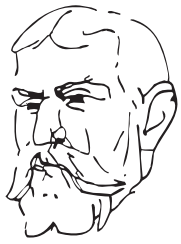
Wiadomości Lekarskie

Official journal of the Polish Medical Association



Memory of
dr Władysław
Biegański

VOLUME LXXIV, ISSUE 10 PART 1, OCTOBER 2021

Since 1928

ALUNA Publishing House

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

Wiadomości Lekarskie
monthly journal

You can order the subscription for the journal from Wydawnictwo Aluna by:
prenumerata@wydawnictwo-aluna.pl
Wydawnictwo Aluna
Z.M. Przesmyckiego 29
05-510 Konstancin-Jeziorna
Poland

Place a written order first.

If you need, ask for an invoice.
Payment should be done to the following account of the Publisher:
account number for Polish customers (PLN):
82 1940 1076 3010 7407 0000 0000
Credit Agricole Bank Polska S. A., SWIFT: AGRIPLPR

account number for foreign customers (EURO):
57 2490 0005 0000 4600 7604 3035
Alior Bank S. A.: SWIFT: ALBPPLPW

Subscription of twelve consecutive issues (1-12):
Customers in Poland: 480 PLN/year
Customers from other countries: 420 EURO/year

Wiadomości Lekarskie

Distribution and Subscriptions:
Bartosz Guterman prenumerata@wydawnictwo-aluna.pl
Graphic design / production:
Grzegorz Sztank www.red-studio.eu

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

FOR AUTHORS

1. The monthly "Wiadomości Lekarskie" Journal is the official journal of the Polish Medical Association. Original studies, review papers as well as case reports are published.
2. The publication of the manuscript in "Wiadomości Lekarskie" is paid. The cost of publishing the manuscript is PLN 1,000 plus 23% VAT (for foreign authors 250 Euro). If the first author of the manuscript is a member of the Editorial Board, we do not charge a fee for printing the manuscript, and if she or he is the next co-author – the fee is PLN 500 plus 23% VAT. The publisher issues invoices. The fee should be paid after receiving positive reviews, and before publishing the manuscript. Membership of the Polish Medical Association with documented paid membership fees for the last 3 years is also the exempt from publication fee.
3. Only papers in English are accepted for publication. The editors can help in finding the right person for translation or proofreading.
4. Papers should be sent to the editor via the editorial panel (Editorial System), available on the journal's website at https://www.wiadlek.pl. In order to submit an article, free registration in the system is necessary. After registration, the author should follow the instructions on the computer screen.
5. All editorial work is under control and using the editorial panel. This applies in particular to sending manuscripts, correspondence between the editor and author and the review process. In special cases, the editor may agree to contact outside the panel, especially in case of technical problems.
6. Acceptable formats for individual elements of the article are as follows:
   A) Content of the article – doc, docx, rtf, odt.
   B) Tables – doc, docx, rtf, odt
   C) Figures – JPG, GIF, TIF, PNG with a resolution of at least 300 dpi
   D) Captions for figures and tables.
   These elements are sent to the editor separately using the editorial panel. References and article metadata such as titles, keywords, abstracts etc. are supplemented by the author manually in the editorial panel in appropriate places.
7. The volume of original papers – including figures and references – must not exceed 21,600 characters (12 pages of typescript), and review papers – up to 28,800 characters (16 pages).
8. The original manuscript should have the following structure: Introduction, Aims, Material and methods, Results, Discussion and Conclusions which cannot be a summary of the manuscript.
9. When using abbreviations, it is necessary to provide the full wording at the first time they are used.
10. In experimental manuscripts in which studies on humans or animals have been carried out, as well as in clinical studies, information about obtaining the consent of the Ethics Committee should be included.
11. The Editorial Board follow the principles contained in the Helsinki Declaration as well as in the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing and Education, published by the New York Academy of Sciences Ad Hoc Committee on Animal Research. All papers relating to animals or humans must comply with ethical principles set out by the Ethics Committee.
12. The abstract should contain 150-250 words. Abstracts of original, both clinical and experimental, papers should have the following structure: Aims, Material and methods, Results, Conclusions. Do not use abbreviations in the title or the abstract. The abstract is pasted or rewritten by the authors into the appropriate field in the application form in the editorial panel.
13. Keywords (3-5) should be given according to MeSH (Medical Subject Headings Index Medicus catalogs – http://www.nim.nih.gov.mesh/MBrower.html). Keywords cannot be a repetition of the title of the manuscript.
14. Illustrative material may be black and white or color photographs, clearly contrasting or drawings carefully made on a white background. With the exception of selected issues, the Journal is printed in shades of gray (black and white illustrations).
15. The content of the figures, if present (e.g. on the charts), should also be in English
16. Links to all tables and figures (round brackets) as well as references (square brackets) the author must place in the text of the article.
17. Only references to which the author refers in the text should be included in the list of references ordered by citation. There should be no more than 30 items in original papers and no more than 40 items in review papers. Each item should contain: last names of all authors, first letters of first names, the title of the manuscript, the abbreviation of the journal title (according to Index Medicus), year, number, start and end page. For book items, please provide: author's (authors') last name, first letter of the first name, chapter title, book title, publisher, place and year of publication. It is allowed to cite websites with the URL and date of use of the article, and if possible the last names of the authors. Each literature item should have a reference in the text of the manuscript placed in square brackets, e.g. [1], [3-6]. Items should be organized as presented in Annex 1 to these Regulations.
18. When submitting the article to the editor, the authors encloses a statement that the work was not published or submitted for publication in another journal and that they take full responsibility for its content, and the information that may indicate a conflict of interest, such as:
    1. financial dependencies (employment, paid expertise, consulting, ownership of shares, fees),
    2. personal dependencies,
    3. academic and other competition that may affect the substantive side of the work,
    4. sponsorship of all or part of the research at the stage of design, collection, analysis and interpretation of data, or report writing.
19. The authors in the editorial panel define their contribution to the formation of scientific work according to the following key:
    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.
20. In the editorial panel along with the affiliation, the author also gives her or his ORCID number.
21. The Journal is reviewed in double, blind review mode. The submitted papers are evaluated by two independent reviewers and then qualified for publishing by the Editor-in-Chief. Reviews are anonymous. The authors receive critical reviews with a request to correct the manuscript or with a decision not to qualify it for publishing. The procedure for reviewing articles is in line with the recommendations of the Ministry of Science and Higher Education contained in the paper "Good practices in review procedures in science" (Warsaw 2011). Detailed rules for dealing with improper publishing practices are in line with COPE guidelines. The publishing review rules are in the Review Rules section.
22. Each manuscript is subject to verification in the anti-plagiarism system.
23. Manuscripts are sent for the author's approval. The author's corrections should be sent within the time limit indicated in the system. No response within the given deadline is tantamount to the author's acceptance of the submitted material. In special cases, it is possible to set dates individually.
24. Acceptance of the manuscript for publishing means the transfer of copyright to the Aluna Publishing House (Aluna Anna Łuczyńska, NIP 5251624918).
25. Articles published on-line and available in open access are published under Creative Common Attribution-Non Commercial-No Derivatives 4.0 International (CC BY-NC-ND 4.0) allowing to download articles and share them with others as long as they credit the authors and the publisher, but without permission to change them in any way or use them commercially.
26. The authors receive a free PDF of the issue in which their mansucript is enclosed, and on request – a printed copy. The printed copy is sent to the address indicated by the authors as the correspondence address.
27. Manuscripts not concordant with the above instructions will be returned to be corrected.
28. The editors do not return papers which have not been commissioned.
29. The editors take no responsibility for the contents of the advertisements.

CONTENTS

ORIGINAL ARTICLES

REVIEW ARTICLES

CASE STUDIES

ORIGINAL ARTICLE

IMPACT OF VACCINATION ON THE COVID-19 PANDEMIC: BIBLIOMETRIC ANALYSIS AND CROSS COUNTRY FORECASTING BY FOURIER SERIES

DOI: 10.36740/WLek202110101

Olha V. Kuzmenko[1], Vladyslav A. Smiianov[1], Lesia A. Rudenko[2], Mariia O. Kashcha[1], Tetyana A. Vasilyeva[1], Svitlana V. Kolomiiets[1], Nataliia A. Antoniuk[1]

[1]SUMY STATE UNIVERSITY, SUMY, UKRAINE

[2]ALUNA PUBLISHING HOUSE, KONSTANCIN-JEZIORNA, POLAND

ABSTRACT

The aim: Is to build a forecast of the COVID-19 disease course, considering the vaccination of the population from particular countries.

Materials and methods: Based on the analysis of statistical data, the article deals with the topical issue of the impact made by vaccination on the prevention of the COVID-19 pandemic. The time series, showing the dynamics of changes in the number of infected in Chile, Latvia, Japan, Israel, Australia, Finland, India, United States of America, New Zealand, Czech Republic, Venezuela, Poland, Ukraine, Brazil, Georgia for the period 07.08. 2020–09.09.2021, are analyzed. Trend-cyclic models of time series are obtained using fast Fourier transform. The predicted values of the COVID-19 incidence rate for different countries in the period from September 10, 2021 to February 2, 2022 were calculated using the constructed models.

Results and conclusions: The results of the study show that vaccination of the population is one of the most effective methods to prevent the COVID-19 pandemic. The proposed method of modeling the dynamics of the incidence rate based on statistical data can be used to build further predictions of the incidence rate dynamics. The study of behavioral aspects of trust in vaccination is proposed to be conducted within the theory regarding the self-organization of complex systems.

KEY WORDS: forecast, COVID-19, Fourier transform, vaccination, trust

Wiad Lek. 2021;74(10 p.I):2359-2367

INTRODUCTION

The issue of public trust in vaccination around the world is highly relevant at the time of the COVID-19 pandemic spread, caused by the coronavirus SARS-CoV-2 and the emergence of new strains of the virus. Vaccination is one of the main ways to protect the population and the full socio-economic development of states during a pandemic. The success of vaccination programs is largely determined by people's willingness to be vaccinated. Deciding on vaccination depends on many factors, including personal worldview, trust in official sources of information, social institutions related to vaccination, social attitudes, previous experience, belonging to certain social groups, etc. The peculiarity of the vaccination decision is its social nature, i.e., decision-making under the influence or interaction with other people, including experts and scientists. In this regard, the study of behavioral aspects of trust in vaccination, analysis of the impact made by the vaccinated population on the COVID-19 pandemic course, the dissemination of scientific information on the morbidity incidence are urgent tasks.

LITERATURE REVIEW

VOSViewerv.1.6.10 software was used to analyze bibliometric sources for 2020-2021 in the Scopus database in the amount of 444 publications. Figure 1 is constructed, in which 8 clusters based on 73 KEY WORDS are highlighted in different colors to systematize and visualize case studies related to the KEY WORDS "COVID-19", "vaccination" and "trust". The most common KEY WORDS are: COVID-19 (89 files), vaccination (32 files), hesitations regarding vaccination (34 files), Sars-cov-2 (18 files), vaccine (22 files), trust (18 files), etc.

The first cluster, the largest, is red and includes 14 KEY WORDS related to disseminating information on vaccination. Among them, there are social media (32 links), various social networks, in particular, Twitter (23 links), Facebook (15 links), emotions (11 links), speed of information dissemination (17 links), infodemia (26 links). It confirms the importance of social networks in disseminating information, which is very often unverified and untrue. The second cluster, green, contains 13 KEY WORDS related to public attitudes towards COVID-19 vaccination.

These include vaccination (35 links), Sars-cov-2 (74 links), immunization program (13 links), and healthcare (7 links). The third cluster, blue, contains 11 KEY WORDS directly related to the COVID-19 pandemic (267 links), that is the reason why it is related to most of the other KEY WORDS from other clusters. The fourth cluster is presented in a light green color. It contains seven KEY WORDS, among which the most important terms

are the vaccine from COVID-19 (20 links), indecision to vaccination (99 links), health workers (20 links). The fifth cluster, purple, contains seven KEY WORDS, including vaccination (66 links), applicants (19 links), demand (6 links), etc. This cluster, in contrast to the fourth and first, focuses on increasing the number of people willing to be vaccinated and increasing trust in vaccination among the population.

The sixth cluster is blue and contains six KEY WORDS – autonomy, ethics, vaccination, pregnancy, vaccine and pandemic. The cluster enables us to conclude that there are certain problems in the implementation of vaccination, on which researchers focus their attention. The seventh cluster, orange, contains communication, education, communication risks, misinformation, and infectious diseases. Only the eighth cluster (brown) includes the keyword used in the inquiry – trust (45 links); in addition, there are such terms as risk, influenza, collective immunity and conspiracy theory. This cluster focuses on the lack of public confidence in the pandemic through conspiracy theory.

The analysis of the obtained clusters allows us to conclude that the issue of increasing public trust in vaccination against COVID-19 is not sufficiently developed in academic scientific sources. It is important to study the behavioral aspects of trust in vaccination during the COVID-19 pandemic and predict the incidence rate, given the number of vaccinated people.

However, scientific research on the impact of the pandemic crisis on the countries' economic and social development is quite common. Comprehensive studies [1-4], which conducted a thorough bibliometric analysis of recent publications using international scientific databases, analyze the quarantine measures consequences, the COVID-19 pandemic impact on human behavior.

In the context of a pandemic crisis, it is important to increase the efficiency of the medical industry and the quality of its regulation. Scientists [5] study the transformations in health insurance, one of the components of public protection in times of epidemiological threats. The search for ways to overcome this is observed in work [6]. The study [7] deals with issues of innovation policy in healthcare. The issue of improving the medical service quality was relevant before the COVID-19 pandemic, in particular in [8,9].

Scientists pay considerable attention to modeling the spread of COVID-19 in various countries in Europe and the world [10]. In particular, the research [11] modeled the propagation of COVID-19 using the fast Fourier transform.

Research is currently relevant to identifying factors determining human behavior during crises, particularly in a pandemic. Thus, the social responsibility of the population is studied in [12], the influence of truthful news provided by the media is analyzed in [13], and in [14] the ability of artificial intelligence to influence society is studied. In addition, scientists [15] warned in the pre-pandemic period that any social challenges are interconnected between different countries, and only joint work, aimed at results in the world, will have the appropriate consequences.

The issues of human resource management in a pandemic are studied in [16, 17]. The study [18], the central aspect of which is to observe the importance of motivation and its impact on effectiveness in a pandemic, is of particular attention.

The studies [19] of scientists who theoretically prove the need for mass vaccination as a powerful tool to combat COVID-19, which necessitates the correction of public policy, are important. The authors [20] investigate the issue of this correction through corporate social responsibility as a means of personnel management. One should mention those scientists [21] who dedicate their research to the post-pandemic period and focus on the crucial role of university accelerators in influencing public opinion.

The COVID-19 pandemic demonstrated different response of countries to crises, the different behavior of socio-economic systems after passing the bifurcation point. Predicting scenarios of development of various socio-economic systems in a nonlinear environment, in times of crisis, in particular bifurcation analysis of socio-economic systems, has always attracted the scientists' attention [22,23]. The research [24] developed a method for risk assessment of information management and knowledge loss.

Scientists have always paid considerable attention to the study of the individual's social behavior during crises; in particular, these are works [25-27]. These studies are extremely important because adequate management of the vaccination process is impossible without considering the behavioral aspects of public trust in vaccination. The issue of trust in vaccination has been of concern to the WHO since the vaccine investigation. In particular, the WHO Technical Advisory Group on Behavioral Sciences and Health has published a report containing many recommendations for increasing public trust in vaccination [28]. According to experts, vaccination should be a quick, inexpensive and straightforward procedure since one of the forms of human reaction to certain difficulties related to the vaccination procedure can be the refusal of vaccination and a negative attitude towards it. Qualitatively organized vaccination conditions, particularly the convenient location of vaccination centers, convenient working hours, and quality of service contribute to higher vaccination rates. The combination of proper organization of the vaccination procedure with the purposeful dissemination of truthful information from authoritative sources about the importance, usefulness, speed of the vaccination process should help increase the share of the vaccinated population.

Secondly, an essential factor is the use of mechanisms of society's influence. Placement of vaccination centers not only in medical institutions but also in specially prepared central public places, the opportunity for people to inform about the vaccination in the family circle and a wide range of people via social networks, personal communication make it possible to bring vaccination to the social norm.

Third, the peculiarity of the COVID-19 pandemic is the emergence of huge flows of information and misinformation, which has led to a real global "infodemia". Researchers [28] strongly believe that only consistent, transparent, meaningful, active informational and explanatory work on risks, uncertainties and the availability of vaccines will form the public trust in vaccination.

The analysis of research on trust in vaccination from COVID-19 allowed to identify two current scientific problems: forecasting the COVID-19 incidence based on the number of vaccinated people and the study of the behavioral aspects of trust in vaccination.

Table I. Dynamics of change in the number of vaccinated population

Country	19.04.2021 (%)	15.07.2021 (%)	Growth rate (%)	Country	19.04.2021 (%)	15.07.2021 (%)	Growth rate (%)
Chile	41,10	75,63	84	Japan	1,06	31,75	2895
Israel	61,87	66,29	7	Australia	5,45	27,99	414
Finland	23,86	63,81	167	India	7,95	22,72	186
United States of America	39,56	55,93	41	New Zealand	2,86	17,41	509
Czech Republic	15,62	50,18	221	Venezuela	0,90	10,20	1033
Poland	17,70	46,27	161	Ukraine	1,06	5,66	434
Brazil	11,66	41,08	252	Georgia	0,76	4,40	479
Latvia	12,00	37,07	209				

Table II. Comparison of the dynamics of change in the number of infected population with and without vaccination

Country	Estimated number	Actual quantity	Growth rate	Country	Estimated number	Actual quantity	Growth rate
Australia	6,9	19,6	+185%	Japan	3054,2	3548,5	+16%
Brazil	75045,1	61090,8	-19%	Latvia	742,5	288,3	-61%
Chilie	7337,3	5400,4	-26%	New Zealand	3,6	1,8	-50%
Czehia	8991,5	614,7	-93%	Poland	16946,0	1484,6	-91%
Finland	643,1	155,7	-76%	Ukraine	11547,3	2885,1	-75%
Georgia	1677,5	935,4	-44%	USA	45259,6	22492,4	-50%
India	87585,9	200845,5	+129%	Venezuela	1826,3	1260,5	-31%
Israel	6354,2	122,1	-98%				

THE AIM

The aim of the article is to build a forecast of the COVID-19 disease course, considering the vaccination of the population from particular countries.

MATERIALS AND METHODS

A sample of countries with different vaccination levels as of July 16, 2021, was formed for the study. Analysis of the number of vaccinations in various countries showed that the maximum percentage of vaccinated people within one country is 79.9%. For showing the representativeness of the sample, all countries were ranked according to the vaccinated population ratio up to 79.9%, and one country from each five percent interval was selected [29]. Thus, the object of the study includes 15 countries: Chile, Latvia, Japan, Israel, Australia, Finland, India, United States of America, New Zealand, Czech Republic, Venezuela, Poland, Ukraine, Brazil, Georgia, which are presented in the table I.

There was a time series to study the vulnerability level of each country to COVID-19. It demonstrates the dynamics of changes in the number of infected people for the period 16.04.2020–09.09.2021. The COVID Tracking Project site was selected as an information database [30].

RESULTS AND DISCUSSION

Vaccination worldwide began at about the same time, but its rate varies from country to country. In particular, the highest vaccination growth rate in Japan (29 times increase), the lowest – Israel, since in the first four months since the vaccination start, more than 50% of the population were vaccinated.

Irwin's method was used to check for abnormal emissions that distorted the study results. In terms of the number of anomalous emissions of the time series, the leaders are New Zealand – 114 and Brazil – 94. All detected anomalous emissions are the emissions of the first kind. They can be eliminated by replacing the arithmetic mean of the previous and next values.

The method of checking the differences of the mean levels was used to check the stationarity of the studied series. The analysis of the study regarding stationarity allows us to conclude that the inpatient series include the dynamics of changes in the COVID-19 incidence in New Zealand and Japan. The series are not stationary for the rest of the studied countries.

Previous stages of determining the stationarity and periodicity of time series allow us to conclude that the studied time series are non-stationary and have cyclic components. The least-squares method highlights the trend for each time series. Analysis of the trend component of time series before and after vaccination allows us to conclude that for Brazil, Chile, the Czech Republic, Finland, Georgia, Israel, Japan, Latvia, New Zealand, Poland, Ukraine, the United States, there was a significant change in the trend in the direction of reducing the number of COVID-19 diseases with the

vaccination of the population. For such countries as Australia, India, and Venezuela, there has been a transformation in the other direction. The insufficient level of vaccinated population causes this phenomenon in combination with the emergence of a new virus strain.

In the study of time series, the schedule of the time series in the Fourier series is used to identify cyclic or seasonal components. It allows using the harmonic analysis of functions. For a time series, the Fourier series has the form (1), where all coefficients are calculated using discrete values of the time series (2):

$$\tilde{c}(\tau) = a_0^* + \sum_{k=1}^{n/2-1} a_k^* \cos\left(\frac{2\pi k}{T}\tau_i\right) + \sum_{k=1}^{n/2-1} b_k^* \sin\left(\frac{2\pi k}{T}\tau_i\right) + a_{n/2}^*, \quad (1)$$

$$a_0^* = \frac{1}{n}\sum_{i=1}^{n} y_i,\ a_k^* = \frac{2}{n}\cdot\sum_{i=1}^{n} y_i \cos\left(\frac{2\pi}{T}\tau_i\right),\ b_k^* = \frac{2}{n}\cdot\sum_{i=1}^{n} y_i \sin\left(\frac{2\pi}{T}\tau_i\right), \quad (2)$$

In the study of time series, the schedule of the time series in the Fourier series is used to identify cyclic or seasonal components. It allows using the harmonic analysis of functions. For a time series, the Fourier series has the form (1), where all coefficients are calculated using discrete values of the time series.

Analysis of the conducted stage (Table II) regarding the impact of vaccination on COVID-19 incidence allows us to conclude that for most countries, vaccination has significantly reduced the growth of the infected population (Czech Republic, Israel, Poland, Finland, Ukraine, Latvia, New Zealand, United States, Georgia, and Chile). Israel, Finland, the United States, the Czech Republic, Poland, and Brazil are countries where more than 40% of the population has been vaccinated, confirming the effectiveness of vaccination. However, Australia, Japan and India have the opposite effect: the predicted values are much lower than the actual statistics, which are explained by new virus strains, which caused unpredictable waves of disease. The vaccinated population in these countries as of 13.07.21 does not exceed 32% [29], which is considered insufficient for the collective immunity.

For non-stationary time series, logarithmation, verification and confirmation of the stationarity of the obtained series were performed, the trend component was selected and it was removed. The result of the rapid Fourier transform application for the number of new COVID-19 cases in the studied countries is presented by models (3) – (15), for which the determination coefficient R^2 varies from 0.76 to 0.99. It indicates a high degree of relationship between the dispersion of the dependent and the independent variables.

