasia, which led to a 5-fold increase in the likelihood of requiring a cesarean section. In addition, severe hypoxia in newborns was significantly more frequently observed in the first minutes of life in cases of moderate and severe UCTD.

Conclusions: The conducted studies have shown that cesarean sections in cases of stress-compromised pregnancies resulting in preterm birth are performed significantly more often in cases of moderate to severe undifferentiated connective tissue dysplasia (UCTD). Moderate and severe UCTD have a substantial impact on obstetric and perinatal outcomes of deliveries, both at present and in the future.

KEY WORDS: Cesarean section, preterm labor, undifferentiated connective tissue dysplasia

Wiad Lek. 2023;76(11):2481-2484

INTRODUCTION

Over the past two decades, cesarean sections, thanks to advances in medical technology, have become one of the most common abdominal interventions in pregnant women. According to the World Health Organization (WHO), an increase in the rate of cesarean sections of more than 15% does not contribute to reducing perinatal morbidity and mortality [1]. On the contrary, deliveries by cesarean section, especially in preterm pregnancies, can lead to postpartum rehabilitation disorders in mothers and disruptions in adaptive capabilities, particularly in preterm newborns [2].

Pregnant women with undifferentiated connective tissue dysplasia (UCTD) occupy a special place in deliveries completed by cesarean section. A significant factor in this context is isthmic-cervical insufficiency as one of the forms of connective tissue dysplasia.The pathogenesis of isthmic-cervical insufficiency (ICI) is of crucial importance, and connective tissue dysplasia plays a triggering role in preterm pregnancies and premature deliveries [3].

Based on the above information, the study of this issue holds significant scientific as well as practical importance.

THE AIM

Assessment of abdominal delivery by cesarean section in preterm pregnancies in women with undifferentiated connective tissue dysplasia.

MATERIALS AND METHODS

A retrospective follow-up analysis was conducted on 23,616 cases of deliveries at the Uzhhorod City Maternity Hospital from 2012 to 2022. During this period, 10,119 pregnant women underwent cesarean section. Among them, 3,371 cases (39.9%) involved preterm births with undifferentiated connective tissue dysplasia

Table I. Distribution of patients into groups according to the scoring scale for assessing undifferentiated connective tissue dysplasia (UCTD).

Groups	Patients	Scores	The severity of (UCTD)
I	466 (13,8 %)	0-3	Absence of (UCTD)
II	798 (23,7 %)	3-6	Mild (UCTD)
III	2107 (62,5 %)	> 6	Moderate and severe(UCDT)

(UCTD). The inclusion criteria for the study were isthmic-cervical insufficiency, which served as the primary stress-compromising factor in preterm pregnancies.

The degree of connective tissue dysplasia (CTD) in patients was assessed based on the follow-up data, taking into account a combination of various external and visceral markers of undifferentiated connective tissue dysplasia (UCTD).

If there were signs of CTD, it was assessed as '1,' and its absence as '0.' Scores for each sign were summed, and based on the total score, conclusions were drawn regarding the severity of CTD according to the scale proposed by E.V. Fotin and colleagues (2021) [4].Scoring 0-3 points indicated the absence of UCTD symptoms; 3-6 points indicated mild UCTD, and >6 points indicated moderate to severe UCTD

The distribution of patients into groups based on the severity of UCTD is presented in Table I.

Statistical analysis of the research materials was conducted using Excel for Windows and Statistics 7.0 for Windows packages. Differences were considered statistically significant at $p < 0.05$.

RESULTS

A clinical-statistical analysis revealed that out of 23,616 deliveries at the third level of medical care provision for pregnant women at the Uzhhorod City Maternity Hospital from 2012 to 2022, the rate of cesarean section was 42.8% of the total number of deliveries. Out of 10,107 deliveries through cesarean section, 8,429 women (83.3%) were found to have external and visceral markers of undifferentiated connective tissue dysplasia (UCTD).Furthermore, in 3,371 cases (39.9%) of UCTD, cesarean sections were performed in pregnant women with a history of preterm stress-compromised pregnancies. In 427 cases (12.7%), it was associated with habitual miscarriages, and in 242 cases (7.8%), it occurred due to premature deliveries between 28 to 33 weeks of gestation.

Based on the follow-up data, it was determined that one of the significant stress-limiting factors in UCTD was isthmo-cervical insufficiency (ICI).

The clinical and anamnestic characteristics of the 3,371 cases of preterm cesarean section delivery in women with UCTD indicate a significant increase in extra genital pathology at moderate and severe levels of connective tissue dysplasia.

It was found that in the absence of UCTD signs (Group I), extra genital pathology was diagnosed in only 154 cases (33.1%). In cases of mild UCTD (Group II), it was present in 671 cases (84.0%), and in cases of moderate and severe UCTD (Group III), it was present in all 20,107 cases (100%).

Gynecological pathology in patients from all the mentioned groups was represented as follows: 1,459 (43.3%) women had cervical ectropion, 673 (20.0%) had endometrial pathology in the form of polyposis, 293 (8.7%) exhibited ovarian-menstrual cycle disorders, 279 (8.3%) were diagnosed with endometriosis, and 225 (6.7%) women had inflammatory diseases of the pelvis. In patients with a more pronounced UCTD (Group III), ectopic of the cervical cylindrical epithelium was more frequently detected, with a frequency of $71.9 \pm 8.3\%$. In cases of mild UCTD (Group II), this indicator of dysplasia was $31.9 \pm 5.3\%$ ($p < 0.05$).

When assessing obstetric history, it was found that for 445 (13.2%) patients, this pregnancy was their first, while for 2,926 (86.8%), it was a subsequent pregnancy. The first deliveries occurred in 1,342 (39.8%) patients, and repeat deliveries occurred in 2,029 (60.2%) patients. Cesarean section delivery in the first group occurred in 61 (13.1%) cases, while in the third group, it occurred in 1,504 (71.4%) cases, which is five times more frequent. Cesarean section was performed for preterm pregnancies with isthmo-cervical insufficiency (ICI) correction, using both surgical and combined methods. It should be noted that in patients in the second and third groups, with more pronounced UCTD, cesarean section was performed more frequently, as most patients had a complicated obstetric history - a previous cesarean section and extra genital pathology in their medical history.

The most common indications for abdominal delivery were as follows:inability of the uterine scar in 802 cases (23.8%),fetal malpresentation in the pelvis in 643 cases (19.1%),preterm separation of a normally positioned placenta in 165 cases (4.9%),placenta previa in 162 cases (4.8%),severe pre-eclampsia in 105 cases (3.1%).

When evaluating the perinatal outcomes of delivery, there was an increase in the frequency of moderate and severe asphyxia in newborns during the first minute of life when the mothers had a 2nd and 3rd-degree severity of UCTD.

DISCUSSION

The conducted study has shown the necessity for more detailed examination of pregnant women with a history of miscarriages and preterm deliveries, especially in cases of uterine scars with UCTD. As indicated by research [5], this pathology should be considered a molecular-genetic disorder that may manifest more prominently in pregnant women.

Patients with UCTD more frequently have a complicated obstetric history, including miscarriages, preterm deliveries following cesarean sections, as demonstrated in our study. Some authors even categorize a group of obstetric markers of UCTD, including isthmo-cervical insufficiency, weak labor activity, and preterm labor.

In the study of the possible role of UCTD in women who had cesarean section deliveries due to stress-compromised pregnancies with a history of miscarriages and preterm deliveries, it was found that, with the same prevalence of the UCTD phenotype, the degree of connective tissue dysplasia plays a significant role. This observation is supported by other authors as well [6].

"Operated uterus," as shown by our research, is one of the factors that determines a less favorable perinatal prognosis in UCTD with moderate and severe degrees of connective tissue dysplasia in women with uterine scars due to stress-compromised pregnancies with a history of miscarriages and preterm deliveries. The clinical manifestation of these processes includes an increase in the frequency of incomplete uterine scarring, preterm separation of normally positioned placentas, and chronic intrauterine fetal hypoxia, especially in cases of moderate and severe UCTD. The number of women with placental abruption, a complication directly related to the functional state of the uterus, is significantly higher in cases of moderate and severe UCTD.

The obtained data suggest that for optimizing perinatal outcomes in women with a history of UCTD, there is a rationale for conducting secondary prevention based on the personalized of pre-gravid preparation, in-depth assessment of the psychological and emotional state with the involvement of a psychologist. This is supported by other authors as well [7-9].

Therefore, the data obtained allow us to emphasize that the presence of uterine scars, especially in the context of stress-compromised pregnancies with a history of preterm deliveries, creates additional risks for uterine rupture, placental dysfunction, and the development of fetal distress.

CONCLUSIONS

The conducted studies have shown that cesarean sections in cases of stress-compromised pregnancies resulting in preterm birth are performed significantly more often in cases of moderate to severe undifferentiated connective tissue dysplasia (UCTD). Moderate and severe UCTD have a substantial impact on obstetric and perinatal outcomes of deliveries, both at present and in the future.

REFERENCES

1. Kesarevo sechenie dolzhno vypolnyat'sya tol'ko pri nalichii medicinskih pokazanij.[Caesarean section should only be performed if there is a medical indication]. Press-realiz.VOZ.-2015. https://www.who.int/ru/news/item/09-04-2015-caesarean-sections-should-only-be-performed-when-medically-necessary. [date access 23.05.2023] (In Russian)
2. Huang X, Lei I, Tan H. Cesarean delivery for first pregnancy and neonatal morbidity in second pregnancy. European Journal Of Obstetrics, Gynecology A. Reproductive Biology. 2011;158(2): 201-208. doi:10.1016/j.ejogrb.2011.05.006.
3. Au Sh, Cullinane F, Savarirayan R, Grover SR. Midtrimester pregnancy loss and cervical incompetence-Is there an association with connective tissue laxity? Open Journal of Obstetrics and Gynecology. 2014; 4(1): 28-32. doi: 10.4236/ojog.2014.41006.
4. Fotina EV, Zakhirova RR, Aleksenkova MV, Panina OB. Displaziya soedinitel'noj tkani v geneze istmiko-cervikal'noj nedostatochnosti. [Dysplasia of connective tissue in the genesis of clinical cervical insufficiency]. 2021. doi: 10.17749/2313-7347/ob.gyn.rep.2021.131. (in Russian)
5. Zabolotnov VA. Chastota kesarevykh roztyniv ta obgruntovanist vyboru metodu rozrodzhennia. [The frequency of cesarean sections and the validity of the choice of delivery method]. Zbirnyk naukovykh prats Asotsiatsii akusheriv-hinekolohiv Ukrainy. 2008, . http://aagu.com.ua/publisher/downloads/zbirnyk_2012_1.pdf. [date access 23.05.2023] (in Ukrainian)
6. Nazarenko LH, Nedorezova KM, Dubrova L. Stan fetoplatsentarnoho kompleksu u zhinok z kesarevym roztynom v anamnezi [The state of the fetoplacental complex in women with a history of caesarean section]. Zbirnyk naukovykh prats Asotsiatsii akusheriv-hinekolohiv Ukrainy. K: «Polihraf. 2014. http://www.irbis-nbuv.gov.ua/cgi bin/irbis_nbuv/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=znpaagu_2014_1-2_74. [date access 23.05.2023] (in Ukrainian)
7. Sammaritano LR, Bermas BL, Chakravarty EE et al. American College of Rheumatology Guideline for the Management of Reproductive Health in Rheumatic and Musculoskeletal Diseases. Arthritis Care Res. (Hoboken). 2020;72:461–488. doi: 10.1002/acr.24130.
8. Tani C, Monacci F, Elefante E et al. Pregnancy and Undifferentiated Connective Tissue Disease: Outcome and Risk of Flare in 100 Pregnancies. Rheumatolog(Oxford). 2020;59(6): 1335–1339. doi:10.1093/rheumatology/kez440.

9. Bermas BL. Safety of Rheumatic Disease Medication Use during Pregnancy and Lactation. 2023. https://medilib.ir/uptodate/show/7998. [date access 23.05.2023]

ORCID and contributionship:
Oksana Korchynska: 0000-0001-7265-4829 [A-C]
Vasyl Maliar: 0000-0001-9950-5014 [B,C]
Vitalii Maliar: 0000-0002-1310-535X [D,E]
Volodymyr Maliar: 0000-0003-0350-3255 [E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Vasyl Maliar
Uzhhorod National University
1 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380976645458
e-mail: mvasa@ukr.net

Received: 26.05.2023
Accepted: 27.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

IMMUNOLOGICAL DISORDERS AND COLON DYSBIOSIS IN OBESE PATIENTS WITH HYPOTHYROIDISM

DOI: 10.36740/WLek202311123

Yelyzaveta S. Sirchak, Mikola M.Voloshin, Ivan I. Kohutych, Oksana M. Moskal, Vasyl V. Palapa
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To investigate the peculiarities of immunological changes and their relationship with colon dysbiosis in obese patients with HT.

Materials and methods: The examined patients included 48 patients with HT and obesity (group 1) and 34 patients with obesity (group 2). Patients underwent fecal analysis for dysbiosis. The levels of complement, namely C3 and C4 and the concentration of immunoglobulins (IgA, Ig M, IgG) were determined by means of chromogenic analysis.

Results: During the clinical examination, constipation and flatulence were more often diagnosed in patients of group I (58.3% and 66.7%, respectively - $p<0.001$), while in patients of group 2 with increased BMI without thyroid dysfunction, a tendency to diarrhea was more often found, accompanied by periodic pain along the colon (50.0% and 32.3% of patients, respectively - $p<0.001$). Changes in the immunological status of patients in both groups were found. In patients with HT and increase of BMI an increase in serum IgA, IgM, IgG levels were found. An increase in serum immunoglobulins (A, M and G) was also diagnosed in group 2 of examined patients too.

Conclusions: 1. In patients with obesity decrease in the concentration of *Bifidobacterium, Lactobacillus* and increase in the number of *Staphylococcus*, *Clostridium*, *Proteus* and *Klebsiella* were detected, which is more pronounced in patients with a combination of obesity and hypothyroidism. 2. Impairment distinct of immunological status in patients with hypothyroidism and obesity was diagnosed, which was manifested by increased levels of immunoglobulins, namly (A, M, G), as well as a decrease in blood serum complements (C3, C4). 3. The level of IgA, G directly depends on the decrese of *Bifidobacterium*, *Lactobacillus* and increse of *Staphylococcus*, *Clostridium* and *Klebsiella* in patients with obesity, which is more pronounced in patients with a combination of obesity and hypothyroidism.

KEY WORDS: hypothyroidism, obesity, body mass index, immunological disorders, colon dysbiosis

Wiad Lek. 2023;76(11):2485-2490

INTRODUCTION

Obesity is gaining increasing attention around the world and is seen as a significant public health problem due to its widespread negative effects on human health, such as an increased risk of diabetes, cardiovascular disease and other internal organ diseases [1]. Due to the increasing prevalence of obesity worldwide, it is important to determine the prevalence of obesity-related conditions, including thyroid dysfunction [2]. Obesity and hypothyroidism (HT) are two closely related pathological conditions. Many researchers believe that hypothyroidism is the cause of obesity. However, the cause-and-effect relationship between the two is ambiguous. There is a growing body of evidence that changes in thyroid-stimulating hormone (TSH) may well be secondary to obesity [3].

In patients with a combination of hypothyroidism and obesity, research for possible exacerbating mechanisms, including the study of immunological processes formed against a background of impaired gut microbiota, is currently an important link in both experimental and clinical medicine.

THE AIM

The aim of this work was to investigate the peculiarities of immunological changes and their relationship with colon dysbiosis in obese patients with HT.

MATERIALS AND METHODS

The study was conducted at the Department of Propaedeutics of Internal Medicine of Uzhhorod National University. We examined 48 patients with HT and obesity or overweight (1 group of patients).This research work was conducted with the consent of patients, and its methodology was in line with the Helsinki Declaration (1975, 1983) and Ukrainian legislation.

Table I. Distribution of examined patients with depending on BMI

Indicator	Examined patients	
	group 1 (n=48)	group 2 (n=34)
Overweight (BMI: 25.0–29.9)	14.6 %	14.7 %
Class 1 obesity (BMI: 30.0 - 34.9)	31.2 %	32.3 %
Class 2 obesity (BMI: 35.0 – 39.9)	41.7 %	41.2 %
Class 3 obesity (BMI: over 40,0)	12.5 %	11.8 %

Table II. Clinical signs of intestinal lesions in the examined patients

Clinical symptoms	Examined patients	
	group 1 (n=48)	group 2 (n=34)
Constipation	58.3 %**	12.5 %
Diaarrhoea	22.9 %	50.0 %*
Flatulence	66.7 %**	23.5 %
Pain along the colon	16.7 %	32.3 %*

Note: the differences between the indicators in patients of groups 1 and 2 are significant: * - $p<0.01$; ** - $p<0.001$.

Patients of 1 group were aged from 18 to 70 years, the average age was 44.7 ± 6.5 years; there were 28 females (58.3 %) and 20 males (41.7 %). The comparison group (2 group) included 34 patients with and obesity and normal levels of thyroid hormones (among them were 20 females (58.8 %) and 14 males (41.2 %) with the average age of 43.8 ± 4.7 years. The control group consisted 30 healthy individuals (among them were 56.7 % females and 43.3 % males with the average age of 45.1 ± 5.8 years).

Patients were examined using a general clinical method. According to WHO recommendations, patients were divided by BMI, with BMI of 18.5-24.9 corresponding to normal weight; 25.0-29.9 to overweight; 30.0-34.9 to class 1 obesity; 35.0-39.9 to class 2 obesity; 40.0 and more to class 3 obesity [4].

All patients underwent ultrasoundwere examination. Standard general and biochemical blood serum tests were performed with a focus on lipid and carbohydrate metabolism. All patients were assigned the serum levels of thyroid hormones (free trioxine (T4), triiodothyronine (T3)) and TSH determined.

The diagnosis of DMD was made in accordance with the NICE guidelines (National Institute for Health and Care Excellence, 2019), the European Thyroid Association (ETA) and American Thyroid Association (ETA) Guidelines - 2012-2014. The criteria for evaluating unified clinical protocols and local protocols were also taken into account[5, 6]. The faeces were examined for dysbiosis. The severity of intestinal dysbiosis was assessed according to the classification of Kuvaieva IB, Ladodo KS (1991 year).

Complement (C3, C4) and immunoglobulin (Ig) A, M, G levels were studied by means of chromogenic analysis on the Sysmex 500 and 560 (Japan), using Siemens reagents.

The exclusion criteria were: patients with normal body weight, hyperthyroidism.

The statistical calculation of patient outcomes was performed using the computer program STATISTICA 10.0 (StatSoftInc, USA).

RESULTS

After an anthropometric examination, the examined patients were divided according to BMI (Table I).

According to the results obtained, the distribution of the examined patients in groups 1 and 2, depending on BMI, was homogeneous.

The analysis revealed predominantly class 2 and class 1 obesity in patients of both groups. Overweight was observed in 14.6-14.7 % of the subjects, and Class 3 obesity in 12.5-11.8 % of patients, respectively.

Clinical examination of all patients in both groups revealed complaints indicating changes in the intestines, with the vast majority of patients in group I having complaints of constipation. The results are shown in Table II.

During the clinical examination, constipation and flatulence were more often diagnosed in patients of group I (58.3% and 66.7%, respectively - $p<0.001$), while in patients of group 2 with increased BMI without thyroid dysfunction, a tendency to diarrhea was more often found, accompanied by periodic pain along the colon (50.0% and 32.3% of patients, respectively - $p<0.001$).

Colon dysbiosis was detected in the examined patients of both groups. This was reflected in a decrease in the number of *Bifidobacterium, Lactobacillus,*

Table III. Changes in the quantitative and qualitative composition of the colonic microflora in the examined patients and control group in the examined patients and control group

Indicator	Examined patients	
	group 1 (n=48)	group 2 (n=34)
Bifidobacterium:	Control group 100.0 % (8.44±0,17)	
frequency (%)	79.2 %*	88.2 %*+
lg CFU/g	5.91±0.12**	6.78±0.14**+
Lactobacillus:	Control group 100.0 % (6.86±0.21)	
frequency (%)	75.0 %**	85.3 %*
lg CFU /g	4.87±0.23**	5.45±0.08*+
Escherichia:	Control group 100.0 % (7.76±0.11)	
frequency (%)	79.2 %*	91.2 %+
lg CFU /g	6.03±0.12**	6.63±0.08*+
Enterococcus:	Control group 100.0 % (7.76±0.09)	
frequency (%)	52.1 %**	70.6 %*++
lg CFU /g	6.06±0.12**	6.54±0.10*+
Enterobacter:	Control group 23.3 % (1.23±0.07)	
frequency (%)	43.8 %*	61.8 %**++
lg CFU /g	3.08±0.11**	2.77±0.15**
Citrobacter:	Control group 30.0 % (1.47±0.12)	
frequency (%)	58.3 %*++	35.3 %
lg CFU /g	2.64±0.12**	2.08±0.15*
Staphylococcus:	Control group 30.0 % (3.15±0.09)	
frequency (%)	66.7 %**++	32.4 %
lg CFU /g	5.03±0.16**	4.52±0.15*
Klebsiella:	Control group 16.7 % (1.21±0.08)	
frequency (%)	50.0 %**++	29.7 %*
lg CFU /g	3.56±0.17**+	2.89±0.16**
Clostridium:	Control group 16.7 % (4.21±0.12)	
frequency (%)	45.8 %**+	29.7 %*
lg CFU /g	5.88±0.09*+	5.07±0.12
Proteus:	Control group 13.3 % (0.38±0.08)	
frequency (%)	37.5 %**+	20.6 %*
lg CFU /g	2.86±0.16***+	1.89±0.14**
Candida:	Control group 3.3 % (2.88±0.16)	
frequency (%)	25.0 %***++	11.8 %**
lg CFU /g	4.77±0.09**+	4.01±0.08*

Note: the differences between the indicators in the control group and in patients of groups 1 and 2 are significant: * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$; differences between indicators in patients of groups 1 and 2 are significant: + - $p < 0.05$; ++ - $p < 0.01$.

Escherichia, Enterococcus and an increase in the number of *Citrobacter*, *Staphylococcus*, *Klebsiella*, *Clostridium*, *Proteus* and *Candida* compared with the control group in examined patients – Table III.

More pronounced disorders in the qualitative and quantitative composition of colonic microflora were established in patients of group 1 (patients with HT and increase of BMI). At patients in group 1 a decrease in the number of *Bifidobacterium* (by 9.0 % - $p<0.05$) *Lactobacillus* (by 10.3% - $p<0.05$), *Escherichia* with normal enzymatic properties (by 12.0 % - $p<0.05$) and *Enterococcus* (by 18.5 % - $p<0.01$) were detected significantly more often compared with patients in group 2. In patients of group 1 an increase in the number of *Staphylococcus* (by 34.3 % - $p<0.01$), *Citrobacter* (by 23.0 % - $p<0.01$), *Klebsiella* (by 20.3 % - $p<0.01$), *Clostridium*

Table IV. Change in serum immunological status in the examined patients and control group

Indicator	Control group (n=30)	Examined patients	
		group 1 (n=48)	group 2 (n=34)
IgA, g/L	2.17 ± 0.08	13.15 ± 0.18***+	9.45 ± 0.09***
IgM, g/L	2.14 ± 0.06	4.97 ± 0.11**+	4.05 ± 0.07**
IgG, g/L	12.07 ± 0.11	23.48 ± 0.17**+	19.67 ± 0.10*
C3, g/L	1.48 ± 0.09	0.68 ± 0.05**	0.83 ± 0.07*+
C4, g/L	0.28 ± 0.03	0.17 ± 0.03*	0.22 ± 0.05

Note: the differences between the indicators of the control group and the examined group are significant: * - p <0.05; ** - p <0.01; *** - p <0.001; differences between indicators in patients of groups 1 and 2 are significant: + - p <0.05.

Table V. Comparison of indicators of immunological status with the species composition of the colonic microflora in the examined patients

Indicators of the microbial composition of the colon	Indicators of immunological status					
	Examined patients					
	group 1			group 2		
	IgA	IgG	C3	IgA	IgG	C3
Bifidobacterium	r=0.92; p<0.01	r=0.78; p<0.01	r=0.68; p<0.05	r=0.82; p<0.01	r=0.56; p<0.05	r=0.54; p<0.05
Lactobacillus	r=0.88; p<0.01	r=0.64; p<0.05	r=0.66; p<0.05	r=0.76; p<0.01	r=0.52; p<0.05	r=0.48; p<0.05
Staphylococcus	r=0.80; p<0.01	r=0.66; p<0.05	r=0.64; p<0.05	r=0.78; p<0.01	r=0.66; <0.05	_
Clostridium	r=0.64; p<0.05	r= 0.68; p<0.05	_	r=0.54; p<0.05	–	–
Klebsiella	r= 0.70; p<0.01	_	_	_	_	–

(by 16.1 % - p <0.05) and *Candida albicans* (by 13.2 % - p<0.01) was found significantly more often compared with the data of group 2.

Changes in colonic microflora in those examined were also accompanied by changes in immunoglobulin levels and complement parameters in serum. At the same time, more pronounced changes in the immunological status were found in patients of group I (Table IV).

Changes in the immunological status of patients in both groups were found. In patients with HT and increase of BMI an increase in serum IgA, IgM, IgG levels were found, with the most pronounced in patients of group 2. An increase in serum immunoglobulins (A, M and G) was also diagnosed in group 2 of examined patients too. A significant increase in immunoglobulin levels was found in patients with HT and obesity. Increased levels of IgA and G are evidence of activation of the humoral immune system, which was found in obese patients regardless of thyroid damage. Decreases in the parameters of the complement (C3, C4) system were also observed in the examined patients.