Australia	$\ln f(t) = 0{,}59\cos\left(\frac{5\pi t}{256} + 1{,}13\right) + 1{,}33\cos\left(\frac{3\pi t}{256} + 1{,}62\right) + 1{,}48\cos\left(\frac{\pi t}{256} - 0{,}65\right) + 1{,}49\cos\left(\frac{\pi t}{64} + 1{,}04\right) + 0{,}0015t + 2{,}95,\ R^2 = 0{,}76$	(3)
Brazil	$\ln f(t) = 0{,}66\cos\left(\frac{\pi t}{128} - 2{,}58\right) + 0{,}34\cos\left(\frac{3\pi t}{256} + 3{,}01\right) + 0{,}35\cos\left(\frac{\pi t}{256} + 3{,}0\right) + 0{,}41\cos\left(\frac{73\pi t}{256} + 0{,}5\right) + 0{,}0022t + 9{,}85,\ R^2 = 0{,}94$.	(4)
Chilie	$\ln f(t) = 0{,}71\cos\left(\frac{\pi t}{128} - 2{,}36\right) + 0{,}46\cos\left(\frac{3\pi t}{256} - 2{,}41\right) + 0{,}47\cos\left(\frac{\pi t}{256} + 2{,}05\right) + 0{,}36\cos\left(\frac{\pi t}{64} - 2{,}24\right) + 0{,}0003t + 7{,}715,\ R^2 = 0{,}99.$	(5)
Czehia	$\ln f(t) = 0{,}47\cos\left(\frac{5\pi t}{256} + 1{,}39\right) + 0{,}79\cos\left(\frac{3\pi t}{256} - 0{,}16\right) + 2{,}61\cos\left(\frac{\pi t}{256} + 3{,}12\right) + 0{,}43\cos\left(\frac{73\pi t}{256} + 0{,}63\right) + 0{,}0022t + 5{,}99,\ R^2 = 0{,}98.$	(6)
Finland	$\ln f(t) = 1{,}08\cos\left(\frac{\pi t}{128} + 0{,}93\right) + 1{,}11\cos\left(\frac{3\pi t}{256} - 0{,}21\right) + 0{,}81\cos\left(256\frac{\pi t}{256} + 2{,}87\right) + 0{,}25\cos\left(\frac{7\pi t}{256} - 2{,}31\right) + 0{,}0067t + 3{,}118,\ R^2 = 0{,}98.$	(7)
Georgia	$\ln f(t) = 1{,}30\cos\left(\frac{\pi t}{128} + 0{,}88\right) + 0{,}69\cos\left(\frac{3\pi t}{256} - 1{,}10\right) + 1{,}46\cos\left(\frac{\pi t}{256} + 2{,}93\right) + 0{,}2\cos\left(\frac{7\pi t}{256} - 1{,}47\right) + 0{,}013t + 2{,}214,\ R^2 = 0{,}99.$	(8)
India	$\ln f(t) = 1{,}28\cos\left(\frac{\pi t}{128} + 2{,}86\right) + 0{,}36\cos\left(\frac{3\pi t}{256} - 1{,}74\right) + 0{,}41\cos\left(\frac{\pi t}{256} - 2{,}44\right) + 0{,}35\cos\left(\frac{\pi t}{64} + 0{,}61\right) + 0{,}0039t + 9{,}478,\ R^2 = 0{,}99.$	(9)
Israel	$\ln f(t) = 0{,}85\cos\left(\frac{\pi t}{128} - 0{,}11\right) + 1{,}85\cos\left(\frac{3\pi t}{256} + 1{,}35\right) + 1{,}49\cos\left(\frac{\pi t}{256} - 2{,}41\right) + 0{,}69\cos\left(\frac{5\pi t}{256} + 1{,}89\right) + 0{,}0011t + 6{,}279,\ R^2 = 0{,}99$	(10)

Latvia	$\ln f(t) = 0{,}74\cos\left(\frac{\pi t}{128} - 0{,}31\right) + 0{,}77\cos\left(\frac{3\pi t}{256} - 1{,}17\right) + 2{,}01\cos\left(\frac{\pi t}{256} + 2{,}9\right) + 0{,}4\cos\left(\frac{5\pi t}{256} + 0{,}14\right) + 0{,}0096t + 1{,}776, R^2 = 0{,}99.$	(11)
Poland	$\ln f(t) = 0{,}41\cos\left(\frac{\pi t}{128} - 1{,}55\right) + 1{,}01\cos\left(\frac{3\pi t}{256} - 0{,}91\right) + 2{,}37\cos\left(\frac{\pi t}{256} + 2{,}99\right) + 0{,}45\cos\left(\frac{\pi t}{64} + 1{,}39\right) - 0{,}0001t + 7{,}26, R^2 = 0{,}99.$	(12)
Ukraine	$\ln f(t) = 0{,}25\cos\left(\frac{73\pi t}{256} - 0{,}1\right) + 0{,}48\cos\left(\frac{3\pi t}{256} - 0{,}83\right) + 1{,}38\cos\left(\frac{\pi t}{256} + 3{,}14\right) + 0{,}38\cos\left(\frac{\pi t}{64} \mp 1{,}14\right) + 0{,}0025t + 7{,}24, R^2 = 0{,}99$	(13)
USA	$\ln f(t) = 0{,}57\cos\left(\frac{\pi t}{128} - 0{,}165\right) + 0{,}31\cos\left(\frac{5\pi t}{256} + 1{,}5\right) + 0{,}76\cos\left(\frac{\pi t}{256} - 2{,}82\right) + 0{,}6\cos\left(\frac{\pi t}{64} + 1{,}04\right) + 0{,}0005t + 10{,}78, R^2 = 0{,}99$	(14)
Venezuela	$\ln f(t) = 0{,}96\cos\left(\frac{\pi t}{128} + 2{,}96\right) + 0{,}23\cos\left(\frac{5\pi t}{256} + 2{,}28\right) + 0{,}62\cos\left(\frac{\pi t}{256} - 2{,}32\right) + 0{,}39\cos\left(\frac{3\pi t}{256} + 2{,}14\right) + 0{,}0063t + 4{,}502, R^2 = 0{,}98$	(15)

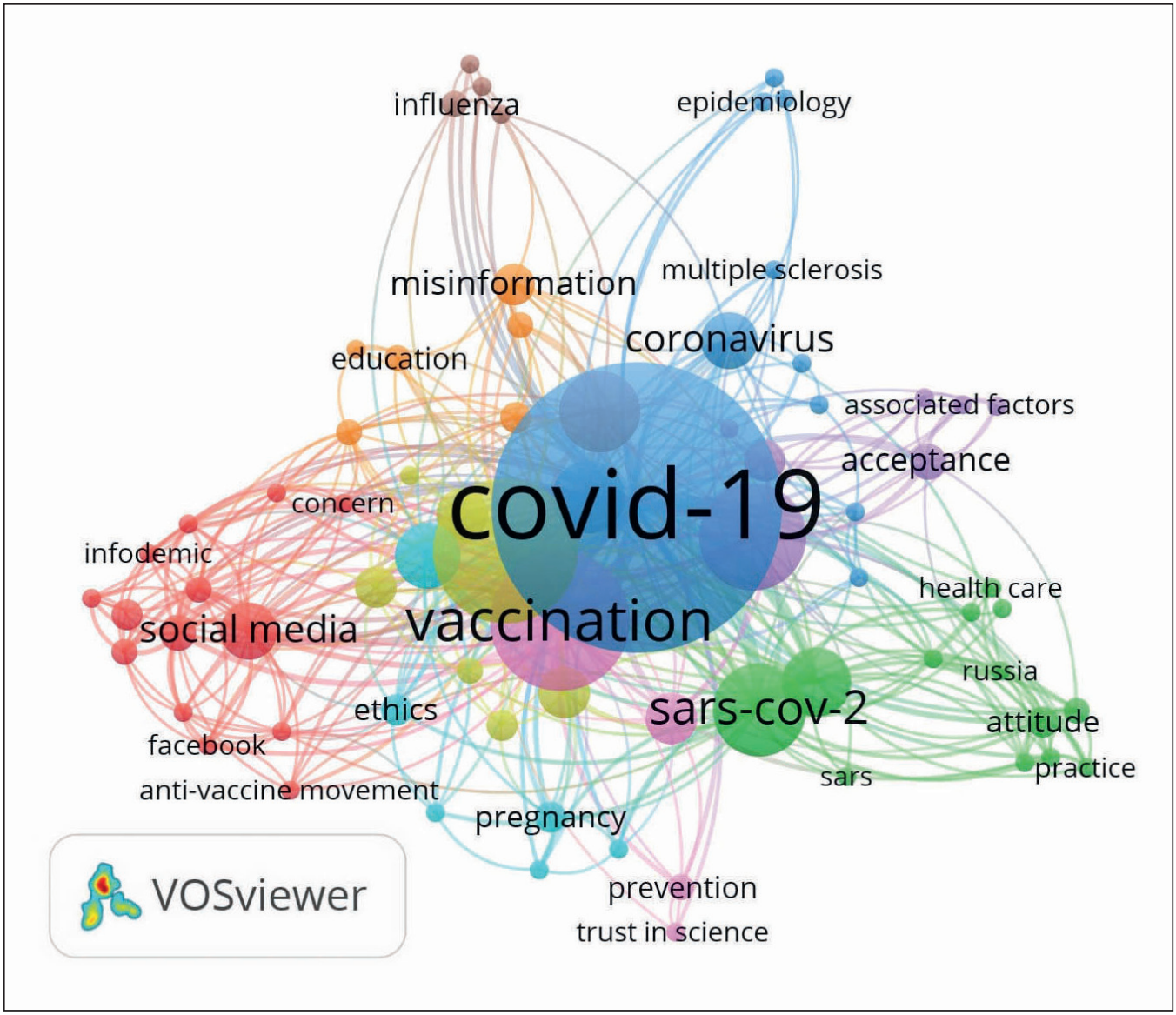


Fig. 1. Distribution of concepts Covid-19, vaccination, trust and related terms in publications are built using VOSViewerv.1.6.10

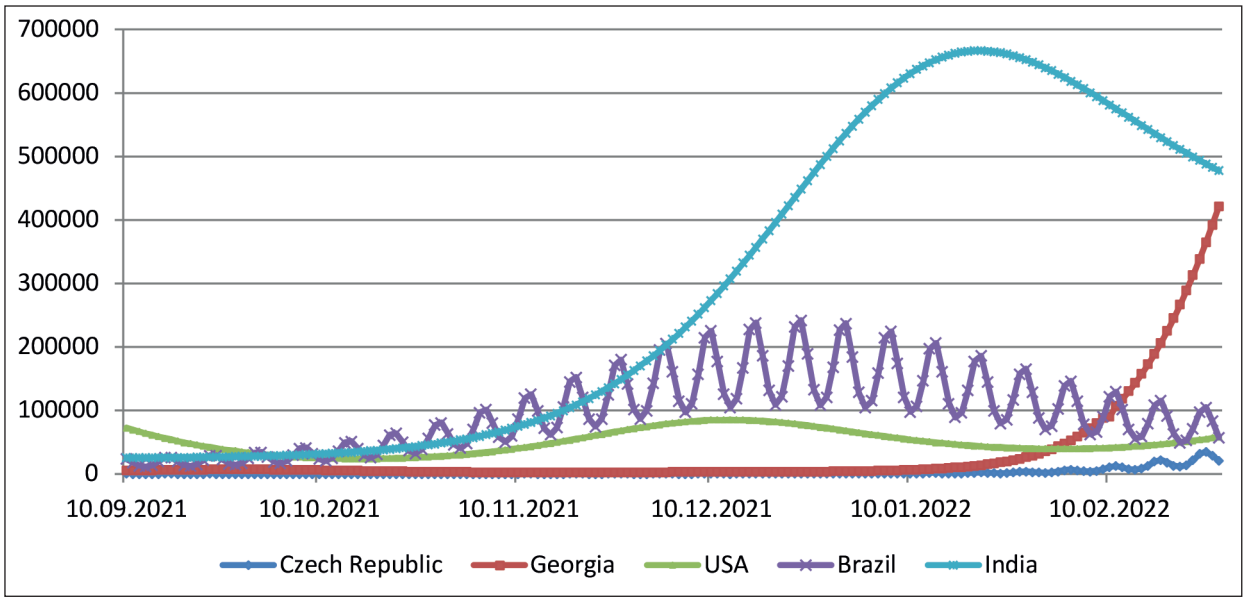


Fig. 2. Predicted values of the number of new infected with Covid-19 in 10.09.21-27.02.22 for the Czech Republic, Georgia, USA, Brazil and India.

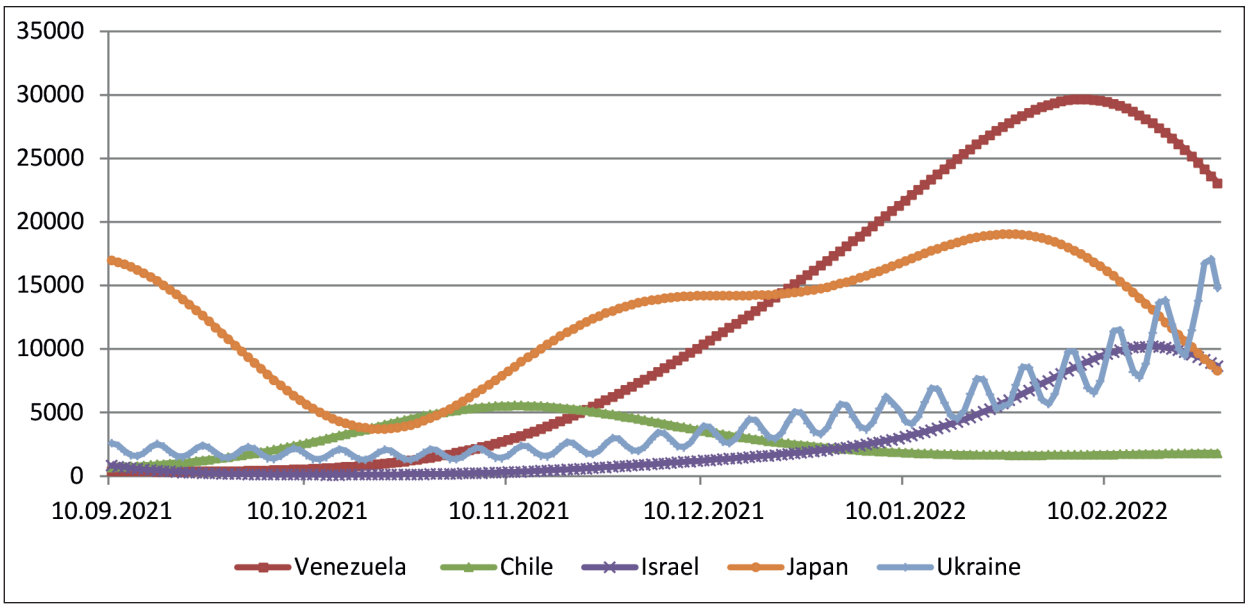


Fig. 3. Predicted values of the number of new infected with Covid-19 in 10.09.21-27.02.22 for Venezuela, Chile, Israel, Japan and Ukraine.

No logarithmation was performed for Japan and New Zealand because the series are stationary. Using a similar methodology, the following models were obtained (16) – (17).

Japan

$$f(t) = 2970\cos\left(\frac{\pi t}{128} - 0{,}063\right) + 2811\cos\left(\frac{5\pi t}{256} + 1{,}46\right) + 2985\cos\left(\frac{\pi t}{256} - 0{,}59\right) + 3387\cos\left(\frac{\pi t}{64} + 0{,}524\right) + 20{,}189t - 2037{,}2,\ R^2 = 0{,}89 \quad (16)$$

New Zealand

$$f(t) = 7{,}45\cos\left(\frac{7\pi t}{256} + 1{,}07\right) + 8{,}32\cos\left(\frac{3\pi t}{256} + 0{,}36\right) + 8{,}27\cos\left(\frac{\pi t}{256} + 1{,}99\right) + 8{,}036\cos\left(\frac{\pi t}{64} + 0{,}44\right) + 0{,}0212t + 7{,}517, R^2 = 0{,}7 \quad (17)$$

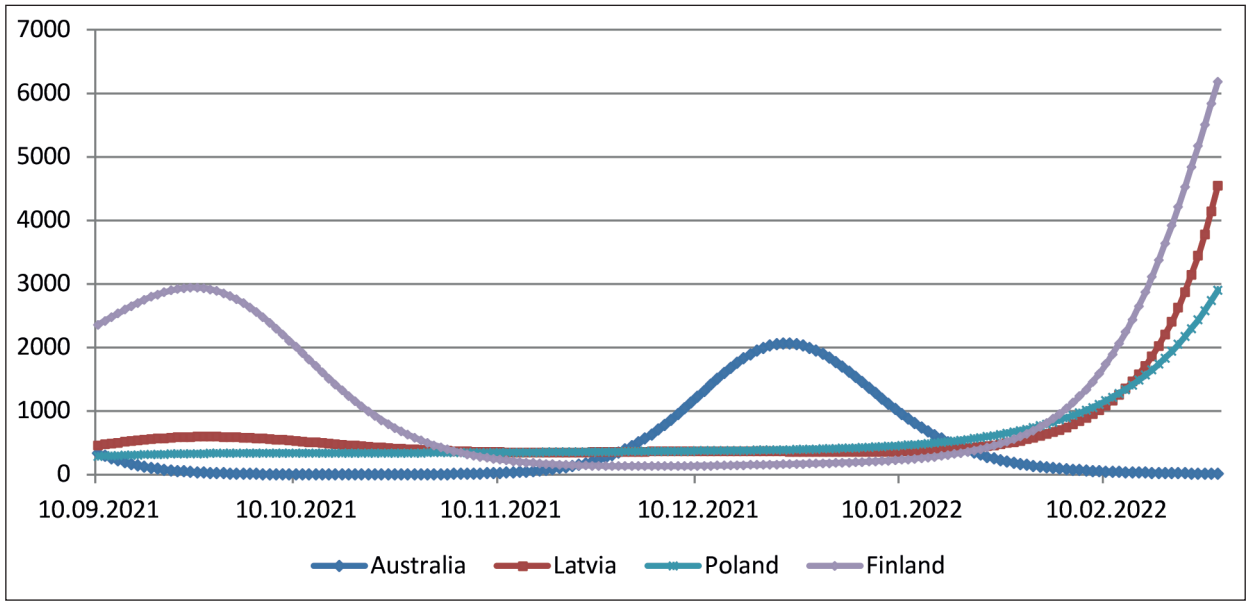


Fig. 4. Predicted values of the number of new infected with Covid-19 in 10.09.21–27.02.22 for Australia, Latvia, Poland and Finland.

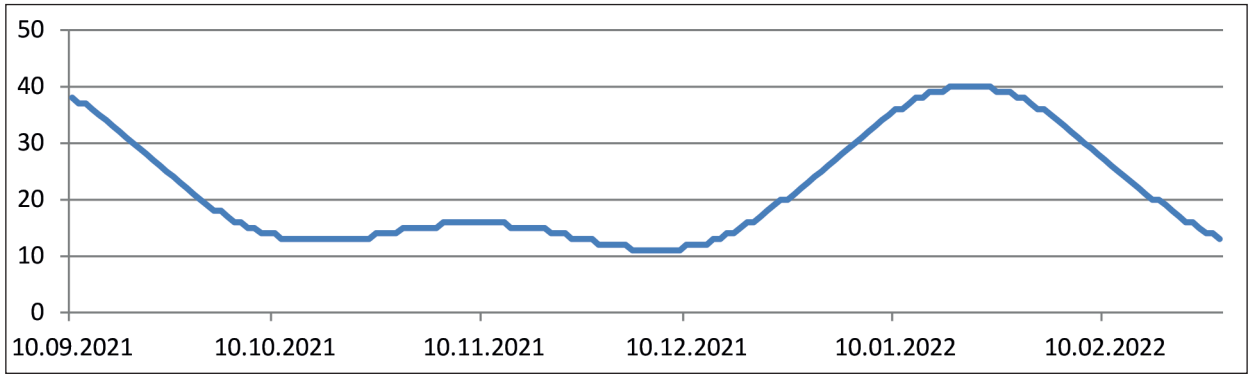


Fig. 5. Predicted values of the number of new infected with Covid-19 in 10.09.21-27.02.22 for New Zealand.

The results of calculating the number of people infected with Covid-19 in the period from 10.09.21 to 27.02.22 are presented in Figures 2-5.

Analysis of the obtained forecast regarding the dynamics of the COVID-19 incidence for different countries from September 10, 2021, to February 2, 2022 shows that with the trend of wave formation for each country, we can expect an increase in the number of diseases. In particular, for Finland, Chile, Brazil and the United States, an increase in morbidity may be observed in early or mid-October; in Australia, India and Venezuela – in early November, the peak incidence will occur in mid-January – early February; in Israel and Ukraine – an increase in early December; in January the incidence may increase in Poland, the Czech Republic and Georgia. Latvia has the most time until the next wave compared to other countries – a possible outbreak of COVID-19 occurs in early February. For New Zealand, an increase in the incidence rate is in early December, and the peak incidence will occur in mid-January, for Japan, an increase from late October, and the peak – early December.

Mathematical modeling of the COVID-19 disease dynamics in 15 countries, taking into account the vaccination level, confirmed that vaccination of the population is one of the most effective means to prevent the pandemic extension.

CONCLUSIONS

Forecasting the dynamics of the incidence rate in particular countries allows you to develop an action plan to accelerate the vaccination pace. The primary states' tasks on the vaccination process organization are to coordinate efforts of responsible institutions to ensure timely and reliable vaccine logistics; provide infrastructure capacity for efficient storage, transport and administrate the vaccine; ensure a sufficient number and even distribute the vaccine among the country's population; involve sufficient medical staff for vaccinations; ensure communication with the country's population on the need for vaccination, creating conditions for increasing public confidence in vaccination, etc.

Effective implementation of the state policy to prevent the spread of the COVID-19 pandemic must consider

the behavioral aspects of trust in vaccination and the psycho-emotional state of people. One of the areas of research on the behavioral aspects of trust in vaccination is the use of the theory of complex system self-organization. The study of society as a complex nonlinear system will allow effective control of the vaccination process. The essence of this approach is that the control of the vaccination process should be focused on own laws of self-organization and evolution of society as a complex system. The important fact is not the governing influence magnitude but its consistency with their trends in the development of the individual and society.

REFERENCES

1. Tiutiunyk, I., Humenna, Yu., & Flaumer, A. Covid-19 impact on business sector activity in the EU countries: digital issues. Health Economics and Management Review.2021; 2(1): 54-66. doi:10.21272/hem.2021.1-06
2. Antonyuk, N., Plikus, I., & Jammal, M. Sustainable business development vision under the Covid-19 pandemic. Health Economics and Management Revie. 2021;2(1): 37-43. doi:10.21272/hem.2021.1-04
3. Keliuotytė-Staniulėnienė, G., Daunaravičiūtė, K. The Global Green Bond Market in the Face of the COVID-19 Pandemic. Financial Markets, Institutions and Risks.2021; 5(1): 50-60. doi:10.21272/fmir.5(1).50-60.2021
4. Smiianov, V. A., Lyulyov, O. V., Pimonenko et al. The Impact Of The Pandemic Lockdown On Air Pollution. Health And Economic Growth: System Dynamics Analysis. Wiad Lek. 2020; 73(11):2332-2338. doi: 10.36740/WLek202011102
5. Hinrichs, G., Bundtzen, H. Impact of COVID-19 on personal insurance sales – Evidence from Germany. Financial Markets, Institutions and Risks. 2021$;5(1): 80-86. doi:10.21272/fmir.5(1).80-86.2021
6. Kadar, B., & Reicher, R. Zs. Innovations in Health Care Management: the Effect of the Pandemic on the Labour Market Change. Marketing and Management of Innovations.2020; 4: 120-130. doi:10.21272/mmi.2020.4-09
7. Vasylieva, T., Kuzmenko, O., Rashid, M. N. et al Innovations in government management of the healthcare system: forecasting of covid-19 consequences in social, investment and business development. Marketing and Management of Innovations.2020; 4: 11-25. doi:10.21272/mmi.2020.4-01
8. Smiianov, V. A., Smiianova, O. I., Gruzieva, T. S.et al. Study of motivational factors in doctors in respect of healthcare quality improvement. Wiad Lek. 2017; 70(1): 27-31.
9. Grshybowskyj, J. L., Smiianov, V. A., Myronyuk, I. M. et al. Ten indicators which characterize medical-demographic processes in adjacent regions of ukraine and poland. Wiad Lek. 2019; 72(5 cz 1): 868-876.
10. John P.A. Ioannidis, Sally Cripps, Martin A. Tanner. Forecasting for COVID-19 has failed, International Journal of Forecasting.2020. doi:10.1016/j.ijforecast.2020.08.004.
11. Kascha, M., Palienko, M., Marchenko, R. Forecast of COVID-19 progress considering the seasonal fluctuations. Health Economics and Management Revie. 2021; 2(2): 71-82. doi:10.21272/hem.2021.2-07
12. Formankova, S., Trenz, O., Faldik, O.et al. The future of investing-sustainable and responsible investing. Marketing and Management of Innovations. 2018; 2: 94-102. doi:10.21272/mmi.2018.2-08
13. Dutta, P., Dutta, U., Hasan, S. Et al. Educate, Empower And Protect People Through Timely Authentic Information: Explore How Bangladeshi Newspapers Response To COVID-19 Pandemic. SocioEconomic Challenges. 2020; 4(3): 93-103. doi:10.21272/sec.4(3).93-103.2020
14. Lopez, B.S., Alcaide, A.V. Blockchain, AI and IoT to Improve Governance, Financial Management and Control of Crisis: Case Study COVID-19. SocioEconomic Challenges. 2020: 4(2): 78-89. doi:10.21272/sec.4(2).78-89.2020.
15. Nelson, E.M. Socioeconomic Challenges – A Global Perspective Evaluating Invisible Connections-Resolutioning Necessary Global Collaborative. SocioEconomic Challenges.2017; 1(3): 116-119. doi: 10.21272/sec.1(3).116-119.2017
16. Tomcikova, L., Svetozarovova, N., Coculova, J. Challenges and Priorities in Talent Management During the Global Pandemic Caused by COVID–19. Marketing and Management of Innovations.2021; 2: 94-103. doi:10.21272/mmi.2021.2-08
17. Gallo, P., Mihalcova, B., Vegsoova, O.et al.. Innovative Trends in Human Resources Management: Evidence for the Health Care System. Marketing and Management of Innovations.2019; 2: 11-20. doi:10.21272/mmi.2019.2-01
18. Tovmasyan, G., Minasyan, D. The Impact of Motivation on Work Efficiency for Both Employers and Employees also During COVID-19 Pandemic: Case Study from Armenia. Business Ethics and Leadership.2020; 4(3): 25-35. doi:10.21272/bel.4(3).25-35.2020
19. Ray, A. Containing Airborne Transmission of COVID-19 and Its Implications for Global Economic Recovery. Business Ethics and Leadership.2021; 5(1): 81-88. doi:10.21272/bel.5(1).81-88.2021
20. Alkubaisy, A. Corporate Social Responsibility Practice in the Gulf Cooperation Council Countries amidst the COVID-19 Pandemic. Business Ethics and Leadershi. 2020; 4(4): 99-104. doi:10.21272/bel.4(4).99-104.2020
21. Moskovicz, A. Post-pandemic Scenario for University Startup Accelerators. Financial Markets, Institutions and Risk. 2021; 5(2): 52-57. doi:10.21272/fmir.5(2).52-57.2021
22. Kuzmenko, O., Šuleř, P., Lyeonov, S. Data mining and bifurcation analysis of the risk of money laundering with the involvement of financial institutions. Journal of International Studies. 2020; 13(3): 332-339. doi:10.14254/2071-8330.2020/13-3/22
23. Lyeonov, S., Żurakowska-Sawa, J., Kuzmenko, O. et al. Gravitational and intellectual data analysis to assess the money laundering risk of financial institutions. Journal of International Studies.2020;13(4): 259-272. doi:10.14254/2071-8330.2020/13-4/18
24. Yarovenko, H., Bilan, Y., Lyeonov, S., et al. Methodology for assessing the risk associated with information and knowledge loss management. Journal of Business Economics and Management.2021; 22(2): 369-387. doi:10.3846/jbem.2021.13925
25. Lyeonov, S., Liuta, O. Actual problems of finance teaching in ukraine in the post-crisis period. The financial crisis: Implications for research and teaching.2016;p. 145-152 doi:10.1007/978-3-319-20588-5_07
26. Didenko, I., Paucz-Olszewska, J., Lyeonov, S. Et al. Social safety and behavioral aspects of populations financial inclusion: A multicountry analysis. Journal of International Studies.2020; 13(2):347-359. doi:10.14254/2071-8330.2020/13-2/23
27. Brychko, M., Bilan, Y., Lyeonov, S. Trust crisis in the financial sector and macroeconomic stability: A structural equation modelling approach. Economic Research-Ekonomska Istrazivanja.2021; 34(1): 828-855. doi: 10.1080/1331677X.2020.1804970
28. Behavioural considerations for acceptance and uptake of COVID-19 vaccines: WHO Technical Advisory Group on Behavioural Insights and Sciences for Health, meeting report, 15 October 2020 Available at: https://apps.who.int/iris/bitstream/handle/10665/337335/9789240016927-eng.pdf?sequence=1&isAllowed=y (accessed 16.06.2021)

29. Our World in Data, Available at: https://ourworldindata.org/covid-vaccinations, (accessed 16.07.2021)
30. The COVID Tracking Project, Available at: https://covidtracking.com/data/api (accessed 09.09.2021)

Acknowledgment:
The study was carried out within the theme "Economic and mathematical modeling and forecasting, development of methodological principles of creating a roadmap for reforming the health care system in Ukraine taking into account behavioral, social, economic and legal determinants" (basic funding of the Ministry of Education and Science, order of 16. 04. 2021 № 434, Treaty BF / 24-2021).

ORCID and contributionship:
Olha V. Kuzmenko: 0000-0001-8575-5725 [A, C, F]
Vladyslav A. Smiianov: 0000-0002-4240-5968 [A, E, F]
Lesia A. Rudenko: 0000-0003-0556-8263 [B, E, F]
Mariia O. Kashcha: 0000-0001-9055-8304 [A – D, F]
Tetyana A. Vasilyeva: 0000-0003-0635-7978 [A, E, F]
Svitlana V. Kolomiiets: 0000-0002-7832-8415 [B, D, F]
Nataliia A. Antoniuk: 0000-0001-8610-3219 [B, E, F]

CORRESPONDING AUTHOR
Mariia O. Kashcha
Economic Cybernetics Department,
Sumy State University
2, Rimskogo Korsakova Str., 30000 Sumy, Ukraine
tel: +380667509188
e-mail: m.kascha@uabs.sumdu.edu.ua

Received: 05.07.2021
Accepted: 28.09.2021

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

IMMUNOHISTOCHEMICAL AND MORPHOMETRIC CHARACTERISTICS OF CHORIONIC TROPHOBLAST AND DECIDUAL CELLS OF FETAL MEMBRANES IN CASE OF ANTE-INTRANATAL FETAL DEATH

DOI: 10.36740/WLek202110102

Sergiy O. Sherstiuk, Olha S. Zats, Liudmila L. Sherstiuk, Stanislav I. Panov
V.N. KARAZIN KHARKOV NATIONAL UNIVERSITY, MEDICAL SCHOOL, KHARKIV, UKRAINE

ABSTRACT

The aim: To identify immunohistochemical and morphometric features of chorionic trophoblast cells and fetal membranes derived decidual cells, which were obtained from stillbirths associated with pre-eclampsia, iron deficiency anemia, and acute chorioamnionitis.

Materials and methods: The study included 58 fetal membranes of fetuses, who died in the ante-intranatal period. The membranes were divided into 6 obstetric history-based groups: premature (n = 8) and full-term (n = 8) stillbirths complicated by preeclampsia; premature (n = 8) and full-term (n = 8) stillbirths complicated by iron deficiency anemia, premature (n = 10) and full-term (n = 16) stillbirths complicated by chorioamnionitis. A control group consisted of 8 membranes obtained from physiological pregnancies followed by the birth of a live full-term baby. Samples (rupture site) were probed with cytokeratin to identify the fetal trophoblast layer of the chorion and with vimentin for further identification of the decidual cells. The thickness of the trophoblastic layer, expression levels of cytokeratin and vimentin were determined.

Results: A decrease of the cytokeratin expression by the chorionic trophoblasts and a thinning of the chorionic trophoblast cell layer due to an increasing gestational age were shown in case of the mentioned pathological conditions. In comparison with the control group, the level of vimentin expression by decidual cells was increased in case of full-term pregnancy complicated by preeclampsia, decreased in pregnancies complicated by chorioamnionitis, and remained unchanged in case of accompanying anemia.

Conclusions: The changes in the studied immunohistochemical parameters are more pronounced in case of chorioamnionitis, which indicates more severe morphological and functional changes.

KEY WORDS: preeclampsia, anemia, chorioamnionitis, fetal membranes, immunohistochemistry

Wiad Lek. 2021;74(10 p.I):2368-2373

INTRODUCTION

Throughout the entire period of pregnancy, an intrauterine development and survival of a fetus largely depend on a morphological and functional state of fetal membranes. They are responsible for trophic, respiratory, mechanical, immune, and endocrine functions, promotion of fetal growth, and protection from adverse environmental conditions [1].

Fetal membranes include the amnion and the chorion. The avascular amnion, which lies between the amniotic fluid and the chorion, consists of 5 layers: a continuous monolayer of the cubic epithelium, a basement membrane, a compact layer, a fibroblast layer, and a spongy layer. The chorion, in turn, is represented by the reticular layer and the trophoblast region, which are interconnected through the pseudo-basal membrane [2]. The chorionic mesoderm is separated from the cells of the extravillous trophoblast by the basal plate, adjacent to the decidua [3]. It is believed that the amnion acts as a structural barrier, it plays a major role in ensuring the mechanical function of the fetal membranes [4]. The chorion provides endocrine function and acts as an immunological buffer, preventing the amnion degradation and protects a fetus from a maternal immune system [5]. At the same time, the amnion and the chorion collectively ensure the integrity of the membranes, since each of them individually is less resistant to an increasing mechanical stretching due to the growth of a fetus during pregnancy [6].

At present, perinatal mortality remains high, ranging from 55 out of 1000 to 520 out of 1000 births, in low- and middle-income countries, which is mainly explained by prematurity and infectious complications [7,8]. Among the factors contributing to miscarriage, an important role is played by pathological conditions affecting pregnant women, primarily preeclampsia (PE) and iron deficiency anemia (IDA). In the case of PE, preterm childbirth is recorded in 20-30% of cases, perinatal morbidity comprises 56%, and perinatal mortality is 3-4 times higher than the average one, reaching 12%. PE increases the risk of placental abruption, massive bleeding, placental insufficiency, fetal growth retardation syndrome, and antenatal fetal death [9]. Concerning IDA, miscarriage occurs in 20–42%, placental insufficiency in 25%, prolonged labor in 42%, hypotonic bleeding in the postpartum period in 47%, and purulent septic complications in 12% of cases.

Accordingly, the risk of antenatal fetal death increases in pregnant women with IDA [10]. In general, the cause of one-third of all preterm births is the premature rupture of the fetal membranes, which increases the risk of perinatal mortality and morbidity and is associated with the early gestational age of a fetus [11]. Most often, the precocious rupture of the membranes is combined with chorioamnionitis (CA) and bleeding due to the premature placental abruption [12].

In this regard, the study of the morphology of the fetal membranes under normal conditions and during complicated pregnancy using modern research methods is of particular importance.

THE AIM

To reveal the immunohistochemical and morphometric features of the chorionic trophoblast cells and fetal membranes derived decidual cells, which were obtained from stillbirths associated with pre-eclampsia, iron deficiency anemia, and acute chorioamnionitis.

MATERIALS AND METHODS

The material was collected between January 2016 and December 2019 from the Pathology Department of Municipal Non-Commercial Institution "City Perinatal Center" of Kharkiv City Council. Based on medical histories of pregnancy and childbirth and the protocols of postmortem examination of the fetuses and placentas, 58 cases of ante-intranatal fetal death at the gestational age ranging between 30-41 weeks were selected and the following groups were formed: premature birth complicated by preeclampsia (PPE group) (n = 8), full-term pregnancy complicated by preeclampsia (FPE group) (n = 8), premature birth complicated by iron deficiency anemia (PIDA group) (n = 8), full-term pregnancy complicated by iron deficiency anemia (FIDA group) (n = 8), premature birth complicated by acute chorioamnionitis (PCA group) (n = 10), full-term pregnancy complicated by acute chorioamnionitis (FCA group) (n = 16). Chorioamnionitis was diagnosed histologically using the standardized diagnostic criteria proposed by Redline et al [13]. Cases of congenital malformations and multiple pregnancies were excluded from the study. The control group (CG) consisted of 8 observations of normal pregnancies followed by the birth of a live full-term baby.

In each observation, 4-5 mm thick strips of the fetal membranes were excised near the place of their rupture (n = 66) for further morphological examination. Paraffin-embedded tissue blocks were prepared using standard procedure. Immunohistochemistry assay (IHC) was performed following the standardized protocols using monoclonal antibodies (mAb) to cytokeratin pan (AE-1 / AE-3, DAKO, Denmark) – for identification of epithelial cells and to vimentin (V9, DAKO, Denmark) – for identification of mesenchymal cells with the DAKO EnVision imaging system (Denmark). The slides were studied using an Olympus BX-41 microscope (Japan). The thickness of the layer of reactive trophoblasts was measured using video microscopic morphometry (Olympus DP-Soft, Version 3.1) and Microsoft Excel, the expression level of each IHC marker was measured by cytophotometric evaluation of the optical density of cellular cytoplasm [14].

Statistical analysis was performed using the Statistica 6.0 and Microsoft Excel 2003 software package. Nonparametric methods were used to compare the parameters (Mann-Whitney U-test, Kruskal-Wallis test). The significance of differences between the average values of the indicators was taken at the level of $p < 0.05$.

The management of the study was conducted in full compliance with the ethical principles contained in the "Human Rights Declaration" adopted in Helsinki, which follows the Good Practice Rules in the Clinical Study and Legal Regulations and with the approval of the Ethics Committee of the V.N. Karazin Kharkiv National University.

RESULTS

In all studied groups, IHC assay with anti-cytokeratin pan (AE-1 / AE-3) mAb, revealed cytokeratin-positive cells, whose cytoplasm was stained brown. Cytokeratin was expressed by the single-row amniotic epithelial cells and metabolically active chorionic trophoblast cells.

The layer of cytokeratin-positive chorionic cells was detected in the trophoblastic region between the reticular layer of the chorion and the adjacent decidual layer. In the PE, IDA full-term and premature pregnancies groups as well as in the CG, trophoblast cells in the regions adjacent to the reticular layer formed a continuous layer and were located compactly in several rows. In the distal parts of the trophoblastic region, the cells were located less compactly, cytokeratin-negative decidual cells were visualized between them. Individual cells or groups of trophoblast cells showed signs of apoptosis — a pyknotic nucleus and an optically empty cytoplasm Fig. 1. (Chorionic trophoblast layer. Individual cells are apoptotic. CG. IHC with anti-cytokeratin mAb, x400.). In the CA groups, the layer of trophoblasts was sharply thinned or destroyed due to the leukocytic infiltration Fig. 2 (Thinning and discontinuity of the trophoblast layer at the site of leukocytic infiltration of the chorion. FCA group. IHC with anti-cytokeratin mAb, x400.) at the inflammatory sites. In the same (CA) groups, apoptotic altered cells were more often visualized.

When measuring the thickness of the trophoblastic layer, the value of this indicator varied depending on the gestational age and the existing complication of pregnancy (Table I). When assessing the level of trophoblastic cytokeratin expression, the average value of the optical density of the marker in the cytoplasm of the studied cells depended on the gestational age and the pathological condition complicating the course of pregnancy. We observed high values of the average thickness of the trophoblast layer and the elevated level of expression of cytokeratin by its cells in the premature pregnancy groups, these indicators significantly exceeded the average values of the CG and other corresponding full-term pregnancy groups. In the full-term pregnancy groups, there was a significant decrease in the

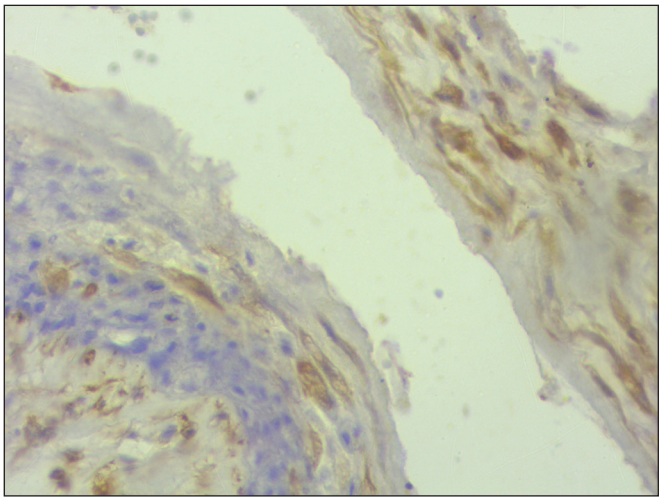


Fig. 1. Chorionic trophoblast layer. Individual cells are apoptotic. CG. IHC with anti-cytokeratin mAb, x400.

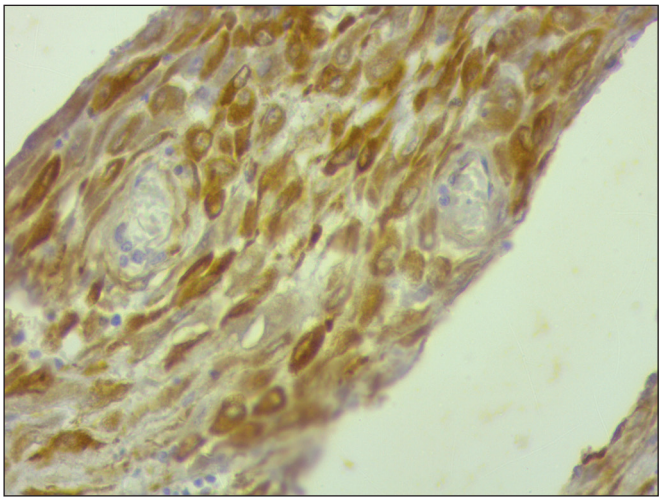


Fig. 2. Thinning and discontinuity of the trophoblast layer at the site of leukocytic infiltration of the chorion. FCA group. IHC with anti-cytokeratin mAb, x400.

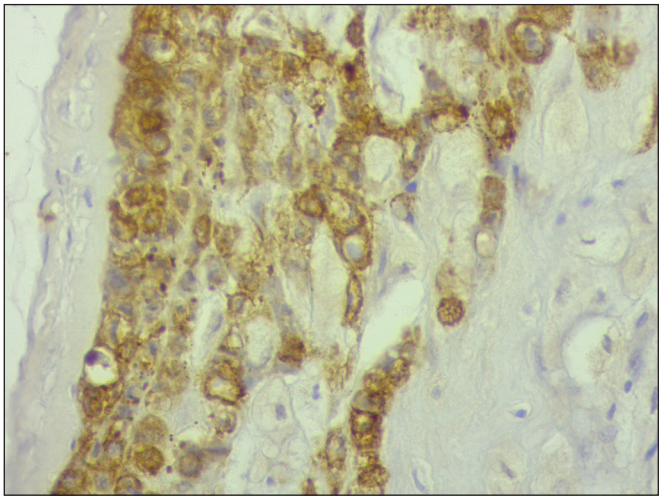


Fig. 3. Vimentin-positive stromal and decidual cells of the chorion and decidua. CG. IHC with anti-vimentin mAb, x400.

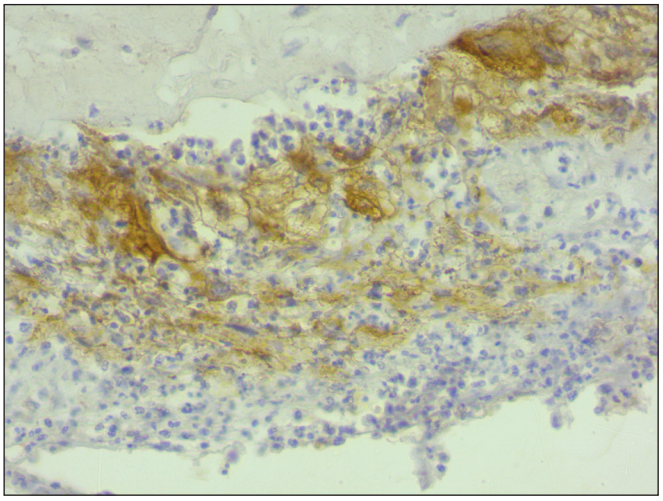


Fig. 4. Intensely vimentin-positive fusiform decidual cells. FPE group. IHC with anti-vimentin mAb, x400.

mean values of both IHC parameters in comparison with the CG, at the same time the lowest values were recorded in the CA group Table I.

Evaluation of the IHC reaction with anti-vimentin (V9) mAb in all groups revealed the expression of the marker in the cytoplasm of a thin layer of fibroblast cells of the spongy layer of the amnion, stromal cells of the reticular layer of the chorion, decidual cells of the cytotrophoblastic region of the chorion and decidua, as well as vascular endothelium cells Fig. 3 (Vimentin-positive stromal and decidual cells of the chorion and decidua. CG. IHC with anti-vimentin mAb, x400.). Vimentin-positive decidual cells were visualized in the distal parts of the cytotrophoblastic region of the chorion, there were both single positive cells as well as their groups, separated by vimentin-negative trophoblast cells. Some decidual cells acquired a fusiform shape, intense vimentin expression was observed in their cytoplasm and the number of such cells rose with the increasing gestational age Fig. 4 (Intensely vimentin-positive fusiform decidual cells. FPE group. IHC with anti-vimentin mAb, x400.). Individual decidual cells demonstrated apoptotic changes – they had a pyknotic nucleus and vimentin-negative cytoplasm.

Finally, we assessed the level of vimentin expression by decidual cells in the study groups (Table I). In the case of premature childbirth, it was significantly lower in contrast to all other full-term complicated pregnancy groups and the CG. The full-term pregnancy groups, compared with CG, showed significantly higher mean value of optical density of the cytoplasm of decidual cells in preeclampsia, while in CA group it was significantly lower, and in case of IDA, it did not differ significantly.

DISCUSSION

In this study, the immunohistochemical and morphometric changes in chorionic trophoblast cells and decidual cells of fetal membranes near the site of their rupture were

Table I. Values of the IHC morphometric parameters in the studied and control groups

Group	Parameters		
	The thickness of the layer of cytokeratin-positive trophoblast cells, × 10^{-6}m	Level of cytokeratin expression by chorionic trophoblast cells, conventional units	Level of vimentin expression by decidual cells, conventional units
PPE	124.14 ± 2, 24 p_2 <0.001	0.170 ± 0.011 p_2 <0.05	0.231 ± 0.008 p_2 <0.001
FPE	89.00 ± 7.89 p_1 <0.001, p_2 <0.001	0.092 ± 0.006 p_1 <0.01, p_2 <0.001	0.294 ± 0.003 p_1 < 0.001, p_2 <0.05
PIDA	153.71 ± 4.83 p_2 <0.05	0.207 ± 0.011 p_2 <0.001	0.219 ± 0.009 p_2 <0.001
FIDA	130.17 ± 4.53 p_1 <0.001, p_2 <0.001	0.119 ± 0.004 p_1 <0.01, p_2 <0.05	0.282 ± 0.006 p_1 <0.001, p_2> 0.05
PCA	110.53 ± 2.41 p_2 <0.001	0.145 ± 0.004 p_2 <0.05	0.203 ± 0.012 p_2 <0.001
FCA	60.71 ± 6.75 p_1 <0.001, p_2 <0.001	0.079 ± 0.009 p_1 <0.001, p_2 <0.001	0.260 ± 0.003 p_1 <0.01, p_2 <0.05
CG	174.45 ± 6.75	0.135 ± 0.005	0.276 ± 0.002

Note: p_1 - statistical significance of comparison of the premature birth and full-term pregnancy groups complicated by the similar pathologies, p_2 - statistical significance of values compared with the CG.

studied. Based on the IHC findings in the PE, IDA, and CA full-term and premature pregnancies groups, the layer of metabolically active chorionic cells becomes thinner with increasing gestational age. According to the literature data, chorionic thinning reflects the process of the fetal membranes remodeling during pregnancy and their preparation for childbirth and is carried out by the apoptosis of chorionic cells [15, 16]. At the same time, in the full-term pregnancy groups, we found a significant decrease in the thickness of the trophoblast layer, in comparison with not only premature pregnancy groups but also with the CG. In the case of IDA, the average value of the indicator decreased 1.3 times, within the PE group – 2 times, the CA group – almost 3 times. Moreover, in the groups with chorioamnionitis, both in premature and full-term pregnancies, the layer of chorial cells was not focally detected. According to B. Fortner et al (2014), in chorioamnionitis, the chorion undergoes pathological thinning (probably due to the increased cellular apoptosis), which reduces the tensile strength or weakens the physical barrier and bacterial resistance [17]. Inflammatory mediators are one of the important elements of the process leading to the premature rupture of the membranes. Their biosynthesis is a part of the mother's body physiological defense mechanism in response to an invasion of pathogenic microorganisms. These substances include prostaglandins, cytokines (interleukins (IL), tumor necrosis factor (TNF)), proteinases (matrix metalloproteinases (MMP), elastases, cathepsins), they play a leading role in the process of membranes' thinning membranes and apoptosis [18].

The average level of cytokeratin expression by the chorionic cells in corresponding groups with different gestational terms decreased significantly in the case of a full-term pregnancy. At the same time, in comparison with the control group value, the level of cytokeratin expression was 1.1 times lower in the FIDA group, 1.5 times in the FPE group, and 1.7 times in the FCA group. The obtained data indicate that with an increase in the gestational age, the metabolic activity of cytotrophoblast cells decreases, which reflects the physiological involutive-dystrophic processes of the placenta tissues. Under the studied pathological pregnancy conditions, this process is accelerated due to the influence of hypoxia, as well as oxidative stress accompanied by the inflammatory damage and premature aging of cells in case of chorioamnionitis [19, 20].

It is now known that decidual cells are of maternal origin and are formed as a result of the transformation of stromal endometrial fibroblasts [21]. Thus, during the IHC reaction with anti-vimentin (V9) mAb, the cytoplasm of the decidual cells was vimentin-positive. Analysis of the average values of the marker expression level by the decidual cells in all studied full-term pregnancy groups significantly exceeded the average value in the premature childbirth groups. Decidual cells have the properties of fibroblasts, they express various proteins of the extracellular matrix, such as fibronectin, emilin-1, decorin, fibulins, collagens, and laminins (22-24), and vimentin deficiency leads to a decrease in their ability to synthesize collagen [25]. In our study, we observed an acquisition by some of the decidual cells of a fibroblast-like fusiform shape with intense vimentin expression, which may indicate their functional activity, the number of such cells raised with an increase of gestational age. In the FPE and FIDA groups, the increase in the average value of vimentin expression was observed compared to the CG, which is due to the stimulating effect of hypoxia, which develops under these pathological conditions, particularly, in the FPE group, the indicator

significantly differed from the value in the CG, and in the FIDA it tended to increase, which is apparently due to a more severe hypoxic damage of the tissues. In the FCA group, in comparison with the CG, there was a significant decrease in the average value of the vimentin expression level, which indicated a decrease in their functional activity under conditions of an acute inflammatory process.

CONCLUSIONS

During ante-intranatal fetal death in full-term and premature pregnancies, complicated by PE, IDA, and CA, several structural changes are observed in the chorion of the membranes and decidua. In the chorion, with an increase of gestation age, the layer of trophoblast cells becomes thinner and the level of cytokeratin expression decreases, while in full-term pregnancies, the average values of indicators are significantly lower than those in the control group, which indicates a decrease in the metabolic activity of chorionic cells and may be one of the predictors of violation of the integrity of the membranes. With an increase in the gestational age of the studied groups, the content of cytoplasmic vimentin increases in the decidual cells due to an increasing proportion of fibroblast-like decidual cells characterized by intense IHC reaction. In full-term pregnancies complicated by preeclampsia, this average value was significantly higher, however, in the case of chorioamnionitis, it was significantly lower compared with the control group. The change in the intensity of vimentin expression indicates the degree of functional activity of cells, therefore, it is advisable to study the distribution and content of stromal collagens in the fetal membranes and decidual tissue in the future.

REFERENCES

1. Menon R. Human fetal membranes at term: dead tissue or signalers of parturition? Placenta. 2016; 44:1e5
2. Richardson L.S., Vargas G., Brown T. et al. Discovery and Characterization of Human Amniochorionic Membrane Microfractures. Am J Pathol. 2017; 187: 2821-2830.
3. Benirschke K., Kaufmann P. Pathology of the human placenta. New York; Springer-Verlag. 2000:116; 281–297
4. Verbruggen S.W. Oyen M.L., Phillips A.T.M., Nowlan N.C. Function and failure of the fetal membrane: modelling the mechanics of the chorion and amnion. PLoS ONE. 2017;12: e0171588. doi:10.1371/ journal. pone.0171588.
5. Oyen M.L., Calvin S.E., Landers D.V. Premature rupture of the fetal membranes: Is the amnion the major determinant? Am J Obstet Gynecol. 2006; 195(2):510–5.
6. Khwad M.E., Pandey V., Stetzer B. et al. Fetal Membranes From Term Vaginal Deliveries Have a Zone of Weakness Exhibiting Characteristics of Apoptosis and Remodeling. JAMA. 2006; 13(3):191–5.
7. Stewart C.J., Tregoning S.K., Moller G., Wainwright H. Preterm prelabour rupture of the membranes before 28 weeks: better than feared outcome of expectant management in Africa. Eur J Obstet Gynecol Reprod Biol. 2006;126 (2):186–192.
8. Obi S.N., Ozumba B.C. Pre-term premature rupture of fetal membranes: the dilemma of management in a developing nation. J Obstet Gynaecol. 2007;27(1):37–40.
9. Simanov I.V. Features of the course of pregnancy at the preeclampsia of varying severity at the present stage. VFSnegirev Archives of Obstetrics and Gynecology. 2020;7(1):47-52.
10. Bencaiova G., Burkhardt T., Breymann C. Anemia – prevalence and risk factors in pregnancy. Eur J Intern Med. 2012; 23(6):529–533.
11. Joyce E.M., Moore J.J., Sacks M.S. Biomechanics of the fetal membrane prior to mechanical failure: review and implications. Eur J Obstet Gynecol Reprod Biol. 2009;144 (1): S121-S127.
12. Menon R., Fortunato S.J. Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Cl Ob. 2007; 21(3):467–78.
13. Redline R.W., Faye-Petersen O., Heller D. et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6: 435–448.
14. Patent na korysnu model' № 46489 Ukrayina G01N33/00. Sposib kil'kisnoho vyznachennya vmistu antyhenu v biolohichnykh tkanynakh / Hubina-Vakulyk H.I., Sorokina I.V., Markovs'kyy V.D., Kykhtenko O.V., Kupriyanova L.S., Sydorenko R.V. zayavnyk ta patentovlasnyk «KHNMU». – №u 200906730; zayavl. 26.06.2009; opubl. 25.12.2009. Byuleten' № 4.
15. Reti N.G., Lappas M., Riley C. et al. Why do membranes rupture at term? Evidence of increased cellular apoptosis in the supracervical fetal membranes. Am J Obstet Gynecol. 2007;196:484.e1-484.e10.
16. George R.B., Kalich J., Yonish B., Murtha A.P. Apoptosis in the chorion of fetal membranes in preterm premature rupture of membranes. Am J Perinatol. 2008;25: 29–32.
17. Fortner K.B., Grotegut C.A., Ransom C.E. et al. Bacteria Localization and Chorion Thinning among Preterm Premature Rupture of Membranes. PLoS ONE. 2014;9(1): e83338.
18. Tchirikov M., Schlabritz-Loutsevitch N., Maher J. et al. Mid-trimester preterm premature rupture of membranes (PPROM): etiology, diagnosis, classification, international recommendations of treatment options, and outcome. J Perinat Med. 2018;46(5):465–488.
19. Dutta E.H., Behnia F., Boldogh I. et al. Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes. Mol Hum Reprod. 2016;22(2):143–157.
20. Charlton F., Bobek G., Stait-Gardner T. et al. The protective effect of apolipoprotein in models of trophoblast invasion and preeclampsia. Am J Physiol Regul Integr Comp Physiol. 2017; 312(1):40-48.
21. Gellersen B., Brosens I.A., Brosens J.J. Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. Semin 27. Reprod Med. 2007; 25:445–53.
22. Schatz F., Guzeloglu-Kayisli O., Arlier S. et al. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Hum Reprod Update. 2016; 22:497–515. doi: 10.1093/humupd/dmw004.
23. Xu G., Guimond M.J., Chakraborty C., Lala P.K. Control of proliferation, migration, and invasiveness of human extravillous trophoblast by decorin, a decidual product. Biol Reprod. 2002;67:681–9.
24. Winship A., Cuman C., Rainczuk K., Dimitriadis E. Fibulin-5 is upregulated in decidualized human endometrial stromal cells and promotes primary human extravillous trophoblast outgrowth. Placenta. 2015; 36:1405–11.
25. Eckes B., Colucci-Guyon E., Smola H. et al. Impaired wound healing in embryonic and adult mice lacking vimentin. J. Cell Sci. 2000; 113: 2455–2462.