Statistical analysis established a relationship between the composition of the colon microflora and the immunological parameters of the body in the subjects – Table V.

The relationship between the number of bifidus and lactobacilli and the level of IgA, IgG and the level of complement C3 in patients with both group were found. There was a correlation between the number of Staphylococcus in colon microflora and the level of immunoglobulins (A, G) and complement C3 in patients of group 1. Clostridium levels also affect IgA levels in the serum in both groups of examined patients and with level of IgG in patients of group 1. There was also a correlation between Klebsiella and IgA levels in patients of group 1.

DISCUSSION

The gut microbiota plays an important role in the pathogenesis and progression of obesity. Abdominal obesity significantly increases the risk of metabolic syndrome, cardiovascular disease, diabetes mellitus, and non-alcoholic fatty liver disease [7]. Recent studies have also shown that the gut microbiome is associated with thyroid diseases (Graves' disease, Hashimoto's disease, thyroid cancer) [8].

Our research has found changes in the qualitative and quantitative composition of the colonic microflora in patients with HT and obesity on the background of metabolic changes.

It is known that the gut microbiota not only has a positive effect on the activity of the immune system, but also on thyroid function. Thyroid and intestinal diseases often coexist and are often accompanied by other pathological conditions, such as celiac disease

and food intolerance. This occurs as a result of damage to the intestinal barrier and a subsequent increase in intestinal permeability. In this case, antigens pass the intestinal barrier more easily and negatively affect the immune system. In addition, the composition of the intestinal microbiota affects the absorption of essential trace elements for the thyroid gland (iodine, iron, copper, selenium, zinc), which are crucial for the synthesis of thyroid hormones and are necessary for the conversion of T4 to T3. The levels of these trace elements are often reduced in patients with thyroid disease. Taking into account changes in the composition of the gut microbiota, multifactorial therapeutic and preventive strategies for the treatment of these patients could be developed. However, further research is needed to assess the impact of changes in the gut microbiota on thyroid function and disease [9].

CONCLUSIONS

1. In patients with obesity decrease in the concentration of *Bifidobacterium*, *Lactobacillus* and increase in the number of *Staphylococcus*, *Clostridium*, *Proteus* and *Klebsiella* were detected, which is more pronounced in patients with a combination of obesity and hypothyroidism.
2. Impairment distinct of immunological status in patients with hypothyroidism and obesity was diagnosed, which was manifested by increased levels of immunoglobulins, namly (A, M, G), as well as a decrease in blood serum complements (C3, C4).
3. The level of IgA, G directly depends on the decrese of *Bifidobacterium*, *Lactobacillus* and increse of *Staphylococcus*, *Clostridium* and *Klebsiella* in patients with obesity, which is more pronounced in patients with a combination of obesity and hypothyroidism.

REFERENCES

1. Song R, Wang B, Yao Q et al. The Impact of Obesity on Thyroid Autoimmunity and Dysfunction: A Systematic Review and Meta-Analysis. Front. Immunol. 2021; 10: 2349. doi: 10.3389/fimmu.2019.02349.
2. Sanyal D, Raychaudhur M. Hypothyroidism and obesity: An intriguing link. Indian Journal of Endocrinology and Metabolism. 2016; 20 (4): 554-557. doi: 10.4103/2230-8210.183454.
3. Mahdavi M, Amouzegar A, Mehran L et al. Investigating the prevalence of primary thyroid dysfunction in obese and overweight individuals: Tehran thyroid study. BMC Endocrine Disorders. 2021; 21: 89. doi:10.1186/s12902-021-00743-4.
4. WHO: Global Database on Body Mass Index. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [date access 17.05.2023]
5. Kirpach G. Subclinical hypothyroidism, outcomes and recommendations for treatment: an overview and update of the latest literature. Health of Ukraine. 2023; 2: 10-11. doi:10.1080/03007995.2023.2165811.
6. Saveljeva-Kulik NA. Pervynnyy ta subklinichnyy hipotyreoz: klinichna nastanova NICE 2019. [Primary and subclinical hypothyroidism: NICE clinical guideline 2019]. The Ukrainian medical journal. 2019. www.umj.com.ua/uk/novyna-168435-pervinnij-ta-subklinichnij-gipotireoz-klinichna-nastanova-nice-2019 [date access 17.05.2023]
7. Hang Y, Qin Q, Chen J et al. Gut Microbione Alterations in Patiens with Visceral Obesity based on Qyantitative Computed Tomography. Front. Ceii. Infect. Microbiol. 2021. doi:10.3389/fcimb.2021.823262.
8. Su X, Zhao Y, Li Y et al. Gut dysbiosis is associated with primary hypothyroidism with interaction on gut-thyroid axis. Clin Sci (Lond). 2020; 134 (12): 1521–1535. doi:10.1042/CS20200475.
9. Knezevic J, Starchl Ch, Tmava Berisha A, Amrein K. Thyroid-Gut-Axis: How Does the Microbiota Influence Thyroid Function? Nutrients. 2020;12(6):1769. doi:10.3390/nu12061769.

The study was performed within the framework of the scientific topics "Polymorbid Pathology of Digestive System Diseases, Features of Pathogenesis and the Possibility of Correction" (state registration number 0118U004365) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University" and "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177).

ORCID and contributionship:
Yelyzaveta S. Sirchak: 0000-0001-6738-0843 [A, F]
Mikola M.Voloshin: 000 9 -0004-9 451 -6178 [B- E]
Ivan I. Kohutych: 0000-0002-3064-8292 [B]
Oksana M. Moskal: 0000-0001-8615-9933 [B]
Vasyl V. Palapa: 0009-0009-3626-3172 [B]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Yelyzaveta S. Sirchak
Uzhhorod National University
1 Narodna sqr., 88000 Uzhhorod, Ukraine
tel: +380509761794
e-mail: sirchakliza777@gmail.com

Received: 19.05.2023
Accepted: 27.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

HISTOLOGICAL AND MORPHOLOGICAL CHANGES OF THE VASCULAR BED OF THE THYMUS IN WHITE RATS UNDER THE INFLUENCE OF MONOSODIUM GLUTAMATE

DOI: 10.36740/WLek202311124

Mykhailo Yu. Kochmar, Mykhailo M. Havrylets, Kalyna M. Hoblyk, Illya P. Zayachuk, Vasyl I. Griga
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To evaluate the effect of 28-day oral administration of MSG at the rate of 30 mg/kg of body weight on histological and morphometric parameters of the vascular bed of the thymus in rats.

Materials and methods: The scientific experiment was conducted on 20 white non-linear rats of reproductive age (4-5 months) weighing from 220 to 280 g, which were divided into two groups (10 rats each). Depending on the term of decapitation, the experimental animals were divided into two groups (10 rats in each group). We studied the effect of 14 and 28 days of MSG administration on the body of rats (I and II groups of experimental rats). The experimental animals were daily orally treated with MSG at a dose of 30 mg/kg body weight, which was dissolved in 0.5 ml of dechlorinated tap water at room temperature. Control rats of III and IV groups (5 rats in each of the control groups) were injected with a placebo (0.5 ml of dechlorinated tap water at room temperature) for 14 and 28 days. Intact animals of III and IV groups were also decapitated on the 14th and 28th days of the experiment, respectively.

After the end of the experiment, animals were decapitated under light ether anesthesia. After decapitation, the animals were dissected into the chest cavity to remove the thymus. Histological preparations were studied using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out using VideoTest-5.0, KAARA Image Base and Microsoft Excel programs on a personal computer.

Results: During the microscopic examination of histological preparations of the retrosternal gland in experimental animals of the 1st group (daily administration of MSG at the rate of 30 mg/kg of body weight for 14 days), it was established that the lumen of the arteries is moderately filled with blood elements. The veins are dilated with a changed shape and filled with blood. The following ultrastructural changes were detected in the experimental animals of group I: the lumen of arteries, arterioles and venules is slightly expanded, the nuclei of endotheliocytes are enlarged, occupy a significant part of the cytoplasm, the karyolem forms intussusceptions. The plasmolemma of the lumenal surface of endotheliocytes forms numerous microvilli. At the same time, organelles in the cytoplasm of endotheliocytes lose their contours. After 28 days of exposure to MSG at a dose of 30 mg/kg of body weight in rats (II group of experimental animals), structural changes in the vascular bed of the thymus worsened. The wall of arteries and arterioles is more thickened and swollen, collagen fibers are stratified. In their lumen, there are many uniform elements attached to the vascular wall and testify to thrombus formation. Perivascular edema is determined. The diameter of hemocapillaries is increased, their basal membrane is swollen. Veins and venules are also dilated, full blood, interendothelial contacts in the vessel wall are dilated, the basement membrane is damaged. This contributes to the diapedesis of blood plasma through the vessel wall, which leads to perivascular edema.

Conclusions: Administration of MGS to rats at a dose of 30 mg/kg of body weight for 14 days leads to violations of the morphometric indicators of the vascular bed in the thymus, namely, to an increase in the outer and inner diameter of the arteries, an increase in the area of the middle membrane and the lumen of the vessels, which tend to progress with maximum indicators on the 28th day of the experiment. 2. The study of the vascular bed of the thymus against the background of taking MSG in a dose of 30 mg/kg of the weight of rats indicates the most pronounced changes in hemocapillaries, mainly on the 28th day of the experiment, which is manifested by an increase in their outer diameter. In the lumen of the hemocapillaries, deformed erythrocytes are identified, arranged in the type of "coin columns".

KEY WORDS: monosodium glutamate, thymus, vessels

Wiad Lek. 2023;76(11):2491-2496

INTRODUCTION

The flavor additive of monosodium glutamate (MSG) is widely used in the industry for the production of food products, medicines, fodder and feed additives for industrial livestock production of whey [1]. Studies have shown that even minimal doses of monosodium glutamate (0.6 and 1.6 mg/g of body weight for two weeks or 100-500 mg/kg of body weight for three weeks) can cause harmful effects on the body of humans and laboratory animals. animals, in particular rodents [2].

The effect of monosodium glutamate on the body of animals and humans manifests itself mainly in the form of metabolic disorders. Increasing the daily dose of MSG even by 1 g. significantly increased the risk of

developing metabolic syndrome and obesity in people, regardless of lifestyle (diet, physical activity). Studies on laboratory animals have shown an increase in body weight, the development of insulin resistance and other hormonal disorders, changes in serum biochemical indicators [1, 2].

Monosodium glutamate has a complex mechanism of action, which can be conditionally divided into indirect and direct. The indirect effect of MSG on the body is realized by affecting the neuroendocrine system and damage to the hypothalamus. As a result of damage to the autonomic nervous system, the work of controlled organs and systems is disrupted. The direct effect of MSG is the occurrence of inflammatory processes in tissues, infiltration by lymphoid cells, edema and microcirculation disorders. As a result, hypoxia and fibrosis develop in tissues. Damage to the endothelium of vessels causes disruption of histo-hematic barrier functions and hemorrhage [3].

The the study of the influence of MSG on changes in the vascular bed in various organs and systems is an urgent task of modern medical science.

THE AIM

The aim is to evaluate the effect of 28-day oral administration of MSG at the rate of 30 mg/kg of body weight on histological and morphometric parameters of the vascular bed of the thymus in rats.

MATERIALS AND METHODS

The scientific experiment was conducted on 20 white non-linear rats of reproductive age (4-5 months) weighing from 220 to 280 g, which were divided into two groups (10 rats each). Experimental animals were kept in vivarium conditions in compliance with all regulations, namely the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), the Law of Ukraine No. 3447–IV "On the protection of animals from cruel treatment", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001). Experimental and control animals were housed in separate boxes in the vivarium. All experimental animals were kept in the conditions of the vivarium of the Lviv National Medical University named after Danylo Halytskyi.

Depending on the term of decapitation, the experimental animals were divided into two groups (10 rats in each group). We studied the effect of 14 and 28 days of MSG administration on the body of rats (I and II groups of experimental rats). The experimental animals were daily orally treated with MSG at a dose of 30 mg/kg body weight, which was dissolved in 0.5 ml of dechlorinated tap water at room temperature. Control rats of III and IV groups (5 rats in each of the control groups) were injected with a placebo (0.5 ml of dechlorinated tap water at room temperature) for 14 and 28 days. Intact animals of III and IV groups were also decapitated on the 14th and 28th days of the experiment, respectively.

After the end of the experiment, animals were decapitated under light ether anesthesia, with the animals previously weighed. After decapitation, the animals were dissected into the chest cavity to remove the thymus. After dissection of the ventral wall of the chest cavity and the front surface of the neck, the mammary gland was removed. The obtained material was processed according to generally accepted methods. For histological examination, tissue blocks of the thymus were fixed in a 10.0% solution of neutral formaldehyde. After fixation, the material was washed, dehydrated in a series of alcohols of increasing concentration, passed through chloroform and poured into paraplast. Sections of tissue with a thickness of 5-7 μm were prepared on a rotary microtome, placed on glass, stained with hematoxylin-eosin according to the generally accepted method. Histological preparations were studied using a MICROmed SEO SCAN light microscope and a Vision CCD Camera. Morphometric studies were carried out using VideoTest-5.0, KAARA Image Base and Microsoft Excel programs on a personal computer. The research was carried out in the specified terms of the experiment in preparations stained with hematoxylin and eosin. The relative area of the cortical substance of the thymus lobe, the relative area of the medullary substance of the thymus lobe, the cortical-cerebral index, the thickness of the capsule, the number of thymocytes per unit area in the cortical and medullary substances of the thymus lobe, as well as the diameter of small thymocytes in the compositions of the cortical and medullary substance of the thymus lobe were studied morphometrically.

In accordance with the purpose of the scientific work, the arteries of the muscular type of small and medium caliber were studied. The values of the external (D_1) and internal (D_2) diameters, the area of the middle membrane (media) (Sm) and the area of the lumen (Sl) were determined. The assessment of the functional state of the blood vessels was carried out by calculating the Voogenvoort arterial permeability coefficient (VC), which is the ratio of the wall area to the area of the lumen - VC= (Sm/Sl) x 100%.

The outer diameter (D) of the blood capillaries of the cortical and medullary substances of the thymus lobes

was also measured.

The analysis and processing of the results of the examination of patients was carried out by the computer program Statistics 10.0 (StatSoftInc, USA) for Windows, using parametric and non-parametric methods of evaluating the obtained results.

RESULTS

The parameters of the arteries, that supply blood to the thymus in rats were studied (interlobular and intralobular arteries of the retrosternal gland, from which the arcuate arteries that form the vessels of the hemomicrocirculatory bed of the organ branch out).

During the microscopic examination of histological preparations of the retrosternal gland in experimental animals of the 1st group (daily administration of MSG at the rate of 30 mg/kg of body weight for 14 days), it was established that the lumen of the arteries is moderately filled with blood elements. The veins are dilated with a changed shape and filled with blood. The following ultrastructural changes were detected in the experimental animals of group I: the lumen of arteries, arterioles and venules is slightly expanded, the nuclei of endotheliocytes are enlarged, occupy a significant part of the cytoplasm, the karyolem forms intussusceptions. The plasmolemma of the lumenal surface of endotheliocytes forms numerous microvilli. At the same time, organelles in the cytoplasm of endotheliocytes lose their contours.

During the microscopic examination of the histological preparations of the first group of animals, an increase in the lumen of the veins, which are filled with blood-forming elements, was established. The general increase in the diameter of the vessels of the microcirculatory bed is determined. At the same time, erythrocytes in the lumens of hemocapillaries are arranged in the type of "coin columns". Single erythrocytes are detected in the intercellular space, which indicates damage to the vascular wall. The lumen of the capillaries is slightly narrowed, which occurs as a result of the swelling of the endotheliocytes and the protrusion of the plasmolemma into the lumen of the vessels. The lumen of arterioles is also slightly expanded. The basement membrane of the venules is thickened in some places.

After 28 days of exposure to MSG at a dose of 30 mg/kg of body weight in rats (II group of experimental animals), structural changes in the vascular bed of the thymus worsened. The wall of arteries and arterioles is more thickened and swollen, collagen fibers are stratified. In their lumen, there are many uniform elements attached to the vascular wall and testify to thrombus formation. Perivascular edema is determined. The diameter of hemocapillaries is increased, their basal membrane is swollen. Veins and venules are also dilated, full blood, interendothelial contacts in the vessel wall are dilated, the basement membrane is damaged. This contributes to the diapedesis of blood plasma through the vessel wall, which leads to perivascular edema.

The release of formed blood elements into the parenchyma is also determined. In the lumen of the hemocapillaries, erythrocytes are deformed, also arranged in a "coin column" type, as in animals of the 1st group. Erythrocytes often attach to the lumenal surface of endotheliocytes. The wall of arteries and arterioles is thickened due to swelling of endotheliocytes. Initial signs of sclerosis in arteries and arterioles are determined.

The most pronounced microscopic changes in animals of the II group were found in the blood capillaries, namely, an increase in their outer diameter, the basal membrane of hemocapillaries was significantly thickened and stratified. In the lumen of hemocapillaries, deformed clusters of erythrocytes are determined. In animals of the II group, electron microscopy revealed enlarged nuclei of endotheliocytes of an irregular elongated shape with protrusions and depressions in the wall of hemocapillaries. The cytoplasm of endotheliocytes is thinned, enlightened. The lumen of the hemocapillaries is reduced due to the fact that the plasmalemma. of the lumenal surface of endotheliocytes forms numerous protrusions and microvilli in the hemocapillary lumen.

The parameters of the arteries supplying blood to the thymus in rats were also assessed by the morphometric method. Their outer diameter was determined, which is 50.12±1.12 μm in animals of III and 49.95±1.07 μm in rats of IV groups, the inner diameter is 25.70±1.02 μm in III and 25, 63±1.14 μm in animals of the IV group, the area of the middle membrane (1428.23±11.46 μm2 in animals of the III and 1441.25±10.74 μm2 in rats of the IV group), the area of the lumen (513.56±5, 37 μm2 in animals of III and 514.02±4.06 μm2 in rats of IV groups), the Vogenvoort coefficient, which was 290.67±3.15% in animals of III group and 289.77±5.56% in rats of IV group. The indicator of the outer diameter of the blood capillaries in the thymus lobules was as follows in the groups of control animals: in the cortical substance - 12.07±0.55 μm in animals of III and 12.23±0.61 μm in rats of IV group; in the brain substance, 24.06±1.07 μm in III animals and 24.21±1.18 μm in IV group rats (Table I). As indicated by the results of the statistical analysis, we did not establish a significant difference between the indicators in rats of III and IV groups. Accordingly, their indicators were taken as the norm, with which the obtained results of the I and II groups of experimental rats were subsequently compared.

Table I. Morphometric indicators of the vascular bed of the retrosternal gland in experimental rats

Indicators	Groups of studied animals			
	Experimental groups		Control groups	
	I group (n=10)	II group (n=10)	III group (n=5)	IV group (n=5)
D_1 arteries, μm	53,04±1,05*	54,88±1,14*	50,12±1,12	49,95±1,07
D_2, μm	26,87±1,12*+	22,04±0,86*	25,70±1,02	25,63±1,14
Sm arteries, μm^2	1603,21±12,55**	1785,35±14,78**++	1428,23±11,46	1441,25±10,74
Sl arteries, μm^2	560,23±5,48*+	535,64±4,12*	513,56±5,37	514,02±4,06
VC, %	289,12±3,26*	328,77±3,08*+	290,67±3,15	289,77±5,56
D hemocapillaries of the cortical substance, μm	16,02±0,71*	18,95±0,46**+	12,07±0,55	12,23±0,61
D hemocapillaries of the brain substance, μm	29,88±0,27*	34,12±0,45**+	24,06±1,07	24,21±1,18

Note: the difference between the indicators of rats of experimental groups (I and II groups) and control groups (III-IV groups) is significant: * – $p<0.05$; ** – $p<0.01$; the difference between the indicators in rats of the I and II groups is significant: + - $p<0.05$; ++ - $p<0.01$.

The analysis of the results of the morphometric parameters of the vascular bed of the thymus in rats of the I group (after 14 days of taking MSG) indicates a significant increase in the indicator of the outer diameter of the arteries (up to 53.04±1.05 μm - $p<0.05$). The maximum pronounced increase of this indicator was established on the 28th day of the experiment in rats of the II group (increase to 54.88±1.14 μm). In rats of both experimental groups, the external diameter of arteries was significantly different from this indicator of intact rats of III and IV groups - $p<0.05$. On the contrary, the internal diameter of the arteries was maximally increased in rats of the I group and significantly differed from this indicator in the rats of the II group ($p<0.05$). In rats of the II group, the inner diameter of the arteries was lower than this indicator of the control animals, namely - by 3.66±0.16 μm from this indicator in the rats of the III group and by 3.59±0.28 μm from the indicator in the animals of the IV group - $p<0.05$.

A significant increase in the area of the middle membrane and the area of the lumen of the arteries in the retrosternal gland was established in rats of both experimental groups in comparison with such indicators in the animals of the control groups. At the same time, if the maximum pronounced deviation from the norm in the analysis of the area of the middle layer of arteries was found in animals of the III group (its increase to 1785.35±14.78 μm2 - $p<0.01$), then on the contrary - the area of the lumen of the arteries increased maximally in animals of I group (560.23±5.48 μm2 - $p<0.05$), in comparison with the data of control animals. If on the 14th day of the experiment, the VC in the animals of the I group actually did not differ from that in the rats of the control groups, then on the 28th day of receiving MSG, it was found to have significantly increased in comparison with the animals of the control groups (up to 328.77±3.08 % - $p<0.05$).

During the morphometric study of histological preparations, a reliable gradual increase in the outer diameter of hemocapillaries was established in both the cortical and medullary substances of the thymus with the maximum values in rats of the III group – up to 18.95±0.46 μm in the cortical substance of the lobules and up to 34.12±0 .45 μm in the brain substance of the lobules, which, accordingly, significantly exceed these indicators of intact animals ($p<0.01$).

So, already 14 days after the introduction of MSG to rats at a dose of 30 mg/kg of body weight, changes in the vascular bed of the thymus in experimental animals are determined, which have a tendency to progress with maximally pronounced changes on the 28th day of the experiment.

DISCUSSION

Various experimental and clinical studies are conducted to determine the impact of food additives on health. The most common of them is monosodium glutamate. The food supplement, which has been known as a "flavor enhancer" for more than 100 years, is the fifth type of taste in Japan - "umami", after sour, bitter, salty and sweet. It was first discovered by a Japanese scientist, Professor Kikunae Ikeda and introduced into mass production as one of the most common food additives after salt and pepper. In the professional literature, information about the negative impact of MSG on the structural organization of organs, in particular the nervous, digestive, immune and other systems, is increasingly appearing [4].

Experimental studies indicate that in glutamate-induced obesity, excess fat accumulates in adipose tissue as a result of increased cholesterol levels, which leads to cardiovascular pathology [5]. The preliminary data obtained by us also indicate a violation of lipid me-

tabolism in rats when MSG is administered at a dose of 30 mg/kg of body weight against the background of structural changes in organs, including the thymus. In an experimental study by Aghajani M. et al. (2017), it was established that the introduction of MSG in combination with a high-calorie diet led to oxidative stress due to increased levels of nitric oxide. This, in turn, increases the area of damage during myocardial infarction in experimental animals [6].

The data obtained by us also confirm the negative effect of MSG on the vascular structures of the thymus in rats receiving the dietary supplement at a dose of 30 mg/kg body weight. Already on the 14th day of the experiment, the expansion of the lumen of the arteries and arterioles of the thymus was established, which indicates vasodilatation. The lumen of the veins and venules is increased, they are full of blood. Two-week administration of GN contributes to the narrowing of the lumen of the hemocapillaries of the thymus in rats.

Destructive changes in the vascular bed of the thymus after 28 days of taking GN in a dose of 30 mg/kg body weight of rats deepen even more. Veins and venules are more dilated, full-blooded. The basement membrane is swollen. The wall of arteries and arterioles are thickened, swollen, there are many shaped elements in their lumen, which is a sign of thrombus formation. Perivascular edema increases. Hemocapillaries increase in diameter, both in the cortical and medullary substances of the thymus, their basal membrane is significantly thickened.

Therefore, as indicated by the results of our experimental study, structural changes in the vascular bed of the thymus occur against the background of taking MSG in rats. This is manifested by a change in the lumen of blood vessels, vasodilatation, as well as sclerotic changes, which tend to progress depending on the term of appointment of this dietary supplement.

CONCLUSIONS

Administration of MGS to rats at a dose of 30 mg/kg of body weight for 14 days leads to violations of the morphometric indicators of the vascular bed in the thymus, namely, to an increase in the outer and inner diameter of the arteries, an increase in the area of the middle membrane and the lumen of the vessels, which tend to progress with maximum indicators on the 28th day of the experiment.

2. The study of the vascular bed of the thymus against the background of taking MSG in a dose of 30 mg/kg of the weight of rats indicates the most pronounced changes in hemocapillaries, mainly on the 28th day of the experiment, which is manifested by an increase in their outer diameter. In the lumen of the hemocapillaries, deformed erythrocytes are identified, arranged in the type of "coin columns".