This work is a fragment of a comprehensive research program of the Human Anatomy Department of the V.N. Karazin Kharkiv National University: "The impact of maternal pa-

thology on the fetal and newborn organism development". State Registration Number: 0117U004838. This work was funded according to the plan of that program. The authors did not receive any financial support from the manufacturers of medical instruments and drugs.

ORCID and contributionship:
Sergey O. Sherstiuk: 0000-0001-6062-7962 [A,E,F]
Olha S. Zats: 0000-0003-4005-4428 [B,C,D]
Liudmila L. Sherstiuk: 0000-0002-2993-2843 [B,C,E]
Stanislav I. Panov: 0000-0001-9264-0088 [B,C,E]

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

CORRESPONDING AUTHOR
Olha S. Zats
V. N. Karazin Kharkiv National University
6 Svobody Sq., 61022 Kharkiv, Ukraine
tel: +38 067 399 33 11
e-mail: Olgse@ukr.net

Received: 05.04.2021
Accepted: 08.08.2021

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

OSTEOPLASTIC PROPERTIES OF MULTIPOTENT MESENCHYMAL STROMAL CELLS OF ADIPOSE TISSUE

DOI: 10.36740/WLek202110103

Andriy Bambuliak, Nataliia Kuzniak, Valentyna Honcharenko, Marianna Ostafiychuk, Alina Palamar
BUKOVINIAN STATE MEDICAL UNIVERSITY, CHERNIVTSI, UKRAINE

ABSTRACT

The aim: Determining the ability of samples based on MMSC – AT differentiating in the osteogenic direction.

Materials and methods: The study was conducting at Bukovinian State Medical University, Chernivtsi, Ukraine. Adipose tissue samples were obtaining from the neck of 60 experimental animals (white Wistar rats). Multipotent mesenchymal stromal cells of adipose tissue were obtained by grinding adipose tissue of rats in 0.1% collagenase 1A . Alkaline phosphatase activity was assessing by using the Alkaline Phosphatase Detection Kit (Sigma, USA) according to the manufacturer's protocol. Osteopontin gene expression was determining by immunocytochemical method. To determine the mRNA used the PCR method, which is associated with reverse transcription (RT-PCR) in the area of quantification of gene expression to the marker BGP.

Results: On the 21st day of observations, the expression of mRNA encoding the BGP gene decreased in samples № 1 and № 3 to 35,800 ± 420.0 copies and to 35,000 ± 400.0 copies, $p1<0.01$, $p>0.05$. Also was observing growth of copies of the BGP gene in samples № 2 and № 4 in 2.1, $p<0.01$ and 2.2 times, $p\text{-}p2<0.05$, relative to the data in sample № 1.

Conclusions: Comparative study of osteoplastic properties samples MMSC-AT showed that a larger number of cells differentiate into the osteoblasts in samples containing MMSC-AT + PRP (№ 2) and MMSC-AT + PRP + «Kolapan» (№ 4). This has been proven higher alkaline phosphatase activity, higher levels osteopontin expression, and higher levels BGP gene expression.

KEY WORDS: alkaline phosphatase, osteopontin, multipotent mesenchymal stromal cells of adipose tissue, BGP

Wiad Lek. 2021;74(10 p.I):2374-2378

INTRODUCTION

In medical practice, doctors often face injuries of various aetiologies, which require replacement of bone tissue with biocompatible material [1]. The primary goal of reconstructive surgery is the correct choice of material that has the ability quickly repair damaged tissue in a short period of time [2]. In modern medicine, the use of stem cells in reconstructive surgery and in particular in dental practice is promising. This is possible by phenomenal discoveries in biology and biotechnology, which are basing on the ability of stem cells after their introduction into the recipient's body to get to the site of tissue damage and restore their cellular structure [3, 4].

The optimal source of multipotent stem cells is adipose tissue [5]. This is due to the availability of methods for obtaining material, low invasiveness for the body, the ability to obtain cellular material in sufficient quantities and at the right time [6, 7]. Multipotent mesenchymal stromal cells of adipose tissue are able to differentiate in adipogenic, osteogenic, chondrogenic, endothelial, myogenic, hepatogenic, epithelial and neurogenic directions [8, 9].

The ability to osteogenic differentiation is manifesting by increased levels of markers of bone formation. One of the main markers of bone formation is alkaline phosphatase [10]. It, localized in osteoblasts, plays an important role in the processes of bone mineralization, as it catalyzes the transfer of phosphoric acid ions from the ether to the components of the organic matrix of bone. Increased activity of this enzyme in the blood as a marker of bone formation indicates the activation of bone remodelling [11].

Given the development of modern medicine, in particular dentistry, the search for new materials that would promote osteogenesis and improve bone mineralization processes remains relevant [12]. All of the above prompted us to conduct this study.

THE AIM

Determining the sample based on the MMSC-AT that most differentiates in the osteogenic direction.

MATERIALS AND METHODS

The study was conducting at Bukovinian State Medical University, Chernivtsi, Ukraine. Adipose tissue samples were obtaining from the neck of 60 experimental animals (white Wistar rats) [13]. Multipotent mesenchymal stromal cells of adipose tissue (MMSC-AT) were obtained by grinding adipose tissue of rats in 0.1% collagenase 1A [14]. For the toxicological experiment, which allows to establishing the direct influence of factors in the contact of implantation material at the cellular level, were selected four samples. Sample

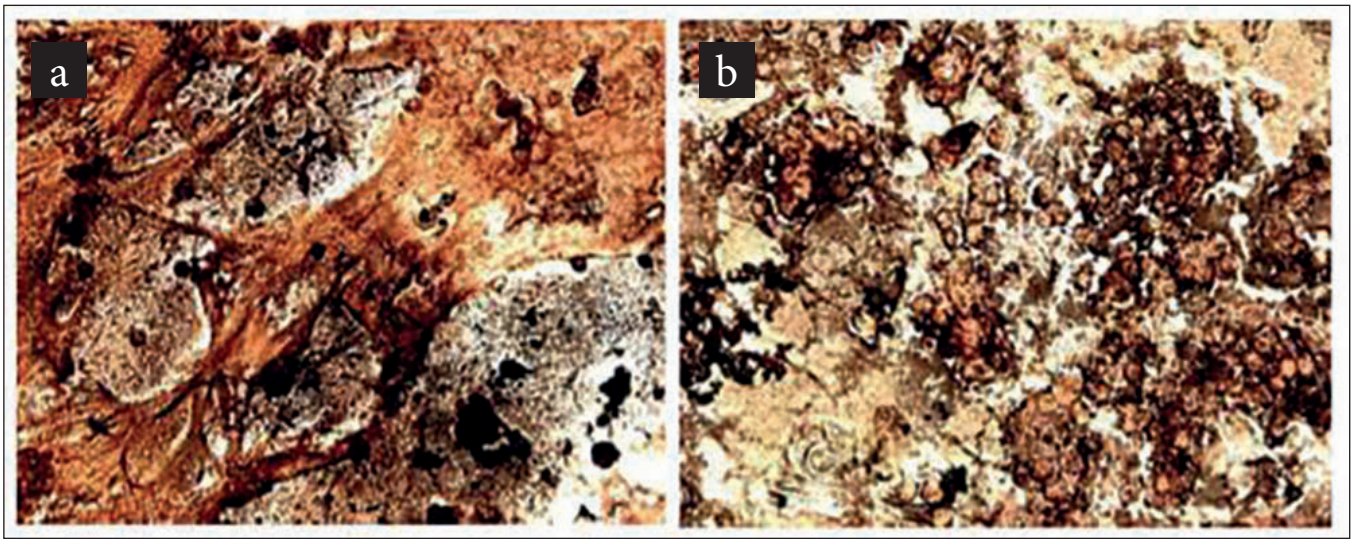


Fig. 1. Osteogenic cell differentiation according to Von Kossa: a – osteoblasts differentiated from MMSC-AT (sample № 1); b – osteoblasts, differentiated from MMSC-AT + PRP + "Kolapan" (sample № 4)

Table I. Alkaline phosphatase activity of osteoblasts in different samples of MMSC-AT

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	0,25±0,03	0,28±0,04	0,26±0,03	0,27±0,04
14 days	1,38±0,04	1,52±0,05°°	1,37±0,04**	1,54±0,05°,•
21 days	3,56±0,04	3,92±0,09°	3,60±0,08**	4,15±0,08°,•

Notes:
1. °p<0.01; °°p<0,05 – significant difference in values relative to the sample № 1;
2. *p_1<0.01; **p_1<0,05 – significant difference in values relative to the sample № 2;
3. •p_2<0.01; ••p_2<0,05 – significant difference in values relative to the sample № 3.

№ 1 – MMSC-AT, passed osteogenic differentiation; sample № 2 – MMSC-AT+ PRP (platelet-enriched blood plasma); sample № 3 – MMSC – AT + "Kolapan"; sample № 4 – "Kolapan" + MMSC-AT+ PRP. Alkaline phosphatase activity was assessing by using the Alkaline Phosphatase Detection Kit (Sigma, USA) according to the manufacturer's protocol [15]. Osteopontin gene expression was determining by immunocytochemical method [16]. To determine the mRNA used the PCR method, which is associated with reverse transcription (RT-PCR) in the area of quantification of gene expression to the marker BGP [17, 18]. For demonstrating calcium precipitates on the surface of cultured cells was performed Von Kossa analysis. Statistical processing of research results was carrying out using commonly used methods of variation statistics.

RESULTS

One of the biochemical markers of osteoblast differentiation and bone tissue formation is the activity of the alkaline phosphatase (AF) enzyme secreted by osteoblasts and take part in osteoid mineralization. Activity AF in the samples was studying on the seventh, 14th and 21 days of observations (table I).

On the 7th day of the experiment, the activity of AF in the samples studied was the same and ranged from the minimum values of 0.25 ± 0.03 mmol / min • 105 in the sample № 1 to the maximum data of 0.28 ± 0.04 mmol / min • 105 in sample № 2, p – p_2>0,05.

On the 14th day of observations, AF activity increased in all samples, but minimal values was marking in sample № 1 (1.38 ± 0.04 mmol / min • 105) and in sample № 3 (1.37 ± 0.04 mmol / min • 105, p1<0.05). At the same time, in samples № 2, p<0.05 and № 4, p, p2<0.01, p1>0.05, the activity of AF was higher in relation to the data in sample № 1 and № 3, on average by 10.14%.

On the 21st day of the experiment, the highest AF activity was determined in samples № 2 containing a combination of MMSC-AT + PRP – 3.92 ± 0.09 mmol / min • 105, p<0.01, and in sample № 4, which combined MMSK -AT + PRP + "Kolapan" – 4,15 ± 0,08 mmol / min • 105, p, p_2<0,01, p_1>0,05. At the same time, in samples № 1 and № 3 the activity of AF was significantly lower and ranged from 3.56 ± 0.04 mmol / min • 105 to 3.60 ± 0.08 mmol / min • 105, p_1<0, 05, respectively, which indicated on the less metabolic activity of cells in these samples.

To confirm the differentiation of MMSC-AT in the studied samples into osteoblasts, the expression of osteopontin genes was conducting by reverse transcriptase polymerase chain reaction (RT-PCR) in real time. To do this, we used primers for osteopontin – the main protein of bone tissue:

Table II. Expression of osteopontin mRNA in the dynamics of the cultivation of MMSC-AT on the environment with factors of cell differentiation into osteoblasts in different samples

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	0,87±0,04	1,41±0,06°	0,89±0,04*	1,40±0,06°,•
14 days	1,38±0,04	2,47±0,05°	1,52±0,03°°,*	2,50±0,06°,•
21 days	2,96±0,06	3,45±0,07°	2,73±0,05°°,*	3,49±0,07°,•

Notes:
1.°p<0,01; °°p<0,05 – significant difference in values relative to the sample № 1.
2.*p_1<0,01 – significant difference in values relative to the sample № 2.
3.•p_2<0,01 – significant difference in values relative to the sample № 3.

Table III. The number of copies of cDNA encoding BGP bone protein in osteoblasts in different samples

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	20.000±365,0	24.500±376,0°	22.500±370,0°,*	25.000±385,0°,•
14 days	40.500±560,0	55.000±575,0°	43.000±570,0°,*	60.000±720,0°,*,•
21 days	35.800±420,0	75.000±620,0°	35.000±400,0*	80.500±790,0°,*,•

Notes:
1.°p<0,01 – significant difference in values relative to the sample № 1.
2.*p_1<0,01 – significant difference in values relative to the sample № 2.
3.•p_2<0,01 – significant difference in values relative to the sample № 3.

direct -5'-AAGGCGCATTACAGCAAACACTCA-3' and reverse – 5TCATCGGACTCCTGGCTCTTCAT-3'.

It should be noting that on the 7th day of the experiment (table II) the lowest mRNA level was recorded in sample № 1 – 0.87 ± 0.04 and in sample № 3 – 0.89 ± 0.04, p1<0, 01. At the same time, in sample № 2, which contained a combination of MMSC-AT + PRP, and in sample № 4, which combined MMSC-AT + PRP + "Kolapan", the mRNA level ranged from 1.41 ± 0.06, p<0.01 to 1.40 ± 0.06, p, p2<0.01, p1>0.05, respectively, and was, on average, 62.06% higher than the data in samples № 1 and № 3.

On the 14th day of the study, an increase in the level of osteopontin expression was observing in all experimental samples; however, in the culture of MMSC-AT (sample № 1) the mRNA level was probably lower, with a value of 1.38 ± 0.04 than in the other samples. At the same time, the level of osteopontin expression exceeded the value sample № 1: by 78.98% in sample № 2, p<0.01, by 10.14% in sample № 3, p<0.05 and by 81.16% in samples № 4, p, p2<0.01, p1>0.05.

On the 21st day of observations, a further increase in mRNA levels in the studied cultures was determined. The minimum level of osteopontin expression was investigating in sample № 3, which contained MMSC-AT + "Kolapan" – 2.73 ± 0.05, p<0.05, p1<0.01. The contents of the studied parameter in the sample № 1 – 2.96 ± 0.06 was slightly higher. At the same time, in samples № 2 and № 4 the mRNA level was, on average, 11.22%, p1<0.01 and 27.11% higher, p2<0.01, than in cultures № 1 and № 3, in accordance.

Von Kossa staining (Figure 1) qualitatively confirmed the production salts of calcium and phosphates by osteoblasts, which was differentiationing from MMSC-AT.

It was found that in sample № 1, which contained MMSC-AT with osteogenic differentiation, significantly fewer cells capable of producing calcium precipitates were studied in contrast to sample № 4, which included MMSC-AT + PRP + "Kolapan", where after staining was found massive fragments of mineralized matrix.

Osteogenic differentiation of MMSC-AT into osteoblasts in the studied samples was confirming by quantitative assessment of gene expression to the BGP marker (bone gla-protein). BGP-bone glutamine protein (osteocalcin) is a small protein that is most widely present in the bone matrix, participates in calcification processes and is a marker of osteoblast activity, which is 15% of extracted non-collagenous proteins.

It was found that the expression of mRNA encoding BGP manifests itself as follows (table III): on the 7th day of observations, the minimum number of copies of the BGP gene was examined in sample № 1 – 20,000 ± 365.0 copies and slightly more in sample № 3 – 22,500 ± 370.0 copies, p, p1<0.01. At the same time, the maximum expression of mRNA encoding the BGP gene was investigated in samples № 2 and № 4, which was 1.2, p<0.01 and 1.3 times, p, p2<0.01, p1>0 , 05, higher respectively to the values of the sample № 1.

On the 14th day of research in all studied samples investigated the further increase in the expression of the studied parameter. However, in samples № 2 and № 4, the number of copies of the BGP gene exceeded the data in sample № 1 by 1.4 times, p<0.01 and 1.5 times, p_2 p0.01, respectively.

On the 21st day of observations, the expression of mRNA encoding the BGP gene decreased in samples № 1 and № 3 to 35,800 ± 420.0 copies and to 35,000 ± 400.0 copies,

p1<0.01, p>0.05. Also was observing growth of copies of the BGP gene in samples № 2 and № 4 in 2.1, p<0.01 and 2.2 times, p-p2<0.05, relative to the data in sample № 1.

DISCUSSION

The modern development of biotechnology does not allow the creation of complex organs de novo [19] and is largely limited to the stimulation of the innate abilities of the body's regeneration, which can be supplemented by the replacement of individual tissue sites and the regenerative cascade induction [20]. The existing strategy of tissue engineering usually consists in the in vivo expansion of populations of multipotent cells, such as MMSC, with their subsequent transplantation in the form of a cell suspension or scaffolds inhabited by MSCs into damaged areas [21]. Due to their unique regenerative potential and immunomodulatory properties, MMSC have great prospects in tissue engineering and reconstructive therapy, not only due to their direct participation in tissue regeneration, but also due to a modulating effect on the recipient's immunogenesis in response to the introduction of a foreign body (implant) [22].

Bone is a dynamic tissue characterized by its ability to recover from injury without scarring [23]. The differentiation of MMSC into osteoblasts plays a decisive role in bone regeneration and remodelling. MSCs obtained from bone marrow are considered to be an adequate source for tissue engineering of bones due to their ability for osteogenic differentiation [24]. MMSC can also be isolated from umbilical cord blood, placenta, adipose tissue, etc. The efficiency of osteogenic differentiation of various human MMSC has been demonstrated when populating biocompatible polymer matrices with them. At the same time, it was found that MMSC obtained from bone marrow demonstrated greater efficiency of differentiation into osteoblasts than other types of MMSC [25]. These cells are usually transplanted into three-dimensional porous scaffolds that provide the necessary extracellular environment that contains physical and chemical signals for tissue development and regeneration [26]. Despite the fact that strategies based on various types of biomaterials and stem cells have been developed over the years, modern tissue engineering has not found wide application in clinical settings [27]. Achieving this goal will require a deep understanding of the normal physiological processes of tissue development and the mechanisms underlying the interaction between MMSC and biomaterials during tissue formation, since many important details remain unclear [28]. Biomaterials play a decisive role in the creation of tissue-engineered structures [29]. The material must be able to maintain its structure and integrity for predictable periods of time to allow new tissue formation and maturation even under stress conditions [30, 31].

Thus, MMSC play an important role in bone regeneration, both by regulating the formation of osteoclasts and by negatively affecting the effectors of inflammation and osteclastogenesis [32]. MMSC have the ability to regenerate mesenchymal tissues, regulate bone metabolism, and modulate inflammation, making them attractive candidates for cell technologies in regenerative medicine. Modern strategies for the creation of tissue-engineered constructs are actively using MMSC s to improve the integration of implants and prevent immunological rejection. Preclinical studies have shown that the colonization of biocompatible materials with MMSC significantly increases osteoconductivity and improves implant integration [33, 34]. The first clinical trials of scaffolds inhabited by MMSC confirm their effectiveness [35]. This indicates that the use of tissue-engineered constructs based on biocompatible scaffolds populated with MMSC for bone regeneration is promising.

CONCLUSIONS

So, a comparative study of osteoplastic properties samples MMSC-AT showed that a larger number of cells differentiate into the osteoblasts in samples containing MMSC-AT + PRP (№ 2) and MMSC-AT + PRP + "Kolapan" (№ 4). This has been proven higher alkaline phosphatase activity, higher levels osteopontin expression, and higher levels BGP gene expression. Thus, our study proves the effectiveness of the use of samples №2 and №4 based on MMSC – AT to replace bone defects.

REFERENCES

1. Gomillion C.T., Burg K.J. Stem cells and adipose tissue engineering. Biomaterials. 2006;27(36):6052-6063.
2. Bandrivsky Y., Bandrivska O., Gnid R. et al. Indicators of markers of bone metabolism in patients with generalized periodontitis depending on blood group. Arch Balk Med Union 2019;54(1):72-77. doi:10.31688/ABMU.2019.54.1.10
3. Mazurkevych A. I., Kharkevych Yu. O., Maliuk M. O. et al. Influence of mesenchymal stem cells of bone marrow and embryonic fibroblasts of rats on the course of reparative processes in their skin. Scientific bulletin of the National University of Biological Resources and Prorodokoristuvannya of Ukraine. 2017; 151 (1): 197–205.
4. Coleman S. R., Mazzola R. F. Fat Injection: from filling to regeneration. 2009, 800 p.
5. Mazurkevych A. I., Maliuk M. O., Tkachenko S. M., Kharkevych Yu. O. Study of biocompatibility of hemostatic sponges with thebarrel cages of marrow of rabbit during cultivation of in vitro. Bulletin of the Sumy National Agrarian University. 2015;1 (34): 7 – 11.
6. Mazurkevych A. I., Maliuk M. O., Danilov V. B. Methodical recommendations "Use of mesenchymal stem cells for the correction of reparative processes in the organism of animals-recipients". K. 2014; 42: 157
7. Aggarwal S., Pittenger M.F. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815-22.
8. Augello A., Tasso R., Negrini S.M. et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2015;35(5):1482-90.
9. Mangashetti L.S., Khapli S.M., Wani M.R. IL-4 inhibits bone-resorbing activity of mature osteoclasts by affecting NF-kappa B and Ca2+ signaling. J Immunol. 2016;175(2):917-25.
10. Skrypa O. L., Bandrivsky Y.L. Determining the frequency of functional disorders of the tmj in patients with mandible fractures depending on the location. Wiad Lek. 2020:2:245 – 249.

11. Quarto R., Mastrogiacomo M., Cancedda R. et al. Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med. 2012;344(5):385-6
12. Pittenger M.F., Mackay A.M., Beck S.C. et al. Multilineage potential of adult human mesenchymal stem cells. Science. 2019;284(5411):143-7.
13. Gimble J., Guilak F. Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy. 2003;5(5):362-9.
14. . Amabile G., Meissner A. Induced pluripotent stem cells: current progress and potential for regenerative medicine. Trends Mol Med. 2014;15(2):59-68.
15. Friedenstein A.J., Petrakova K.V., Kurolesova A.I., Frolova G.P. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 2016;6(2):230-47.
16. Jaiswal R.K., Jaiswal N., Bruder S.P. et al. Adult human mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase. J Biol Chem. 2010 ; 275(13):9645-52.
17. Johnstone B., Hering T.M., Caplan A.I. et al. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res. 2011;238(1):265-72.
18. Zuk P.A., Zhu M., Ashjian P. et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2012;13(12):4279-95.
19. Senatov F.S., Zadorozhnyy M.Yu., Niaza K.V. et al. Shape memory effect in 3D-printed scaffolds for self-fitting implants. Eur. Polymer J. 2017;93(1):222-231.
20. Feinberg A.W. Engineered tissue grafts: opportunities and challenges in regenerative medicine. Wiley Interdiscip. Rev. Syst. Biol. Med. 2012;4(2):207-220.
21. Griffith L.G., Naughton G. Tissue engineering – current challenges and expanding opportunities. Science. 2002;295(5556):1009-1014.
22. Harrison R.H., St-Pierre J.P., Stevens M.M. Tissue engineering and regenerative medicine: a year in review. Tissue Eng. Part B Rev. 2014; 20(1):1-16.
23. Yagi H., Soto-Gutierrez A., Parekkadan B. et al. Mesenchymal stem cells: mechanisms of immunomodulation and homing. Cell Transplant. 2010;19(6): 667-679.
24. Lin G.L., Hankenson K.D. Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. J. Cell Biochem. 2011;112(12): 3491-3501.
25. Xue R., Qian Y., Li L. et al. Polycaprolactone nanofiber scaffold enhances the osteogenic differentiation potency of various human tissue-derived mesenchymal stem cells. Stem Cell Res. Ther. 2017;8(1): 148.
26. Dawson J.I., Oreffo R.O. Bridging the regeneration gap: stem cells, biomaterials and clinical translation in bone tissue engineering. Arch. Biochem. Biophys. 2008;473(2):124-131.
27. Hanson S., D'Souza R.N., Hematti P. Biomaterial-mesenchymal stem cell constructs for immunomodulation in composite tissue engineering. Tissue Eng. Part A. 2014;2(15): 2162-2168.
28. Discher D.E., Mooney D.J., Zandstra P.W. Growth factors, matrices, and forces combine and control stem cells. Science. 2009; 324(5935): 1673-1677.
29. Hanker J.S., Giammara B.L. Biomaterials and biomedical devices. Science. 1988;242(4880): 885-892.
30. Guarino V., Causa F., Ambrosio L. Bioactive scaffolds for bone and ligament tissue. Expert. Rev. Med. Devices. 2007;4(3): 405-418.
31. Roszer T. Understanding the Mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm. 2015; 1:1-16.
32. Chena F-M., Liu X. Advancing biomaterials of human origin for tissue engineering. Prog. Polym. Sci. 2016;53(1): 86-168.
33. Johnson V., Webb T., Norman A. et al. Activated mesenchymal stem cells interact with antibiotics and host innate immune responses to control chronic bacterial infections. Sci. Rep. 2017;7(1):9575.
34. Liu W.H., Liu J.J., Wu J. et al. Novel mechanism of inhibition of dendritic cells maturation by mesenchymal stem cells via interleukin 10 and the JAK1/STAT3 signaling pathway. PLoS ONE. 2013;38(1): e55487. doi: 10.1371/journal.pone.0055487.
35. Fisher M.B., Mauck R.L. Tissue engineering and regenerative medicine: recent innovations and the transition to translation. Tissue Eng. Part B Rev. 2013;19(1): 1-13.