REFERENCES

1. Kinash OV, Yeroshenko GA, Shevchenko KV et al. Effects of sodium glutamate on human and animal. Herald of problems of biology and medicine. 2021; 3 (161): 49-52. doi: 10.29254/2077-4214-2021-3-161-49-52.
2. Sodomora OO. Monosodium Glutamate: Mechanisms of Action and Role in the Development of Structural Changes of Organs and Systems (Literature Review). Ukrainian Journal of Medicine, Biology and Sports. 2022;7(2): 40-48. doi:10.26693/jmbs07.02.040.
3. Kinash OV, Chupryna OB, Donets IM et al. Mekhanizmy diyi hlutamatu natriyu na orhany ta systemy [Mechanisms of monosodium glutamate impact on organs and systems]. Aktual'ni problemy suchasnoyi medytsyny: Visnyk Ukrayins'koyi medychnoyi stomatolohichnoyi akademiyi. 2021; 4 (21): 178-183. doi: 10.31718/2077-1096.21.4.178. (in Ukrainian)
4. Vashcheniuk MI. Sodium glutamate: mechanisms of influence on different organs and body systems (Literature rewiew). Art of Medicine. 2021; 1 (25): 101-105. doi: 10.21802/artm.2023.1.25.101.
5. Triantafyllou E, Woollard KJ, McPhail MJ et al. The role of monocytes and macrophages in acute and acute-on-chronic liver failure. Front Immunol. 2018; 9: 2948. doi: 10.3389/fimmu.2018.02948.
6. Aghajani M, Imani A, Faghihi M et al. Does increased nitric oxide production and oxidative stress due to high fat diet affect cardiac function after myocardial infarction? J Cell Mol Anesth. 2017; 2(1): 3-8. doi:10.22037/jcma.v2i1.14288.

The study was performed within the framework of the scientific topics "Polymorbid Pathology of Digestive System Diseases, Features of Pathogenesis and the Possibility of Correction" (state registration number 0118U004365) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University" and "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177).

ORCID and contributionship:
Mykhailo Yu. Kochmar: 0000-0002-0219-0552 [A, E, F]

Mykhailo M. Havrylets: 0000-0002-5995-4576 [B-D]
Kalyna M. Hoblyk: 0009-0005-0583-5693 [B, D]
Illya P. Zayachuk: 0000-0002-5728-2980 [B]
Vasyl I. Griga: 0000-0003-3666-8910 [B, D]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Mykhailo Yu. Kochmar
Uzhhorod National University
1 Narodna Square, 88000 Uzhhorod, Ukraine
tel: +380509135074
e-mail: kaf-anatomy@uzhnu.edu.ua

Received: 14.05.2023
Accepted: 22.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

THE IMPACT OF TOCILIZUMAB ON THE BIOCHEMICAL MARKER PROCALCITONIN DURING COVID-19 INFECTION

DOI: 10.36740/WLek202311125

Maysaa Ali Abdul Khaleq
DEPARTMENT OF PHARMACY, AL-MAARIF UNIVERSITY COLLEGE, AL-ANBAR, IRAQ

ABSTRACT

The aim: The primary objective of our study was to track the TCZ's impact on PCT levels in a cohort of COVID-19 patients who received dexamethasone daily from admission to the day of discharge.

Materials and methods: There were two groups: a treatment group of 40 patients who received tocilizumab and a 40-patient control group that did not receive the medication. Both groups' daily blood culture results and serum procalcitonin biochemical indicators were observed for 20 days, or until discharge or death. After 10 days, non-parametric univariate and linear mixed model analyses were used to compare the two groups' differences.

Results: Tocilizumab is administered on Day 5 and greatly reduces procalcitonin. The two groups did not differ in the percentage of positive blood cultures.

Conclusions: Procalcitonin levels in COVID-19 individuals who have received tocilizumab maybe not a dependable predictor of superinfection with bacteria.

KEY WORDS: COVID-19, tocilizumab, clinical biochemical marker, procalcitonin

Wiad Lek. 2023;76(11):2497-2501

INTRODUCTION

The coronavirus disease (COVID-19) global pandemic has been around since 2019 [1, 2]. The most common side effect of the disease is adult respiratory distress syndrome (ARDS) [3], that characterized by widespread viral particles and inflammatory cells in the lungs within Pneumocystis type II [4]. Tocilizumab (TCZ), also called rheumatoid arthritis medication, which is a monoclonal antibody against the IL-6 receptor and reduces cytokine storm, has generated interest as an active drug for severe COVID-19 [5, 6]. Early studies in 2020 showed that TCZ survived COVID-19 in severe cases [7]. The REMAP-CAP study, which evaluated TCZ compared with Sarilumab [8], was published in late February 2021 and is the largest trial to date. Patients taking the IL-6 receptor inhibitor compared favorably to the control group, spending an average of 10 days without organ support in the TCZ group against 11 fewer days in the Sarilumab group. According to the three-month analysis, the survival rate of the treated patients was better than that of the control group [9]. As more and more studies are published proving the benefits of TCZ on survival, physicians are becoming more concerned about the potential disadvantages of TCZ. Known side effects include immunosuppression, liver injury, neutropenia, and thrombocytopenia. However, we think there may be a more significant indirect impact on clinical care that needs to be addressed in further research [10]. Approximately 20% of hospital admissions for COVID-19 are affected by bacterial coinfection on admission, and up to 50% of these admissions are at risk of developing hospital-acquired sepsis within weeks of their ICU stay [11]. Before the pandemic, there was information [12] showing that TCZ could independently lower each and every sign of infection that physicians use to keep an eye on the prognosis and development of bacterial sepsis and decide whether to intensify or reduce antimicrobial therapy. The use of TCZ in the treatment of severe COVID-19 infection is an interesting topic given the impact of TCZ on biochemical and clinical markers of infection. Commonly used Procalcitonin (PCT) is one such indicator of infection, CRP, and white blood cell count (WCC) [13]. There are even fewer data on how TCZ affects markers of infection in people who are regularly treated with corticosteroids, since it only became commonplace treatment for COVID-19 after June 2020 [14]. Hariyanto and Kurniawan examined the effects of TCZ on multiple biochemical markers of infection in a rapid study of nine studies and 577 patients. CRP, ferritin, and D-dimer decreased and lymphocytes increased after TCZ injection. This review

Table I. Detection of procalcitonin in COVID-19 patient groups under study

Parameter	Group	No.	Mean± SD	p-value
Procalcitonin (pg/ml)	Control (group 1)	40	180.522±166.538	0.000*
	Treated with TCZ (group 2)	40	63.941±14.317	

* P< 0.05

Table II. Relationship between COVID-19 severity and procalcitonin

Test variable	Accuracy	Area (AUC)	Asymptomatic significance	Asymptomatic 95% confidence interval	
				Lower bound	Upper bound
Procalcitonin	Excellent	0.916	0.000*	0.861	0.971

* p<0.05

Table III. Differences in PCT biochemical markers between the two groups at days 5, 10 and 15 with matching of p-values

	Control Group	Treatment Group	p-value
Number of patients	40	40	
Any positive blood culture	22 (55%)	26 (65%)	0.58
Date of the event	8 [5 - 11]	5.5 [3 - 8]	0.15
Fungal cultures	12 (30%)	8 (20%)	0.45

found that PCT also decreased after TCZ treatment [15]. A sudden increase in oxygen consumption and admission to the ICU are red flags. Patients with slower disease progression or serum alanine aminotransferase levels greater than one-fifth of the higher bound of the standard were not taken into account for TCZ treatment. Eligible candidates received only one intravenous dose of 8 mg/kg TCZ up to a prescribed dose of 800 mg [16, 17].

THE AIM

The primary objective of our study was to track the TCZ's impact on PCT levels in a cohort of COVID-19 patients who received dexamethasone daily from admission to the day of discharge.

MATERIALS AND METHODS

It was a retrospective single-center study conducted at the Al-Yarmouk teaching hospital in Baghdad, Iraq, in a general intensive therapy unit (ITU). The treatment group was comprised by the first 50 continuously admitted COVID-19 patients to the ITU who received TCZ. The 50 patients not given TCZ in the control group for this trial were admitted to the ITU due to COVID-19. From the day when TCZ was administered until death, discharge from the ITU, or 20 days after administration, whichever came first, blood panel results for PCT at 6 am every day were documented. The first set noted was frequently taken in this group during admission and shortly before the TCZ dose was administered.

STATISTICAL ANALYSIS

The statistical analysis for this study was completed using Microsoft Excel 2013 and the statistical package for social sciences (SPSS) version 19.0. The Chi-square test was used to describe the relationship between these data. The Fisher exact test was used if there were 25% fewer cells than predicted. Numerical data were described using the mean, standard error of the mean, and standard deviation. The independent sample t-test was used to compare the two groups. The null hypothesis was tested, and the P-values were used to assess the significance of the testing. A P value of less than 0.05 was used to determine if a result was significant.

RESULTS

When PCT was measured in COVID-19 patients who participated in this study, the results revealed a substantial increase in PCT that was correlated with the severity of the infection (P value = 0.000). Day 5, using the treatment group's PCT values, univariate analysis revealed that TCZ administration had an impact on the PCT level. The mean±SD of PCT was 63.941±14.317, and 180.522±166.538 in the control group (Table I).

The investigation evaluated the reliability of the PCT test, and it revealed a statistically significant relationship between PCT and the severity of COVID-19 infection in study participants. The area under the curve for PCT was (0.916), and the 95% confidence interval was equal to 0.861-0.971 (Table II).

MICROBIOLOGY

The results of blood cultures for cultures of bacteria and fungi from the TCZ group and the control group are detailed in Table III, along with the relevant p-values.

The proportion of patients in each group who had a positive blood culture (without contaminants) was comparable: 65% in Tocilizumab group *vs* 55% in Controls, p-value=0.58. In patients receiving TCZ, Optimistic results tended to emerge during the ITU stay earlier, however, this did not reach statistical significance (Table III). The frequency of fungal cultures was the same in both groups.

DISCUSSION

The primary objective of this study was to investigate the significance of PCT levels following TCZ administration to COVID-19 pneumonia patients with severe illness. Our results show that TCZ therapy significantly reduces PCT for up to five days following the dose. After this stage, there was a statistically significant fall in the median PCT. Studies conducted abroad have previously drawn some conclusions concerning results comparable to those reached by our group. Results of the COVID-19 research showed that in those who did not require mechanical ventilation, TCZ therapy dramatically decreased PCT and CRP [18-20]. Patients with COVID-19 who took part in this study's PCT detection results showed a significant rise in PCT that was associated with the severity of the infection. The results are in line with several studies, such as Rue Hua et al., which demonstrate how PCT can be utilized as a measure of disease severity and to gauge the severity of COVID-19 patients. Regular PCT readings may also help determine the prognosis [21]. Additionally, a subset of COVID-19 individuals who are at risk for experiencing a severe illness and having negative outcomes may be found using Natalee Hazzans et al. high PCT levels [22]. Another meta-analysis study by Lippi G and Plebian et al., found that bacterial illnesses boost extra thyroidal PCT synthesis and preserve PCT activity due to greater quantities of IL-1, TNF-ά and IL-6, but viral infections decrease PCT production due to interferon. Serum PCT concentrations in COVID-19 patients remain normal because there are no issues, but higher values in severe cases may indicate bacterial co-infection [23]. PCT is primarily created during inflammation by two different pathways: a direct channel induced by lipopolysaccharide (LPS) or other toxic bacteria-derived metabolites, and an indirect avenue induced by other inflammatory mediators like IL-6, TNF-ά, and others. The fact that PCT is created in bacterial septicemia through several different pathways, either directly or indirectly, may help to explain why PCT is elevated in severe COVID [24]. The results of this study were not supported by other investigations, which included those by Huang C et al., Wan S et al., Wang D et al., Barraza H et al., Chen T et al., and Langford BJ et al. which found no statistically significant connection between higher PCT and illness severity or outcomes. Potential confounding factors include a small sample size, various severity classification criteria, the PCT threshold, and/or the proportion of patients with severe disease [25-28]. Less research has been done on other elements, and conflicting reports seem to exist. Other studies that were stated at the beginning of the study showed a variety of favorable impacts on survival results and weaning off mechanical breathing [9].

CONCLUSIONS

Procalcitonin and C-reactive protein levels are significantly lowered in critically ill patients treated with tocilizumab. Blood culture results and patients with COVID-19 are unaffected by this medicine in the days following therapy. Because it shows that PCT might no longer be a reliable sign of superinfection in patients getting TCZ treatment for severe COVID-19 pneumonia. This study is important as TCZ still carries a minor risk of bacterial infection, even though it appears to help patients with COVID-related ARDS most of the time. While watching from the onset of contamination, additional markers, for example, WCC and cultures of bacteria, maybe greater significance than the commonly used PCT and CRP values. Given that microbial resistance must be considered, it is currently unclear if to lessen the likelihood of an undetected superinfection, preventive antibiotics should be given along with TCZ. The study's shortcomings include the inclusion of only one site and the very small sample size. All patients received dexamethasone, which might have been a confusing element that masked more pronounced disparities between the two groups.

RECOMMENDATION

Repercussions of TCZ medication and the ideal strategy to shield these individuals from the issues associated with bacterial superinfection require more study. Larger studies and lengthier investigations are necessary to more fully comprehend the precise impacts and processes of TCZ function in severe COVID-19 before explicitly proposing a change in clinical practice. Examine the impact of TCZ on additional markers, such as the number of white blood cells, neutrophils, lymphocytes, etc.

REFERENCES

1. Emeksiz HC, Hepokur MN, Şahin SE, et al. Immunogenicity, safety and clinical outcomes of the SARS-CoV-2 BNT162b2 vaccine in adolescents with type 1 diabetes. Front Pediatr. 2023;11:1191706. doi:10.3389/fped.2023.1191706
2. Gonzalez-Isunza G, Jawaid MZ, Liu P, et al. Using machine learning to detect coronaviruses potentially infectious to humans. Sci Rep. 2023;13(1):9319. doi:10.1038/s41598-023-35861-7
3. Tzotzos SJ, Fischer B, Fischer H, et al. Incidence of ARDS and outcomes in hospitalized patients with COVID-19: a global literature survey. Crit Care. 2020;24(1):516. doi:10.1186/s13054-020-03240-7
4. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8(4):420-422. doi:10.1016/S2213-2600(20)30076-X.
5. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052
6. Zhang C, Wu Z, Li JW, et al. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55(5):105954. doi:10.1016/j.ijantimicag.2020.105954
7. Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A. 2020;117(20):10970-10975. doi:10.1073/pnas.2005615117.
8. Versus Arthritis. Sarilumab. https://www.versusarthritis.org/about-arthritis/treatments/drugs/sarilumab. [Access: Jun. 18, 2021].
9. REMAP-CAP Investigators, Gordon AC, Mouncey PR, et al. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. N Engl J Med. 2021;384(16):1491-1502. doi:10.1056/NEJMoa2100433
10. Wadud N, Ahmed N, Shergil M, et al. Improved survival outcome in SARs-CoV-2 (COVID-19) acute respiratory distress syndrome patients with Tocilizumab administration. MedRxiv. 2020, https://www.medrxiv.org/content/10.1101/2020.05.13.20100081v1 [Access: May 20 2021]. doi: 10.1101/2020.05.13.20100081
11. Elabbadi A, Turpin M, Gerotziafas GT, et al. Bacterial coinfection in critically ill COVID-19 patients with severe pneumonia. Infection. 2021;49(3):559-562. doi:10.1007/s15010-020-01553-x
12. Berman M, Ben-Ami R, Berliner S, et al. The Effect of Tocilizumab on Inflammatory Markers in Patients Hospitalized with Serious Infections. Case Series and Review of Literature. Life (Basel). 2021;11(3):258. doi:10.3390/life11030258
13. Magrini L, Gagliano G, Travaglino F, et al. Comparison between white blood cell count, procalcitonin and C reactive protein as diagnostic and prognostic biomarkers of infection or sepsis in patients presenting to emergency department. Clin Chem Lab Med. 2014;52(10):1465-1472. doi:10.1515/cclm-2014-0210
14. Crothers K, DeFaccio R, Tate J, et al. Dexamethasone in hospitalised COVID-19 patients not on intensive respiratory support. Eur Respir J. 2022;60(1):2102532. doi:10.1183/13993003.02532-2021
15. Ivan Hariyanto T, Kurniawan A. Tocilizumab administration is associated with the reduction in biomarkers of coronavirus disease 2019 infection. J Med Virol. 2021;93(3):1832-1836. doi:10.1002/jmv.26698
16. The National Institute for Health and Care Excellence. COVID-19 rapid Guideline: Managing COVID-19. https://app.magicapp.org/#/guideline/L4Qb5n/section/ERY1zn. [Access: Mar. 25, 2021].
17. Salama C, Han J, Yau L, et al. Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. N Engl J Med. 2021;384(1):20-30. doi:10.1056/NEJMoa2030340
18. Antonelli M, Penfold R, Merino J, et al. Post-vaccination SARS-CoV-2 infection: risk factors and illness profile in a prospective, observational community-based case-control study. 2021;22(1):43-55. doi: https://doi.org/10.1016/S1473-3099(21)00460-6.
19. Rhee C. Using Procalcitonin to Guide Antibiotic Therapy. Open Forum Infect Dis. 2016;4(1):ofw249. Published 2016 Dec 7. doi:10.1093/ofid/ofw249
20. Hofmaenner DA, Wendel Garcia PD, Ganter CC, et al. What every intensivist should know about Tocilizumab. Crit Care. 2021;25(1):262.. doi:10.1186/s13054-021-03696-1
21. Hu R, Han C, Pei S, et al. Procalcitonin levels in COVID-19 patients. Int J Antimicrob Agents. 2020;56(2):106051. doi:10.1016/j.ijantimicag.2020.106051
22. Vazzana N, Dipaola F, Ognibene S. Procalcitonin and secondary bacterial infections in COVID-19: association with disease severity and outcomes. Acta Clin Belg. 2022;77(2):268-272. doi:10.1080/17843286.2020.1824749
23. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. Clin Chim Acta. June 2020;505:190-191. doi: 10.1016/j.cca.2020.03.004
24. Müller B, White JC, Nylén ES, et al. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab. 2001;86(1):396-404. doi:10.1210/jcem.86.1.7089
25. Wan S, Xiang Y, Fang W, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J Med Virol. 2020;92(7):797-806. doi:10.1002/jmv.25783
26. Barrasa H, Rello J, Tejada S, et al. SARS-CoV-2 in Spanish Intensive Care Units: Early experience with 15-day survival in Vitoria. Anaesth Crit Care Pain Med. 2020;39(5):553-561. doi:10.1016/j.accpm.2020.04.001

27. Chen T, Dai Z, Mo P, et al. Clinical Characteristics and Outcomes of Older Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective Study. J Gerontol A Biol Sci Med Sci. 2020;75(9):1788-1795. doi:10.1093/gerona/glaa089
28. Langford BJ, So M, Simeonova M, et al. Antimicrobial resistance in patients with COVID-19: a systematic review and meta-analysis. Lancet Microbe. 2023;4(3):e179-e191. doi:10.1016/S2666-5247(22)00355-X

ORCID and contributionship:

Maysaa Ali Abdul Khaleq: 0000-0003-3548-7835 [A-F]

Conflict of interest:

The Author declare no conflict of interest.

CORRESPONDING AUTHOR

Maysaa Ali Abdul Khaleq
Department of Pharmacy
Al-Maarif University College
Al-Anbar, Iraq
e-mail: dr.maysaa.ali@uoa.edu.iq

Received: 27.07.2023
Accepted: 29.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

ECHOCARDIOGRAPHIC ASPECTS OF ASSESSMENT OF MITRAL INSUFFICIENCY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION WITH REDUCED LEFT VENTRICULAR EJECTION FRACTION

DOI: 10.36740/WLek202311126

Liubov R. Stetsiuk[1], Ivan M. Klishch[1], Ihor O. Stetsiuk[2], Borys M. Todurov[2], Mykhailo B. Todurov[3], Larysa Ya. Fedoniuk[1]

[1]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

[2]KYIV HEART INSTITUTE OF THE MINISTRY OF HEALTH OF UKRAINE, KYIV, UKRAINE

[3]P.L. SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KYIV, UKRAINE

ABSTRACT

The aim is to analyze patients with acute myocardial infarction with reduced ejection fraction in order to determine risk factors for mitral insufficiency.

Materials and methods: The study included 149 patients with acute myocardial infarction. Among the patients, there were 113 males (75.8%) and 36 females (24.2%). The age of the patients ranged from 43 to 86 years. Echocardiography was performed using a Phillips Epiq 7 machine. Patients were examined three times: upon admission to the hospital, after revascularization, and six months after discharge. Patients received transthoracic echocardiography, which was used to determine the presence and degree of mitral regurgitation.

Results: According to the results of the study, it was found that the presence of concomitant somatic pathology worsens the course of an acute myocardial infarction, in turn increasing the likelihood of valvular pathology. An increase in left ventricular volume indicators, such as end-diastolic volume, end-systolic volume, end-diastolic index, and the index of contractile function (ejection fraction) contributes to the development of mitral valve insufficiency.

Conclusions: The presence of mitral regurgitation in patients with acute myocardial infarction and reduced left ventricular ejection fraction worsens the course of the disease and negatively affects the prognosis.

KEY WORDS: myocardial infarction, mitral regurgitation, echocardiography

Wiad Lek. 2023;76(11):2502-2509

INTRODUCTION

Mitral regurgitation is a frequent complication of acute myocardial infarction (AMI). If a mitral regurgitation occurs, it can manifest itself in the different degrees of severity, from clinically and hemodynamically obvious to clinically latent and detected only as an incidental finding during the echocardiography. Surely, mitral valve insufficiency is diagnosed using a Doppler diagnostics and it occurs in 39% of patients with myocardial infarction (MI) [1]. Papillary muscle dysfunction and associated dysfunction of the affected wall of the left ventricle are thought to be the most common cause of mitral regurgitation (MR) in post-AMI patients [2, 3, 4], and MR tends to be a more frequent complication of inferior than anterior infarction.

Echocardiography is the gold standard for evaluating valvular diseases of the heart, in particular mitral regurgitation [5]. Two-dimensional (2D) and three-dimensional (3D) echocardiography are used mainly to identify the etiology and the mechanism of the MR, while a Doppler method provides an accurate assessment of MR severity. Moreover, the integration of other ancillary findings, such as left ventricular size and function, the coexistence of significant tricuspid regurgitation, and pulmonary artery pressure, play a significant role in the decision-making process regarding the type and timing of intervention in severe MR. Echocardiographic assessment of IMR should also include assessment of global and regional LV function, LV ejection fraction, LV dimensions, LV wall motion abnormalities, and pulmonary hypertension [6].

A thorough complex echocardiographic analysis of anatomical structures and functional changes will make it possible to timely and correctly establish the

diagnosis of mitral insufficiency, and most importantly - to understand the cause of its occurrence. Diagnosis of the genesis of valvular dysfunction is the main point in choosing the correct treatment tactics for the patient.

THE AIM

To investigate the prevalence of mitral regurgitation in patients with acute myocardial infarction and examine the risk factors associated with its occurrence.

MATERIALS AND METHODS

149 AMI-patients, who were treated at the Kyiv Heart Center, took part in the research. In the period from March 03, 2021 to November 16, 2022, the medical histories of patients who were admitted to a medical institution with acute myocardial infarction of type I and received percutaneous coronary intervention (PCI) were retrospectively studied. All patients received transthoracic echocardiography before the PCI, after the intervention, and 6 months after the occurrence of myocardial infarction.

Patients were divided into 2 groups: without mitral insufficiency (n=48) and with mitral insufficiency (n=101).

An echocardiographic research was performed on a Philips Epiq7 ultrasound diagnostic device. To assess the degrees severity of mitral insufficiency, were used the flow convergence assessment method (PISA, EROA), measuring the size of the vena contracta.

Convergence of proximal blood flow was assessed in an apical 4-chamber projection, allowing measurement of proximal isovolumetric surface area (PISA) with reduced smoothing speed and increased penetration depth. The effective regurgitation orifice area (EROA) was obtained from the continuous-wave Doppler profile of the MR jet in the apical 4-chamber position.

The width of the vena contracta (VC) was assessed on the long-axis parasternal image. MR was classified as mild, moderate, or severe according to established criteria [7]. The dimensions of the left atrium (LA) and LV were indexed according to Mosteller's Body Surface Area calculation. LV ejection fraction (LVEF) was calculated by the Simpson's Method of Disc. The volume of the LA was calculated by the two-plane method based on the images obtained from the apical four-chamber and apical two-chamber images at the end of systole. LA dilatation was defined as the indexed left atrial volume (LAVi) > 34 ml/m2 [8].

RESULTS

As a result of the study, 149 AMI-patients were examined. All patients underwent transthoracic echocardiography. Patients are divided into 2 groups: 1st group - 48 patients without mitral valve insufficiency and 2nd group - 101 patients with mitral valve insufficiency (Table I). In both groups of patients, men prevailed, 38 (79.17%) in the 1st group and 75 (74.26%) p=0.516. The average age of patients was higher in the group of patients with mitral valve insufficiency - 65.59±1.01, and in the 2nd group - 63.44±1.49 (p=0.233). Hypertensive disease was observed in almost all patients of both groups (48 patients (100%) and 100 patients (99.01%) p=0.492). Diabetes mellitus was more often noted in patients of the 2nd group - 34 (33.66%), and in the 1st group - 14 people (29.17%) p=0.586.

Patients with mitral insufficiency often have chronic kidney disease (38 (37.62%) and 12 (25%) p=0.129). When comparing the body mass index in patients, no significant difference was noted (28.41±0.57 kg/m2 and 28.31±0.45 kg/m2 (p=0.898)). In the assessment of previous cerebral circulation disorders, the second group had higher indicators than the first group (12 (11.88%) and 5 (10.42%) p=0.794)). When patients were admitted to the department, they were evaluated according to the NYHA scale, according to the obtained results, class III prevailed in both groups (48 (100%) and 99 (98.02%) p=0.330)).

All patients received echocardiographic examination three times - before surgery, after surgery and 6 months after surgery.

When analyzing the data of the first echocardiography before stenting (Table II), a larger end-diastolic volume of the left ventricle was found in the patients of the second group (147.51±3.77 and 138.75±5.41 (p=0.187)). The indicator of end-systolic volume did not have a significant difference - 58.69±1.90 in the first group and 57.60±1.30 in the second (p=0.634). The end-diastolic index is higher in patients with mitral valve insufficiency 76.44±1.88, and in patients without mitral insufficiency - 70.06±2.38 (p=0.047). The ejection fraction was lower at the time of admission to stenting in the second group of patients (39.79±0.68 and 43.31±0.97 (p=0.004).

Patients underwent a second echocardiography the day after coronary arteries stenting (Table III). According to the research data: the end-diastolic volume of the left ventricle in patients of the second group remained larger than in the first group (146.11±3.84 and 143.79±5.38 (p=0.730)), increased in both groups, comparing with the previous indicators of echocardiography. End-systolic volume prevailed in patients with mitral insufficiency (78.60±3.00 and 75.46±4.32 (p=0.552)). The end-diastolic index was higher in the second group (78.09±1.75 and 72.06±2.38 (p=0.048), the results increased in both groups compared to the

Table I. Basic clinical characteristics of patients

Indicator		Group 1 n=48	Group 2 n=101	p
Age		**63,44±1,49**	**65,59±1,01**	**0,233**
Gender	male	38 (79,17)	75 (74,26)	*0,516*
	female	10 (20,83)	26 (25,74)	
risk factors	hypertension	48 (100)	100 (99,01)	*0,492*
	DM	14 (29,17)	34 (33,66)	*0,586*
	CKD	12 (25,00)	38 (37,62)	*0,129*
	BMI	28,41±0,57	28,31±0,45	*0,898*
	BSA	1,97±0,03	1,93±0,02	*0,287*
	ACVA	5 (10,42)	12 (11,88)	*0,794*
NYHA scale	III	48 (100)	99 (98,02)	*0,330*
	IV	0	2 (1,98)	

Table II. Preoperative echocardiographic characteristics of the LV

Indicator	Group 1 n=48	Group 2 n=101	p
EDV	138,75±5,41	147,51±3,77	*0,187*
ESV	58,69±1,90	57,60±1,30	*0,634*
EDI	70,06±2,38	76,44±1,88	*0,047*
EF	43,31±0,97	39,79±0,68	*0,004*

Table III. Postoperative echocardiographic characteristics of the LV

Indicator	Group 1 n=48	Group 2 n=101	p
EDV	143,79±5,38	146,11±3,84	*0,730*
ESV	75,46±4,32	78,60±3,00	*0,552*
EDI	72,06±2,38	78,09±1,75	*0,048*
EF	44,729±1,28	40,48±0,67	0,002

first study. The ejection fraction increased after revascularization in all patients, but the 1st group had higher data (44.729±1.28 and 40.48±0.67 (p=0.002)).

In addition to the indicators of the left ventricle, the function of the mitral valve was evaluated (Table IV).

Pathological mitral regurgitation was absent in 48 patients (group 1), and MR was noted in 101 patients (Table V). All patients, in addition to the visual assessment of color Doppler data, were assessed the effective regurgitation orifice area (EROA), vena contracta to objectify these degrees of mitral insufficiency. The following data were obtained during the classification of mitral insufficiency in the second group of patients: 1st degree of MI in 68 patients (67.33%) p=0.874; 2nd degree – in 26 (25.74%) p=0.137; 3rd degree – in 7 patients (6.93%) p=0.062.

After 6 months, the end-diastolic volume and left ventricular ejection fraction were re-analyzed in patients who were dismissed from the hospital in satisfactory condition (Table VI). EDV indicators remained higher in patients of the second group (148.93±3.75 and 144.60±7.70 (p=0.570)), slightly increased in comparison with postoperative studies. The LV ejection fraction remained lower in patients with MR (39.59±0.82 and 41.96±0.91 (p=0.080)), slightly decreased compared to the indicators obtained 6 months ago.

Therefore, comparing the echocardiographic characteristics of patients with an acute myocardial infarction with a reduced left ventricular ejection fraction, it becomes clear that an increase in the volume indicators of the left ventricle and a decrease in the ejection fraction contribute to the development of mitral insufficiency.

Table IV. Echocardiographic characteristics of the severity of mitral insufficiency

Degree of severity	Grade I	Grade II	Grade III
Width of Vena Contracta (mm)	<3	3 – 6	≥7
EROA (mm2)	<20	20-39	≥40

Table V. Echocardiographic assessment of MV insufficiency after revascularization

Indicator		Group 1 n=48	Group 2 n=101	p
EROA		0,13±0,00	0,22±0,02	*0,003*
Vena contracta		1,54±0,08	4,24±0,17	*0,145*
MI degree of severity	0	48 (100,00)	0	*0,874*
	1	0	68 (67,33)	*0,004*
	2	0	26 (25,74)	*0,137*
	3	0	7 (6,93)	*0,062*

Table VI. Echocardiographic characteristics after 6 months

Indicator	Group 1 n=40	Group 2 n=97	p
EDV	144,60±7,70	148,93±3,75	*0,570*
EF	41,96±0,91	39,59±0,82	*0,080*

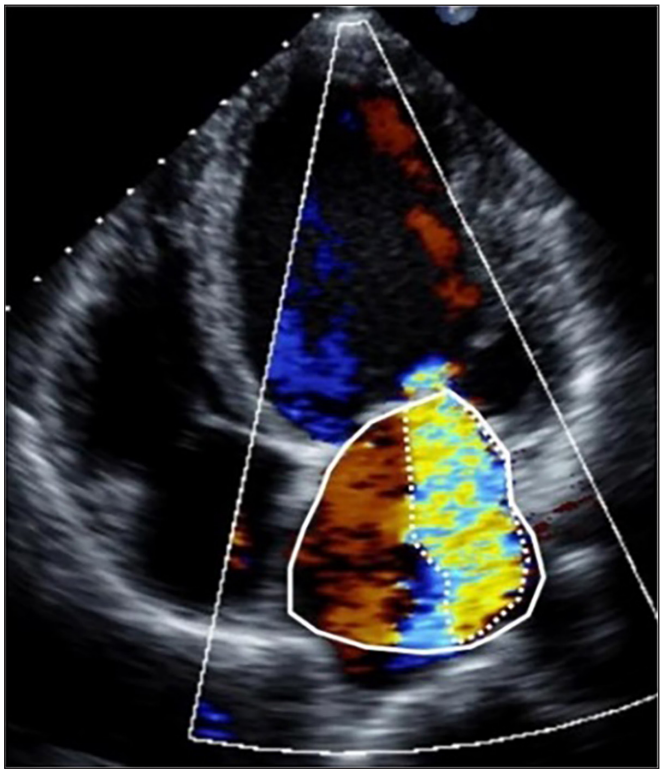


Fig. 1. MR quantification by the ratio of the maximum distal flow area to the left atrium area [10].

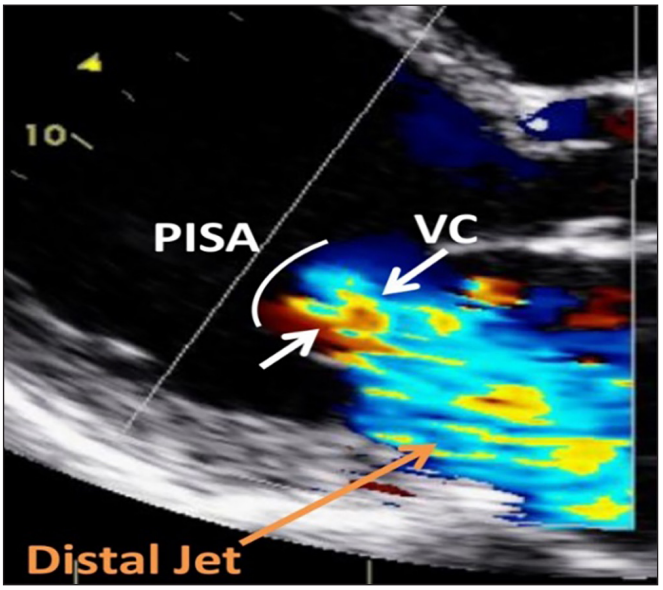


Fig. 2. Vena contracta measurement. VC (white arrows) of the MR flow is measured from the parasternal view along the long-axis as the narrowest part of the proximal flow at the level of the tips of the leaflets or slightly distal to them [10].

DISCUSSION

One of the complications of acute myocardial infarction is mitral valve insufficiency. The prognosis of the disease depends on several factors, including age, Killip classification during the acute phase, delay in receiving specialized care, chosen treatment strategy, extent of coronary vessel involvement, heart valve pathology, left ventricular ejection fraction (LVEF), the presence of comorbidities such as diabetes, kidney insufficiency, family history, and other aspects.

Mitral valve insufficiency is diagnosed using echocardiography. Timely detection of insufficiency and its degree is important for the patient's prognosis.

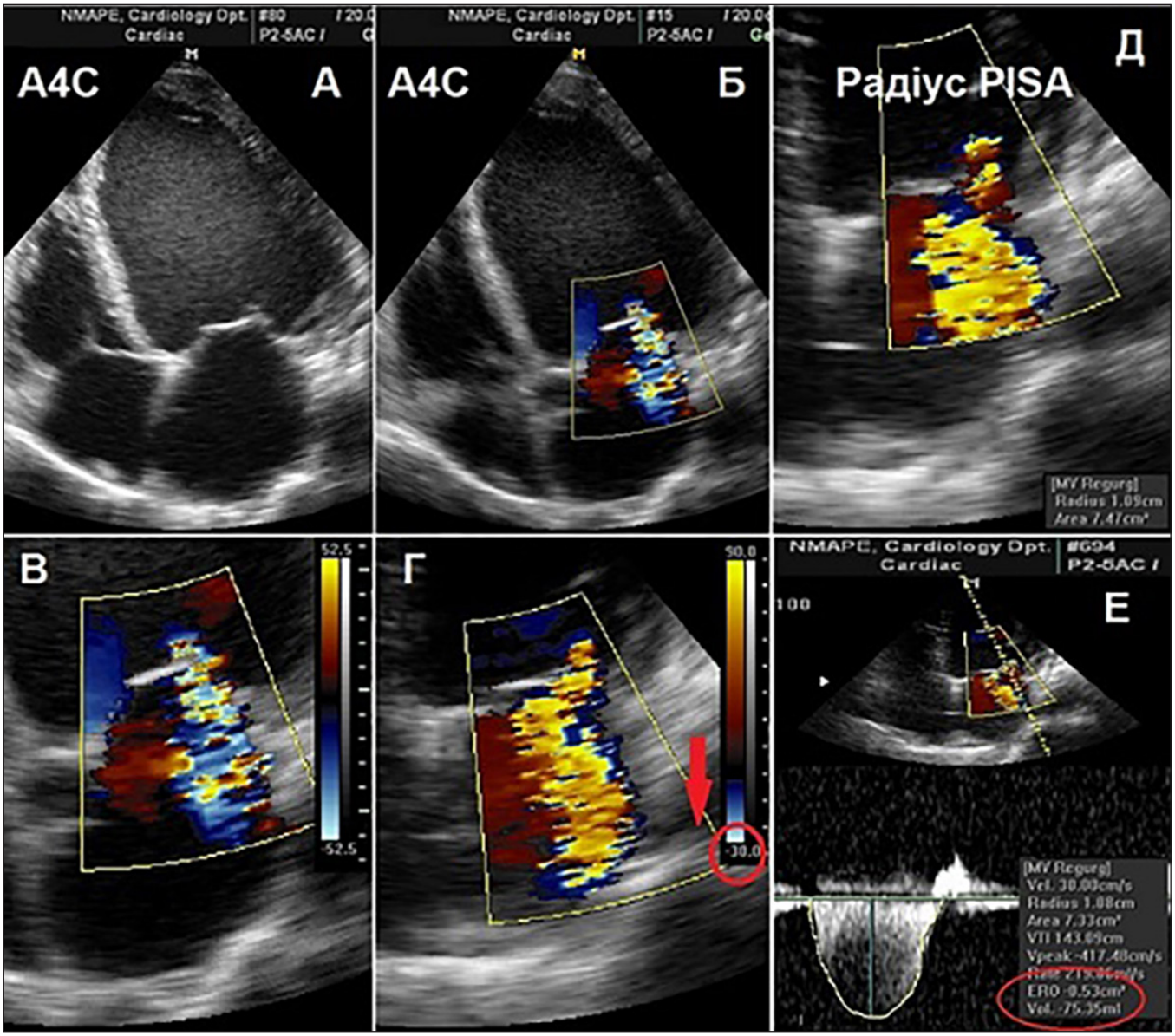


Fig. 3. Quantitative evaluation of the difficulty of MR using the PISA method: A. A4C position; B. Doppler color flow mapping (CFM); B. "Zoom" mode of the area of interest; D. Lowering the zero line of the colored speed scale to obtain the PISA hemisphere; D. Determination of the radius of PISA using the first eleasing; E. Continuous-wave MR spectrum: manual contouring to determine VTI, allowing calculation of EROA and Rvol. (VTI – Velocity Time Integral) [13].

MR should be evaluated using an integrative approach that includes multiple Doppler techniques for direct quantification, as well as ancillary data (left atrial size, LV chamber size, pulmonary vein flow pattern) in the overall assessment [9].

Color Doppler techniques include:

1.Quantitative assessment of MR by the ratio of the plot of maximum distal flow to the left atrium area. Assessment of MR severity based on the plot of the regurgitation area includes tracing the flow area (dashed white line) on the image of the four chambers of the apex and comparing the ratio of the area of the jet to the area of the left atrium (solid white line) (Fig. 1) [10].

2. Vena contracta (VC) measures the linear size of the neck of the MR jet as it enters the regurgitation orifice at the level of the leaflets. The VC is a simple linear measurement of the regurgitation orifice and is relatively independent of load conditions. The VC is measured in the long-axis parasternal plane with a magnified image, and the depth and size of the sector are optimized for color Doppler resolution (Fig. 2). Magnification is critical for accurate vena contracta classification, as small differences in measurement can change the classification category. Since reference ranges for VC have been defined in the long-axis planes, dual-chamber VC measurement should be avoided.

3. A method for evaluating flow convergence. The flow convergence assessment technique (PISA) (Fig. 3)

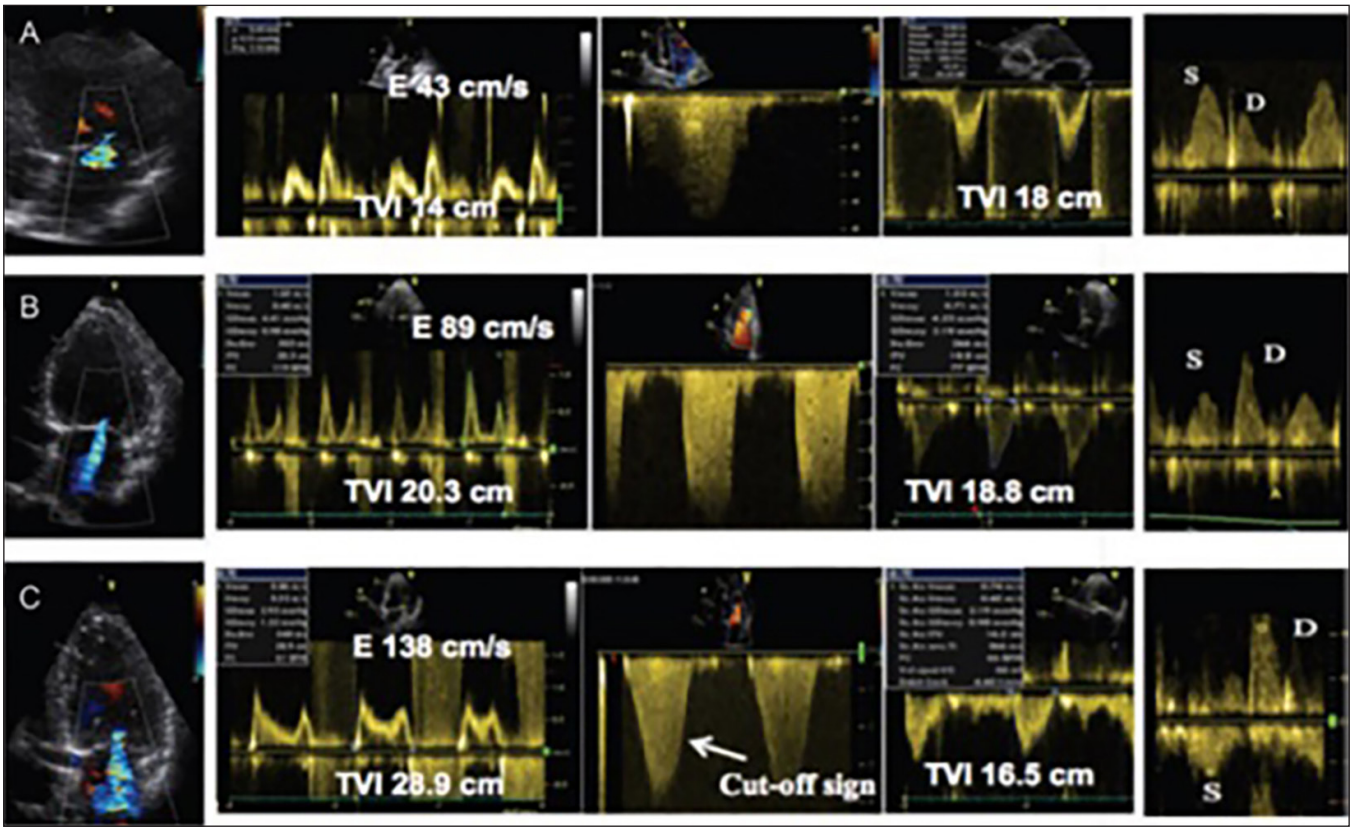


Fig. 4. Three examples of different grades of MR: mild (A), moderate (B) and severe (C). As the severity of MR increases, the area of the regurgitant flow (EROA) and the speed of the early-diastolic transmitral wave E increase. In severe MR, the slow-wave (SW) spectrum of the regurgitant flow is truncated, triangular, and intense. A notch (barb) on the CWD spectrum ("cutoff" sign) can be observed with severe MR. VTI is the integral of the linear velocity of the flow. Mild MR - normal flow contour in the pulmonary vein; moderate MR – decrease in the rate of anterograde systolic flow in the PV; severe MR – reversion of systolic flow in the pulmonary vein (S – systolic wave; D – diastolic wave) [11].

today is the most recommended quantitative method in all cases where it can be used (Fig. 4) [11]. Traditionally, the four-chamber (A4C) position is recommended for optimal PISA imaging. However, the left ventricular long axis (PLAX) position is also often useful for imaging in anterior mitral valve prolapse, where the regurgitant flow jet is directed under the posterior valve and along the posterior wall of the left atrium (LA). The region of interest is optimized by reducing the scan depth and lowering the zero line of Doppler color flow mapping (CFM) to the Nyquist limit to a value of ~15-40 cm/s. The PISA radius is measured in mid-systole to the first spectrum reversal line. Upon that, the values of regurgitation volume (Rvol.) and effective regurgitation orifice area (EROA) are obtained using standard formulas. The presence of PISA at the established Nyquist limit of 50-60 cm/s indicates the need to rule out the severe MR. Grades of MR include mild, moderate, and severe. Moderate MR is divided into "mild-moderate" (EROA 20-29 mm2 or Rvol. 30-44 ml) and "moderate-severe" or "expressed" (EROA 30-39 mm2 or Rvol. 45-59 ml). Primary MR is considered severe with EROA ≥40 mm2 and Rvol. ≥60 mL. In secondary MR, the threshold values for severe regurgitation, which have prognostic value, are EROA = 20 mm2 and Rvol. = 30 ml [12]. EROA is the most powerful parameter that is a marker of MR severity. A large EROA can lead to a large kinetic energy of regurgitation (a large Rvol.), but also to a potential energy with a small Rvol., but with a high pressure in the LA [13].

Ischemic mitral regurgitation (IMR) occurs as a result of left ventricular (LV) remodeling following a myocardial infarction [14, 15, 16]. This form of mitral valve insufficiency occurs without changes in the structure of the mitral valve leaflets and is the result of pathological processes in the myocardium [17, 18]. IMR presents a complex diagnostic and therapeutic challenge, and currently, there are no clear recommendations for choosing the optimal treatment method, whether it be a pharmacological approach, revascularization, or surgical intervention [19, 20]. There are currently a range of surgical, interventional, and electrophysiological methods available for the treatment of IMR [21, 22, 23]. However, there are no clear guidelines for selecting one treatment method over another for IMR, and research into specific factors contributing to the development of this insufficiency using visualization methods is ongoing.

Due to results of our research, the presence of comorbid somatic pathologies such as obesity, diabetes, chronic kidney disease, chronic heart failure, and a history of acute cerebrovascular events complicates the course of acute myocardial infarction and increases the likelihood of ischemic mitral regurgitation.

CONCLUSIONS

It is important to rely on objective indicators during an echocardiographic research and assessment of the function of the heart valves. By comparison of groups of patients with acute myocardial infarction and with mitral insufficiency and without mitral insufficiency, it can be said that the first ones have higher indicators of LV contractile function, such as EDV, ESV, EDI. In patients with MR, indicators of LV contractile function are lower than in the absence of MR. Therefore, the presence of MR on the background of acute myocardial infarction with reduced LV ejection fraction complicates the course of the patient's disease and worsens the prognosis.

REFERENCES

1. Lamas GA, Mitchell GF, Flaker GC, SmithJr SC, Gersh BJ, Basta L, et al. Clinical Significance of Mitral Regurgitation after Acute Myocardial Infarction. Circulation. 1997;96:827-833. doi: 10.1161/01.CIR.96.3.827.
2. Kono T, Sabbah HN, Stein PD, Brymer JF, Khaja F. Left ventricular shape as a determinant of functional mitral regurgitation in patients with severe heart failure secondary to either coronary artery disease or idiopathic dilated cardiomyopathy. Am J Cardiol.1991;68:355-359.
3. Hetmaniuk IB, Fedoniuk LYa, Zhulkevych IV, Andreychyn SM, Hudyma AA, Hanberher II, et al. Microscopic and submicroscopic structure of the heart atria and auricles in condition of the experimental thermal trauma. Biointerface Research in Applied Chemistry. 2020;10(2):5237-5242.
4. Rudenko AV, Vitovskyi RM, Isaienko VV, Krykunov OA, Beshliaha VM, Salo SV, et al. The mitral valve abscess, which simulated the left ventricle tumor. Klinichna khirurhii. 2007;9:58-60.
5. Zoghbi WA, Adams D, Bonow RO, Enriquez-Sarano M, Foster E, et al. Recommendations for noninvasive evaluation of native valvular regurgitation: a report from the american society of echocardiography developed in collaboration with the society for cardiovascular magnetic resonance. J Am Soc Echocardiogr. 2017;30:303-71.
6. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP, et al. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the american college of cardiology/american heart association task force on practice guidelines. J Am Coll Cardiol. 2014;63:e57-185.
7. Zoghbi WA, Adams D, Bonow RO, Enriquez-Sarano M, Foster E, Grayburn PA, et al. Rec- ommendations for noninvasive evaluation of native valvular regurgita- tion: a report from the American society of echocardiography developed in collaboration with the society for cardiovascular mag- netic resonance. J Am Soc Echocardiogr. 2017;30:303-371.
8. Lancellotti P, Moura L, Pierard LA, Agricola E, Popescu BA, Tri- bouilloy C, et al. European Association of Echocardi- ography recommendations for the assessment of valvular regurgita- tion. Part 2: mitral and tricuspid regurgitation (native valve disease). Eur J Echocardiogr. 2010;11:307-332.
9. Zoghbi WA, Enriquez-Sarano M, Foster E, Grayburn PA, Kraft CD, Levine RA, et al. Recommendations for evaluation of the severity of native valvular regurgitation with two-dimensional and doppler echocardiography. J Am Soc Echocardiogr. 2003;16:777-802.
10. Dudzinski DM, Hung J. Echocardiographic assessment of ischemic mitral regurgitation. Cardiovascular Ultrasound. 2014;12:46.
11. Enriquez-Sarano M, Miller FA Jr, Hayes SN, Bailey KR, Tajik AJ, Seward JB. Effective mitral regurgitant orifice area: clinical use and pitfalls of the proximal isovelocity surface area method. J Am Coll Cardiol 1995;25:703-9.
12. Bouma W, van der Horst IC, Wijdh-den Hamer IJ, et al. Chronic ishaemic mitral regurgitation. Current treatment results and new mechanism-based surgical approaches. Eur. J. Cardiothorac. Surg. 2010;37:170-185.
13. Rekomendatsiyi shchodo ekhokardiohrafichnoyi otsinky rehurhitatsiyi na natyvnykh klapanakh. Chastyna I. Atrioventrykulyarna rehurhitatsiya. Rekomendatsiyi robochoyi hrupy z funktsional'noyi diahnostyky Ukrayins'koyi asotsiatsiyi kardiolohiv ta Vseukrayins'koyi asotsiatsiyi spetsialistiv z ekhokardiohrafiyi [Recommendations for echocardiographic assessment of regurgitation on native valves. Part I. Atrioventricular regurgitation. Recommendations of the working group on functional diagnostics of the Ukrainian Association of Cardiology and the All-Ukrainian Association of Specialists in Echocardiography]. (Ukrainian) https://amosovinstitute.org.ua/wp-content/uploads/2018/11/Rekomendatsiyi-EhoKG-MN-TN-1.pdf [data access 10.08.2023]
14. Maistrenko AD. Surgical treatment of ischemic mitral insufficiency of ischemic genesis. Vestnik Khirurgii im. I. I. Grekova. 2014;173(3):103-106.
15. Bouma W, van der Horst IC, Wijdh-den Hamer IJ, et al. Chronic ischaemic mitral regurgitation. Current treatment results and new mechanism-based surgical approaches. Eur J Cardiothorac Surg. 2010;37:170-185.
16. Fedoniuk LYa, Malyk YuYu. Typical mitral valve chordae tendineae and abnormally located chordae tendineae of the human heart left ventricle. Svit medytsyny ta biolohii. 2015;1(48):173-178.
17. Calafiore AM, Iaco AL, Gallina S, et al. Surgical treatment of functional mitral regurgitation. Int J Cardiol. 2013;166:559-571.