The work is a fragment of scientific research work of the Department of Surgical Dentistry and Maxillofacial Surgery of the Higher State Educational Institution of Ukraine "Bukovinian State Medical University" " Multidisciplinary approach to the diagnosis, treatment and prevention of major dental diseases, with the preservation of regenerative properties of tissues and the restoration of the protective properties of anatomical structures in residents of Northern Bukovina." (state registration number 0116U002929).

ORCID and contributionship:
Andriy Bambuliak: 0000-0002-6383-9327 [B,D]
Nataliia Kuzniak: 0000-0002-4020-7597 [F]
Valentyna Honcharenko: 0000-0001-7276-9052 [C]
Marianna Ostafiychuk: 0000-0001-7820-3295 [A]
Alina Palamar: 0000-0002-8935-3552 [E]

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

CORRESPONDING AUTHOR
Andriy Bambuliak
Bukovinian State Medical University
6/74 Kopernika st., 58000 Chernivtsi, Ukraine
tel: +380979372029
e-mail: mocart_83@mail.ru

Received: 01.10.2020
Accepted: 02.08.2021

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

CONDITION OF RENAL OXYGENATION IN PRETERM INFANTS WITH HEMODINAMICALLY SIGNIFICANT PATENT DUCTUS ARTERIOSUS

DOI: 10.36740/WLek202110104

Tamara P. Borysova[1], Denis M. Surkov[2], Olha Y. Obolonska[1], Aleksey I. Obolonskiy[2]
[1] STATE INSTITUTION «DNIPROPETROVSK MEDICAL ACADEMY OF THE MINISTRY OF HEALTH OF UKRAINE» (SI «DMA»), DNIPRO, UKRAINE
[2] REGIONAL CHILDREN'S CLINICAL HOSPITAL, DNIPRO, UKRAINE

ABSTRACT

The aim: To study the condition of renal oxygenation ($RrSO_2$) and fractional tissue oxygen extraction (FTOE) in the kidneys of premature infants with HSPDA.

Materials and methods: 74 preterm newborns (gestational age 29-36 weeks) were divided into three groups: I – 40 children with HSPDA, II – 17 children with patent ductus arteriosus (PDA) without hemodynamic disorders, III – 17 children with closed ductus arteriosus. Renal oxygen saturation ($RrSO_2$) was assessed during the whole day on the first, third and tenth day of life with near-infrared spectroscopy. FTOE was calculated according to the formula: $FTOE = (SpO_2 - RrSO_2)/SpO_2$.

Results: With HSPDA on the first and third days of life, there was a significant decrease in $RrSO_2$ and a significant increase in FTOE by the kidney tissue in comparison with children with PDA without hemodynamic disorders and children with a closed ductus arteriosus. The results obtained can be explained by the "phenomenon of the systemic circulation stealing" and the development of hypoperfusion, ischemia of the kidney tissues, which leads to an increase in the need for oxygen in the parenchyma.On the tenth day of life, premature infants who had HSPDA on the first day showed an increase in $RrSO_2$ and a decrease in FTOE.

Conclusions: Non-invasive monitoring of renal oxygenation using can be used as a screening tool to identify the phenomenon of "ductal stealing" in HSPDA.

KEY WORDS: renal oxygenation, near-infrared spectroscopy, hemodinamically significant patent ductus arteriosus, premature infants

Wiad Lek. 2021;74(10 p.I):2379-2383

INTRODUCTION

Ductus arteriosus is one of the main components of the fetus circulation that connects the left pulmonary artery and descending aorta. In healthy term newborn soon after labor ductus arteriosus spasms and closes spontaneously. The rate of spontaneous closure of the ductus arteriosus in preterm infants is delayed. The ductus arteriosus remains open at the age of 4 days in about 10% of premature babies with a gestational age of 30 to 37 weeks, 80% of premature babies with a gestation of 25-28 weeks, 90% of those born at 24 weeks of gestation. By the 7th day after birth, these indexes decrease to about 2%, 65% and 87%, respectively [1].

Hemodinamically significant patent ductus arteriosus (HSPDA) – is a state in which the ductus arteriosus is not only open, but also the volume of transductal shunt is big enough to cause hemodynamic disorders [2]. The blood shunts from left to right which promotes excessive circulation in lungs and the development of "ductal stealing phenomenon" with perfusion depletion of the organs situated postductal, like kidneys and gastro-intestinal tract [2-5].

Perfusion depletion of the organs lowers its regional oxygenation. A new method of non-invasive monitoring of tissue oxygenation in vivo in real time such as Near-Infrared Spectroscopy (NIRS) may provide information about the degree of the disorder [6-9].

NIRS of kidneys is a reliable parameter for renal oxygenation ($RrSO_2$) and reflects the local oxygen saturation. Taking into account the peculiarities of this research method, the level of venous blood oxygen saturation (venous saturation) is actually determined, i.e. the amount of oxygen left in the blood after it passes through the kidneys and oxygen is extracted [10].

Using the NIRS data and the oxygen saturation of the arterial blood it is possible to calculate fractional tissue oxygen extraction (FTOE) [7]. This index reflects the balance between the delivery and consumption of oxygen in tissues and, therefore, can be used as an index of insufficient perfusion and tissue oxygenation.

THE AIM

The aim is to study the condition of regional tissue oxygenation and fractional tissue oxygen extraction in the kidneys of premature infants with hemodynamically significant patent ductus arteriosus.

MATERIALS AND METHODS

A cohort, prospective study was conducted in 2018-2019 on the basis of the Department of Anesthesiology and Intensive Care of Newborns in the Dnepropetrovsk Regional Children's Clinical Hospital and was approved by the regional commission on medical ethics.

Inclusion criteria: preterm infants at 29-36 weeks gestation with a closed ductus arteriosus, patent ductus

Table I. Clinical characteristics of the examined children

Index	I group, n=40	II group, n=17	III group, n=17	pI-II<	pI-III<	pII-III<
Gestational age, M±s (Me; Q1-Q3) in weeks	32,6±1,93 (33; 32-34)	32,8±2,28 (33; 31,5-34,5)	33,9±1,22 (34; 33-35)	ns	0,03	ns
• 35 – 36 w, n (P)	7 (17,5 %)	4 (23,5 %)	6 (35,3 %)	ns	ns	ns
• 32 – 34 w, n (P)	24 (60,0 %)	9 (52,9 %)	11 (64,7 %)	ns	ns	ns
• 29 – 31 w, n (P)	9 (22,5 %)	4 (23,5 %)	0 (0,0 %)	ns	0,04	0,04
Weight, M±s (Me; Q1-Q3) in g	2037,8±552,60 (1950; 1620-2437,5)	1856,5±424,63 (1900; 1485-2175)	2047,1±356,58 (1980; 1825-2300)	ns	ns	ns
• More than 2400 g, n (P)	10 (25,0 %)	2 (11,8 %)	3 (17,6 %)	ns	ns	ns
• 1501 – 2400 g, n (P)	23 (57,5 %)	9 (52,9 %)	13 (76,5 %)	ns	ns	ns
• ≤ 1500 g, n (P)	7 (17,5 %)	6 (35,3 %)	1 (5,9 %)	ns	ns	0,04
Boys, n (P)	28 (70,0 %)	8 (47,1 %)	7 (41,2 %)	0,05	0,05	ns
Girls, n (P)	12 (30,0 %)	9 (52,9 %)	10 (58,8 %)			
Apgar score on 1 minute, M±s (Me; Q1-Q3) in points	6,1±1,28 (7; 5-7)	5,7±1,21 (6; 5-7)	6,5±0,51 (6; 6-7)	ns	ns	ns
Apgar score on 5 minute, M±s (Me; Q1-Q3) in points	6,8±1,04 (7; 6-8)	6,5±0,87 (7; 6-7)	7,0±0,61 (7; 7-7)	ns	ns	ns
Respiratory distress syndrome	27 (67,5 %)	14 (82,4 %)	15 (88,2 %)	ns	ns	ns
Intrapartum asphyxia	7 (17,5 %)	3 (17,6 %)	0 (0,0 %)	ns	ns	ns
Intrauterine infection	6 (15,0 %)	0 (0,0 %)	2 (11,8 %)	ns	ns	ns
PDA size on day 1, M±s (Me; Q1-Q3) in mm	2,36±0,834 (2,1; 1,7-2,7)	1,11±0,154 (1; 1-1,25)	—	0,001	—	
PDA size on day 3, M±s (Me; Q1-Q3) in mm	0,50±0,816 (0; 0-1)	0,06±0,243 (0; 0-0)		0,03		

Note. Mann-Whitney U-test, the χ2 test and Fisher's exact test are used («ns» – significant difference not observed).

arteriosus (PDA), and HSPDA, signed informed parental consent to participate in the study.

Exclusion criteria: congenital malformations, intraventricular and intracranial hemorrhages of III-IV degree, sepsis, severe asphyxia, skin diseases, intrauterine growth retardation.

We examined 74 preterm infants. All children were admitted to observation in the first day of life. The examined patients were divided into three groups depending on the presence of PDA and its hemodynamic significance: I group – 40 children with HSPDA, II group – 17 children with PDA without hemodynamic disorders, III group – 17 children with a closed ductus atreriosus.

The observation period was 10 days. 8 children dropped out of the study because of the developed exclusion criteria: 4 – grade III-IV intraventricular hemorrhage, 4 – neonatal sepsis.

During the study 40 children with HSPDA were treated with ibuprofen for the PDA closure. 19 children were received ibuprofen in a high dose (20–10–10 mg/kg/day) [11] and 21 children – in a standard dose (10–5–5 mg/kg/day) [12].

Clinical-laboratory examination included gestational age, weight, physical examination, CBC, arterial oxygen saturation (SpO_2), echocardiography, regional renal oxygenation ($RrSO_2$), and fractional tissue extraction of oxygen (FTOE) in the kidneys. All children were examined on the first, third and tenth days of life.

Echocardiographic and Doppler examination with broadband microconvex probe with a frequency of 5-8 MHz («TOSHIBA» Nemso XG model SSA-580A (Japan) was made to all children at admission to the department (5-11 hour of life) and further daily to determine the PDA,

Table II. Indexes of regional renal oxygenation ($RrSO_2$), arterial blood oxygenation (SpO_2) and fractional tissue oxygen extraction in preterm infants according to study groups, M±s (Me; Q1-Q3)

Index	Day	I group, n=40 (34)	II group, n=17 (17)	III group, n=17 (15)	p_{I-II}<	p_{I-III}<	p_{II-III}<
$RrSO_2$, %	I	59,4±8,08 (56; 54-62,5)	87,0±4,74 (88; 84-90)	87,5±3,50 (88; 86,5-89,5)	0,001	0,001	ns
	III	76,2±7,56 (75; 70,25-83,5)***	83,6±5,91 (87; 77-89)	85,2±4,82 (87; 80-89)	0,001	0,001	ns
	X	92,6±3,11 (93; 91-95) *** ^^^	80,4±4,64 (83; 75-85) ** ^	80,5±3,85 (81; 77-84) ***^	0,001	0,001	ns
SpO_2, %	I	96,7±1,84 (97; 95-98)	96,8±1,39 (97; 96-98)	96,4±1,94 (96; 95,5-98)	ns	ns	ns
	III	97,4±1,55 (98; 96,3-98,8) *	95,7±1,57 (96; 94,5-96)	96,4±1,58 (97; 95-98)	0,002	0,04	ns
	X	97,7±1,51 (98; 96,8-99)	96,3±1,21 (96; 95-97)	97,0±1,13 (97; 96-98) ^	0,002	ns	ns
Fractional tissue oxygen extraction (FTOE)	I	0,385±0,0855 (0,42; 0,34-0,44)	0,101±0,0544 (0,09; 0,05-0,15)	0,093±0,0339 (0,08; 0,07-0,11)	0,001	0,001	ns
	III	0,218±0,0769 (0,23; 0,15-0,28) ***	0,126±0,071 (0,08; 0,06-0,2)	0,116±0,0552 (0,09; 0,07-0,18)	0,001	0,001	ns
	X	0,053±0,0282 (0,05; 0,03-0,07) *** ^^^	0,165±0,0524 (0,15; 0,11-0,22) ** ^	0,170±0,0359 (0,18; 0,13-0,2) *** ^	0,001	0,001	ns

Notes: The sample size for the tenth day is given in parentheses.
Mann-Whitney U-test was used for independent samples comparison («ns» – significant difference not observed).
*, **, *** – significant difference from the level on day 1;
^, ^^, ^^^ – from level on day 3, respectively $p<0,05$, $p<0,01$ and $p<0,001$ according to Wilcoxon signed rank test.

its sizes and hemodynamic significance. Echocardiographic and Doppler criteria of HSPDA are: large ductal size (>1.5 mm in newborn with weight < 1500 g, >1.4 mm/kg in newborn with weight ≥ 1500 g), demonstration of left to right shunt, growing or pulsatile shunt pattern, increased left atrial to aortic root ratio (LA:Ao>1.4), retrograde descending aortic flow [13, 14].

Regional renal oxygen saturation was assessed with NIRS device «Somanetics INVOS 5100 C "(USA). Neonatal transducers were placed over the region of the right kidney with preliminary ultrasound imaging. Daily monitoring of $RrSO_2$ was performed on the first (5–11 hour of life), third and tenth day after birth. The daily variability of $RrSO_2$ parameters was less than 9%. The average daily $RrSO_2$ value was calculated for each child, as well as for a group of patients. Normal indexes of $RrSO_2$ were considered in the first day of life – 88-95%, on the third day – 85-90%, tenth day – 75-80% [10].

Arterial blood oxygenation (SpO_2) was determined with patients monitor Datascope Passport–2 (USA).

Fractional tissue oxygen extraction was calculated according to the formula: $FTOE = (SpO_2 – RrSO_2)/ SpO_2$ [7].

To solve the above-mentioned tasks and test the initial assumptions, a set of statistical research methods was used, namely: the Mann-Whitney U-test, as a nonparametric analogue of the one-way analysis of variance method for independent samples, the Wilcoxon signed rank test for related samples, the $\chi 2$ test and Fisher's exact test for contingency tables. The normal distribution of quantitative samples was checked using the Kolmogorov-Smirnov test. Data analysis was performed using the statistical software package IBM SPSS Statistics 23.

RESULTS

The clinical characteristics of the examined children are presented in Table I. Distribution by sex: boys – 43 (58.1%), girls – 31 (41.9%). It is interesting to note the significant predominance of boys in the group with HSPDA. The average gestational age was 32.9 ± 0.22 weeks. The largest number of children had a gestational age of 32-34 weeks. The number of premature babies with a gestational age of 29–31 weeks was the same in the group with PDA and HSPDA. There were no children with such gestational age in the third group, which can be explained by the fact that with a gestational age of 29-31 weeks almost all premature babies have PDA. The average body weight at birth was 1998.2 ± 56.55 g, there was no significant difference between the groups. More than half of the children examined had low body weight. Very low body weight (≤ 1500 grams) was observed in almost every fifth infant, and much more often in samples with PDA. There were no differences in Apgar scores at the first and fifth minutes between the study groups. Respiratory distress syndrome was observed in 75.7%, newborn asphyxia – in 13.5%, intrauterine infection – in 10.8% of children. The

incidence of these diseases between the study groups did not differ significantly.

The size of PDA on the first day in children of the first group (Table I), on average, exceeded that in the second group by more than two times (p<0.001). On the third day of life, the size of the PDA significantly decreased in both groups. At the same time, the size of the PDA in the first group was still significantly higher than that in the second group (p<0.03). On the tenth day of life, ductus arteriosus was closed in all patients.

The dynamics of indexes of arterial blood oxygen saturation, regional renal oxygenation, fractional tissue extraction of oxygen in the kidneys is presented in Table II.

The average $RrSO_2$ index on the first day in all examined patients was 72.2 ± 1.79%. Regional renal oxygenation in the first day of life varied significantly depending on the presence of HSPDA: because of hemodynamic disorders, the $RrSO_2$ level was significantly lower than the index of not only the third, but also the second group (p<0.001). Regional renal oxygenation on the first day in children without HSPDA generally corresponded to normal values (88-95% according to McNeill S et al. [10]) and did not differ significantly between the second and third groups.

On the third day of life in the first group, the ductus arteriosus was already closed in 67.5% of children and a significant dynamics of $RrSO_2$ (p<0.001) was observed – the average index increased to 76.2 ± 7.56%, but still remained below the level of the second and the third group (p<0.001).

On the tenth day of life in the group I, a significant increase in $RrSO_2$ continued, on average to 92.6 ± 3.11% (p<0.001). The following paradox attracted attention – $RrSO_2$ on the tenth day of life in children of the first group was significantly higher than that in the second and third groups. At the same time, significant negative dynamics of $RrSO_2$ was noted in the second and third groups.

Oxygen saturation (SpO_2) throughout the observation period in all groups was maintained within the normal range (Table 2). In addition, on the third day the SpO_2 index in the first group was even significantly higher than in the second group (97.4 ± 1.55% versus 95.7 ± 1.57%; p <0.002), while the $RrSO_2$ index was significantly lower (76.2 ± 7.56 versus 83.6 ± 5.91%, p<0.001).

Fractional tissue oxygen extraction (FTOE) by kidney tissue on the first day of life had a significantly higher level in children with HSDPA than in children of the second and third groups (0.385 ± 0.0855 versus 0.101 ± 0.0544, p <0.001 and 0.093 ± 0.0339, p <0.001). This trend persisted on the third day of life: FTOE in the group of children with HSPDA significantly decreased to 0.218 ± 0.0769 (p<0.001) and remained significantly higher than in the second and third groups. On the tenth day, the FTOE in the first group decreased to 0.053 ± 0.0282 (p<0.001) and a reverse trend was observed in relation to the indexes of the second and third groups, namely: this index became significantly lower than in the second and third groups.

DISCUSSION

Hemodinamically significant patent ductus arteriosus leads to the development of "ductal stealing phenomenon" with perfusion depletion of the organs situated postductal, like kidneys [2, 3, 4, 5]. Kidneys perfusion depletion lowers its regional oxygenation which could be clarified by a method of non-invasive monitoring of tissue oxygenation in vivo in real time – Near-Infrared Spectroscopy (NIRS) [6-9]. A few studies have shown a decrease in renal oxygenation in premature infants with congenital heart defects and having a left-right shunt [14, 15].

Kidney is an organ with a large venous blood flow and, therefore, high regional saturation values and low oxygen extraction [16, 17].

The results of this study showed that in premature infants with HSPDA, on the first day of life, a reduced level of renal oxygenation and an increase in fractional oxygen extraction by the kidney tissue were observed in comparison with premature infants with PDA without hemodynamic disorders or with a closed duct. On the third day of life in children with HSPDA, because of the closure of the ductus arteriosus, a significant, but not sufficient improvement in renal oxygenation was observed.

The decrease in the indices of tissue oxygenation of the kidneys in premature infants with HSPDA in the first day of life can be explained by the "phenomenon of systemic circulation stealing" and the development of hypoperfusion, ischemia of the kidney tissue. This leads to an increased demand for oxygen in the renal parenchyma [18], which was reflected in our results – low renal oxygenation and high fractional tissue oxygen extraction in the kidneys.

On the tenth day of life in premature infants who had HSPDA on the first day, the opposite pattern was observed. The $RrSO_2$ index increased significantly, and the fractional tissue extraction of oxygen decreased in comparison with the data on the first and third days. The results obtained can be explained by the mechanism that prevents the development of the kidney tissue hyperoxia and their subsequent damage. The presence of arterio-venous shunts in the kidney provides a mechanism by which oxygen, which is excessive for the metabolic needs of the kidneys, can bypass the renal microcirculation [19]. This was reflected in the high level of $RrSO_2$, which actually reflects the degree of venous saturation. Low fractional oxygen extraction indicates a decrease in oxygen consumption of the damaged kidney parenchyma as a result of hypoxia.

CONCLUSIONS

The presence of HSPDA in premature infants leads to a decrease in renal oxygenation due to renal hypoperfusion. A low $RrSO_2$ index and a high level of fractional oxygen extraction by the kidneys in the first day of life are a marker of HSPDA. On the tenth day of life, premature infants who had HSPDA on the first day showed an increase in $RrSO_2$ and a decrease in fractional tissue extraction of oxygen. Therefore, non-invasive monitoring of renal oxygenation using NIRS can be used as a screening tool to identify and assess the effect of "ductal stealing" on kidney oxygenation in HSPDA.

REFERENCES

1. Benitz W.E. Committee on Fetus and Newborn, American Academy of Pediatrics. Patent Ductus Arteriosus in Preterm Infants. Pediatrics. 2016;137(1):e20153730. doi:10.1542/peds.2015-3730.
2. Rios D.R., Bhattacharya S., Levy P.T., McNamara P.J. Circulatory Insufficiency and Hypotension Related to the Ductus Arteriosus in Neonates. Front Pediatr. 2018;6:62. doi:10.3389/fped.2018.00062.
3. Clyman R.I., Couto J., Murphy G.M. Patent ductus arteriosus: are current neonatal treatment options better or worse than no treatment at all? Semin Perinatol. 2012;36(2):123-129. doi:10.1053/j.semperi.2011.09.022.
4. El Hajjar M., Vaksmann G., Rakza T. et al. Severity of the ductal shunt: a comparison of different markers. Arch Dis Child Fetal Neonatal Ed. 2005;90(5):F419-422. doi: 10.1136/adc.2003.027698.
5. Slaughter J.L., Cua C.L., Notestine J.L. et al. Early prediction of spontaneous Patent Ductus Arteriosus (PDA) closure and PDA-associated outcomes: a prospective cohort investigation. BMC Pediatr. 2019;19(1):333. doi: 10.1186/s12887-019-1708-z.
6. Dempsey E.M., El-Khuffash A.F. Objective cardiovascular assessment in the neonatal intensive care unit. Arch Dis Child Fetal Neonatal Ed. 2018;103(1):F72-F77. doi:10.1136/archdischild-2017-313837.
7. Sood B.G., McLaughlin K., Cortez J. Near-infrared spectroscopy: applications in neonates. Semin Fetal Neonatal Med. 2015;20(3):164-172. doi: 10.1016/j.siny.2015.03.008.
8. Hazle M.A., Gajarski R.J., Aiyagari R. et al. Urinary biomarkers and renal near-infrared spectroscopy predict intensive care unit outcomes after cardiac surgery in infants younger than 6 months of age. J Thorac Cardiovasc Surg. 2013;146(4):861-867.e1. doi:10.1016/j.jtcvs.2012.12.012.
9. Ruf B., Bonelli V., Balling G. et al. Intraoperative renal near-infrared spectroscopy indicates developing acute kidney injury in infants undergoing cardiac surgery with cardiopulmonary bypass: a case-control study. Crit Care. 2015;19(1):27. doi:10.1186/s13054-015-0760-9.
10. McNeill S., Gatenby J.C., McElroy S., Engelhardt B. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. J Perinatol. 2011;31:51-57. doi:10.1038/jp.2010.71.
11. Dani C., Vangi V., Bertini G. et al. High-dose ibuprofen for patent ductus arteriosus in extremely preterm infants: a randomized controlled study. Clin Pharmacol Ther. 2012;91(4):590-596. doi: 10.1038/clpt.2011.284.
12. Hirt D., Van Overmeire B., Treluyer J.M. et al. An optimized ibuprofen dosing scheme for preterm neonates with patent ductus arteriosus, based on a population pharmacokinetic and pharmacodynamic study. Br J Clin Pharmacol. 2008;65(5):629-636. doi: 10.1111/j.1365-2125.2008.03118.x.
13. Shepherd J.L., Noori S. What is a hemodynamically significant PDA in preterm infants? Congenit Heart Dis. 2019;14(1):21-26. doi: 10.1111/chd.12727.
14. Altit G., Bhombal S., Tacy T.A., Chock V.Y. End-organ saturation differences in early neonatal transition for left- versus right-sided congenital heart disease. Neonatology. 2018;114:53-61. doi: 10.1159/000487472.
15. Harer M.W., Chock V.Y. Renal Tissue Oxygenation Monitoring – An Opportunity to Improve Kidney Outcomes in the Vulnerable Neonatal Population. Front Pediatr. 2020;14(8):241. doi: 10.3389/fped.2020.00241.
16. Cerbo R.M., Cabano R., Di Comite A. et al. Cerebral and somatic rSO2 in sick preterm infants. J Matern Fetal Neonatal Med. 2012;25(4):97-100. doi: 10.3109/14767058.2012.715030.
17. Cerbo R.M., Maragliano R., Pozzi M. et al. Global perfusion assessment and tissue oxygen saturation in preterm infants: where are we? Early Hum Dev. 2013;89(1):S44-46. doi: 10.1016/S0378-3782(13)70014-8.
18. Redfors B., Bragadottir G., Sellgren J. et al. Acute renal failure is NOT an «acute renal success»--a clinical study on the renal oxygen supply/demand relationship in acute kidney injury. Crit Care Med. 2010;38(8):1695-1701. doi: 10.1097/CCM.0b013e3181e61911.
19. Evans R.G., Gardiner B.S., Smith D.W., O'Connor P.M. Intrarenal oxygenation: unique challenges and the biophysical basis of homeostasis. Am J Physiol Renal Physiol. 2008;295(5):F1259-1270. doi: 10.1152/ajprenal.90230.2008.

ORCID and contributionship:

Tamara P. Borysova: 0000-0001-8347-4348 [A,D,F]
Denis M. Surkov: 0000-0002-6456-8493 [A,B,E]
Olha Y. Obolonska: 0000-0001-9863-1828 [A-F]
Aleksey I. Obolonskiy: 0000-0002-3190-0402 [B]

Conflict of interest:

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olha Y. Obolonska
State Institution «Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine
9 Vernadsky st., 49044, Dnipro, Ukraine
tel: +380981227449
e-mail: o_obolonskaja@ukr.net

Received: 23.10.2020
Accepted: 02.08.2021

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

LEVELS OF OSTEOCALCIN AND PROCOLLAGEN I N-TERMINAL PROPEPTIDE (PINP) IN MEN SUFFERING FROM ANKYLOSING SPONDYLITIS

DOI: 10.36740/WLek202110105

Oksana Pavliuk[1], Sergii Shevchuk[1,2]

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

[2]SCIENTIFIC RESEARCH INSTITUTE FOR REHABILITATION OF DISABLED PERSONS OF THE NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

The aim: To evaluate osteocalcin and PINP levels in men suffering from AS and to compare them with structural and functional state of bone tissue and clinical course of illness.

Materials and methods: The study included 82 patients suffering from AS with an average age of 40,9±0,9 years. Osteocalcin level was determined in 82 patients, and PINP level was determined in 79 patients. Control group included 22 apparently healthy persons. Disease activity was assessed through CRP level, ASDAS and BASDAI scores, while functional ability was assessed through the BASFI score. Osteocalcin and PINP levels were determined by immunoenzymatic method for the purpose of evaluating the metabolic state of bone tissue.

Results: Average osteocalcin and PINP levels were not significantly different in patients suffering from AS and patients in the control group and did not show any significant correlation with ASDAS, BASDAI, BASFI and CRP scores. In patients with spinal ankylosis, average osteocalcin values (14,3 ng\ml) and PINP (747,2 pg\ml) were higher compared to patients with single syndesmophytes (11,0 ng\ml; 711,8 pg\ml) and patients without syndesmophytes (10,4 ng\ml; 537,7 pg\ml respectively).

Conclusions: Osteocalcin and PINP levels are not related to age, disease duration, BMI, glucocorticoids load and inflammatory process activity, however, they are closely related to the presence of bone growths.

KEY WORDS: ankylosing spondylitis, bone density, osteoporosis, osteocalcin

Wiad Lek. 2021;74(10 p.I):2384-2391

INTRODUCTION

It is known that the reduction of bone mineral density in the form of osteoporosis/osteopenia is a widely known complication of ankylosing spondylitis (AS) and is frequently observed at early stages of the disease [1]. Loss of bone mass in this cohort of patients significantly increases the risk of compression fractures of vertebrae [2-3]. Over the period of 30 years after establishing the diagnosis of AS this risk makes up 14%-30% which is reliably higher than in practically healthy persons [4-5]. Pathogenesis of the reduction of bone mineral density actively correlates with activity of inflammatory process and, as a result, with intensification of resorption processes in bone tissue (N-telopeptide, C-telopeptide) and the reduction of bone formation markers (osteocalcin, PINP). Remodelling of bone tissue in case of AS is particularly characterized by the fact that in different regions of the skeleton the processes of formation and resorption of bone tissue take place in parallel. Thus, syndesmophytes are formed as a result of local inflammation with increased synthesis of bone matrix by osteoblasts in the points of tendon attachment and the presence of chronic inflammation which is supported by excessive amount of proinflammatory (TNF-alpha, IL-6, IL-7 etc.) cytokines which activate osteoclastogenesis and bone tissue resorption [6]. For this reason, the determination of bone metabolism markers would give us an opportunity to evaluate bone metabolism state in patients suffering from AS. Literary data on this matter are contradictory. A range of studies conducted on patients suffering from AS show an increase in the osteocalcin level in blood serum [7-8], according to the data of other studies, osteoporosis develops on the background of normal osteocalcin level [9], however, some studies show low concentration of the markers of bone tissue biosynthesis [10-11].

As for the Ukrainian cohort of patients suffering from AS, no studies were conducted on this matter. The role of disease course in the formation of bone metabolism disorders is also unknown.

THE AIM

The aim of our study was to examine metabolic state of the bone, in the context of bone formation markers (osteocalcin, PINP), evaluate their connection to the structural and functional state of the bone according to the data of X-ray densitometry and compare the identified disorders with disease course in men suffering from AS.

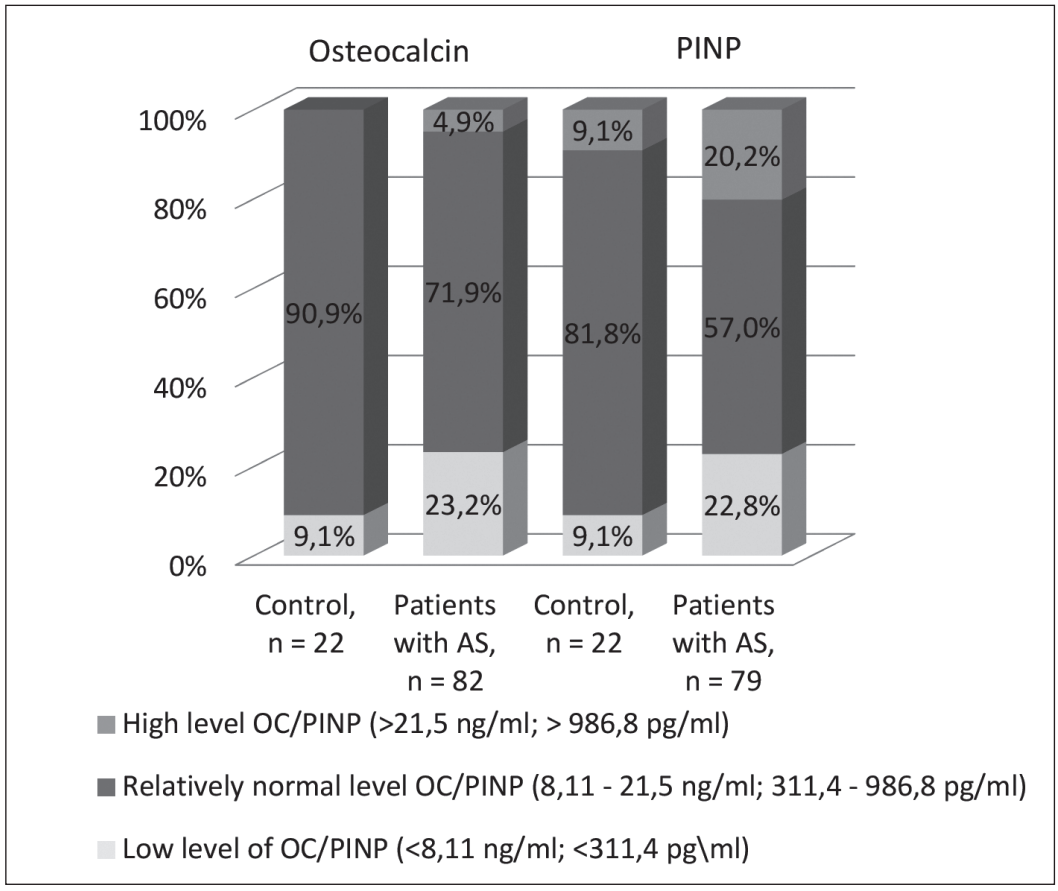


Fig 1. Levels of osteocalcin and PINP in blood serum in men suffering from AS

MATERIALS AND METHODS

The study included 82 patients suffering from AS (100% of them were men), their average age was 40,9±0,9 years, disease duration made 9,02±0,6 years. Osteocalcin level was determined in 82 patients, and PINP level was determined in 79 patients. Control group included 22 practically healthy persons without any rheumatic pathology. As a result of the expert examination conducted by the bioethics committee of Vinnytsia National Medical University it has been established that research methods do not contradict the basic norms of bioethics under the Declaration of Helsinki and do not violate any human rights under current laws of Ukraine. The diagnosis of AS was established on the basis of ASAS criteria [12]. All the patients were assessed on the basis of their age, disease duration, intake of glucocorticoids (GC), calcium preparations and vitamin D. For each patient, cumulative dose and duration of treatment with GC was determined.

Disease activity was evaluated on the basis of BASDAI score and ASDAS-CRP score (<1,3 – inactive ankylosing spondylitis; 1,3-2,1 – moderate activity; 2,1 -3,5 – high activity; >3,5 – very high activity) and functional activity was evaluated using the Bath Ankylosing Spondyloarthritis Functional Index (BASFI). The level of markers of inflammatory process activity, erythrocyte sedimentation rate and C-reactive protein (CRP) were analysed using standard laboratory methods at a medical institution. Markers of bone remodelling (osteocalcin and PINP) were evaluated by an immunoenzymatic method using the sets «N-MID Osteocalcin ELISA Kit» (Immunodiagnostic Systems, Great Britain) and «Human PINP (Procollagen I N-Terminal Propeptide) ELISA Kit» (Ela science, USA).

Bone mineral density (BMD) was measured using dual-energy X-ray absorptiometry on the device «Hologic Discovery Wi» (S/N 87227) at the level of the lumber spine and the femoral neck. For patients aged 50 years old or older, the following terms of the World Health Organization (WHO) were used with respect to osteopenia and osteoporosis: osteopenia, T-score <-1 up to> -2,5 and osteoporosis, T-score ≤ -2,5 SD. For patients aged up to 50 years Z-score ≤ -2,0 SD was considered to be lower than expected for this age [13].

Statistical analysis of the results was performed using personal computer applications Microsoft office, in particular, Microsoft Excel 2010 and using the program "SPSS-10.0.5 for Window" (licensed № 305147890). The following statistical characteristics were used: the number of observations (n), the arithmetic mean (M), standard error of the mean (m), median range (maximum-minimum), relative values (abs.,%). The normality of the distribution of indicators was determined by the Shapiro-Wilk test. In our studies, there was a normal distribution of indicators, so the significance of the differences was determined by Student's t-test, to determine the relationships between indicators – Pearson's correlation analysis (r). P-value ≤ 0,05 was considered significant statistically. To compare the

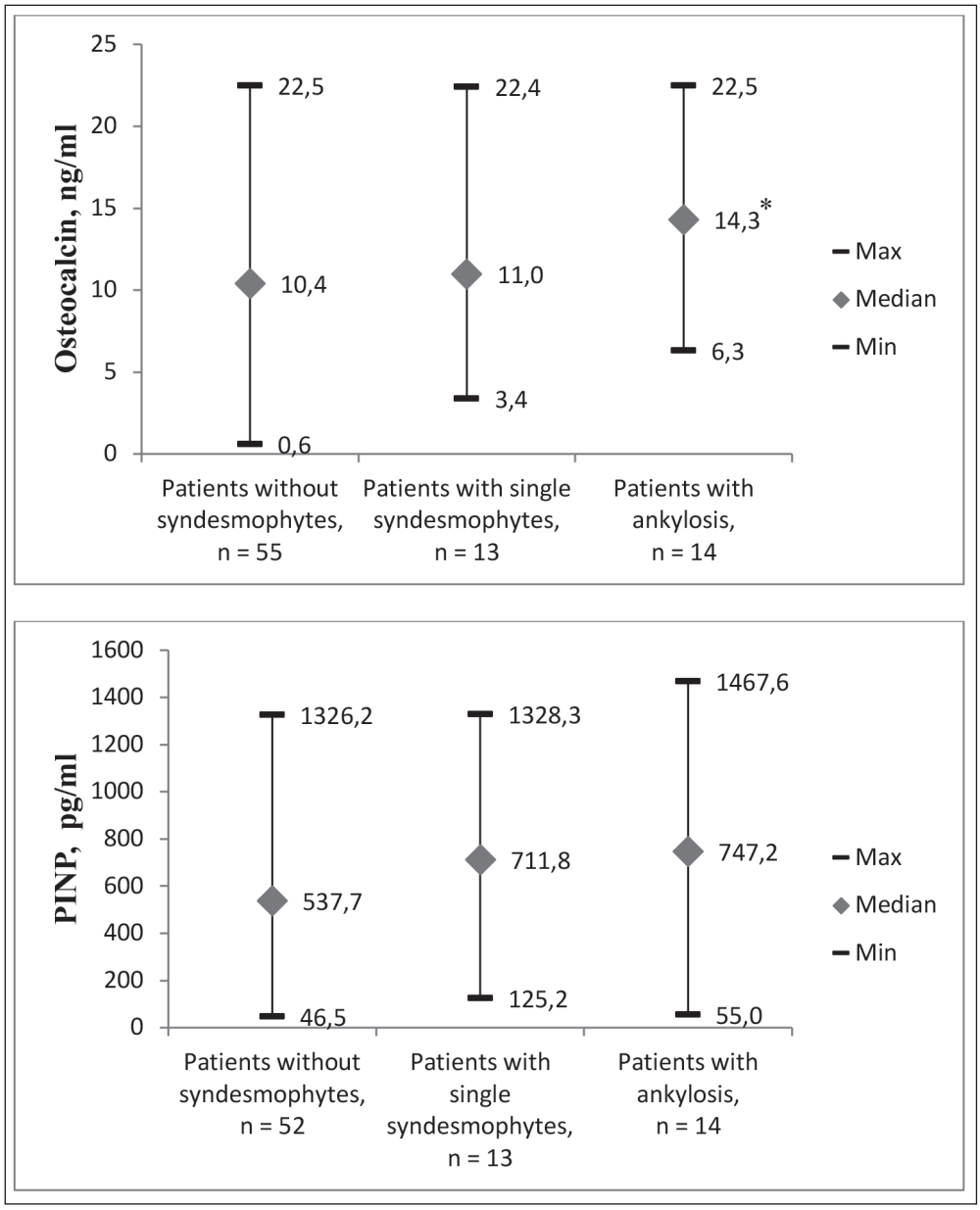


Fig 2. Osteocalcin and PINP levels depending on the presence of syndesmophytes
Note: symbol * – means reliable differences when compared to a group of patients without syndesmophytes,
t-test was used for intergroup comparison.

significance of the differences between the relative values used the exact Fisher method. The percentile method was used to establish standards for laboratory test results, such as the level of osteocalcin, the N-terminal propeptide of type I procollagen.

RESULTS

The conducted study has shown that the content of bone metabolism markers, i.e. osteocalcin (OC) and procollagen I N-terminal propeptide (PINP), was not significantly different in men suffering from AS and in persons from the control group. In the control group, the level of osteocalcin was 12,9±0,9 ng/ml, while in men with AS was 12% lower and made 11,3±0,6 ng/ml. As for PINP level, in the control group they made 622,6±46,9 pg\ml and in the main group they made 606,7±42,1 pg\ml, i.e. were 2,5% lower. Ranging the levels of bone formation markers showed (Figure 1) that low levels of osteocalcin and procollagen type 1 N-terminal propeptide were found in 9,1% of persons in the control group, while 81,8-90,9% persons had a relatively normal level of these markers. Low levels of bone metabolism markers were found in 23% of patients suffering from AS and relatively normal levels were found in 57-71,9% of patients. The study showed that from 4 to 20% of patients suffering from AS had relatively high levels of osteocalcin and procollagen type 1 N-terminal propeptide, while the control group had no patients with such levels of OC and only 9,1% of patients with such level of PINP.

The study did not find a connection between the age and changes in levels of markers of bone tissue synthesis (Table I). Thus, the highest share of patients with low level of osteocalcin was found in the age group of 35-50 years and included 16 patients (30,8%), in the group aged up to 35 years, low level of OC was registered in 31,6% of patients and in the group of patients older than 50 years it was registered in 27,3 % of patients. Similar results were obtained after analysing PINP levels,

Table I. Levels of osteocalcin and PINP depending on the age of patients, disease duration, BMI and glucocorticoids load

Group	Bone metabolism markers			
	Osteocalcin, n (%)		PINP, n (%)	
	M±m	Low level (<8,11 ng\ml)	M±m	Low level (<311,4 pg\ml)
Control, n=22	12,9±0,9	2 (9,1%)	622,6±46,9	2 (9,1%)
Patients with AS, n=82/79	11,3±0,6	19 (23,2%)	606,7±42,1	18 (22,8%)
Depending on the age				
<35 years n=19/19	11,0±1,0	6 (31,6%)	520,5±75,8	6 (31,6%)
35-50 years n=52/49	11,2±0,8	16 (30,8%)	588,9±52,7	14 (28,6%)
>50 years n=11/11	11,5±1,4	3 (27,3%)	811,6±132,6	2 (18,2%)
r	0,07		0,23	
Depending on disease duration				
up to 5 years n=13/13	12,6±1,0	1 (7,7%)	653,8±104,6	2 (15,4%)
5-10 years n=42/41	10,5±0,9	15 (35,7%)	558,9±56,5	13 (31,7%)
> 10 years n=27/25	11,5±1,1	9 (33,3%)*	650,4±81,0	7 (28%)
r	0,14		0,11	
Depending on BMI				
BMI<25 n=45/44	10,8±0,9	15 (33,3%)	545,9±58,0	17 (38,6%)
BMI > 25 n=37/35	11,7±0,8	10 (27,02%)	675,8±60,5	5 (14,3%)#
r	0,19		0,19	
Depending on glucocorticoids load				
Cumulative dose of GC < 21,6 g (n=55/53)	11,1±0,7	17 (30,9%)	611,3±50,5	12 (22,6%)
Cumulative dose of GC > 21,6 g (n=27/26)	11,4±1,2	8 (30,7%)	587,5±78,6	10 (38,4%)

Note: symbol * means reliable difference from the group of patients with disease duration of up to 5 years;
symbol # means reliable difference from the group of patients with BMI < 25.
n - the number of observations, M - the arithmetic mean, m - standard error of the mean (m), M ± m means - mean ± standard error of the mean (SEM), r- Pearson's correlation analysis. To compare the significance of the differences between the relative values used the exact Fisher method.

as the largest share of patients (28,6%) with low level was also found in the age group of 35-50 years. Disease duration also had no influence on OC and PINP levels in blood serum. In the group of patients with disease duration of 5-10 years and more than 10 years, low levels of osteocalcin and PINP were found practically in each second patient.

The study did not show any significant connection between bone metabolism markers and BMI, however, there was a distinct tendency towards a decrease of OC and PINP levels in proportion to the reduction of body mass. Levels of osteocalcin and PINP also had no relation to glucocorticoids (GC) load. Specifically, practically similar share of patients with low level of OC was registered in patients with cumulative dose of GC > 21,6 g when compared to patients with GC dose < 21,6 g. It should be also noted that in patients with cumulative dose of GC > 21,6 g, the average content of PINP as well as the share of patients with low levels of the studied index were not also reliably different.

Levels of markers of bone tissue synthesis grew in parallel to the increase in the activity of inflammatory process (Table II). Specifically, in patients with very high activity of inflammatory

Table II. Connection of OC and PINP with activity indices based on ASDAS-CRP and BASDAI scores, BASFI functional index and CRP

	Level	Bone metabolism marker			
		Osteocalcin, n (%)		PINP, n (%)	
		M±m	Low level (<8,11 ng/ml)	M±m	Low level (<311,4 pg\ml)
ASDAS	<3,5 (n=42/41)	10,3±0,9	15 (36,5%)	583,9±59,4	13 (31,7%)
	> 3,5 (n=40/38)	12,1±0,7	10 (26,3%)	624,5±61,1	9 (23,7%)
	r	-0,15		-0,19	
BASDAI	<4 (n=24/23)	10,9±1,2	8 (33,3%)	596,9±78,0	7 (30,4%)
	> 4 (n=58/56)	11,3±0,7	17 (30,3%)	597,9±50,7	15 (26,8%)
	r	-0,12		-0,10	
BASFI	< 4 (n=23/22)	11,5±1,2	7 (30,4%)	688,1±72,5	5 (22,7%)
	> 4 (n=59/57)	11,1±0,7	18 (30,5%)	570,8±51,3	17 (29,8%)
	r	-0,18		-0,08	
CRP	< 5,4 (n=20/19)	10,3±1,4	8 (40,0%)	571,4±98,3	6 (31,6%)
	5,4-13,4 (n=41/40)	11,4±0,8	12 (29,3%)	602,1±59,3	10 (25,0%)
	> 13,4 (n=21/20)	11,5±1,1	5 (23,8%)	627,5±76,3	6 (30,0%)

n - the number of observations, M ± m means mean ± standard error of the mean (SEM), r- Pearson's correlation analysis.

process (ASDAS >3,5), a share of patients with low level of osteocalcin and PINP was 8-10% lower than in the group of patients with moderate activity of inflammatory process (ASDAS < 3,5). Similar trends were observed also with respect to BASDAI score. Average levels of OC and PINP in the group of patients with high activity (BASDAI > 4) were practically comparable to patients with low activity (BASDAI < 4). A share of patients with low levels of bone tissue synthesis was not also reliably different in groups depending on the functional ability determined on the basis of BASFI score. While performing correlation analysis, no reliable correlations were found between high activity of inflammatory process (based on ASDAS-CRP and BASDAI scores) and low functional ability (BASFI score) with metabolic state of bone tissue determined on the basis of OC and PINP levels.

Ranging CRP levels as optimal, high and very high it was shown that in the group of patients with optimal level of CRP a share of patients with low levels of markers of bone tissue synthesis probably no different than in the groups of patients with high and very high CRP. Based on average values of OC and PINP, there were also significant differences in groups of patients. In groups with very high levels of CRP, concentration of OC and PINP was practically 9-11% higher than in the group with optimal level of CRP.

The study showed that serum levels of OC and PINP in patients suffering from AS were associated with presence of osteophytes (Figure 2). Thus, average level of OC in patients with syndesmophytes made 11,0±1,4 ng/ml and in patients without syndesmophytes it made 10,4±0,7 ng/ml, i.e. it was 5,5% lower. Such trend was also observed with respect to PINP level where in the group of patients without syndesmophytes, low level of PINP was found in 17 patients and in the group with syndesmophytes it was found only in 3 patients (23,1%), while the average level of PINP was 24,5% higher. In patients with compete spinal ankylosis, average levels of the mentioned markers of bone tissue synthesis were even higher, and with respect of OC level in blood serum, in general, the values were reliable when compared to patients without syndesmophytes.

In the next part of the study, we tried to analyse the differences between patients depending on X-ray changes (ankylosis, syndesmophytes) in the spine (Table III). It was found that in patients with complete spinal ankylosis, besides reliably higher levels of OC and PINP, the average age of the patient was also reliably higher when compared to patients without syndesmophytes. Patients with spinal ankylosis when compared to patients without spinal ankylosis showed a tendency towards an increase in disease duration as well as activity of inflammatory process and cumulative GC dose was higher. It was established that patients with complete ankylosis had a higher BMD index of the lower spine when compared to patients without ankylosis and, on the contrary, they had a lower BMD index of the femoral neck, which can be obviously connected to the place (trabecular or cortical part of the vertebral bodies) of BMD

Table III. Peculiarities of AS disease course in patients without syndesmophytes, with single syndesmophytes and spinal ankylosis

Indexes	Patients without syndesmophytes, n= 55	Patients with single syndesmophytes, n = 13	Patients with spinal ankylosis, n=14
OC levels, ng/ml	10,4±0,7	11,0±1,4	14,3±1,5*
PINP levels, pg/ml	537,7±48,5	711,8±116,9	747,2±105,2
Age, years	38,6±1,2	48,3±1,3*	43,5±1,5*#
Disease duration	7,7±0,6	9,7±1,3	9,7±1,3
Cumulative GC dose,g	14,5±1,8	16,7±2,7	16,0±2,9
ASDAS, points	3,5±0,1	3,4±0,1	3,5±0,2
BASDAI, points	5,2±0,2	5,3±0,4	5,6±0,4
BASFI, points	4,9±0,3	5,6±0,5	5,2±0,4
CRP levels, mg/l	10,7±0,9	11,5±1,8	14,7±2,8
ESR levels, mm/h	23,1±1,4	23,1±4,6	26,2±3,4
BMD of the lumbar spine, g/cm^2	0,93±0,02	0,95±0,04	0,98±0,04
Z- score of the lumbar spine, SD	-1,38±0,2	-0,68±0,4	-0,36±0,6
BMD of the femoral neck, g/cm^2	0,75±0,02	0,73±0,03	0,73±0,04
Z-score of the femoral neck, SD	-0,87±0,01	-1,07±0,2	-1,14±0,1

Note: symbol * means reliable differences when compared to the group of patients without syndesmophytes,
symbol # means reliable differences when compared to the group of patients with single syndesmophytes.

determination. As for Z-score, in the region of the femoral neck it was reliably higher in patients with complete spinal ankylosis when compared to patients without syndesmophytes.

DISCUSSION

Thus, by analysing metabolic state of bone tissue (levels of bone synthesis markers), it was established that an insignificant reduction of osteocalcin and PINP levels were observed in men suffering from ankylosing spondylitis when compared to patients from the control group. And specifically, average concentration of osteocalcin in patients suffering from AS was 12% lower than in practically healthy persons and made 11,3±0,6 ng/ml in comparison to 12,9±0,9 ng/ml. Average values of PINP in the main group made up 606,7±42,1 pg/ml and 622,6±46,9 pg/ml in the control group which was only 2,5% more. Literary data on this matter are contradictory. Thus, according to the data of Ö. Altindag et al [14], average level of OC in patients suffering from AS was 43% lower than in the control group. Osteocalcin concentration of blood serum was lower than in the study by Huang WN et al [15]. However, there also exist such studies, where OC levels in patients suffering from AS in comparison to patients from the control group were not different or were even 57% higher [16]. The opinion of researchers on the bone formation marker PINP is contradictory. Thus, in the study by Perpétuo IP et al [17], serum level of P1NP was lower in patients suffering from AS when compared to the control group, and, on the contrary, in the study by Pnar Borman et al [18], PINP level was significantly higher in patients suffering from AS and grew in proportion to the disease activity.

The study did not show any influence of age and disease duration on the concentration of bone metabolism markers in blood serum. However, the study by Huang J et al [9] found a correlation between osteocalcin and disease duration in patients suffering from AS (r = 0,323, p = 0,034).

Levels of the mentioned bone formation markers also had no relation to BMI and GC load. According to the data of Yushina SA et al [19], patients who systematically took GC showed stable reduction of osteocalcin level.

It is known that one of the unfavourable pathogenic factors of bone metabolism is a systemic inflammatory process related to excessive activation of proinflammatory factors, such as tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6 and IL-17 which lead to excessive destruction of bone tissue, primarily, due to hyperactivation of osteoclasts and decreased synthesis of osteoblasts [20]. Ankylosing spondylitis is uniquely characterized by the fact that due to multiple inter-relations and interactions of bone tissue and the immune system, active inflammation, on the one hand, contributes to bone resorption by way of intensified differentiation of osteoclasts and, on the other hand, leads to local bone formation, primarily, through TNF-alpha and IL-17 hyperproduction which cause excessive activation of osteoblasts and synthesis of pathologically new bone formations and in the future lead to the formation of syndesmophytes and spinal ankylosis [21-22].