18. Calafiore AM, Refaie R, Iaco AL, et al. Chordal cutting in ischemic mitral regurgitation: a propensity-matched study. J Thorac Cardiovasc Surg. 2014;148:41-46.
19. Cheung A, Webb J, Verheye S, et al. Shortterm results of transapical transcatheter mitral valve implantation for mitral regurgitation. J Am Coll Cardiol. 2014;64:1814-1819.
20. Liel-Cohen N, Guerrero JL, Otsuji Y, et al. Design of a new surgical approach for ventricular remodeling to relieve ischemic mitral regurgitation: insights from three-dimensional echocardiography. Circulation 2000;101:2756-2763.
21. Madaric J, Vanderheyden M, Van Laethem C, et al. Early and late effects of cardiac resynchronization therapy on exercise induced mitral regurgitation: relationship with left ventricular dyssynchrony, remodelling and cardiopulmonary performance. Eur Heart J. 2007;28:2134-2141.
22. Mihos CG, Yucel E, Santana O. The role of papillary muscle approximation in mitral valve repair for the treatment of secondary mitral regurgitation. Eur J Cardiothorac Surg. 2017;51:1023-1030.
23. Nappi F, Lusini M, Spadaccio C, et al. Papillary Muscle Approximation Versus Restrictive Annuloplasty Alone for Severe Ischemic Mitral Regurgitation. J Am Coll Cardiol. 2016;67:2334-2346.

The study was conducted as a fragment of the complex scientific projects of the Department of Functional and Laboratory Diagnostics (I. Horbachevsky Ternopil National Medical University) «Comorbidity in the clinic of internal diseases and in the practice of a family doctor: peculiarities of the course of diseases, the strategy of providing medical care during global civil challenges» (state registration number 0123U100061).

ORCID and contributionship:
Liubov R. Stetsiuk: 0009-0002-3286-4869 [A,D]
Ivan M. Klishch: 0000-0001-6226-4296 [A,F]
Ihor O. Stetsiuk: 0000-0002-1865-9976 [C]
Borys M. Todurov: 0000-0001-6647-4469 [F]
Mykhailo B. Todurov: 0009-0008-6299-8319 [A,B]
Larysa Ya. Fedoniuk: 0000-0003-4910-6888 [E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Liubov R. Stetsiuk
I. Horbachevsky Ternopil National Medical University
Maidan Voli, 1, 46001 Ternopil, Ukraine
tel: +380975190648
e-mail: lstetsyuk97@gmail.com

Received: 30.05.2023
Accepted: 15.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

ORIGINAL ARTICLE

EXPERIMENTAL BACKGROUND FOR HORMONE-VITAMIN COMPLEX USING IN COURSE OF REHABILITATION AFTER IONIZING RADIATION

DOI: 10.36740/WLek202311127

Gennadiy F. Stepanov, Rooslan S. Vastyanov
ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE

ABSTRACT

The aim: To determine the efficacy of the original hormone-vitamin complex in terms of biochemical activity enhancement and muscle system functional activity restoration in the irradiated rat's descendents.

Materials and methods: The activity of NADP-dependent malatedehydrogenase and the content of ATP, ADP and AMP were determined in the blood, myocardium and thigh muscles of rats exposed to ionizing gamma-radiation. The rats were also checked in the forced swimming test. The efficacy of the hormone-vitamin complex was determined in all mentioned indexes.

Results: Our results testify the expressed changes in muscle tissue functioning in an irradiated person, which was expressed by the dysfunction of biochemical reactions aimed at synthetic energy processes, and by the macroergic compounds level depletion together with physical performance minimization. Our data showed the hormone-vitamin complex injection to irradiated animals and their descendants improved the muscle energy resources due to glycolytic substrate phosphorylation enhancement and due to tricarboxylic acids cycle oxidative potential strengthening.

Conclusions: Original scheme of post-radiation lesions complex pharmacological correction prevented the development of tissues providing with macroergic compounds, anaerobic processes strengthening, metabolic acidosis, weakening of both substrate phosphorylation and tricarboxylic acids cycle. The original scheme of ionizing radiation-induced energetic disorders pharmacological corrections in the irradiated animals' descendents we consider as an experimental basis for the reasonability of these compound radioprotective effects testing during the physiotherapeutic treatment of persons exposed to ionizing radiation.

KEY WORDS: ionizing radiation, muscle dysfunction, energetic exhaustion, physical performance, hormone-vitamin complex, treatment efficacy, rehabilitation

Wiad Lek. 2023;76(11):2510-2516

INTRODUCTION

The concept of medical support of rehabilitation in response to ionizing radiation provoked damage of the human body acquires not only medical and industrial but also economic, social and state importance [1, 2]. A significant period of time which separates us from the terrible nuclear disaster in Chernobyl significantly reduced the severity of the problem, but already the accident at the Fukushima nuclear power plant reminded us of the extremely dangerous impact of ionizing radiation on the biological organism [3]. The military aggression currently taking place against our country urgently raises the question of improving existing and developing new methods of rehabilitation after ionizing radiation, since the enemy is resolutely trying to destroy the Zaporizhzhya nuclear power plant, and depleted uranium ammunition is increasingly being used on the battlefield [2, 4].

The medical side of this problem allows us to recall the development of degenerative-destructive changes at all structural and functional levels of organization and systemic regulation of the activity of a biological organism after exposure to ionizing radiation [5, 6]. Rehabilitation schemes that were used had the resulting effect on the ionizing factor harmful effects elimination and the body's energy reserve direct stimulation (or activation) [7]. We have been conducting experimental trials aimed at elucidating the details of the radioprotective effect of the original hormone-vitamin complex [8, 9]. We chose as the main concept the activation of the body's protective adaptive resources with an emphasis on pharmacologically-induced muscle system activity increase during the rehabilitative effect implementation. Our attention is focused on the descendents of irradiated animals which allows us to follow the depth of damage and pathophysiological mechanisms of

Table I. Changes of NAD-dependent malatedehydrogenases activity in the tissues of 1-month-old rats born from animals irradiated with different doses and exposed to radiation at a dose of 1.0 Gy as s result of hormone-vitamin complex influence

N	Enzymes	Myocardium		Skeletal muscle		Blood
		Cytoplasm	Mitochondria	Cytoplasm	Mitochondria	
Intact rat pups, n=10						
1	NAD-MDH (direct reaction)	0.58±0.05	0.29±0.02	0.21±0.01	47.37±3.24	1.93±0.16
2	NAD-MDH (reverse reaction)	2.46±0.02	0.22±0.03	1.07±0.03	54.18±3.61	4.37±0.36
1-month-old rats born from animals once irradiated by 0.5 Gy and exposed to irradiation at a dose of 1.0 Gy, n=10						
Before correction						
1	NAD-MDH (direct reaction)	0.66±0.18	0.24±0.01	0.28±0.01	40.36±2.80	2.24±0.19
2	NAD-MDH (reverse reaction)	2.46±0.15	0.27±0.05	1.45±0.07[a]	68.24±2.92	4.62±0.31
After correction						
1	NAD-MDH (direct reaction)	0.63±0.18	0.23±0.01	0.26±0.01	43.28±3.20	1.79±0.18
2	NAD-MDH (reverse reaction)	2.08±0.14	0.25±0.06	1.28±0.06	66.48±2.86	4.28±0.31
1-month-old rats born from animals once irradiated by 1.0 Gy and exposed to irradiation at a dose of 1.0 Gy, n=10						
Before correction						
1	NAD-MDH (direct reaction)	0.98±0.36	0.18±0.01[a]	0.31±0.01[a]	26.56±1.41[a]	1.25±0.13[a]
2	NAD-MDH (reverse reaction)	3.81±0.24[a]	0.29±0.09	1.84±0.10[a]	78.52±3.64[a]	5.72±0.39[a]
After correction						
1	NAD-MDH (direct reaction)	0.69±0.20[b]	0.25±0.02	0.28±0.01	41.94±3.21[b]	1.92±0.19[b]
2	NAD-MDH (reverse reaction)	1.99±0.13[b]	0.28±0.07	1.29±0.07	68.42±2.92	4.55±0.34

Notes: [a] – $p<0.05$ – studied indexes significant differences compared with the same in intact rat pups; [b] – $p<0.05$ – studied indexes significant differences compared with the same before pharmacological correction.

radio-induced changes in the organism of the second generation of animals and to determine the necessary level of sanogenetic restorative effects implementation.

THE AIM

The aim of the present work was to determine the efficacy of the original hormone-vitamin complex in terms of biochemical activity enhancement and muscle system functional activity restoration in the irradiated rat's descendents.

MATERIALS AND METHODS

This study was conducted in accordance with the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013). We used the recommendations of the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985) and the rules of humane treatment of experimental animals and conditions approved by the Bioethics Commission of the Odessa National Medical University (protocol No. 32D dated 03/17/2016).

Experimental trials were carried out on 66 Wistar rats, weighing 200-250 g. Rats were kept at a constant temperature of 24-26°C, 55-60% humidity, 12/12 hr light/dark cycle and on a standard vivarium diet. The cages were changed weekly, and rats' fluids were changed every two day.

The mature rats were exposed to a single total gamma irradiation of ^{60}Co in the morning on an empty stomach on the "Agat" telegammatherapy unit (the distance to the device was 75 cm, the dose rate was 0.54 Gy/min, the absorbed dose was 0.5 Gy and 1.0 Gy).

The rats were randomly divided into 7 groups: the 1st group (n=10) – 1-month-old rats born from intact animals; the 2nd and the 3rd groups (n=2x10) – 1-month-old rat pups born from animals once totally irradiated by 0.5 Gy and 1.0 Gy; the 4th and the 5th groups (n=2x10) – 1-month-old rat pups born from animals once totally irradiated by 0.5 Gy and by 1.0 Gy and exposed to a dose of 1.0 Gy; the 6th and the 7th groups (n=2x8) – 1-month-old rat pups born from animals once totally irradiated by 0.5 Gy and by 1.0 Gy and exposed to a dose of 1.0 Gy, which were administered with a hormone-vitamin complex (HVC).

The HVC included tocopherol acetate (i.m., 50 mg/kg, 30 min after irradiation), retabolil (i.m., 2.5 mg/kg, 3 hrs after irradiation), cocarboxylase (s.c., 5 mg /kg) and nicotinamide (s.c., 10 mg/kg), which were administered 1 day after irradiation dissolved in 0.5 ml of saline. The HVC was administered during 12 days [10].

After euthanasia (i.v., propofol, 60 mg/kg) the animals' blood was collected, the heart (2/3 of the heart`s apex) and the frontal group of thigh muscles (mainly quadriceps femoris and sartorius muscle) were

Table II. Changes of ATP, ADP and AMP content in the tissues of 1-month-old rats born from animals irradiated with different doses and exposed to radiation at a dose of 1.0 Gy as s result of hormone-vitamin complex influence

N	Investigated compounds	Skeletal muscle	Myocardium
Intact rat pups, n=10			
1	ATP	2.86±0.24	4.93±0.37
2	ADP	0.39±0.04	0.24±0.02
3	AMP	0.22±0.02	0.10±0.01
1-month-old rats born from animals once irradiated by 0.5 Gy and exposed to irradiation at a dose of 1.0 Gy, n=10			
Before correction			
1	ATP	2.29±0.19	4.53±0.35
2	ADP	0.77±0.07[a]	0.44±0.04[a]
3	AMP	0.29±0.02[a]	0.16±0.02[a]
After correction			
1	ATP	2.83±0.23	4.93±0.37
2	ADP	0.40±0.04[b]	0.24±0.02[b]
3	AMP	0.23±0.02	0.11±0.01[b]
1-month-old rats born from animals once irradiated by 1.0 Gy and exposed to irradiation at a dose of 1.0 Gy, n=10			
Before correction			
1	ATP	1.17±0.14[a]	2.45±0.44[a]
2	ADP	0.52±0.05[a]	0.57±0.05[a]
3	AMP	0.40±0.04[a]	0.18±0.02[a]
After correction			
1	ATP	2.79±0.22[b]	4.81±0.35[b]
2	ADP	0.40±0.04	0.25±0.02[b]
3	AMP	0.23±0.02[b]	0.11±0.01[b]

Notes: [a] – $p<0.05$ – studied indexes significant differences compared with the same in intact rat pups; [b] – $p<0.05$ – studied indexes significant differences compared with the same before pharmacological correction.

removed. We determined the activity NADP-dependent malatedehydrogenase in both direct and reverse reactions and the content of ATP, ADP and AMP in one sample using combined reactions [11]. All indexes of energy metabolism were expressed in μmol per 1 g of the studied tissue.

The physical working capacity of the animals was determined in the forced swimming test with a load and was evaluated by the swimming duration with a load of 10% of body weight attached with a rubber ligature to the root of the animal's tail [12]. Swimming was carried out in the time interval from 10.00 to 12.00 in glass vessels with an inner diameter of 30 cm and a height of 50 cm. The moment of the trial finish was considered the animal's fatigue in the form of an inability to rise to the surface of the water for 8 s or the animal's refusal to swim (immersion to the bottom for more than 10 s).

The data obtained were presented as mean (x) and the standard error of the mean (SE). $\chi 2$ criterion was used to detect the significant differences between the investigated groups $p<0.05$ was considered as statistically significant difference.

RESULTS

The activity of NAD-dependent MDH in the cytoplasm of cardiac and skeletal muscles of descendents born from irradiated animals in different doses and exposed to radiation 1.0 Gy after the HVC introduction is comparable to the same index in the control animals ($p>0.05$; Table I). The activity of the direct NAD-dependent MDH reaction in the mitochondria of cardiac and skeletal muscles of descendents born from irradiated animals in different doses and exposed to radiation 1.0 Gy after the HVC injection is reduced, and the lowest values are observed in rats born from irradiated animals by 1.0 Gy and exposed to irradiation at a dose of 1.0 Gy ($p<0.05$). The pharmacological correction, however, results in these indexes being identical to corresponding ones in intact rats ($p>0.05$).

Direct NAD-dependent MDH reaction activity in the blood of descendents born from irradiated animals in different doses and exposed to radiation 1.0 Gy after the HVC administration is reduced. The lowest index is observed in rats born from animals irradiated by 0.5 Gy and exposed to radiation at a dose of 1.0 Gy after pharmacological correction.

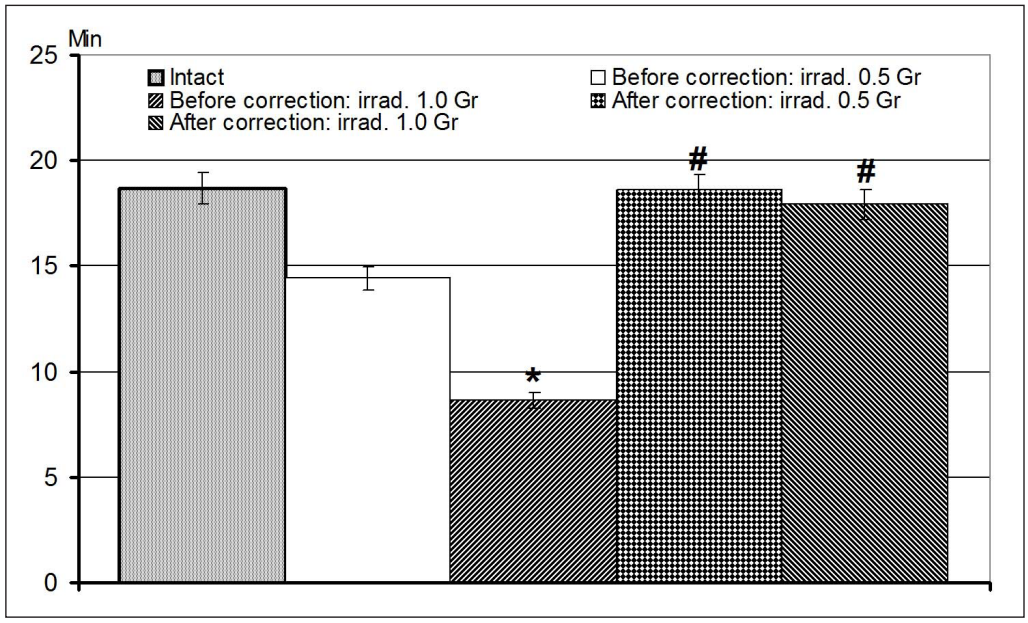


Fig. 1. Changes of forced swimming time of 1-month-old rats born from animals irradiated with different doses and exposed to radiation at a dose of 1.0 Gy as s result of hormone-vitamin complex influence.
Notes: * – $p<0.05$ – studied indexes significant differences compared with the same in intact rat pups; # – $p<0.05$ – studied indexes significant differences compared with the same before pharmacological correction.

The activity of NAD-dependent MDH in the cytoplasm of myocardium of descendents born from irradiated animals in different doses and exposed to radiation by 1.0 Gy after the HVC injection is lower vs the same control index. The lowest index of its activity is observed in the cytoplasm of the myocardium in rats born from animals irradiated at a dose of 1.0 Gy and exposed to irradiation by of 1.0 Gy after the HVC administration ($p>0.05$).

The activity of NAD-dependent MDH (reverse reaction) in the mitochondria of cardiac and skeletal muscles, as well as in the blood of descendents born from irradiated animals in different doses and exposed to radiation by 1.0 Gy after the HVC introduction is comparable to such control indexes. The indexes obtained depend on the dose exposed to parents.

The ratio direct/reverse NAD-dependent MDH reaction in descendents born from irradiated animals in different doses and exposed to radiation by 1.0 Gy after the HVC injection increases drastically. This becomes significant in rats born from animals once irradiated by 1.0 Gy and exposed to radiation at a dose of 1.0 Gy, compared with such indexes in descendents born from irradiated animals in different doses and exposed to radiation by 1.0 Gy without the pharmacological correction ($p<0.05$).

Adenylnucleotides content change was recorded in rat pups born from irradiated animals at a different doses and exposed to irradiation by 1.0 Gy ($p<0.05$; Table II).

We registered both ADP and AMP content significant increase due to which a stable ATP concentration is maintained in the muscles of 1-month-old rats born from animals irradiated at a dose of 0.5 Gy and exposed to irradiation by 1.0 Gy, and an acute ATP content decrease (by 2.0-2.4 times; $p<0.05$) within the skeletal and cardiac muscle, respectively, and an acute ADP and AMP levels increase ($p<0.05$) in muscle tissue of 1-month-old rats born from parents once irradiated at dose 1.0 Gy and then exposed to radiation at the same dose. Pharmacological correction of these metabolic disorders resulted in ATP concentrations increase in both rat pups born from animals once irradiated at a doses of 0.5 Gy and 1.0 Gy and exposed to radiation by 1.0 Gy (in both groups $p<0.05$).

The values of ADP and AMP content in the studied groups of animals exceeded the same indexes in 1-month-old rats and were comparable to them, which indicated the pronounced changes development as the result of HVC use ($p<0.05$).

Physical activity of the descendents born from irradiated animals and exposed to a maximal dose of radiation significantly differed from the same in the intact rats and, moreover, it was changed after the HVC administration (Fig. 1). Swimming time of 1-month-old rats born from animals once irradiated at a dose of 0.5 Gy and exposed to irradiation by 1.0 Gy was 23% less compared with the same index in intact rats and 30% less compared with the same index in non-irradiated rat pups born from animals once irradiated by 0.5 Gy ($p<0.05$).

We registered the muscle tissue functioning significant changes in rats born from once irradiated at a dose of 1.0 Gy and exposed to irradiation at the same dose after the HVC pharmacological correction. These rats demonstrated swimming time significantly less by more than 2 times compared with the same control index ($p<0.05$) and by 32% compared with the same index in non-irradiated rat pups born parents once irradiated at a dose of 1.0 Gy.

DISCUSSION

Thus, the obtained results testify the expressed changes in muscle tissue functioning in an irradiated person, which was expressed by the dysfunction of biochemical reactions aimed at synthetic energy processes, and by the macroergic compounds level depletion together with physical performance minimization, which, in a systemic analysis, illuminates pathogenetic mechanisms intended to destructive, degenerative and necrotic directions, the result of which should be a regulatory "breakdown", systemic-anti-systemic dysfunction and/or energetic exhaustion, the comorbid pathology burden and the death of the organism [6].

It has been proven that HVC introduction for the muscle tissue metabolic disorders correction in irradiated animals and their descendants improved the muscle energy resources due to glycolytic substrate phosphorylation enhancement and due to tricarboxylic acids cycle oxidative potential strengthening on at the stage of MDG action and on the stage catalyzed by succinatedehydrogenase. Our results are confirmed by a significant array of actual data concerning the physical performance increase in the studied rats.

We specially organized such a method of post-radiation lesions complex pharmacological correction to prevent the development of providing tissues with macroergic compounds, anaerobic processes strengthening, metabolic acidosis, weakening of both substrate phosphorylation and tricarboxylic acids cycle as well as cellular genetic apparatus damage cells and strengthening the normalization of regeneration processes [5]. We had already optimistic results of HVC using in animals and their descendants, which proved the energy supply processes restoration in irradiated rats [8].

We consider important to provide the experimental study of muscle tissue dysfunction not only and not so much in irradiated animals, but in their descendants, which has the adequate clinical validity (the second generation is living after the accident at Chernobyl nuclear power plant) and provides an opportunity to investigate thoroughly the pathophysiological mechanisms of radiation-induced morphological, functional, biochemical and regulatory violations, and also allows to comprehensively study the possibilities of rehabilitation effects and establish its efficacy.

We achieved positive results in the case of a full-fledged pharmacological correction of the formed energetic disorders, which should be the background of a complete rehabilitation scheme for the specified contingent of victims, taking into account the multimodal concept of health restoration of military personnel adopted by the medical service of the Military Forces of Ukraine [13]. It is important to understand and be able to change dynamically the composition of the complex pharmacological therapy of radiation-induced functional disorders depending on the clinical condition of the patients. This is possible in case of a clear understanding the original scheme of pharmacocorrection mechanisms of action. It's important to understand that under the specified pathological conditions, the activation of the reverse NAD-dependent MDH occurs in the cytoplasm and in the mitochondria of muscle tissue, as well as the predominance of the reverse NADP-dependent MDH reaction. Reduced forms of $NADH^+$ accumulate in the tissues, which induces the acidosis and creates conditions for competition between aerobic and anaerobic processes, where anaerobic reactions have an advantage. The formation of pronounced epigenetic changes induced by ionizing radiation has also been shown, as a result of which the ATP content decreases due to (a) the delay in the processes of adenyl system phosphorylation from its dephosphorylation, (b) an increase in the methylated ATP derivatives content and (c) this metabolite increased degradation, which will definitely lead to a change in physical performance of an organism [14].

The original treatment scheme includes drugs with antioxidant properties - multivitamin complexes, which additionally strengthen the whole organism resistance to the negative impact of adverse environmental factors [15]. The HVC ensures the coherence of metabolic processes in the body, resistance to the influence of extreme environmental factors, and the ability to adapt the regulatory systems of the body [16]. We believe that this is important in the processes of utilization and resynthesis of metabolites of a carbohydrate nature, which are under complex and multi-stage hormonal and vitamin control, in which the hormones of the pancreas and adrenal glands play a leading role, supporting the metabolism and the functional activity of the bodies vital systems [6, 17].

We consider our idea, consisting in the above-mentioned pharmacological compounds combined use to test their efficacy in the irradiated animals' descendents as an experimental basis for the reasonability of the original scheme radioprotective effects testing during the physiotherapeutic treatment of persons exposed to ionizing radiation.

CONCLUSIONS

1. The data obtained testify the expressed changes in muscle tissue functioning in an irradiated person, who was expressed by the dysfunction of biochemical reactions aimed at synthetic energy processes,

and by the macroergic compounds level depletion together with physical performance minimization.
2. The hormone-vitamin complex introduction in irradiated animals and their descendants improved the muscle energy resources due to glycolytic substrate phosphorylation enhancement and due to tricarboxylic acids cycle oxidative potential strengthening on at the stage of MDG action and on the stage catalyzed by succinatedehydrogenase. We registered also the physical performance increase in the studied rats.
3. Original scheme of post-radiation lesions complex pharmacological correction prevented the development of tissues providing with macroergic compounds, anaerobic processes strengthening, metabolic acidosis, weakening of both substrate phosphorylation and tricarboxylic acids cycle as well as cellular genetic apparatus damage cells and strengthening the normalization of regeneration processes.
4. The original scheme of ionizing radiation-induced energetic disorders pharmacological corrections in the irradiated animals descendents we consider as an experimental basis for the reasonability of these compound radioprotective effects testing during the physiotherapeutic treatment of persons exposed to ionizing radiation.