We did not find any reliable associative connection between the activity of inflammatory process evaluated on the basis of ASDAS-CRP, BASDAI scores and the concentration of bone metabolism markers. Thus, in case of very high activity of the disease and low functional ability (ASDAS >3,5; BASDAI >4; BASFI>4), levels of osteocalcin

and PINP in blood serum were practically comparable to moderate activity and functional ability of the patient. Bone formation markers also had no relationship to the CRP level. Similar results were obtained in the study by Park MC et al [23] where average level of osteocalcin in blood serum was not different in patients and in the control group and showed no significant correlations with BMD and BASDAI scores. No significant connection of disease activity to OC level was also found in the study by Muntean L et al [24]. However, the study by Bugrova OV et al [25] showed reliable associative connections between the level of osteocalcin and ASDAS-CRP score.

Analysis of bone formation markers depending on X-ray changes in the spine showed that in the group of patients with complete spinal ankylosis, OC values were reliably 37,5% higher than in the group without syndesmophytes. Also, in the last group, PINP levels were lower than in the group with single syndesmophytes and complete spinal ankylosis. The study showed that patients with complete spinal ankylosis had a reliably higher age than patients without osteophytes. However, bone mineral density was determined on the basis of BMD score which was calculated in the region of the lumbar spine, and it was found to be lower in the last group when compared to the group with spinal ankylosis; simultaneously, BMD level in the region of the femoral neck was lower in the group of patients with syndesmophytes and spinal ankylosis than in the group without syndesmophytes. As for Z-score, it was slightly higher in the region of the femoral neck in patients with complete spinal ankylosis when compared to patients without syndesmophytes. The data provided by other studies are also contradictory. According to the data of Arends S et al [26], increased levels of OC and PINP in patients suffering from AS were associated with reduced bone mineral density. In the study by Gamez-Nava et al [27], patients with marked syndesmophytes had a higher level of OC than patients without syndesmophytes.

That is, such different results can be explained by the fact that measurement of bone mineral density in the region of the lumbar spine using X-ray absorptiometry is normally performed in anteroposterior projection. For this reason, any type of spinal lesion related to AS, and namely: presence of osteophytes, calcifications, degenerative changes of facet joints, hyperostosis and lesion of the posterior arch of vertebrae can influence the measurement of bone mineral density, i.e. increase BMD value and, thus, BMD levels can be normal or high in patients with osteoporosis as previously shown in studies [28-29]. In our opinion, determination of bone mineral density in the region of the femoral neck is a more sensitive method of osteoporosis evaluation in case of AS in patients with single syndesmophytes as well as in patients with complete spinal ankylosis.

Thus, when analysing our own data and the results of literary data it should be notated that in men suffering from AS, concentration of bone formation markers does not significantly differ from practically healthy persons. OC and PINP levels have no relation to age, disease duration, BMI and GC load. Inflammation process activity also did not significantly affect the synthesis of OC and PINP. In men suffering from AS, a pathologically new formation of bone tissue is observed which shows itself first in the form of syndesmophytes and later in the form of complete spinal ankylosis which is confirmed by credible increase of bone formation markers.

CONCLUSIONS

1. In men suffering from ankylosing spondylitis the level of OC and PINP in blood serum do not significantly differ from patients in the control group. OC and PINP levels have no relation to age, disease duration, BMI and GC load. Inflammation process activity also did not significantly affect the levels of markers of bone tissue synthesis.
2. Patients suffering from AS show pathologically new formation of bone tissue in the form of syndesmophytes and spinal ankylosis which is closely connected to the elevated level of bone formation markers, and is not associated with the course of the disease.

REFERENCES

1. Briot K., Durnez A., Paternotte S. et al. Bone oedema on MRI is highly associated with low bone mineral density in patients with early inflammatory back pain: results from the DESIR cohort. Ann Rheum Dis. 2013;72(12):1914-1919.
2. Malochet-Guinamand S., Pereira B., Tatar Z. et al. Prevalence and risk factors of low bone mineral density in spondyloarthritis and prevalence of vertebral fractures. BMC Musculoskelet Disord. 2017;18(1):357.
3. Leone A., Marino M., Dell'Atti C. et al. Spinal fractures in patients with ankylosing spondylitis. Rheumatol Int. 2016;36:1335–46.
4. Prieto-Alhambra D., Muñoz-Ortego J., De Vries F. et al. Ankylosing spondylitis confers substantially increased risk of clinical spine fractures: a nationwide case-control study. Osteoporos Int. 2015; 26: 85–91.
5. Bessant R., Keat A. How should clinicians manage osteoporosis in ankylosing spondylitis? J Rheumatol. 2002; 29 (7): 1511–9.
6. Weitzmann M.N. Bone and the Immune System. Toxicol Pathol. 2017; 45(7): 911-924.
7. Kwon S.R., Lim M.J., Suh C.H. et al. Dickkopf-1 level is lower in patients with ankylosing spondylitis than in healthy people and is not influenced by anti-tumor necrosis factor therapy. Rheumatol Int. 2012; 32(8): 2523–2527.
8. Visvanathan S., van der Heijde D., Deodhar A. et al. Effects of infliximab on markers of inflammation and bone turnover and associations with bone mineral density in patients with ankylosing spondylitis. Ann Rheum Dis. 2009; 68:175–182.
9. Huang J., Song G., Yin Z. et al. Alteration of Bone Turnover Markers in Canonical Wingless Pathway in Patients With Ankylosing Spondylitis. Arch Rheumatol. 2016; 31(3): 221–228.
10. Korczowska I., Przepiera-Bedzak H., Brzosko M. et al. Bone tissue metabolism in men with ankylosing spondylitis. Adv Med Sci. 2011; 56(2): 264–269.
11. Franck H., Keck E. Serum osteocalcin and vitamin D metabolites in patients with ankylosing spondylitis. Ann Rheum Dis. 1993;52(5):343–346.
12. Rudwaleit M., van der Heijde D., Landewé R. et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part II): validation and final selection. Ann Rheum Dis. 2009; 68(6): 777–783.

13. Baim S., Binkley N., Bilezikian J.P. et al. Official Positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Position Development Conference. J Clin Densitom. 2008;11(1):75-91.
14. Altindag Ö., Karakoç1 M., Soran N. et al. Bone Mineral Density in Patients with Ankylosing Spondylitis. Rheumatism. 2008; 23: 42-5.
15. Huang W.N., Tso T.K., Kuo Y.C., Tsay G.J. Distinct impacts of syndesmophyte formation on male and female patients with ankylosing spondylitis. Int J Rheum Dis. 2012; 15(2):163–168.
16. Chi-Chien Niu, Song-Shu Lin, Li-Jen Yuan et al. Correlation of blood bone turnover biomarkers and Wnt signaling antagonists with AS, DISH, OPLL, and OYL BMC Musculoskeletal Disorders. 2017; 18: 61.
17. Perpétuo I.P., Caetano-Lopes J., Vieira-Sousa E. et al. Ankylosing Spondylitis Patients Have Impaired Osteoclast Gene Expression in Circulating Osteoclast Precursors. Front Med (Lausanne). 2017;4:5.
18. Borman P., Bodur H., Bingöl N. et al. Bone mineral density and bone turnover markers in a group of male ankylosing spondylitis patients: relationship to disease activity. J Clin Rheumatol. 2001; 7(5): 315–321.
19. Yushina S.A., Bugrova O.V., Ovchinnikova N.A., Aleksandrova I.A. Osobennosti metabolizma kostnoy tkani u bolnykh ankiloziruyushchim spondiloartritom. Osteoporosis and Bone Diseases. 2011;14(2):15-18.
20. Perpétuo I.P., Raposeiro R., Caetano-Lopes J. et al. Effect of Tumor Necrosis Factor Inhibitor Therapy on Osteoclasts Precursors in Ankylosing Spondylitis. PLoS One. 2015; 10(12): e0144655.
21. Briot K., Roux C. Inflammation, bone loss and fracture risk in spondyloarthritis. RMD Open. 2015;1(1):e000052.
22. Coury F., Peyruchaud O., Machuca-Gayet I. Osteoimmunology of Bone Loss in Inflammatory Rheumatic Diseases. Front Immunol. 2019;10:679.
23. Park M.C., Chung S.J., Park Y.B., Lee S.K. Bone and Cartilage Turnover Markers, Bone Mineral Density, and Radiographic Damage in Men with Ankylosing Spondylitis. Yonsei Med J. 2008; 49(2): 288-294.
24. Muntean .L, Rojas-Vargas M., Font P. et al. Relative value of the lumbar spine and hip bone mineral density and bone turnover markers in men with ankylosing spondylitis. Clin Rheumatol. 2011;30(5):691–695.
25. Bugrova O.V., Nagornova K.A., Artemova N.E. Bone metabolism and its regulation in patients with ankylosing spondylitis. Nauchno-Prakticheskaya Revmatologiya. 2016; 54(5) 527-529.
26. Arends S., Spoorenberg A., Bruyn G.A. et al. The relation between bone mineral density, bone turnover markers, and vitamin D status in ankylosing spondylitis patients with active disease: a cross-sectional analysis. Osteoporos Int. 2011; 22(5): 1431-1439.
27. Gamez-Nava J.I., de la Cerda-Trujillo L.F., Vazquez-Villegas M.L. et al. Association between bone turnover markers, clinical variables, spinal syndesmophytes and bone mineral density in Mexican patients with ankylosing spondylitis. Scand J Rheumatol. 2016;45(6):480–490.
28. Deminger A., Klingberg E., Lorentzon M. et al. Which measuring site in ankylosing spondylitis is best to detect bone loss and what predicts the decline: results from a 5-year prospective study. Arthritis Res Ther. 2017;19(1): 273.
29. Van der Weijden M.A., van Denderen J.C., Lems W.F. et al. Low bone mineral density is related to male gender and decreased functional capacity in early spondylarthropathies. Clin Rheumatol. 2011;30(4):497-503.

ORCID and contributionship: :
Oksana Pavliuk: 0000-0002-5582-3800 [A-D]
Sergii Shevchuk: 0000-0002-5649-2775 [A,C,E,F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Sergii Shevchuk
National Pirogov Memorial Medical University
56 Pirogova st.,, 21018 Vinnytsia, Ukraine
tel: +380961119123
e-mail: shev.sv76@gmail.com

Received: 15.10.2020
Accepted: 02.08.2021

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

IMPACT OF INFECTIOUS DISEASES ON PUBERTAL TIMING IN UKRAINIAN GIRLS: RESULTS A MULTICENTER STUDY

DOI: 10.36740/WLek202110106

Aidyn G. Salmanov[1,2], Orusia A. Kovalyshyn[3], Roman S. Scoreiko[4], Victor M. Zinchenko[5], Serhiy M. Baksheev[6], Liudmyla V. Manzhula[6], Oleksandr A. Voloshyn[7]

[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY OF THE NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[3]LVIV MEDICAL INSTITUTE, LVIV, UKRAINE
[4]RIVNE MEDICAL ACADEMY, RIVNE, UKRAINE
[5]ACADEMIC MEDICAL CENTER, KYIV, UKRAINE
[6] KYIV CITY MATERNITY HOSPITAL, KYIV, UKRAINE
[7]KYIV REGIONAL MATERNITY HOSPITAL, KYIV, UKRAINE

ABSTRACT

The aim: To determine current age at the initiation of puberty for Ukrainian school-aged girls and infections impact to puberty.

Materias and methods: We performed a multicentre cohort study. The analyses are based on data that were collected and evaluated biannually on Ukrainian school girls aged 6-17 years from 5 regions of Ukraine. Pubertal development was classified according to the Marshall and Tanner criteria.

Results: Overall, 2,784 girls have been included in the study. Mean age of onset of puberty in Ukrainian girls was 10.1±1.0 yrs. Age of onset of pubic hair was 11.0±1.0 yrs and that of axillary hair was 11.6±1.0 yrs. Mean age in girls of menarche was 12.2±0.9 yrs. There were no significant correlations between age at onset of puberty and body mass index, final height, total peak height velocity, duration of puberty, and peak height velocity. A total of 2,420 infectious diseases were diagnosted. Of these, 64.8% were viral and 35.2% bacterial infections, respectively. Ukrainian girls with infectious diseases (especially viral infections) had older age at pubertal onset and positive association of infections in childhood with late age of menarche.

Conclusions: Infectious morbidity (especially viral infections) in girls may be associated with later puberty. The lowering of the number of infections in childhood could be an additional factor that contributes to earlier puberty. To reduce infectious diseases, more attention should be paid to the vaccination of children.

KEY WORDS: school-aged girls, puberty, menarche, infectious diseases, Ukraine

Wiad Lek. 2021;74(10 p.I):2392-2399

INTRODUCTION

Early or later pubertal timing in girls is a major problems for clinical practice and public health. Early puberty is associated with hormone-related cancers [1, 2], cardiovascular disease [2, 3] and its associated risk factors [4], and poor psychosocial adjustment [5]. The literature data from Europe show a sharp decline in mean age at menarche from approximately 17 years in the early nineteenth century to approximately 13 years by the mid-twentieth century, with a minor decrement during the past 25–30 years [6-10]. Average age at puberty has decreased sharply with economic development and is still falling in long-term developed Western countries [2,11], developing populations [12,13], and recently developed populations [14]. Mean age at onset of breast development has also decreased in all ethnic groups [7,8]. The decline in age of pubertal timing has serious public health due to problems of the reproductive system in girls. Reasons for the continuing fall in age of puberty are not well understood, and they could encompass many environmental exposures, including nutrition [9,10] and endocrine disruptors [11, 12]. One possible explanation for the decline is a decrease in exposures to infections [13]. The lowering of the number of infections in early life that accompanies economic development could be an additional factor that contributes to earlier puberty [2].

According literature data, infectious morbidity in girls may be associated with later puberty, perhaps via suppression of the gonadotropic axis [2]. Experience of infections disease is well known to down-regulate the gonadotropic axis, either by reducing sex-steroid production or possibly through regulation of sex-steroid receptors in humans, although the biologic mechanism by which the immune system in school-aged children permanently affects the gonadotropic axis in girls remains to be elucidated. The growth axis is active during puberty and interacts with the immune system. Pubertal onset is later in children with prenatally acquired human immunodeficiency virus infection or inflammatory bowel diseases [14].

While limited studies have explored relationships between pubertal timing and infectious diseases. In girls,

findings on prior infections and age of puberty are limited and seemingly contradictory; more infections in infants or young children, but not in school-aged children are associated with later puberty. The potential impact of infectious diseases on pubertal timing remains relatively unexamined. Every year in Ukraine, a large number of viral and bacterial infections are detected among school-age children. However, in Ukraine, there are no studies examining how the risk of an infectious disease is associated with puberty in girls.

THE AIM

To determine current age at the initiation of puberty for Ukrainian school-aged girls and infections impact to puberty.

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANTS

We performed a multicentre cohort study was based on surveillance data. This study included 2784 girls aged 6-17 years from 5 regions of Ukraine. We have compiled a list of 10 urban secondary schools in five different regions of Ukraine that agreed to participate in the study. All schools are similar in number and age of children. Inclusion criteria were as follows: 1) the study participants were local residents; 2) informed consent was acquired from participants and their guardians, and girls were in puberty aged from 6 to 17 years old. Chronic or debilitating disease were reasons for exclusion. We also excluded children with a diagnosis diseases associated hypothalamic–pituitary congenital malformations, neurological, neurosurgical and/or genetic diseases, psychomotor delay, oncological diseases, other endocrine impairments requiring hormonal treatments, or taking drugs that may interfere with pubertal development in girls.

DEFINITIONS

Pubertal development was classified according to the Marshall and Tanner criteria [15]. The age of pubertal onset was defined as the age at durable Tanner B2 stage. The rate of pubertal progression was taken to be the time from B2 to B3 and/or B4. Skeletal maturation was expressed as BA and evaluated, when possible, as the ratio of the change in BA to the change in chronological age (CA) (BA/CA), and as the difference between CA and BA (CA minus BA) in years. Precocious puberty was defined as the development of pubertal changes at an age that was younger than the accepted lower limits for age of onset of puberty (before the age of 8 years in girls). We defined slow or accelerated pubertal development if the rate of progression from one pubertal stage to another is more or less than 6 months or 1 SD in comparison with the general population. Parents were asked to complete a questionnaire specifying which devices children used, how long they used them for, at what times of the day and whether they were used by the children in their bedrooms during the hours before they went to sleep.

DATA COLLECTION

Data on height growth and pubertal development were collected by biannual visits to 10 secondary level schools. Previously established factors influencing age of pubertal onset were identified through literature searches and questions developed to measure which of these factors relate to puberty in our sample. The paper-based questionnaires were disseminated to participants. The study was explained to all potential participants and an information sheet, consent form and a copy of the questionnaire provided to each prior to them agreeing to participate. All questionnaires were anonymous and on completion were handed to researchers in a sealed envelope. Respondents provided details of current sexuality activity and menarche, and time spent of school and history of infectious diseases and other major childhood illness. The questionnaire utilised identical questions and allowed collection of an independent comparative sample. A total of 4,375 paper questionnaires were distributed with 2,812 questionnaires completed and returned (64.3% response). Overall, 5 questionnaires were excluded as they had been completed by non local residents. A further 21 were excluded as key fields, such as age or sex, were missing or age fell outside the target range. Two cases were excluded on the basis of spoilt returns resulting in final samples for analysis of 2,784 paper based respondents.

Pubertal development was assessed by different physicians at 5 Medical Centers. All participants underwent a physical examination and auxological evaluation. We recorded height, weight, body mass index (BMI), height velocity (HV) and stage and rate of pubertal progression (Tanner scale) [15]. Age-related reference values for height, bone age and BMI were those currently used in Ukraine. Final height (FH) was defined as attainment of a height velocity (HtV) less than 0.5 cm/yr. Breast (B), pubic hair (PH) and axillary hair (AH) development stages were rated in accordance with the Tanner criteria by one observer (RB) throughout the study [15]. Evaluation of pubertal stages was also done at 6 monthly intervals. Attainment of a breast stage of 2 was accepted as the onset of puberty. Duration of puberty was taken as the time period from attainment of a breast stage of 2 to final height. HtV was calculated from the multiple measurements taken on each individual child.

ETHICS

The Shupyk National Healtcare University of Ukraine Ethics Committee approved this study. Informational consent was obtained from the study participants and from the parents of enrolled children. All participants' data were anonymised prior to the analysis.

STATISTICAL ANALYSIS

Statistical analyses were performed with the use of SPSS X software (SPSSX Inc., Chicago, IL, USA). To analyze the data, the children were grouped by their breast stages; and height velocities for each breast stage group were

Table I. The ages of percentiles (ages in years) at attainment of stages of pubertal development in girls (n=2,784) in Ukraine (2017-2019)

Developmental Stage	Percentiles (ages in years)				
	3	10	50	90	97
Breast					
B2	8.0	8.7	10.3	11.4	11.9
B3	9.9	10.5	11.7	12.7	13.4
B4	11.0	11.4	12.3	13.5	13.9
B5	11.3	11.8	12.8	13.9	14.4
Pubic hair					
PH2	8.9	9.5	11.2	12.2	12.6
PH3	10.2	10.8	11.8	13.0	13.6
PH4	10.9	11.4	12.3	13.5	13.9
PH5	11.5	11.8	12.8	13.9	14.5
Axillary hair					
AH2	11.5	10.6	11.6	12.8	13.4
AH3	11.0	11.4	12.5	13.6	14.3
Menarche	10.5	11.0	12.2	13.3	14.0

Table II. Relationships between breast stage and weight, height, height velocity, weight velocity and body mass index in Ukrainian girls.

Breast Stage in girls	Age (yrs)	Weight (kg)	WtV (kg)	Ht (cm)	HtV (cm/yr)	BMI (kg/m^2)
2	10.2±1.0	35.8±6.93	5.5±3.33	141.7±7.63	7.3±3.92	17.8±2.63
3	11.6±0.91	42.4±7.31	4.8±3.62	150.4±6.32	5.3±3.42	18.8±2.72
4	12.5±0.83	48.1±7.12	3.7±3.41	155.6±5.74	3.2±2.43	19.9±2.74
5	12.8±0.84	51.5±6.92		158.0±5.82		20.6±2.63

WtV, weight velocity; HtV, height velocity; Ht, height; BMI, body mass index.

Table III. The auxological and pubertal characteristics of the Ukrainian girls followed to final height

Characteristics	Mean±SD
Duration of puberty (yrs)	
From breast stage 2 to breast stage 5	1.7±0.5
From breast stage 2 to menarche	1.8±0.6
From breast stage 2 to final height	4.9±1.2
Age at menarche (yrs)	12.2±0.9
Height increment during puberty (cm)	
From breast stage 2 to breast stage 5	11.3±3.7
From breast stage 2 to final height	16.0±3.9
Height increment after menarche (cm)	6.4±2.7
Findings relating to peak height velocity	
Amplitude (cm/yr) (range)	8.5±1.0
Chronological age (yr) (range)	11.3±1.5
Final height (cm)	164.3±6.1

calculated. HtV at a certain breast stage was designated as the annual velocity following the attainment of that breast stage. Body mass index (BMI) was calculated as weight (kg)/height (m^2). The characteristics of the study population were described using frequency distributions for categorical variables and mean values. The statistical significance of the continuous variable comparisons was assessed using the Student t test and the Mann-Whitney U test, depending on the distribution of the analyzed variable; the comparison of categorical variables was conducted using the chi square test or Fisher's Exact test if there was a small (< 5) expected cell size. All statistical tests were two-tailed and a $p < 0.05$ was considered statistically significant.

RESULTS

PUBERTAL TIMING IN GIRLS

Between January 2017 and December 2019, data on more than 2,784 girls were collected. Mean age of onset of puberty was 10.1±1.0 yrs. Age of onset of pubic hair was 11.0±1.0 yrs and that of axillary hair was 11.6±1.0 yrs. In this study mean age of menarche was 12.2±0.9 yrs (range 9.6-14.0 yrs). Onset of pubic hair development was earlier than breast development in 23.7% of the girls, breast development being the first sign of puberty in the remaining 76.3%. Age of percentiles at attainment of stages of pubertal development in girls is presented in Table I. Relationships between breast stage (B) and weight, height (Ht), height

Table IV. Infectious diseases prevalence in girls (n=2,784), according to age in Ukraine (2017-2019)

Pathogen/Infection	All cases (n=2,420) n (%)	Age-group of girls					
		6-7 (n=448) n (%)	8-9 (n=421) n (%)	10-11 (n=512) n (%)	12-13 (n=503) n (%)	14-15 (n=488) n (%)	16-17 (n=412) n (%)
Viral infections	1,567 (64,8)	340 (75,9)	418 (96,7)	370 (75,0)	223 (68,6)	112 (74,4)	104 (25,2)
Measles	286 (10,3)	42 (9,4)	112 (26,6)	72 (14,1)	25 (5,0)	19 (3,9)	16 (3,9)
Chickenpox	76 (2,7)	25 (5,6)	27 (6,4)	15 (2,9)	7 (1,4)	2 (0,4)	0
Mumps	74 (2,7)	27 (6,0)	22 (5,2)	12 (2,3)	12 (2,4)	1 (0,2)	0
Rubella	126 (4,5)	31 (6,9)	36 (8,6)	34 (6,6)	15 (3,0)	5 (1,0)	5 (1,2)
Infectious mononucleosis	139 (5,0)	26 (5,8)	29 (6,9)	27 (5,3)	17 (3,4)	22 (4,5)	18 (4,4)
Viral meningitis	122 (4,4)	31 (6,9)	22 (5,2)	27 (5,3)	17 (3,4)	11 (2,3)	14 (3,4)
Rotavirus	211 (7,6)	64 (14,3)	56 (13,3)	49 (9,6)	36 (7,2)	4 (0,8)	2 (0,5)
Influenza	398 (14,3)	77 (17,2)	89 (21,1)	111 (21,7)	73 (14,5)	27 (5,5)	21 (5,1)
Hepatitis A virus	84 (3,0)	17 (3,8)	25 (5,9)	23 (4,5)	13 (2,6)	5 (1,0)	1 (0,2)
Hepatitis B virus	26 (0,9)	0	0	0	2 (0,4)	9 (1,8)	15 (3,6)
Hepatitis C virus	25 (0,9)	0	0	0	6 (1,2)	7 (1,4)	12 (2,9)
Bacterial infections	853 (35,2)	122 (24,1)	140 (3,3)	128 (25,0)	158 (31,4)	125 (25,6)	180 (74,8)
Campylobacteriosis	31 (1,1)	5 (1,1)	12 (2,9)	9 (1,8)	4 (0,8)	1 (0,2)	0
Salmonellosis	211 (7,6)	71 (15,8)	47 (11,2)	6,8	34 (6,8)	18 (3,7)	12 (2,9)
Shigellosis	112 (4,0)	12 (2,7)	19 (4,5)	23 (4,5)	22 (4,4)	25 (5,1)	11 (2,7)
Escherichia coli enteritis	58 (2,1)	5 (1,1)	14 (3,3)	16 (3,1)	11 (2,2)	7 (1,4)	5 (1,2)
Tuberculosis	85 (3,1)	0	1 (0,2)	9 (1,8)	27 (5,4)	23 (4,7)	25 (6,1)
Meningococcal disease	96 (3,4)	11 (2,5)	25 (5,9)	26 (5,1)	31 (6,2)	2 (0,4)	1 (0,2)
Pertussis	57 (2,0)	18 (4,0)	22 (5,2)	15 (2,9)	2 (0,4)	0	0
Syphilis	39 (1,4)	0	0	0	2 (0,4)	9 (1,8)	28 (6,8)
Gonorrhea	136 (4,9)	0	0	1 (0,2)	19 (3,8)	35 (7,2)	81 (19,7)
Leptospirosis	28 (1,0)	0	0	0	6 (1,2)	5 (1,0)	17 (4,1)

velocity (HiV), weight velocity (WtV) and body mass index (BMI) in girls are shown in Table II.

In the subsample of girls who were followed longitudinally to final height, mean age at onset of puberty was 10.6±0.9 yrs. Mean height at onset of puberty in girls was 144.8±6.83 cm at a mean velocity of 7.4±1.53 cm/yrs. Height velocity before the year of onset of puberty was 6.2±1.2 cm/yrs and weight velocity before the year of onset of puberty 4.6±2.8 kg/yrs. These values were similar to those of the total group Ukrainian girls. The auxological and pubertal characteristics of Ukrainian girls who were followed to final height are given in Table III.