REFERENCES

1. Pechyborshch VP, Volyanskyi PB, Yakimets VM et al. Medychnyy zakhyst naselennya v nadzvychaynykh sytuatsiyakh v yedyniy derzhavniy systemi tsyvil'noho zakhystu [Medical protection of the population in emergency situations in the unified state system of civil protection]. Kyiv : Lyudmila Publishing House. 2019, p.693. (Ukrainian)
2. Chekhun VF, Domina EA. Suchasnyy pohlyad na stokhastychni efekty ionizuyuchoho vyprominyuvannya [Modern view on the stochastic effects of ionizing radiation]. Oncology. 2022;24(1):5-10. doi: 10.32471/oncology.2663-7928.t-24-1-2022-g.10339. (Ukrainian)
3. Konoplev A. Fukushima and Chernobyl: Similarities and Differences of Radiocesium Behavior in the Soil–Water Environment Toxics. 2022;10(10):578. doi: 10.3390/toxics10100578.
4. Maidanyuk VP, Volianskyi PB, Pechiborshch VP et al. Viyna y teroryzm: medyko-sanitarni naslidky ta derzhavne rehulyuvannya medychnym zakhystom naselennya [War and terorism: health consequences and state regulation of the population medical protection]. Current Issues of Transport Medicine. 2023;3(73):7-20. doi: 10.5281/zenodo.8254462. (Ukrainian)
5. Burgio E, Piscitelli P, Migliore L. Ionizing Radiation and Human Health: Reviewing Models of Exposure and Mechanisms of Cellular Damage. An Epigenetic Perspective. International Journal of Environmental Research and Public Health. 2018;15(9):1971. doi: 10.3390/ijerph15091971.
6. Moroz VM, Shandra OA, Vastyanov RS, et al. Physiology. Vinnytsia: Nova Knyha. 2016, p.722.
7. Wei Z, Edin J, Karlsson AE, et al. Can gamma irradiation during radiotherapy influence the metal release process for biomedical CoCrMo and 316L alloys? J Biomed Mater Res B Appl Biomater. 2018;106(7):2673-2680.
8. Stepanov GF, Kostina AA, Dimova AA. Porivnialna kharakterystyka terminalnoi lanky hlikolizu v miazakh statevozrilykh tvaryn ta yikhnikh nashchadkiv [Comparative characteristics of the terminal link of glycolysis in the muscles of sexually mature animals and their offspring]. Odesa Medical Journal. 2021;5:9-13. (Ukrainian)
9. Stepanov GF, Vastyanov RS The peculiarities of low dose ionizing radiation influence on muscles metabolism in experimental animals. World of Medicine and Biology. 2023;2(84):233-238.
10. Mardashko OO, Popyk GS, Storchilo OV. Sposib korektsii homeostazu orhanizmu, yakyi zaznav vplyvu radiatsiinoho urazhennia [A method of correcting the homeostasis of an organism affected by radiation damage]. Ukraine Patent UA 68126. 2004, p.4. (Ukrainian) https://sis.ukrpatent.org/uk/search/detail/378445/ [data access 11.04.2023]
11. Kodali ST, Kauffman P, Kotha SR et al. Oxidative Lipidomics: Analysis of Oxidized Lipids and Lipid Peroxidation in Biological Systems with Relevance to Health and Disease. In L. J. Berliner (Eds.) et al. Measuring Oxidants and Oxidative Stress in Biological Systems. Springer. 2020:61-92.
12. Armario A. The forced swim test: Historical, conceptual and methodological considerations and its relationship with individual behavioral traits. Neurosci Biobehav Rev. 2021;128:74-86.
13. Babov KD, Khomenko IP, Tertyshnyi SV et al. Orhanizatsiya etapnoyi reabilitatsiyi viys'kovosluzhbovtsiv z vohnepal'nymy defektamy m"yakykh tkanyn na rivnyakh nadannya medychnoyi dopomohy [Organization of staged rehabilitation of servicemen with gunshot defects of soft tissues at the levels of providing medical assistance]. Medical Perspectives. 2021;26(4):188-195. (Ukrainian)
14. Fix DK, Hardee JP, Bateman TA et al. Effect of irradiation on Akt signaling in atrophying skeletal muscle. Journal of applied physiology. 2016;121(4):917-924.
15. Azzam EI, Colangelo NW, Domogauer JD et al. Is Ionizing Radiation Harmful at any Exposure? An Echo That Continues to Vibrate. Health physics. 2016;110(3):249-251.

16. Stotska SV, Moisienko VV, Kotkova TM et al. Zastosuvannia likarskykh roslyn pry radiohennykh zakhvoryuvanniakh [Use of medicinal plants in radiogenic diseases]. Chornobylska katastrofa. Aktualni problemy, napriamky ta shliakhy yikh vyrishennia. Zhytomyr : Poliskyi universytet. 2021, pp.174-177. (Ukrainian)
17. Liu L, Liang Z, Ma S, et al. Radioprotective countermeasures for radiation injury (Review). Molecular medicine reports. 2023;27(3):66. doi.org/10.3892/mmr.2023.12953.

The study was performed within the framework of the scientific topics "Mechanisms of epigenetic disorders of the leading links of bioenergetics and nitrogen metabolism in irradiated animals and their descendants", state registration No 0121U114601.

ORCID and contributionship:
Gennadiy F. Stepanov: 0000-0002-8242-8689 [A,B,C,D]
Rooslan S. Vastyanov: 0000-0001-8585-2517 [E,F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Gennadiy F. Stepanov
Odesa National Medical University
Valikhovsky lane, 2, 65026, Odesa, Ukraine
e-mail: medchem@ukr.net

Received: 24.05.2023
Accepted: 10.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

REVIEW ARTICLE

LEGAL CONFLICTS IN MEDICAL PRACTICE AND METHODS OF THEIR RESOLUTION

DOI: 10.36740/WLek202311128

Viktor V. Zaborovskyy, Yaroslav V. Stupnyk, Mykhaylo M. Hetsko, Pavlo P. Chervko
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD,UKRAINE

ABSTRACT

The aim: A theoretical and applied study of the essence of legal conflicts in the field of medical activity in order to determine the proper system of ways to protect the interests of patients, doctors and other participants in medical legal relations.

Materials and methods: Various methods of scientific knowledge make up the methodological basis of research. Thus, the comparative legal method was used to compare the features of the legal regulation of the rights protection of medical legal relations subjects in different countries. The essence and classification criteria of both legal conflicts and methods of protection in the field of medical activity were investigated with the help of a system-complex method. The following other methods were used in the study, in particular: formal-logical, dialectical, analysis and synthesis.

Conclusions: The classification of dispute resolution methods in the aspect of medical activity indicates the possibility of distinguishing non-jurisdictional (self-defense) and jurisdictional (special, administrative, judicial protection) forms of patients' rights protection, as well as distinguishing two levels of such protection (pre-trial and judicial), each of which has its distinctive features and the patient himself is able to determine in what way and by what level to protect his rights. An important role in the conflict resolution in the field of health care is primarily played by the pre-trial protection, the defining features of which are the voluntary nature of conflict resolution, the availability, convenience and speed of dispute resolution as well as the possibility of compensation for the damage caused

KEY WORDS: medical activity; health care; legal conflict; forms of patients' rights protection; pre-trial protection level

Wiad Lek. 2023;76(11):2517-2524

INTRODUCTION

There is no doubt that the 21st century is characterized by the rapid development of medicine, regarding both the improvement of medical law norms and the application of modern methods of diagnosis and treatment. On the one hand, this contributes to improving the quality of medical care and creating the possibility of treating very complex diseases, while on the other hand, it does not prevent from arising the conflict situations in terms of providing such care.

THE AIM

The aim of the article wasa comprehensive theoretical and applied study of the essence of legal conflicts that arise in the field of health care, in order to determine the appropriate system of ways to protect the interests of patients, doctors and other participants in medical legal relations.

The issue of determining the essence of legal conflicts as well as procedures and mechanisms for protecting the participants' rights in medical legal relations was being studied by a number of scientists, including Y. Astaykina, S. Bobrovnyk, L. Bokeriya., R. Hryvtsova, O. Drozdova, O. Klymenko, R. Maidanyk, H. Mykhailova, O. Mostovenko, T. Semina, R. Stefanchuk, S. Stetsenko, Y. Shvets, E. Shcheglov and others. At the same time, a comprehensive study of the legal nature of conflicts in the field of medical activity and methods of their resolution was virtually disregarded by the scientists.

MATERIALS AND METHODS

Various methods of scientific knowledge make up the methodological basis of research. Hence, the comparative legal method was used to compare the features of the legal regulation of the rights protection of medical legal relations subjects in different countries. The essence and classification criteria of both legal conflicts and methods of protection in the field of medical activity were investigated with the help of the system-complex method. The following other methods were used in the study, in particular: formal-logical, dialectical, analysis and synthesis.

REVIEW AND DISCUSSION

LEGAL CONFLICT AS A BASIS FOR THE PROTECTION OF SUBJECTS OF MEDICAL LEGAL RELATIONS

Actually, one of the medical activity features is that such professional activity is inextricably linked with a sufficiently large number of potential reasons for patients to remain dissatisfied with the provision of medical care. E. Shcheglov and M. Smutok note that even the slightest deviation from the results expected by the patient can affect the occurrence of a conflict situation, and when it comes to life and health, human consciousness and emotions are in a special state [1]. At the same time, it is necessary to take into account the fact that the doctor's profession is related to medical intervention, the peculiarity of the human body reactions, various manifestations of disease course, the problem of correct and timely diagnosis, etc., and therefore, objectively, the doctor cannot always foresee and do everything [2]. All this indicates an increased possibility of conflict occurrence during the medical care provision, which acquire a special, social significance.

Aiming to reveal the essence of the legal conflict itself as a basis for the protection of medical legal relations subject, it is necessary to pay attention to such an aspect as the protection of the doctor's honour and business reputation. This necessity is due to the fact that the profession of a doctor in recent years has increasingly become the object of social and legal control by society, taking into account, foremost, the rapid increase in the number of claims against medical institutions and doctors, as well as the number of criminal and civil cases initiated in connection with failure to provide or improper provision of medical care [3]. This shows that the legal conflict occurrence in the aspect of medical activity is the basis for the human rights protection in the field of health care not only in relation to patients, but to doctors and other participants in medical legal relations as well.

It should be noted that there is no unified understanding of the concept of "legal conflict" among scientists, however most of them adhere to the viewpoint that such a conflict, which is a type of social conflict, represents a certain confrontation regarding the interests, needs, and views of several subjects. Moreover, a number of such scientists, revealing the essence of the definition of this concept, pay attention to both its negative and positive features [4], pointing out that a legal conflict: can have a positive effect on the process of changing the legal reality, and, accordingly, inhibit or stimulate (be a driving force) social or state transformations [5], be a form of improving society, which enables people to gain experience not only of a negative, but of a positive nature as well (the ability to recognize the nature of conflict, the ability to manage it, and most importantly, to have conflict resolution mechanisms) [6].

Legal conflict must be considered as a dynamic process, and its occurrence and existence requires the presence of the following components: preconditions for the occurrence of a legally significant conflict situation; the existence of a legal conflict situation itself; and actions carried out by one of the parties to defend their interests (the direct content of the conflict), which makes it possible to classify such conflicts according to different criteria. Therefore, researching the types of legal conflicts, Y.O. Astaykina and O.M. Khokhlov come to the conclusion that the most accurate is the classification of these conflicts by law, since conflicts are possible in all spheres of life in society, and mostly conflicts arise from issues related to such spheres as labour, family, civil, administrative, financial, criminal, criminal procedure law, etc [7].

An important place among such conflicts is occupied by legal conflicts in the sphere of rights protection of medical legal relations subjects. The possibility of a legal conflict in the field of medical activity is due to the fact that the purpose of the contract for providing medical services is the provision of a service itself, and not the achievement of the recovery result [8], which is very often forgotten by patients, first of all, in the case when, as a result of providing such services, there was no improvement in their health condition. As rightly noted by O.S. Mostovenko, in practice, the object of legal relations for a doctor is the process of providing medical care (services), while for patients, it is their health, and sometimes their life (sometimes the patient forgets that the medical worker is not responsible for the onset of the patient's illness, and is not aware of the fact that doctors cannot guarantee one hundred percent recovery or improvement in the quality of life, regardless of the country where medical care will be provided, its cost, years of experience of the doctor himself) [9]. Unfortunately, in many cases, a medical error is a precondition for a legal conflict in the field of medical activity. Thus, according to scientists, there is no country where medical errors do not occur, just as there is no single universally accepted definition of such an error, and statistics show that up to 70,000 people die from errors of medical personnel in Great Britain every year, in Italy – about 90 thousand, in the USA – 50-100 thousand people, where, in addition, almost every tenth doctor becomes the object of legal claims from a former patient. In Germany, up to 100,000 cases of incorrect diagnosis of the disease, wrong prescription

of drugs as well as surgical and interventional care are detected annually [3]. A certain precondition for the increase in the number of legal conflicts in the field of medical activity was the increase in the legal literacy of the population in recent years, as a result of which the number of complaints from patients and their relatives, who apply for the protection of their rights, has significantly increased [10].

Having studied the preconditions for the occurrence of legal conflicts in the field of medical activity, it is necessary to reveal their essence, as well as the criteria for the classification of their types. Widespread in the literature is the point of view according to which a conflict in the field of medical activity should be understood as an open confrontation between subjects of legal relations in the field of medical activity, connected with their realization of interests of a mutually exclusive nature, while physical (patient, private doctor) and legal entities (medical institution, health care management body) can act as subjects of a legal conflict in medicine [11]. Other scientists hold an almost identical point of view [12].

Elucidation of the essence of legal conflicts in the field of medical activity is primarily aimed at preventing the occurrence of conflict situations or at mitigating their consequences and ending them. Investigating this question, H.L. Mykhailova rightly points out that, on the one hand, the most effective method of preventing conflict situations related to the provision of medical services is their prevention and resolution at an early stage of development (for this purpose, specialized state and non-state institutions are created) [13], and on the other hand, it requires the existence of an extensive system that will allow the specific features of consideration and resolution of disputes in the field of medical activity, taking into account all their diversity. The proper functioning of the relevant system would contribute not only to the consideration and resolution of conflict situations at the stages of their origin and development, but also to the accelerated process of restoration of violated rights in the field of health care. This necessity is due to the fact that preventing the occurrence of conflict situations will definitely contribute to the improvement of the relationship between the doctor and the patient, which will have a positive effect on the outcome of treatment and will prevent the spread of a negative attitude towards medicine in general, which is becoming threatening.

The variety of disputes that may arise in the field of medical activity implies the possibility of the existence of different criteria for the classification of legal conflicts. Therefore, according to scientists, the classification of legal conflicts in the field of medical activity should be carried out according to several criteria, namely: depending on the subject of legal regulation (administrative-legal, criminal-legal, civil-legal, etc.); by duration (short-term; long-term); and depending on the theoretical features of the legal conflict and the practice of providing medical care (legitimate, i.e., those arising from legal relations during the medical care provision, and illegal – those arising from offenses during the provision of medical care) [14].

The existence of a significant number of disputes in the field of medical activity as well as criteria for the classification of relevant legal conflicts also implies the existence of an extensive system of ways for resolving such conflicts, which necessitates the study of procedures and mechanisms for the rights protection of medical legal relations subjects.

The need for the existence of such an extensive system of ways to resolve legal conflicts between medical legal relations subjects is due to the fact that, on the one hand, a person, his life and health are recognized as the highest social value, and on the other hand, when performing medical activities, in particular, as regards provision of medical services (especially paid ones), as rightly noted by O.V. Klymenko, there is always a possibility of dissatisfaction with their quality, and, therefore, a mechanism for handling complaints, disputes and conflicts must be provided (in the aspect of health care, due to the delicacy of this sphere, the effectiveness of such a mechanism is doubly important) [15].

In the legal literature, there is no single approach to defining the procedure and mechanisms for protecting the rights of medical legal relations subjects, and scientists cite various ways of protecting such rights and criteria for their classification. The most widespread is the criterion of classification depending on the entity to which the medical legal relations participants turn for the protection of their violated rights and interests, according to which the forms of human rights protection in the field of health care are divided into non-jurisdictional (self-defense; alternative conflict resolution; assistance of independent public associations and appeals to professional medical associations) and jurisdiction [16], within which it is necessary to allocate: 1) judicial, namely the human rights protection in the field of health care in civil, criminal, administrative and constitutional proceedings; 2) extrajudicial: administrative form of human rights protection in the field of health care; appeal to the prosecutor's office; appeal to internal affairs bodies; appeal to the Commissioner of the Verkhovna Rada of Ukraine for human rights [12].

A slightly different approach is followed by O.V. Rozhon and A.M. Ustinchenko, who divide the methods of resolving disputes in medical legal relations into tra-

ditional (judicial and extrajudicial (appeal to the head of the medical institution (oral and written); appeal to the health care management body; assistance of independent public organizations and professional associations; complaint to the prosecutor's office and others)) and alternative (being popular and widely used in Europe, the USA, Canada, Australia), which primarily include: negotiations; mediation; arbitration [12].

There are other criteria for the classification of dispute resolution methods in the aspect of medical activity in the legal literature. Thus, O.V. Drozdova indicates the possibility of selection of preventive and suppressive measures (aimed at suspending an action that violates the patients' rights); restorative (aimed at restoring the patients' rights); compensatory (compensation for property and moral damage) [18] methods, while the means of protection are as follows: the patient's appeal to the ombudsman, non-governmental organizations, the mediator, the arbitration court; submitting a complaint to the relevant body in an administrative procedure; a claim to a court of general jurisdiction and an appeal to the European Court of Human Rights [19].

This makes it possible to come to a conclusion about the possibility of distinguishing non-jurisdictional (self-defense) and jurisdictional (special, administrative, judicial protection) forms of patients' rights protection, as well as distinguishing two levels of such protection (pre-trial and judicial), each of which has its own distinctive features and the patient has the right to determine himself in what way and by what level to protect his rights.

PRE-TRIAL LEVEL OF PROTECTION OF THE RIGHTS OF SUBJECTS OF MEDICAL LEGAL RELATIONS

An important role in the conflict resolution in the field of health care is primarily played by the pre-trial protection, the defining feature of which is the voluntary nature of the conflict resolution (voluntary recognition by the guilty party of the violations committed by it and compensation for the damage caused to the patient) [20], and which is simpler and more accessible (compared to a judicial one) [21], as it is less costly in terms of time and financial resources [22], as well as more convenient (does not require special knowledge) and an effective method for dispute resolution and the possibility of damage compensation without bureaucracy (it is sometimes more profitable for medical institutions to compensate materially caused damage than to enter into a legal process, which, in addition to the so-called "drag", will also have a negative impact on the reputation of the health care institution) [9].

The pre-trial level of the rights protection of medical legal relations subjects is widespread in law enforcement practice and is characterized by the availability of a significant number of ways for its implementation. Analyzing this level of protection, the viewpoint of S.B. Buletsa deserves attention. The scientist indicates the following options: appeal to the head of the medical institution (oral and written); appeal to the higher health management body; appeal to the ethical council, if there is one; seeking help from independent public associations and professional associations; application to the licensing and accreditation commission; filing a complaint with the prosecutor's office; mediation [23], as well as singles out such a method as no-fault compensation system (pre-trial compensation without fault, which takes place in Sweden (a special insurance fund is created at the expense of monthly contributions from commercial medical enterprises and private practitioners), Scotland, Finland, Denmark, New Zealand and in some US states, and it is a no-fault compensation system where patients suffering from post-treatment disability do not go to court to receive compensation) [24].

Without diminishing the role of each of the specified methods, in our opinion, an important role is played by the activity of medical arbitrations (commissions) and the proper functioning of medical self-governance, in particular in the aspect of preventing rights violation of both patients and doctors. Thus, O.V. Klymenko draws attention to the essential role of medical arbitrations (commissions), pointing above all to the example of the USA, where medical arbitrations function on the basis of the Rules of Commercial Arbitration in compliance with the law and taking into account the basic rights and freedoms of a person and a patient [15], as well as the existence of medical arbitrations association. The experience of Austria (medical commissions operate on the basis of a statute or a provision adopted in accordance with the resolution of the Chamber of Physicians and the National Medical Association) and Germany (where the first Medical Arbitration Commission was created back in 1975) [25] is also positive. Along with medical arbitrations (commissions), the activity of independent institutions on medical ethics matters is also important (following France, which was the first to create the National Committee on Medical Ethics, similar committees were created in Italy and Denmark, and now such institutions exist in almost all countries of Europe (Hungary, Slovakia, the Czech Republic, etc.). Committees on ethics are created in medical institutions as an alternative to judicial review of decisions of a medical institution, which have an ethical, moral and legal nature [26]. According to S. B. Buletsa, the advantage

of the activity of such a committee in Hungary is that every hospital has representatives of patients' rights, and their task is to help patients in studying their rights, writing complaints, informing medical workers about patients' rights violation, while such a representative is not in an employment relationship with the hospital as well as health care administration, and therefore he is an independent person and this contributes to the correct resolution of controversial issues between the patient and the doctor [24].

In order to protect the patients' rights, in particular those who are characterized by increased vulnerability (for example, in need of compulsory psychiatric care, are incapacitated, etc.), an important role is played by the medico-legal partnership. Positive in this case is the experience of the USA, where such a partnership is developing at a wide pace (today, according to the National Center for Medical-Legal Partnerships, there are more than 300 medical-legal partnerships in 46 states), whose activities are aimed at improving the health and well-being of low-income citizens and other vulnerable segments of the population by meeting their legal needs and promoting the elimination of legal barriers in the field of health care [27].

Along with the protection of patients' rights, an important role is also played by the availability of effective ways for protecting the doctors' rights. The proper functioning of medical self-government is important in this case. We agree that in those countries where membership in self-governing medical associations is mandatory, they have the right to decide on admission to the profession, disciplinary powers over a doctor, up to depriving him of the right to practice medicine (certainly, if there is for good reasons). At the same time, such associations take care of the legal support of the doctor (in cases where a civil suit is brought or criminal proceedings are opened, this is extremely important), given that "medical" cases are recognized worldwide as one of the most difficult categories of cases. Most foreign countries ensure the activity of self-governing organizations at the highest level (for example, in Switzerland, Norway, Spain, Turkey, Bulgaria, Romania, Slovakia, Slovenia, Croatia, Montenegro, it is regulated by special laws, in Poland – by the Constitution of the country, while in Finland, the medical self-governing association performs the function of a medical trade union, i.e. it takes care of issues of protecting the doctors' rights to decent wages, working conditions, etc.) [28]. Medical self-government, apart from the countries of the European Community, is widespread in North and South America, South and East Asia, and a significant part of African countries, where there were adopted laws by which the state transferred regulatory functions in the management of the health care system to medical self-government as well as outlined the organizational principles of the doctors' professional activity [29].

The protection of the doctors' rights is primarily related to the fact that such institutions have professional lawyers (attorneys) who specialize in medical law, in particular in cases of medical errors [30]. The study of their legal status gave us the opportunity to come to the conclusion that a medical lawyer is a person who carries out professional activities related to the protection, representation and provision of other types of legal assistance to a client (in particular, a patient, a doctor, a medical institution, socially vulnerable segments of the population), that requires both the possession of medical knowledge (laws in the field of medicine, standards that regulate the ethical and professional behaviour of doctors, etc.), as well as his awareness of a number of other areas of law, which may also be the subject of lawsuits or other pretentious activities in the field of health care. Their professional activity is important both in the aspect of consulting their client (a patient of a medical institution) before starting to provide assistance, and in the context of protecting the rights and interests of the doctor (if considering the case of bringing the doctor to civil or criminal liability for medical malpractice committed by his mistake), and the medical institution as a whole (performance of "contractual work", participation in pre-trial proceedings (including ethics committees), as well as in court procedures for the settlement of disputes arising between the medical organization and its clients, in particular patients and their relatives [31-33].

CONCLUSIONS

The peculiarity of medical activity is that such professional activity of doctors is characterized by an increased possibility of conflict occurrence during its implementation, which acquire a special social significance. This is due to the fact that such activity, despite the permanent improvement of the quality of medical care and the predominant use of modern methods for diagnosis and treatment, is inextricably linked with a sufficiently large number of potential reasons for patients to remain dissatisfied with the medical care provision. Therefore, the legal conflict occurrence in the aspect of medical activity is the basis for the human rights protection in the field of health care not only of patients, but of doctors and other participants in medical legal relations as well.

Legal conflict in the field of medical activity is a dynamic process, the origin and existence of which requires the need to study the preconditions for its occurrence, the essence of the conflict situation itself,

as well as its content (actions taken by participants to defend their rights and interests). Elucidation of the essence of legal conflicts in the field of medical activity is primarily aimed at preventing the occurrence of conflict situations, solving them at an early stage of development, or mitigating the possible consequences of such conflicts.

The availability of a significant number of disputes in the field of medical activity as well as criteria for the classification of relevant legal conflicts, requires the existence of an extensive system of conflict resolution methods between medical legal relations subjects, the proper functioning of which will contribute not only to the consideration and resolution of conflict situations at the stages of their nature and development, but also the accelerated process of restoration of violated rights in the field of health care, which, above all, will prevent the spread of a negative attitude towards medicine in general, which is becoming threatening.

The classification of dispute resolution methods in the aspect of medical activity indicates the possibility of distinguishing non-jurisdictional (self-defense) and jurisdictional (special, administrative, judicial protection) forms of patients' rights protection, as well as distinguishing two levels of such protection (pre-trial and judicial), each of which has its distinctive features and the patient himself is able to determine in what way and by what level to protect his rights. An important role in the conflict resolution in the field of health care is primarily played by the pre-trial protection, the defining features of which are the voluntary nature of conflict resolution, the availability, convenience and speed of dispute resolution as well as the possibility of compensation for the damage caused. This level of the rights protection of medical legal relations subjects is widespread in law enforcement practice and is characterized by the availability of a significant number of ways for its implementation, among which an important role is played by the activities of medical arbitrations (commissions), independent institutions on medical ethics, as well as the proper functioning of medical self-governance.

REFERENCES

1. Shchehlov YE, Smutok M. Konflikt medychnoho zakladu z patsiyentom: pravovyy aspekt. Praktyka upravlinnya medychnym zakladom. [Conflict between a medical institution and a patient: legal aspect]. 2012. https://www.legalalliance.com.ua/publikacii/konflikt-medicnogo-zakladu-z-pacientom-pravovij-aspekt/ [date access 27.06.2023] (in Ukrainian)
2. Zakhyst prav medychnykh pratsivnykiv: hotuymosya do samooborony? Vashe zdorov"ya. [Protection of the rights of medical workers: should we prepare for self-defense?]. 2017. https://www.vz.kiev.ua/zahyst-prav-medychnyh-pratsivnykiv-gotujmosya-do-samooborony/ [date access 27.06.2023] (in Ukrainian)
3. Bokeriya LA, Semina TV. Pravovyye voprosy v zdravookhranenii: problemy i resheniya. [Legal issues in healthcare: problems and solutions]. Novosti serdechno-sosudistoy khirurgi. 2017;1(3-4):117-122. (in Russian)
4. Bobrovnyk SV. Kompromis i konflikt u pravi: teoretyko-metodolohichne doslidzhennya [Compromise and conflict in law: theoretical and methodological research]: avtoref. dys. na zdob. nauk. stup. dokt. yuryd. nauk: spets.:12.00.01. K. 2013, p.40. (in Ukrainian)
5. Kornelyuk Yu. Pryroda yurydychnoho konfliktu: fenomenolohichnyy dyskurs. [Nature of legal conflict: phenomenological discourse]. Visnyk Natsional'noho universytetu "L'vivs'ka politekhnika". Yurydychni nauky. 2019; 22: 74-80. https://science.lpnu.ua/sites/default/files/journal-paper/2019/nov/19955/kornelyuk.pdf [date access 27.06.2023] (in Ukrainian)
6. Zabnenkova YuA. Tipy yuridicheskikh konfliktov. [Types of legal conflicts]. Yevraziyskiy nauchnyy zhurnal. 2016; 5:157-159. (in Russian)
7. Astaykina YuA, Khokhlova YeM. Tipy yuridicheskogo konflikta. [Types of legal conflict]. Yevraziyskiy nauchnyy zhurnal. 2016; 5:280-282. (in Russian)
8. Maydanyk R. Pytannya tsyvil'no-pravovoyi vidpovidal'nosti za dohovorom pro nadannya medychnykh posluh. [The issue of civil liability under the contract for the provision of medical services]. Pravo Ukrayiny. 2011; 11(12): 82-90. (in Ukrainian)
9. Mostovenko OS. Zakhyst prav patsiyenta pid chas nadannya medychnykh posluhu konteksti tsyvil'no-pravovoyi vidpovidal'nosti. [Protection of the patient's rights during the provision of medical services in the context of civil liability. Scientific bulletin of public and private law]. Naukovyy visnyk publichnoho ta pryvatnoho prava. 2018;1(1): 78-83. (in Ukrainian)
10. Semina TV. Sotsiologiya i yurisprudentsiya v sovremennom zdravookhranenii. [Sociology and jurisprudence in modern health care]. Byulleten' NTSSSKH im. A.N. Bakuleva RAMN. 2018;19 (1):28-36. doi: 10.24022/1810-0694-2018-19-1-28-36. (in Russian)
11. Stetsenko SG. Yuridicheskaya reglamentatsiya meditsinskoy deyatel'nosti v Rossii (istoricheskiy i teoretiko-pravovoy analiz) [Legal regulation of medical activity in Russia (historical and theoretical and legal analysis)]: dis. . . . dokt. yurid. nauk: 12.00.01. S.-Pb. 2002, p. 453. (in Russian)
12. Bern I, Ezer T, Koen Dzh et al. Prava cheloveka v sfere okhrany zdorov'ya: prakticheskoye posobiye [Human rights in health care: a practical guide]. L'vov: Izd-vo LOBF «Meditsina i pravo». 2012, p.580. https://www.academia.edu/15244914. [date access 27.06.2023] (in Russian)

13. Mikhaylova KhL. Mezhdunarodnyye i natsional'nyye standarty pri uregulirovanii sporov v sfere predostavleniya meditsinskikh uslug [International and national standards in the settlement of disputes in the provision of medical services]: avtoref. dis. na soisk. nauchn. step. kand. yurid. nauk: spets.: 12.00.03. M. 2009, p.27. (in Russian)
14. Suvorova KS. Sudovi formy zakhystu prav patsiyentiv. [Judicial forms of protection of patients' rights]. Yurydychnyy naukovyy elektronnyy zhurnal. 2015;1:141-142. (in Ukrainian)
15. Klymenko OV. Mekhanizmy roz·hlyadu konfliktiv v okhoroni zdorov'ya. [Mechanisms for consideration of conflicts in health care]. Derzhavne upravlinnya: udoskonalennya ta rozvytok. 2013;8. http://nbuv.gov.ua/UJRN/Duur_2013_8_9 [date access 27.06.2023] (in Ukrainian)
16. Shvets' YuYu. Realizatsiya osoboyu konstytutsiynoho prava na okhoronu zdorov"ya: porivnyal'no-pravove doslidzhennya [Implementation of a person's constitutional right to health care: a comparative legal study]: dys. . . . dokt. yuryd. nauk: spets. 12.00.02. Uzhhorod. 2019, p.496. https://www.uzhnu.edu.ua/uk/infocentre/get/19675 [date access 27.06.2023] (in Ukrainian)
17. Rozhon OV, Ustinchenko AM. Sposoby vyrishennya konfliktiv u medychnykh pravovidnosynakh. [Methods of conflict resolution in medical legal relations]. Visnyk Kharkivs'koho natsional'noho universytetu imeni V.N. Karazina. Seriya: Pravo. 2016;22:123-126. http://nbuv.gov.ua/UJRN/VKhIPR_2016_22_31 [date access 27.06.2023] (in Ukrainian)
18. Stefanchuk RO. Osobysti nemaynovi prava fizychnykh osib (ponyattya, zmist, systema, osoblyvosti zdiysnennya ta zakhystu). [Personal non-property rights of natural persons (concept, content, system, features of implementation and protection)]: monohrafiya. K.: KNT. 2008, p.626. (in Ukrainian)
19. Drozdova OV. Dyferentsiatsiya sposobiv i zasobiv zakhystu prav patsiyenta. [Differentiation of methods and means of protection of patient's]. Naukovyy visnyk Khersons'koho derzhavnoho universytetu. Seriya: Yurydychni nauky. 2016; 1: 109-113. (in Ukrainian)
20. Kholodova T. Sudebnyye i dosudebnyye sposoby zashchity prav grazhdan na okhranu zdorov'ya i meditsinskuyu pomoshch'. [Judicial and pre-trial methods of protecting the rights of citizens to health care and medical care]. Pravo i ekonomika. 2006; 9:110. URL: (in Russian)
21. Kurmayeva NA, Gorbyleva YuS. Profilaktika yuridicheskogo konflikta. [Prevention of legal conflict]. Nauchnyy al'manakh. 2016;5-1(19): 416-418. doi: 10.17117/na.2016.05.01.416. (in Russian)
22. Moiseyeva MA. Dosudebnyy i vnesudebnyy poryadok uregulirovaniya sporov, voznikayushchikh vsledstviye okazaniya platnykh meditsinskikh uslug. [Pre-trial and out-of-court settlement of disputes arising from the provision of paid medical services]. Vestnik Akademii Sledstvennogo komiteta Rossiyskoy Federatsii. 2017;3:66-68. (in Russian)
23. Buletsa SB. Tsyvil'ni pravovidnosyny, shcho vynykayut' u sferi zdiysnennya medychnoyi diyal'nosti: teoretychni ta praktychni problemy [Civil legal relations arising in the field of medical activity: theoretical and practical problems]: dys. . . . dokt. yuryd. nauk: 12.00.03. Odesa. 2016, p.437. (in Ukrainian)
24. Buletsa SB. Sposoby dosudovoho zakhystu prav medychnoho pratsivnyka ta patsiyenta. [Ways of pre-trial protection of the rights of a medical worker and patient]. Naukovyy visnyk Uzhhorods'koho natsional'noho universytetu. Seriya: Pravo. 2011; 15: 141-147. https://dspace.uzhnu.edu.ua/jspui/handle/lib/3379 [date access 27.06.2023] (in Ukrainian)
25. Sulakshin SS, Buyanova YeE, Kulakov VV et al. Al'ternativnyye sposoby razresheniya sporov mezhdu sub»yektami predprinimatel'skoy deyatel'nosti [Alternative ways to resolve disputes between business entities]: monografiya. M.: Nauchnyy yekspert. 2013, p.272. (in Russian)
26. McGuire AL, Majumder MA, Cheney JR. The Ethical Health Lawyer: The Ethics of Lawyer-Ethicist. The Journal of Law, Medicine & Ethics. 2005;33(3):603-607. doi: 10.1111/j.1748-720X.2005.tb00523.x.
27. Louise TG, Zabawa B., Galowitz P. Transformations in Health Law Practice: The Intersections of Changes in Health Care and Legal Workplaces. Indiana health law review. 2015;12(1): 183-226. doi: 10.18060/18962.
28. Hryvtsova R. Zakhyst prav medychnykh pratsivnykiv. Vashe zdorov"ya. [Protection of the rights of medical workers]. 2017. https://www.vz.kiev.ua/zahyst-prav-medychnyh-pratsivnykiv-gotujmosya-do-samooborony/ [date access 27.06.2023] (in Ukrainian)
29. Kadenko OA. Rozvytok likars'koho samovryaduvannya, yak neobkhidna umova medychnoyi reform [The development of medical self-government as a necessary condition for medical reform]. Pravovi zasady orhanizatsiyi ta zdiysnennya publichnoyi vlady: materialy Vseukrayins'ka naukovo-praktychna internet-konferentsiya (m. Khmel'nyts'kyy, 23 - 30 kvitnya 2018 r.). Khmel'nyts'kyy. https://univer.km.ua/doc/tezy2018/Kadenko_O_A.pdf [date access 27.06.2023] (in Ukrainian)
30. Myronenko NV. Problemy normatyvno-pravovykh aktiv shchodo zakhystu prav likariv i patsiyentiv. Medychna informatyka ta inzheneriya. Problems of normative legal acts on the protection of the rights of doctors and patients. [Medical informatics and engineering]. 2017;1:38-42. http://nbuv.gov.ua/UJRN/Mii_2017_1_9 [date access 27.06.2023] (in Ukrainian)
31. Zaborovskyy V, Buletsa S, Bysaga Yu et al. Professional activity of medical lawyer. Georgian Medical News. 2020; (300): 146-152.
32. Bielov DM, Petsa DD, Svyshcho VY, Novytsky VV. The human right to transplantation of organs and tissues: medicine, ethics and law. Wiadomości Lekarskie. 2022;75(10): 2519-2525. doi: 10.36740/WLek202210138.
33. Bielov DM, Hromovchuk MV, Hreca YaV, Tymchak VV. Essence of somatic human rights in the process of biomedical research. Wiadomości Lekarskie. 2021;74(10): 2663-2668. doi: 10.36740/WLek202110226.

ORCID and contributionship:
Viktor V. Zaborovskyy: 0000-0002-5845-7535 [A, B,F]
Yaroslav V. Stupnyk: 0000-0001-8024-9735 [B, E]
Mykhaylo M. Hetsko: 0000-0001-9001-3531 [B, D]
Pavlo P. Chervko: 0000-0002-5311-324X [A, C]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Viktor V. Zaborovskyy
Uzhhorod National University
14 University St., 88000 Uzhhorod, Ukraine
e-mail: zaborovskyviktor@gmail.com

Received: 28.06.2023
Accepted: 29.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

REVIEW ARTICLE

CONSTITUTIONAL AND LEGAL PRINCIPLES OF THE LIMITS OF PERMISSIBLE INTERVENTION IN CONDUCTING BIOMEDICAL RESEARCH WITH HUMAN PARTICIPATION

DOI: 10.36740/WLek202311129

Yaroslav V. Lazur, Sergiy P. Kozodayev, Vadym M. Roshkanyuk, Alen V. Panov
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To find out the peculiarities of constitutional and legal principles of the limits of permissible intervention in conducting biomedical research with human participation.

Materials and methods: The methodological basis of the study was worldview dialectical, general scientific and specific scientific methods of learning the phenomena of state and legal reality. Common ones were widely used in the work principles of scientific knowledge — comprehensiveness, objectivity, historicism, unity of theory and practice and others. The most important methodological prerequisite was a systemic approach to the issue of protecting human rights when conducting biomedical experiments with his participation, which made it possible to form a holistic view of the object and subject of research. With its help, the human rights affected by biomedical research in the context of the general legal principle of humanism were analyzed, the limits of permissible intervention in the human body were identified, and conclusions were drawn regarding the need for the separation and normative legal regulation of the complex branch of medical law. In the study, general scientific methods were widely used: historical, systematic analysis of the studied phenomena and synthesis of the obtained results, inductive and deductive reasoning; special: formal-logical, sociological, statistical; and also private-scientific: comparative-legal, normative-legal, and others.

Conclusions: 1) A key point in the mechanism of protection of the subject's rights in the process of conducting biomedical research is the establishment of a number of limits (maxims) that allow interference in the human body and can limit the researcher and protect the subject. These maxims should be integral elements of the process of biomedical research with human participation and determine the basic criteria for the protection of citizens' rights when conducting biomedical research. 2) The limits of permissible intervention in the conduct of biomedical research with human participation are the legal and factual consequences of the activity of bodies authorized to conduct biomedical research, which is based on the law and aimed at achieving the goals set by the researcher for conducting biomedical research with human participation, as a result of which the options for permitted by the norms of the law of behavior of the subjects of biomedical research by establishing various limits of such behavior, which necessarily have an exclusively temporary and subjective nature.

KEY WORDS: human rights and freedoms, generation human rights, personal human rights, somatic human rights, medicine, law, biomedical research, human rights protection, permissible intervention

Wiad Lek. 2023;76(11):2525-2530

INTRODUCTION

Freedom of scientific research in the field of biomedicine cannot be justified only by the right of mankind to obtain new knowledge, but also by the fact that their results can lead to significant progress in terms of health and well-being of patients. However, it is necessary to understand that such freedom is not absolute, since in medical research it is limited by human rights, which are defined by international and national legal acts aimed at their protection [1].

The rights and freedoms of a person and a citizen are a fundamental asset of humanity, an important element in the process of formation and socialization of each individual, because they allow him to satisfy his needs and realize his potential in various spheres of social life. The degree of their guarantee is a litmus test for the democratic development of any society. A special place in the system of rights and freedoms of a person and a citizen is occupied by constitutional rights and freedoms, which, together with the corresponding obligations, are established in the basic law of each state [2]. Generally recognized and most important civil (personal), political, economic, social, cultural and other rights and freedoms are concentrated in constitutions. In this way, the constitution-giver determines the legal status of a person and a citizen and regulates the activities of all legal entities [3].

THE AIM

The aim is to find out the peculiarities of constitutional and legal principles of the limits of permissible intervention in conducting biomedical research with human participation.

MATERIALS AND METHODS

The methodological basis of the study was worldview dialectical, general scientific and specific scientific methods of learning the phenomena of state and legal reality.

Common ones were widely used in the work principles of scientific knowledge — comprehensiveness, objectivity, historicism, unity of theory and practice and others. The most important methodological prerequisite was a systemic approach to the issue of protecting human rights when conducting biomedical experiments with his participation, which made it possible to form a holistic view of the object and subject of research. With its help, the human rights affected by biomedical research in the context of the general legal principle of humanism were analyzed, the limits of permissible intervention in the human body were identified, and conclusions were drawn regarding the need for the separation and normative legal regulation of the complex branch of medical law.

In the study, general scientific methods were widely used: historical, systematic analysis of the studied phenomena and synthesis of the obtained results, inductive and deductive reasoning; special: formal-logical, sociological, statistical; and also private-scientific: comparative-legal, normative-legal, and others.

REVIEW AND DISCUSSION

Effective provision of the constitutional rights and freedoms of a person and a citizen is connected with the need for restrictions in their implementation. Today, scientists distinguish two concepts - "limits of the exercise of human rights" and "restrictions of the exercise of human rights." So, in particular, P.Rabinovych notes that the definition of "limits of the exercise of human rights" is a set of all phenomena that outline the content and scope of human rights. Regarding the concept of "limiting the exercise of human rights", this is the activity of competent state bodies to establish limits on the exercise of human rights. Thus, as we can see, these concepts are not identical [4].

Historically, changes in the development of social relations led to the creation of a kind of conceptual base and principles of democratic and legal statehood, which later manifested itself in the processes of expanding the list of rights and freedoms guaranteed at the state level. If at first their presence had the character of belonging only to the privileged classes, then in the future the enrichment of its content and spread to other layers of the population was conditioned by the increase of production capacities and the capabilities of society to actually provide certain benefits. This is seen as one of the important aspects of forming the basis for the further approval of the principle of equality [5].

A gradual expansion of the "individual" (in which state intervention is limited) sphere of man and citizen is observed. It also gradually contributed to the creation of appropriate opportunities for optimizing conditions for the improvement of personal initiative. This, in turn, is seen as one of the important conditions for the progressive development of civilization [6].

At the current stage, there is also a contradiction between state intervention and the need to create appropriate conditions for the personal safety of citizens, which is resolved by restricting rights and freedoms. In the future, the above may cause an actual narrowing of the achieved level of ensuring human and citizen rights and freedoms at the global level (especially if the legislation of the countries of the world develops in this direction).

The question of the rights and freedoms of a person and a citizen under the conditions of the formation and development of civil society in democratic states occupies a central place. For quite a long time and until now, scientists have been concerned with the issue of establishing an optimal balance between human rights and freedoms and the interests of society and the state as a whole. In the aspect of such a ratio, the problem of admissibility of limiting human rights and freedoms arises.

It is necessary, in our opinion, to note that the term "restrictions" itself, as a rule, is used in different meanings that characterize the activities of state authorities. However, the scope of application of this concept is not limited only to the procedure for the exercise of powers by state authorities, but also affects other aspects of the life of society and the state as a whole. In particular, the concept of "restriction" can be related to:

1) the application of criminal punishment, namely: restraint of will, i.e. detention of a person in open-type penal institutions without isolation from society under the conditions of supervision with the mandatory involvement of the convicted person in work;
2) ensuring the sanitary and epidemiological well-being of the population;
3) provision of quarantine measures;
4) realization of property rights (easements, mortgages, lease, seizure of property, etc.);

5) professional activity of the relevant subject;
6) introduction of an extraordinary administrative and legal regime.
7) the definition of limitation of vital activity, i.e. complete or partial loss of the ability of a natural person to perform self-care, move independently, orientate in space, control behavior, perform labor activities.

Considering biomedical research in the legal sphere, I would like to note that until now the question of whether there is a permissible limit of physical and mental harm that can be inflicted on subjects undergoing biomedical research in the name of I progress of medicine.

At the same time, it is necessary to understand that the essence of law, as the scientist V. Protasov rightly notes in this regard, is that it is a way (instrument / form) of establishing a fair balance of the interests of everyone and everyone: individuals, social strata, classes , social communities and organizations. Only taking into account and coordinating the interests of all social subjects (individual and collective) is the real basis and guarantee of the implementation of legal prescriptions [8]. That is why, in our opinion, the balance of the interests of the researcher and the subject of the study becomes extremely important.

In legal provisions relating to the regulation of biomedical research, such limits can be expressed through the elements of the regulatory system, which are represented, in fact, by the very methods of legal regulation. The reasons for the determining role of methods of legal regulation lie in the peculiarities of the regulatory functions of law, one of which is aimed at consolidating the most important social relations and is carried out through permits and prohibitions, and the other, designed to ensure the dynamics and movement of social processes by specific legal means and, therefore, functions through legal obligations sewing [9].

Being the highest social value, a person acting as a subject in biomedical research must be protected from unlawful encroachment, and the limits of intervention in his body must be defined by a number of restrictions. The principle of humanism is the ideological source and immanent component of human rights, which is filled with a new theoretical content at the current stage of the development of human relations. The modern attitude to man allows us to identify a number of conceptual foundations that provide an opportunity to look at the content of the principle of humanism in a new way and reflect its essence when conducting scientific research. Regarding the field of medical activity, these principles are reduced to the limits of permissible intervention in the human body, limited to a number of fundamental principles. With respect to biomedical research, the limits of permissible intervention can be determined through methods of legal regulation using a combination of obligations and prohibitions [10].

As limiting principles related to obligations, a number of domestic scientists single out: respect for human dignity, recognition of individual independence (personal autonomy) in decision-making, voluntary informed consent, usefulness of the influence exerted, justice, truthfulness, privacy, preservation of medical confidentiality [11].

At the same time, legal prohibitions in S. Alekseev's understanding are moral prohibitions translated into legal language and equipped with a legal sanction. Legal prohibitions, as well as prohibitions in general, are characterized by a fixing, fixing function: they are designed to approve, to reduce to the rank of inviolable, inviolable what is - the existing dominant orders and relations. Therefore, from the regulatory side, they are expressed in legal obligations of a passive content, i.e. in obligations to refrain from committing actions of a certain kind [12].

It must be noted that the problem of coordinating the socially significant interests of citizens and the government cannot be solved without achieving a balance of the interests of the individual, civil society, and the state. Such a balance is achieved by imposing some reasonable restrictions both on the power of the state over the citizen and on the rights and freedoms of the individual. In connection with this, a natural, fundamental question arises - what is meant by "reasonable" ("commensurate") restrictions and to what limits the right can be limited.

Within the framework of the subject of our dissertation research, analyzing the category of "restrictions", we can also highlight their signs. As evidenced by the analysis of the scientific works of a number of domestic and foreign scientists (see, for example, the works of P. Rabinovych, I. Pankevych, I. Yagoforov, etc.), the following deserve special attention among them: 1) restrictions represent certain legal and factual consequences in in the form of "inconvenient" conditions for the realization of the legal interests of the relevant subjects (natural and legal entities), whose rights and freedoms are limited, while simultaneously satisfying the "legal interests" of the subject of power, which introduced these restrictions, or the interests of a third party, which is interested in introducing such restrictions; 2) in any case, restrictions always represent a reduction of "free" (that is, permitted by law) behavior (actions); 3) restrictions introduced in connection with the occurrence of emergency situations always have the limits of their implementation (spatial, temporal, etc.) established by legal norms (law); 4) the legal restriction is established only by the relevant authorized subject, in compliance with the procedure defined in the normative legal acts (laws) [13].

The above analysis gives grounds for concluding that the limits of permissible intervention in the conduct of biomedical research with human participation are legal and factual consequences of the activity of bodies authorized to conduct biomedical research, which is based on the law and aimed at achieving the goals set by the researcher for conducting biomedical research with the participation of a person, as a result of which the options for the behavior of subjects of biomedical research permitted by the law are reduced by establishing various limits of such behavior, which necessarily have an exclusively temporary and subjective nature.

A number of practical principles should be attributed to the method of legal regulation of biomedical research. Let's consider each of them in more detail.

1) The principle of recognition of individual independence (personal autonomy) essentially, according to J. Hans, specifies a qualitatively new role that patients and experimental subjects are beginning to play in modern biomedicine. At the same time, the specified principle involves the recognition of the autonomy of the thinking of the person who participates in the research both in the decision-making process and in the assessment of their consequences. In other words, the patient (subject) must be protected from psychological pressure, including in a hidden form, for example, by creating imaginary interest. This approach provides actual freedom of decision-making, which is a prerequisite for human participation in biomedical experiments [14].

2) Ensuring respect for human dignity is a generalized category of moral and ethical plan that reflects interpersonal relations in society and is characterized by such criteria as benevolence, respectful attitude to thoughts and actions, correctness, politeness, etc. As for the field of medicine in general, and biomedical research involving humans in particular, this category reflects the interpersonal relationship between the doctor (researcher) and the patient (researched) and has the same characteristics. Without observing the principle of "respect for human dignity", not only can no biomedical research be conducted with human participation, but it is also impossible to build effective relationships in the field of health care.

3) The principle of utility provides for potential benefit, both for the person participating in the research and for the entire society as a whole. In this regard, the analysis of the ratio of risk and benefit is extremely important. At the same time, the very decision about whether the ratio of risk and benefit is acceptable should be based on a thorough review of information related to all aspects of the research and a systematic consideration of alternative options. It is necessary to take into account all possible types of harm, not only the physical or psychological suffering of the subject [15].

4) The need to obtain voluntary informed consent is a basic principle that not only requires preventing medical tyranny and protecting freedom, but also encourages a rational decision made by the subject, who, ultimately, must live with the consequences of the medical effects on her body, or the lack thereof. At the same time, the concept of informed consent is relatively new in medical ethics. Many scientists of the past centuries disagreed with this position, because the opinion that most of the circumstances related to the disease should be hidden from patients was dominant for a long time. However, today the principle of informed consent is becoming key in the framework of medical science, practical health care and is one of the most important criteria for observing the rights of experimental subjects [16].

5) According to the principle of truthfulness, agreement on controversial issues that conflict with the interests of scientists and subjects is possible only on the basis of truthful trustful communication between all parties. At the same time, on the one hand, all social cooperation depends on true information, on the other hand, informing about everything could lead to unpredictable consequences. We will not tell each person what we think of them for the simple reason that it would destroy human connections. In this case, one should realize two important and interrelated truths: 1) reporting the truth is not the same as reporting the whole truth, 2) there are circumstances when certain matters should be kept confidential. At the same time, it is always difficult to decide what ethically can be hidden and what should be told. That is, the usual approach to truthfulness is based on two basic principles: one cannot lie and it is allowed to tell the truth only to those who have the right to it [17].

6) Speaking about the principle of justice, it should be noted that when choosing test subjects for conducting biomedical research, it is necessary to be guided by such basic legal categories as equality, impartiality and independence. At the same time, the issue of justice is one of the most difficult in the context of conducting biomedical research. A number of global social and interpersonal conflicts arise on the basis of the subjective understanding of the category "justice" and may be caused, in particular, by unfair treatment.

7) The principle of medical confidentiality prohibits the disclosure of professional information obtained as a result of research without the subject's permission. At the same time, in a number of cases, the non-dis-

closure of professional information is even protected by law, when in certain cases a person who received such information in the performance of his professional duties has the right to its non-disclosure even in court. This means that the doctor cannot disclose information about the state of health, features of the disease, received confidentially from his patient (subject), if the patient (subject) does not give his permission. It is obvious that disclosure of professional information can cause the most serious damage [18].

8) The principle of privacy prohibits scientists (even in the interests of science) from interfering in the private life of subjects without obtaining their consent. A person as a person should be characterized not only by various inclusions in social relations, but also by his individuality. Society, protecting the individuality and private life of an individual, recognizes a certain level of freedom. The use of individual freedom and inviolability of private life is one of the indispensable conditions for the functioning of democracy and the rule of law [19].

CONCLUSIONS

1. A key point in the mechanism of protection of the subject's rights in the process of conducting biomedical research is the establishment of a number of limits (maxims) that allow interference in the human body and can limit the researcher and protect the subject. These maxims should be integral elements of the process of biomedical research with human participation and determine the basic criteria for the protection of citizens' rights when conducting biomedical research.
2. The limits of permissible intervention in the conduct of biomedical research with human participation are the legal and factual consequences of the activity of bodies authorized to conduct biomedical research, which is based on the law and aimed at achieving the goals set by the researcher for conducting biomedical research with human participation, as a result of which the options for permitted by the norms of the law of behavior of the subjects of biomedical research by establishing various limits of such behavior, which necessarily have an exclusively temporary and subjective nature.

REFERENCES

1. Turner EV. Human rights suffer under camouflage of national security. http://www.state.ct.us/foi/Articles/human_right_suffer_under.htm [date access 17.04.2023]
2. Irkha Yu. Obmezhennia konstytutsiinykh prav i svobod liudyny i hromadianyna v interesakh natsionalnoi bezpeky Ukrainy v suchasnykh umovakh. [Limitation of the constitutional rights and freedoms of a person and a citizen in the interests of the national security of Ukraine in modern conditions]. Visnyk Konstytutsiinoho Sudu Ukrainy. 2015;5: 78-87. (in Ukrainian).
3. Bielov D, Aristova I, Hromovchuk M. The History of the Paradigm of Constitutionalism at the Present Stage of Development of the Post-Soviet States (On the Example of Ukraine). Studia Universitatis Cibiniensis. Series Historica. 2019;16: 265-273. https://centers.ulbsibiu.ro/ccpisc/language/en/the-history-of-the-paradigm-of-constitutionalism-at-the-present-stage-of-development-of-the-post-soviet-states-on-the-example-of-ukraine/ [date access 17.04.2023]
4. Rabinovych PM. Prava liudyny i hromadianyna [Human and citizen rights]. K. 2004, p.464. http://nbuv.gov.ua/UJRN/vapny_2012_3_3 [date access 17.04.2023] (in Ukrainian).
5. Aristova I, Zapara S, Bielov D et al. Legal Status of Local Self-government Authorities of Ukraine in the Conditions of Information Society Development: Research Methodology, Journal of Advanced Research in Law and Economics. 2019;10(8). doi: 10.14505/jarle.v10.8(46).02.
6. Byelov DM. Zakhyst prav i svobod lyudyny i hromadyanyna orhanamy systemy mizhnarodnoyi kryminal'noyi yustytsiyi. [Protection of human and citizen rights and freedoms by the bodies of the international criminal justice system]. Konstytutsiyno-pravovi akademichni studiyi. 2016;2:16-24. http://nbuv.gov.ua/UJRN/kpac_2016_2_4 [date access 17.04.2023] (in Ukrainian).
7. Bielov DM, Hromovchuk MV, Hreca YaV, Tymchak VV. Essence of somatic human rights in the process of biomedical research. Wiad Lek. 2021;74(10): 2663-2668.
8. Protasov VN. Teoryia prava y hosudarstva. Problemy teoryy prava y hosudarstva: Voprosy y otvety. [Theory of law and the state. Problems of the theory of law and state: Questions and answers]. M. 1999, p.240. https://vidrodgenya.at.ua/_tbkp/protasov_v.n-teorija_prava_i_gosudarstva-problemy_.pdf [date access 17.04.2023] (in Russian).
9. Brovchenko NV. Yurydychni fakty v konstytutsiinomu pravi Ukrainy: avtoref. dys. ... kand. yuryd. nauk. [Legal facts in the constitutional law of Ukraine]: 12.00.02. 2012, p.20 (in Ukrainian).
10. Bielov DM, Petsa DD, Svyshcho VY, Novytsky VV. The human right to transplantation of organs and tissues: medicine, ethics and law. Wiad Lek. 2022;75(10): 2519-2525.
11. Asadulayev MS, Luk'yanov SA, Likhtshangof AZ. Istoricheskiye, bioeticheskiye i pravovyye aspekty primeneniya stvolovykh kletok. [Historical, bioethical and legal aspects of the use of stem cells]. Meditsina i organizatsiya zdravookhraneniya.. 2017;1. https://cyberleninka.ru/article/n/istoricheskie-bioeticheskie-i-pravovye-aspekty-primeneniya-stvolovyh-kletok [date access 17.04.2023] (in Russian).

12. Alekseev SS. Obshchaia teoryia prava [General theory of law]. 2014, p. 440. https://coollib.com/b/246980-sergey-sergeevich-alekseev-obschaya-teoriya-prava-tom-i/read [date access 17.04.2023] (in Russian).
13. Basov AV, Poniattia "obmezhennia" yak yurydychna katehoriia: teoretychnyi aspekt. [The concept of "restriction" as a legal category: theoretical aspect]. Administratyvne pravo i protses: naukovo-praktychnyi zhurnal zasnovanyi Kyivskym natsionalnym universytetom imeni Tarasa Shevchenka 2013;1:34-37. http://www.irbis-nbuv.gov.ua/cgi-bin/irbis_nbuv/cgiirbis_64.exe?I21DBN=LINK&P21DBN=UJRN&Z21ID=&S21REF=10&S21CNR=20&S21STN=1&S21FMT=ASP_meta&C21COM=S&2_S21P03=FILA=&2_S21STR=apip_2013_1_6 [date access 17.04.2023] (in Ukrainian).
14. Hans J. Philosophical Reflections on Experiments with Human Subjects. Experimentation with Human Subjects. ed. by P. A. Fraund. George Braziller Inc. 1979, p.52.
15. Stetsenko SH. Medychne pravo. [Medical law]. K. 2008, p.343. https://library.nlu.edu.ua/POLN_TEXT/KNIGI-2012/medpravo2008.pdf [date access 17.04.2023] (in Ukrainian).
16. Tereshkevych HT. Informovana zghoda ta eksperymentuvannia nad liudynoiu. Medychne pravo Ukrainy: pravovyi status patsiientiv v Ukraini ta yoho zakonodavche zabezpechennia (henezys, rozvytok, problemy i perspektyvy vdoskonalennia). [Informed consent and human experimentation]. Materialy II Vseukrainskoi naukovo praktychnoi konferentsii 17-18.04.2008, m. Lviv. L. 2008, p.511. http://medicallaw.org.ua/uploads/media/02_334_01.pdf [date access 17.04.2023] (in Ukrainian).
17. Kudriavtsev VN, Zakon. Postupok. Otvetstvennost. [Law. Concession Responsibility]. M. 2001, p.448. http://lawlibrary.ru/izdanie15909.html [date access 17.04.2023] (in Russian).
18. Bachynskyi VT. Likarska taiemnytsia: poniattia ta medyko-pravove zabezpechennia v Ukraini. [Medical secrecy: concept and medical and legal provision in Ukraine]. Visnyk VDNZU «Ukrainska medychna stomatolohichna akademiia». 2015;15(4): 293–297. http://nbuv.gov.ua/UJRN/apsm_2015_15_4_71 [date access 17.04.2023] (in Ukrainian).
19. Petrukhyn YL. Lychnye tainy (chelovek y vlast). [Personal secrets (man and power)] M. 1998, p.232. http://lawlibrary.ru/izdanie20427.html [date access 17.04.2023] (in Russian).

ORCID and contributionship:
Yaroslav V. Lazur: 0000-0002-7520-7167 [D, E]
Sergiy P. Kozodayev: 0000-0002-3411-2161 [A, F]
Vadym M. Roshkanyuk: 0000-0002-3083-5231 [B, E]
Alen V. Panov: 0000-0002-8944-0533 [E, F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Yaroslav V. Lazur
Uzhhorod National University
26 Kapitulna st, 88000 Uzhhorod, Ukraine
e-mail: lazurs80@gmail.com

Received: 10.05.2023
Accepted: 27.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

CASE STUDY

COMPREHENSIVE CARDIOVASCULAR THERAPY IN EMERY-DREIFUSS MUSCULAR DYSTROPHY: A CASE REPORT

DOI: 10.36740/WLek202311130

Taras V. Chendey, Mykola V. Rishko, Victoria I. Chendey
UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

A 25-year-old male with known EDMD was referred for the cardiology consultation due to symptoms of heart failure. Echocardiography showed decrease left ventricular ejection fraction (LVEF) and therapy with ramipril, torsemide and rivaroxaban was initiated. Despite initial improvement, the patient later developed presyncope, bradycardia, irregular heartbeat and worsening of dyspnea. Therefore, implantation of resynchronization pacemaker with the function of implantable cardioverter-defibrillator (CRT-D/P) was performed. Ramipril was substituted by sacubitril/valsartan, and mineralocorticoid receptor antagonist and beta-blocker were initiated. Genetic testing found AD mutation in lamin A/C gene LMNA c.746G>A, p.(Arg249Gln). Upon follow-up, the patient demonstrated resolution of dyspnea and reverse remodeling of the left ventricle with complete restoration of the LVEF.

KEY WORDS: Dilated cardiomyopathy, comprehensive treatment, laminopathy

Wiad Lek. 2023;76(11):2531-2534

INTRODUCTION

Emery-Dreifuss muscular dystrophy (EDMD) is a rare, mostly X-linked or autosomal-dominant (AD) disorder, which affects skeletal muscles and heart. Although skeletal muscle dystrophy typically has milder consequences in EDMD than in other inherited muscle dystrophies, the long-term outlook and lifespan in patients with EDMD are largely defined by cardiac involvement [1]. Cardiomyopathy in EDMD has distinct features which include early development of left atrial myopathy, atrial standstill and atrial fibrillation (AF), decreased left ventricular (LV) ejection fraction (EF), absence of marked LV dilatation, and conduction abnormalities leading to bradycardia and need for pacemaker implantation. The cornerstone of heart failure (HF) prevention and treatment in EDMD remains neurohormonal inhibition with beta-blockers, angiotensin-converting enzyme inhibitors (ACEi) and mineralocorticoid receptor antagonists (MRA), although cardiovascular therapy in EDMD is poorly defined due to lack of dedicated studies [2].

CASE REPORT

We describe a case report study of 25-year-old male patient with cardiomyopathy due to EDMD. The subject gave an informed consent for all medical and surgical interventions as well as consented for the anonymous publication of the case. All decisions pertaining patient's care were made by share decision process which involved discussion with patient, patient's family member and multidisciplinary medical team.

A 25-year-old Caucasian male patient sought medical attention at cardiology clinic due to dyspnea, orthopnea, edema and fatigue. At presentation, he denied chest pain, fainting, syncope, rapid or slow heartbeat. He experienced a progressive girdle muscular wasting since late teens, leading to difficulties with walking, and got a clinical diagnosis of EDMD at the age of 22. He was done several orthopedic surgeries to relieve tendon contractures in his knees and elbows and was initiated on oral rivaroxaban for the prevention of the thromboembolism due to decreased mobility. He denied smoking, alcohol or illicit drug use. The patient's mother experienced similar, although milder, muscular symptoms and had heart failure and complete heart block which required pacemaker implantation. She died prematurely at her late thirties. The patient did not have siblings; other known maternal relatives were not affected. The patient's paternal family history was negative for muscular or cardiovascular conditions.

At presentation, he demonstrated gait impairment; significant muscular wasting of arms, shoulders and legs was evident as well as neck hyperextension and contractures of knees and elbows. The patient had

difficulties with standing up from sitting position and sitting down. His blood pressure was 124/96 mm Hg, his heart rate (HR) was 78 bpm, regularly irregular due to premature beats. His lungs were clear bilaterally and there was no jugular vein distension, heart murmurs or gallops. There was a mild ankle edema.

His serum total creatine kinase returned at 605 IU/L (normal range 39.0-308.0), serum creatinine was 57.6 mcmol/L (normal range 62.0-115.0) and serum potassium was 4.8 mmol/L (normal range 3.5-5.1). Echocardiography showed decreased LVEF at 36%, mild LV and left atrium dilatation, moderaltely elevated non-invasive pulmonary artery systolic blood pressure and restrictive pattern of LV diastolic filling. Further details on the initial and follow-up echocardiography measurements shown in table (Table I). The average HR on 24-hour electrocardiogram (ECG) monitoring was 65 bpm, the minimal HR was 33 bpm; supraventricular ectopic beats accounted for 12.1% of all complexes and 0.5% of all complexes were ventricular ectopies. There was one episode of non-sustained monomorphic ventricular tachycardia (VT) and several episodes of non-sustained AF (Fig. 1, 2).

The patient was initiated on ramiril, torsemide, and anticoagulation with rivaroxaban was continued. He was also given supportive treatment with phosphocreatine supplementation. The patient did well on treatment, but after 4 months he developed increased shortness of breath, bradycardia and presyncope. His N-terminal pro-brain natriuretic peptide level around that time was 165.0 pg/ml (normal range 10.0-134.4). On repeat ECG monitoring there was left bundle branch block, episodes of non-sustained VT, four episodes of sustained AF and multiple episodes of bradycardia with minimal HR of 29 bpm and pauses up to 3.1 sec. Therefore, cardiac resynchronization therapy and implantable cardioverter-defibrillator (CRT-D) placement were scheduled. Medical therapy was updated: the patient was switched from ramipril to sacubitril/valsartan; eplerenone and bisoprolol were added after CRT-D implantation. Loop diuretic and phosphocreatine supplementation were maintained. Although CRT-D resulted in conversion to right bundle branch block pattern on ECG, it did not lead to significant decrease in QRS complex duration. On scheduled in-clinic follow-up after 7 weeks from CRT-D insertion, the patient reported resolution of dyspnea and bradycardia, and his LV EF as well LV dimensions improved significantly. Over the next 7 months of follow-up, the patient remained clinically stable and has achieved a complete reverse remodeling of his left chambers (see Table I). Follow-up CRT-D interrogation did not reveal pacemaker malfunction, VT or ventricular fibrillation. Genetic testing found AD mutation in lamin A/C gene LMNA c.746G>A, p.(Arg249Gln), therefore allowing the verification of diagnosis of EDMD type 2. The additional finding of the genetic testing was the discovery of heterozygosity for the likely pathogenic variant SLC22A5 c.131C>T, p.(Ala44Val), which has been reported in patients with primary carnitine deficiency and is inherited in autosomal-recessive mode. Therefore, *ex juvantibus* supplementation with L-carnitine was instituted.

EDMD is a clinical diagnosis that can be made when three criteria are met: scapulo-humero-peroneal myopathy, joint contractures, and dilated cardiomyopathy with conduction abnormalities [1, 2, 4]. These distinct features allowed clinical description of EDMD decades before genetic background of the disorder was elucidated. EDMD is a rare disease with prevalence estimation reported by Orphanet database at 1:400000 (https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=261).

According to the Online Mendelian Inheritance in Man database, there are seven known subtypes of EDMD (EDMD1 through EDMD7) due to mutations in different genes. Genes involved in most of EDMD subtypes encode nuclear envelope proteins, therefore these diseases are designated as envelopathies [4]. Majority of cases of EDMD are due to mutations in two genes: EMD gene encoding emerin with X-linkled inheritance (EDMD1), and LMNA gene encoding lamin A/C, which is inherited in AD mode (EDMD2). In the recent published case series of 4 patients with EDMD1 HF with reduced LV EF was not reported, though AF and conduction defect were common [3]. Our patient was diagnosed with EDMD2 and was found to have mutation in lamin A/C gene c.746G>A, p.(Arg249Gln). We suggest that this mutation was inherited by the patient from his deceased mother, who apparently had a sporadic disease. Muscular symptoms were moderate-to-severe and preceded conduction abnormalities, arrhythmia and symptomatic HF. Our observation falls in line with two reported cases by Vytopil M et al. [5] of same mutation in patients aged 28 and 22 years in whom contractures of elbow, knee, ankle, rigid spine as well as conduction defects, arrhythmia and need for pacemaker implantation were observed, but no overt HF was reported. Interestingly, dilated cardiomyopathy was observed only in one patient in the abovementioned report, although conduction abnormalities and/or arrhythmia were present in 10 out of 15 patients. Unlike reported cases with the same mutation, our patient developed dilated cardiomyopathy and congestive heart failure, although with mild LV dilatation. This underscores known significant

Table I. Initial and follow-up echocardiography parameters.

	12 Oct 2021	13 Apr 2022	31 May 2022	29 Sep 2022	25 Jan 2023
LV EF, %	36	40	48	53	56
LV EDD, mm	56.4	53.0	53.0	53.0	53.0
LV EDV, ml	150	135	124	124	126
LV ESV, ml	92	77	63	58	55
LAD, mm	40.2	37.5	35.0	35.0	35.0
TR pressure gradient, mm Hg	32.0	29.0	24.0	24.0	24.0

*EDD end-diastolic dimension, EDV – end-diastolic volume, ESV – end-systolic volume, EF – ejection fraction, LAD – left atrial dimension, LV – left ventricle, TR – tricuspid regurgitation.

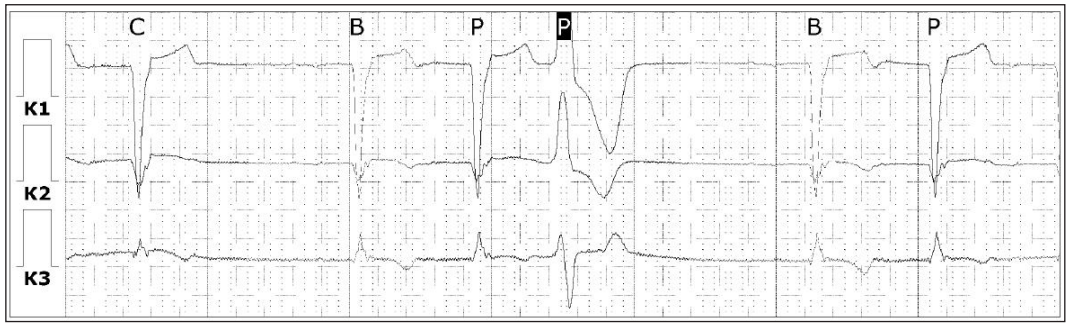


Fig. 1. The fragment of Holter monitoring demonstrating AF.

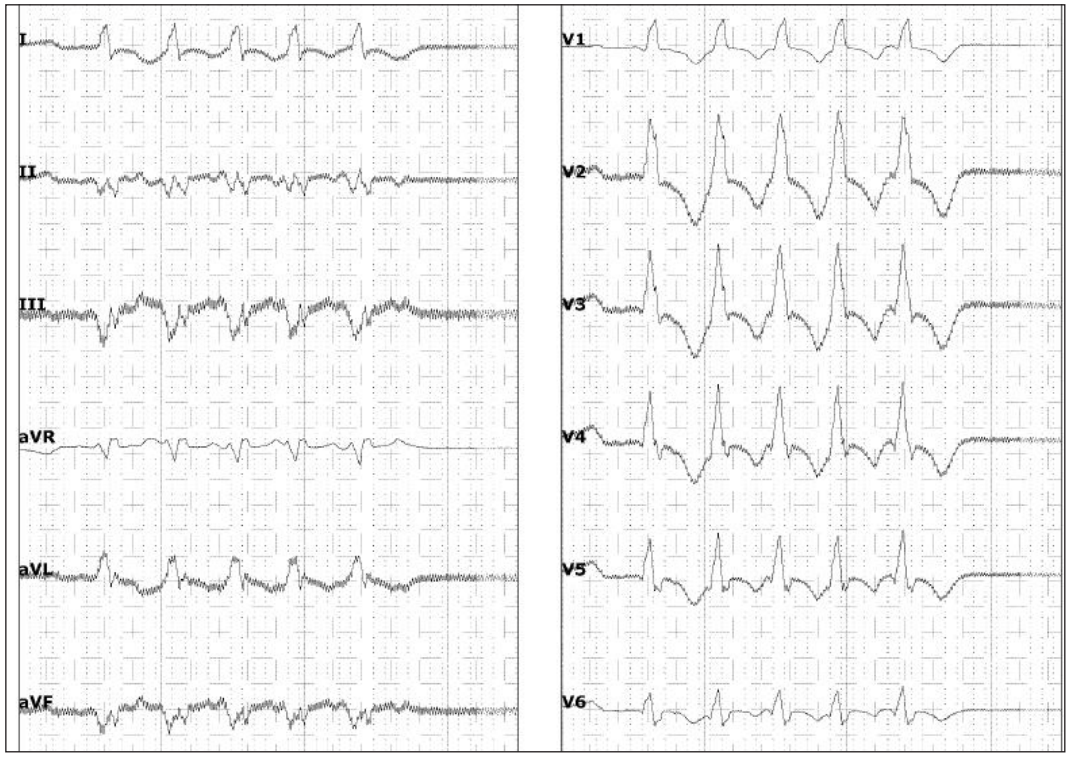


Fig. 2. The fragment of Holter monitoring demonstrating an episode of non-sustained ventricular tachycardia.

heterogeneity in genotype-phenotype correlations in EDMD [3, 4, 6]. Comprehensive medical and device therapy, including neurohormonal blockade with beta-blocker, sacubitril/valsartan, AMR and CRT-D, resulted in clinical improvement and reverse cardiac remodeling in our patient. We found another case reporting 32-year-old male with EDMD2 and LMNA mutation variant c. 136A>G p.(Ile46Val) [7], whose HF deteriorated despite treatment with sacubitril/valsartan and ultimately orthotopic heart transplant was performed. In the time being, our patient is doing well on treatment and is on close follow-up.

CONCLUSIONS

EDMD is a rare, inherited, progressive disorder affecting skeletal muscles and heart. Cardiac dysfunction, when present in EDMD, bears an adverse prognostic

impact and can be effectively controlled using tailored comprehensive medical and device therapy. Standard guideline-derived medical therapy for HF with beta-blocker, sacubitril/valsartan and AMR combined with electrophysiology intervention can result in reverse cardiac remodeling in EDMD.

REFERENCES

1. Heller SA, Shih R, Kalra R, Kang PB. Emery-Dreifuss muscular dystrophy. Muscle Nerve. doi: 10.1002/mus.26782. 2020; 61: 436–448.
2. Wang S, Peng D. Cardiac involvement in Emery-Dreifuss muscular dystrophy and related management strategies. Int Heart J. 2019;60(1):12-18. doi: 10.1536/ihj.17-604.
3. Kashyap N, Nikhanj A, Gagnon LR et al. Cardiac manifestations and clinical management of X-linked Emery-Dreifuss muscular dystrophy: a case series. Eur Heart J Case Rep. 2023;7(1):ytad013. doi: 10.1093/ehjcr/ytad013.
4. Madej-Pilarczyk A. Clinical aspects of Emery-Dreifuss muscular dystrophy. Nucleus. 2018; 9(1): 314-320.
5. Vytopil M, Benedetti S, Ricci E et al. Mutation analysis of the lamin A/C gene (LMNA) among patients with different cardiomuscular phenotypes. J Med Genet. 2003;40(12):e132. doi: 10.1136/jmg.40.12.e132.
6. Iskandar K, Astari FN, Gumilang RA, et al. Autosomal dominant Emery-Dreifuss muscular dystrophy caused by a mutation in the lamin A/C gene identified by exome sequencing: a case report. BMC Pediatrics. 2022;22(1):601. doi: 10.1186/s12887-022-03662-y.
7. Santos J, Almeida I, Pires I, Blanco F. Acute heart failure and bradyarrhythmia in a young male – what hides beneath the surface?: a case report. European Heart Journal - Case Reports. 2021;5(10):ytab413. doi: 10.1093/ehjcr/ytab413.

We would like to thank Dr. Olena Plyska, Dr. Victoria Sabov, Dr. Nataliya Ihnatyshyn, Dr. Volodymyr Komiati and Dr. Myroslav Petkanych from Zakarpattia Regional Clinical Center of Cardiology and Cardiac Surgery in Uzhhrorod for their expertise and invaluable contribution to the patient's care.

ORCID and contributionship:
Taras V. Chendey: 0000-0001-6573-7968 [A,B,D,F]
Mykola V. Rishko: 0000-0002-9624-432X [E]
Victoria I. Chendey: 0009-0001-4708-8009 [B, E]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Taras V. Chendey
Uzhhorod National University
14 University St, 88000 Uzhhorod, Ukraine
tel: +380 97 7903622
e-mail: taras.chendey@uzhnu.edu.ua

Received: 12.05.2023
Accepted: 28.10.2023

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article