IMPACT OF INFECTIOUS DISEASES ON PUBERTAL TIMING

A total of 2420 infectious diseases were diagnosted. (Table IV). Of these, 64.8% (1567/2784) were viral and 35.2% (853/2784) bacterial infections, respectively. Considering all infectious diseases types together, Influenza were most commonly reported, accounting for 14.3% of all infections, followed by Measles (10.3%), Rotavirus (7.6%), Salmonellosis (7.6%), Infectious mononucleosis (5,0%), Gonorrhea (4.9%), Rubella (4.5%), Viral meningitis (4.4%), Shigellosis (4.0%), and Bacterial meningococcal disease (3.4%).

All girls were categorised into either earlier (≤12 years; 44.9%) or later (≥13 years; 55.1%) puberty. Tables V and VI explore associations between infectius diseases and other risk factotrs (relating to life before menarche), and pubertal timing. For girls, having had sex before age 14 years and having drunk alcohol before age 15 years were all associated with earlier menarche. In addition, having had unprotected sex (i.e. without any contraception) under 15 years and smoked under 14 years were significant.

For girls, infectious diseases were all significantly associated with later puberty in the combined analysis. School-aged girls with later pubertal markers reported higher frequencies of viral infections (Influenza, Measles, Rotavirus, Rubella, Infectious mononucleosis and Viral meningitis). Viral Infections was associated with later breast development and age at menarche.

DISCUSSION

To our knowledge, this is the first study that determine current age at the initiation of puberty for Ukrainian

Table V. Associations between risk factors and age of menarche in girls (n=2,784) in Ukraine (2017-2019)

Characteristics of risk factors	All cases n	Age of menarche (years) in girls		*P*
		≤12 (n=1884) %	≥13 (n=900) %	
Drank alcohol	812	59.8	47.4	<0.01
Took drugs	806	8.7	2.9	<0.01
Smoked	570	29.0	22.6	<0.05
Had sex	820	32.8	22.4	<0.001
Had unprotected sex	820	13.5	7.3	<0.05
First pregnant	822	1.3	0.6	0.341
Had an abortion	578	7.9	6.6	0.529
Obesity	112	26.5	15.7	<0.01
Had an infectious disease	2,420	38.1	56.0	<0.001
Viral infections	1,567	15.3	28.6	<0.001
Measles	286	20.9	39.5	<0.001
Chickenpox	76	33.1	30,6	0.494
Mumps	74	42.4	37.0	0.162
Rubella	126	28.8	39.3	<0.01
Infectious mononucleosis	139	26.0	28.3	<0.01
Viral meningitis	122	20.9	33.5	<0.001
Rotavirus	211	52.3	56.1	0.254
Influenza	398	33.4	30.9	0.411
Hepatitis A virus	84	17.3	22,1	0.112
Hepatitis B virus	26	17.4	28.0	0.070
Hepatitis C virus	25	21.7	23.3	0.418
Bacterial infections	853	31.2	48.8	<0.05
Campylobacteriosis	31	19.5	17.4	0,651
Salmonellosis	211	36.2	37.3	0.818
Shigellosis	112	30.6	33.1	0.494
Escherichia coli enteritis	58	17.4	21.7	0.254
Tuberculosis	85	16.6	37.4	<0.001
Meningococcal disease	96	20.8	33.6	<0.001
Pertussis	57	19.9	14.9	0.337
Syphilis	39	19.5	17.4	0.651
Gonorrhea	136	29.2	21.5	0.117
Leptospirosis	28	29.6	33.1	0.651

school-aged girls and infections impact to pubertal timing. In our study mean age of onset of puberty in Ukrainian girls was 10.1±1.0 yrs. Age of onset of pubic hair was 11.0±1.0 yrs and that of axillary hair was 11.6±1.0 yrs. In this study mean age of menarche was 12.2±0.9 yrs (range 9.6-14.0 yrs). Onset of pubic hair development was earlier than breast development in 23.7% of the girls, breast development being the first sign of puberty in the remaining 76.3%. There were no significant correlations between age at onset of puberty and body mass index, final height, total peak height velocity, duration of puberty, and peak height velocity. Age of menarche was negatively correlated with weight at menarche ($p<0.001$), body mass index at menarche ($p<0.001$) and positively correlated with age at onset of puberty ($p<0.002$). Final height was correlated only with height at onset of puberty ($p<0.0001$). Duration of puberty was 4.9±1.2 yrs, when estimated as the time period from B stage 2 to final height. The time period from B stage 2 to menarche was 1.8±0.6 yrs. Mean age of menarche was 12.2±0.9 yrs. Total pubertal height gain was 16.0±3.9 cm. Growth in height continued after menarche and mean gain in height after menarche was 6.4±2.7 cm.

Table VI. Associations between infectious disease and age of pubertal onset in girls (n=2,784) in Ukraine (2017-2019)

Pathogen/Infection	All cases n	Estimated age of pubertal onset (years) in girls		*P*
		≤11 (n=1381) %	≥12 (n=1403) %	
Viral infections	1567	31.5	68.5	<0.001
Measles	286	15.7	26.5	<0.01
Chickenpox	76	6.6	7.9	0.529
Mumps	74	22.4	32.8	<0.001
Rubella	126	7.3	13.5	<0.05
Infectious mononucleosis	139	22.4	32.8	<0.001
Viral meningitis	122	33.1	55.3	<0.001
Rotavirus	211	2.9	8,7	<0.01
Influenza	398	47.4	59.8	<0.01
Hepatitis A virus	84	6.6	7.9	0.529
Hepatitis B virus	26	0.6	1.3	0.341
Hepatitis C virus	25	1.9	8.1	<0.001
Bacterial infections	853	43.4	56.6	<0.05
Campylobacteriosis	31	29.2	20.5	0.115
Salmonellosis	211	16.6	17.6	0.522
Shigellosis	112	21.7	25.3	0.119
Escherichia coli enteritis	58	19.7	21.1	0.193
Tuberculosis	85	23.7	33.1	<0.05
Meningococcal disease	96	2.9	8.3	<0.01
Pertussis	57	3.1	4.3	0.328
Syphilis	39	2.8	8.7	<0.01
Gonorrhea	136	22.8	25.9	0.311
Leptospirosis	28	3.9	5.1	0.347
Total	2420	41.2	58.8	<0.01

Mean final height was 164.3±6.1 cm. Age at onset of puberty correlated negatively with weight, height, with weight before the year of onset of puberty ($p<0.001$) and height velocity before the year of onset of puberty ($p<0.01$).

In literature, relatively few data are available on the accordance between B and pubic hair stages during puberty. Traditionally, breast development is accepted as the initial event in pubertal development in girls [16]. However, PH was observed to occur before breast development in 23.7% of the girls in our study. This finding was 21.5% in Turkish girls, 10% in Dutch girls and approximately 30% in a British study [15-17]. The definition of precocious puberty is based on the age of occurrence of B or pubic hair development in healthy girls in the population. In Dutch girls P3 age for B2 is 8.2 years, a cut-off age close to our findings [17]. Eight years is generally and internationally used as the cut-off age for the definition of precocious puberty in girls. Our findings suggest that this cut-off age can be continued to be used for Ukrainian girls.

The attainment of final height depends on several factors at interplay. One factor may be the differences in height at onset of puberty [16]. The positive correlations found in our study between height at onset of puberty and final height indicate that the smaller the child is at the onset of puberty, the smaller the final height will be, a finding which has been reported in other studies as well [16, 18]. In our study, in compliance with the study on Swiss children [16, 19], nocorrelation was evident between age at onset of puberty and PHV, total pubertal height gain or final height.

Infectious diseases are common in children [20]. Experience of infections is well known to down-regulate the gonadotropic axis, either by reducing sex-steroid production or possibly through regulation of sex-steroid receptors in humans [21-23]. Infectious morbidity in girls may be associated with later puberty, perhaps via suppression of the gonadotropic axis [2]. In our study Ukrainian girls with infectious diseases (especially viral infections) had older age at pubertal onset and positive association of infections in childhood with late age of menarche. In the present cohort of school-aged girls had older age at pubertal onset,

consistent with a previously reported positive association of infections with late age of menarche [13, 14, 24]. Our results suggest a need for prevention efforts that target infections in a broader scope than limited to sexually transmitted diseases.

STUDY LIMITATIONS

The absence of national data in Ukraine compelled us to rely entirely on data from the only multicenter study that determine current age at the initiation of puberty for Ukrainian school-aged girls girls and infections impact to pubertal timing. Pubertal development was classified according to the Marshall and Tanner criteria [15]. A limitation of the study is that it only include 2,784 school-aged girls from five regions of Ukraine. The results this study may not be representative of other regions of Ukraine with different distributions of incidence rate of infectious diseases in school-aged girls. Further research is needed.

CONCLUSIONS

Our studies have shown that Infectious morbidity (especially viral infections) in girls may be associated with later puberty. The lowering of the number of infections in childhood could be an additional factor that contributes to earlier puberty. To reduce infectious diseases, more attention should be paid to the vaccination of children. Further studies are needed to confirm our data and elucidate the relative importance of the various factors at play and how they interact. Clinicians should be aware of the signs of pubertal developmental disorders and should carefully monitor existing girls with infectious diseases for signs of later pubertal timing.

REFERENCES

1. Golub M.S., Collman G.W., Foster P.M. et al. Public health implications of altered puberty timing. Pediatrics. 2008;121(3):S218-30. doi: 10.1542/peds.2007-1813G.
2. Kwok M.K., Leung G.M., Lam T.H. et al. Early Life Infections and Onset of Puberty: Evidence From Hong Kong's Children of 1997 Birth Cohort. Am J Epidemiol. 2011;173(12):1440–1452. doi: 10.1093/aje/kwr028.
3. Lakshman R., Forouhi N.G., Sharp S.J. et al. Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab. 2009;94(12):4953-60. doi: 10.1210/jc.2009-1789.
4. Frontini M.G., Srinivasan S.R., Berenson G.S. Longitudinal changes in risk variables underlying metabolic Syndrome X from childhood to young adulthood in female subjects with a history of early menarche: the Bogalusa Heart Study. Int J Obes Relat Metab Disord. 2003;27(11):1398-404. doi: 10.1038/sj.ijo.0802422.
5. Bellis M.A., Downing J., Ashton J.R. Adults at 12? Trends in puberty and their public health consequences. J Epidemiol Community Health. 2006;60(11):910-1. doi: 10.1136/jech.2006.049379.
6. Sørensen K., Mouritsen A., Aksglaede L. et al. Recent secular trends in pubertal timing: implications for evaluation and diagnosis of precocious puberty. Horm Res Paediatr. 2012;77(3):137–45. doi:10.1159/000336325.
7. Euling S.Y., Herman-Giddens M.E., Lee P.A. et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. Pediatrics. 2008;121(3):S172–91. doi: 10.1542/peds.2007-1813D.
8. Biro F.M., Galvez M.P., Greenspan L.C. et al. Pubertal assessment method and baseline characteristics in a mixed longitudinal study of girls. Pediatrics. 2010;126(3):e583-90. doi: 10.1542/peds.2009-3079.
9. Gunther A.L., Karaolis-Danckert N., Kroke A. et al. Dietary protein intake throughout childhood is associated with the timing of puberty. J Nutr. 2010;140(3):565–571. doi:10.3945/jn.109.114934
10. Rogers IS, Northstone K, Dunger DB, et al. Diet throughout childhood and age at menarche in a contemporary cohort of British girls. Public Health Nutr. 2010;13(12):2052-63. doi: 10.1017/S1368980010001461.
11. Buck Louis G.M., Gray L.E. Jr., Marcus M. et al. Environmental factors and puberty timing: expert panel research needs. Pediatrics. 2008;121(3):S192-207. doi: 10.1542/peds.1813E.
12. Schoeters G., Den Hond E., Dhooge W. et al. Endocrine disruptors and abnormalities of pubertal development. Basic Clin Pharmacol Toxicol. 2008;102(2):168-75. doi: 10.1111/j.1742-7843.2007.00180.x.
13. McDonald J.A., Eng S.M., Dina O.O. et al. Infection and pubertal timing: a systematic review. J Dev Orig Health Dis. 2016;7(6):636-651. doi:10.1017/S2040174416000313
14. Ballinger A.B., Savage M.O., Sanderson I.R. Delayed puberty associated with inflammatory bowel disease. Pediatr Res. 2003;53(2):205-10. doi: 10.1203/01.PDR.0000047510.65483.C9.
15. Marshall W.A., Tanner J.M. Variations in patterns of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303. doi:10.1136/adc.44.235.291.
16. Bundak R., Darendeliler F., Günöz H. et al. Puberty and Pubertal Growth in Healthy Turkish Girls: No evidence for secular trend. J Clin Res Ped Endo. 2008;1(1):8–14. doi:10.4008/jcrpe.v1i1.16
17. Mul D., Fredriks A.M., van Buuren S. et al. Pubertal development in The Netherlands 1965-1997. Pediatr Res. 2001;50(4):479-86. doi: 10.1203/00006450-200110000-00010.
18. Tanaka T., Komatsu K., Takada G. et al. Prediction of adult height in healthy Japanese children. Acta Paediatr Suppl. 1996;417:57-60. doi: 10.1111/j.1651-2227.1996.tb14298.x.
19. Largo R.H., Gasser T., Prader A. et al. Analysis of the adolescent growth spurt using smoothing spline functions. Ann Hum Biol. 1978;5(5):421-34. doi: 10.1080/03014467800003071.
20. Sollai S., Ghetti F., Bianchi L. et al. Infectious diseases prevalence, vaccination coverage, and diagnostic challenges in a population of internationally adopted children referred to a Tertiary Care Children's Hospital from 2009 to 2015. Medicine (Baltimore). 2017;96(12):e6300. doi:10.1097/MD.0000000000006300.
21. Ibrahim I.I., Barakat R.M., Bassiouny H.K. et al. Effect of urinary bilharzial infection on male pubertal development and endocrine functions. Arch Androl. 1983;11(1):59-64. doi: 10.3109/01485018308987461.
22. Mavoungou D., Lansoud-Soukate J., Dupont A. Steroid and gonadotropin hormone levels in young African women with filarial infection.J Steroid Biochem. 1989;34(1–6):577–580.
23. Muehlenbein M.P., Alger J., Cogswell F. et al. The reproductive endocrine response to Plasmodium vivaxinfection in Hondurans.Am J Trop Med Hyg. 2005;73(1):178–187.
24. Blell M., Pollard T.M., Pearce M.S. Predictors of age at menarche in the newcastle thousand families study. J Biosoc Sci. 2008;40(4):563-75. doi: 10.1017/S0021932007002696.

We would like to thank all the nurses and physicians who contributed to the prevalence surveys.

ORCID and contributionship:
Aidyn G. Salmanov: 0000-0002-4673-1154 [A, C- F]
Orusia A. Kovalyshyn: 0000-0002-9710-0694 [B-D, F]
Roman S. Scoreiko : 0000-0001-6986-4972 [B-D,.F]
Victor M. Zinchenko: 0000-0002-5439-8267 [B-D,.F]
Serhiy M. Baksheev: 0000-0003-4144-5965 [B-D, F]
Liudmyla V. Manzhula: 0000-0003-3142-7237 [B-D, F]
Oleksandr A. Voloshyn: 0000 0002- 6586- 5449 [B-D, F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Aidyn G. Salmanov
Shupyk National Healthcare University of Ukraine,
9 Dorohozhytska St., 04112 Kyiv, Ukraine
tel: +380667997631
e-mail: mozsago@gmail.com

Received: 17.07.2021
Accepted: 29.10.2021

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

SEVERITY OF LUNG DAMAGE ASSESSED BY CT-SCAN IN RELATION TO D-DIMER LEVEL IN COVID-19

DOI: 10.36740/WLek202110107

Mustafa Abdul Ridha Alnowfal[1], Nawfal Almubarak[2], Murtadha A. Jeber[3]
[1]BOARD OF ANESTHESIA & ICU, AL-MAWANI GENERAL HOSPITAL, BASRA, IRAQ
[2]HEAD OF ARAB BOARD COUNCIL, COLLEGE OF MEDICINE, UNIVERSITY OF BASRA, BASRA, IRAQ
[3]CONSULTANT INTERNIST, AL-MAWANI GENERAL HOSPITAL, BASRA, IRAQ

ABSTRACT

The aim: This study was targeted to see the correlation between D-Dimer level and severity of lung injury evaluated by CT scan to prevent major complications in early of disease.
Materials and methods: This study was carried out in Al-Mawani Teaching Hospital in Basra – Iraq. The study took place between August to October 2020 included 74 patients from both genders; all patients were admitted to the hospital and serial of investigations were done inform of CT scan, blood chemistry and D-dimer along with vital signs and demographic data were taken at the time of admission.
Results: The correlations between the D-Dimer level and lung damage assessed by CT scan were significantly related with a P-value of less than (0.05) and regression coefficient of (3.016). Age and gender relation with severity of lung involvement were statistically non-significant. All the patients included were beyond surgical classification of ASA.
Conclusions: In COVID-19 infected patients, the Severity of lung injury assessed by chest computed tomography is positively correlated with D-Dimer levels, and it can be considered as an independent predictor of severe cases.

KEY WORDS: COVID-19, D-Dimer, lung damage, anticoagulation therapy, GGO, ARDS

Wiad Lek. 2021;74(10 p.I):2400-2406

INTRODUCTION

An outbreak of an unidentified health storm started back in DEC 2019 in Wuhan – China, which later had been recognized as a type of strange coronaviruses [1]. The disease caused by this virus family called COVID-19 that was referred to the year 2019 [2]. The virus was highly virulent between community and therefore WHO on the 11th Mar 2020 declared this outbreak as a global pandemic [3]. In September 2020 more than 32.750.000 cases were registered across 213 countries. There are so many not registered cases while numbers are continually increasing. And the rate of infectivity and mortality of COVID-19 are still obscured, the death rate in covid-19 is 20 times that of SARS outbreak [4], and 39 times compared with death caused by MERS [5]. Eighty percent of the patients are asymptomatic or have mild symptoms, oxygen requirement was seen in only fifteen percent of the cases and 5% may need ICU. CT scan plays an important role in the assessment of lung involvement, severity, monitoring and progression of patients' outcome [6], the sensitivity of CT scan in diagnosis of lung injury in COVID-19 shows positive in 97% and 75% negative, while specific in 25% (Fig. 1) [7].

In the early course of the disease lung CT scan shows Ground Glass Opacities distributed mainly in the sub pleural area of the lungs. The progress to larger Opacities with crazy paving appearance associated with some consolidations are seen in moderate cases, while bilaterally extensive severe consolidations are observed in severe cases [8]. With increasing severity of lung injury hypoxemia can take place and life-threatening situation may happen with many factors influencing the outcome including coexisting chronic diseases, age, and high D-Dimer level. D-Dimer is a fibrin degradation product of the blood clot break down process that can be assessed by testing a blood sample with a normal value of less than 0.5 mcg/ml (Fig. 2).

The pulmonary capillaries have glycocalyx layer in their endothelium which secrets tissue plasminogen activator that prevents formation of thrombi [9]. In the beginning of the pandemic condition, COVID-19 syndrome was primarily considered as ARDS (acute respiratory distress syndrome) [10], but later on researchers assumed that it's a multisystem disorder [11]. An exaggerated immune response that leads to release of pro-inflammatory markers in form of cytokine elements referred to as cytokine storm syndrome (CSS) because of the many cases who were experiencing thromboembolic events among patients specially in patients who had risk factors for thrombosis including old age, chronic diseases and obesity that increase the morbidity and mortality in these group of people [12]. Other causes of high D-Dimer levels include acute coronary syndrome, massive bleeding, sepsis, DIC (disseminated intravascular coagulation), acute and chronic renal failure, multiple traumatic injury (including RTA – road traffic accidents - and major surgeries), solid

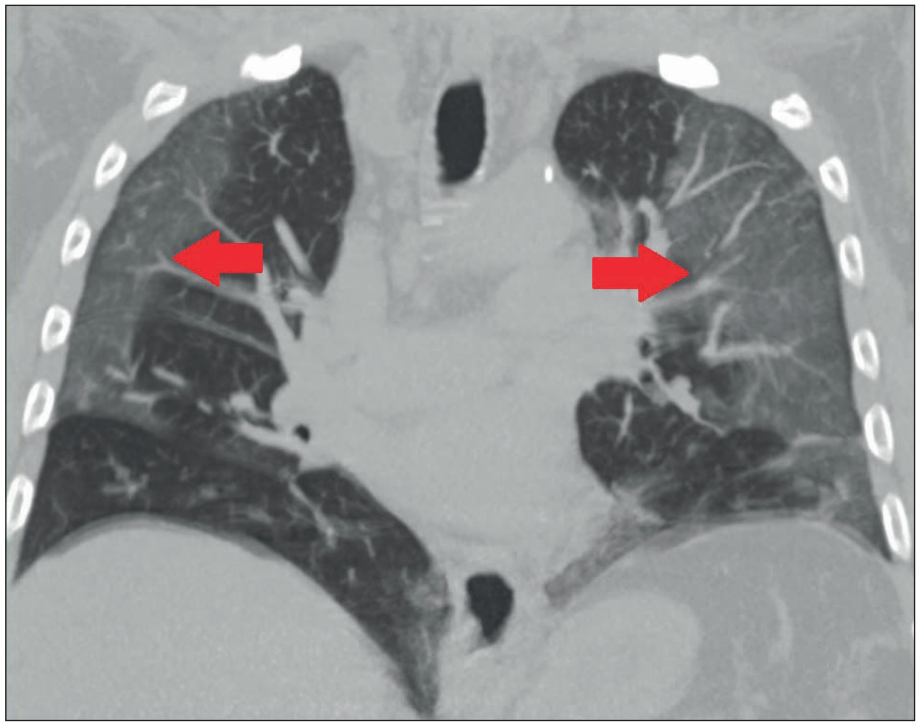


Fig. 1. Coronal section of high-resolution CT of the chest showing ground glass opacities in the periphery of both lungs in a patient with COVID-19 (red arrows).
Source: https://en.wikipedia.org/wiki/Ground-glass_opacity

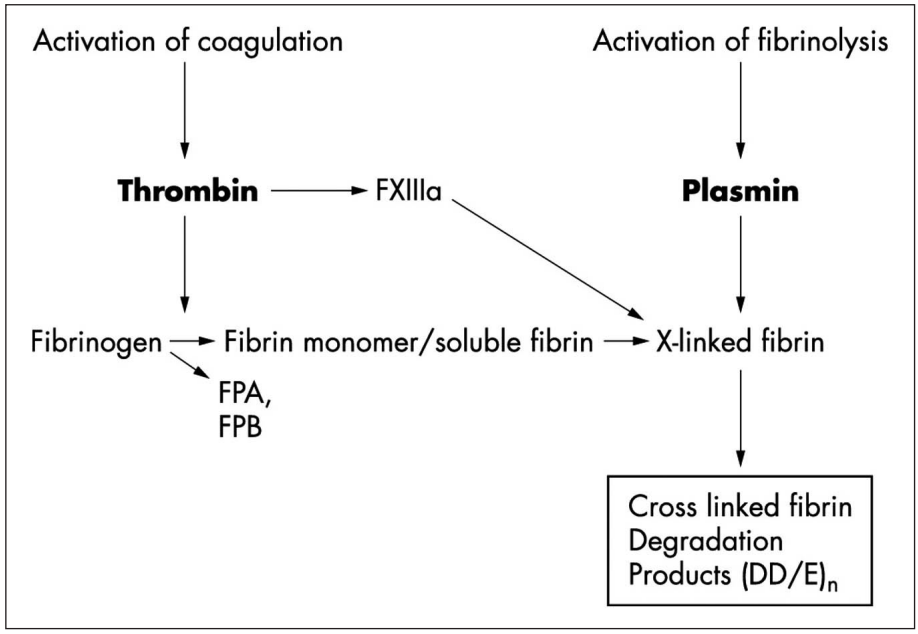


Fig. 2. Simple diagram demonstrates the pathogenesis of D-Dimer.
Source: http://dx.doi.org/10.1136/emj.20.4.319

tumours, leukaemia, severe infections, and thrombolytic therapy. Post mortem studies in COVID and non-COVID ARDS patients showed extensive damage in the alveoli with perivascular infiltration of T-Cells, but in COVID-19 there were some unique changes in the vascular tree including severe injury of the endothelium and alveolar cell membrane distraction with the existence of the virus in both lung tissue and vessels, which was 9 times greater number of micro thrombi and 2.7 times more angiogenesis and microangiopathic than that of non-COVID-19 ARDS (Fig. 3) [13].

Silent hypoxia or happy hypoxia and poor oxygen delivery to the tissues are very common in COVID-19 patients due to minor lung damage considered as pathogneumonic of this novel disease. There is no role of physiological hypoxic pulmonary vasoconstriction therefore lung damage presented as happy hypoxia, and in conclusion there are increasing attention towards thrombotic micro emboli that leads to VQ mismatch in the non-injured lung as underlying cause of hypoxemia [14].

THE AIM

This study was targeted to see the correlation between D-Dimer level and severity of lung injury evaluated by CT scan to prevent major complications in early of disease

Table I. The radiological formula to calculate the severity of lung injury.

Percentages of lung involvements assessed by CT scan (for each lobe) [%]	SCORE
<5	1
5-25	2
25-50	3
50-75	4
>75	5

Table II. Demographical data distribution of the enrolled patients.

Variables		N	[%]
Age (Mean ± SD)		54.36 ±14.26	
Gender	Male	39	52.7
	Female	35	47.3
D-dimer (Mean ± SD) mcg/mL FEU		0.524 ±0.431	
Severity of lung injury	Mild	0	0
	Moderate	52	70.3
	Severe	22	29.7

Table III. Demographical data classification according to the severity of lung injury.

Variables		Moderate lung injury	Sever lung injury	p value
Age (mean ±SD)		53.23±13.99	57.05±14.84	0.296
Gender	Male	29 (55.8%)	10 (45.5%)	0.417
	Female	23 (44.2%)	12 (54.5%)	
D-Dimer (median)		0.235	0.645	<0.05

MATERIALS AND METHODS

STUDY SAMPLING

This is a cross – sectional analytical study in which seventy-four patients with confirmed COVID-19 disease were enrolled, they were admitted to Al-Mawani Teaching Hospital, all patients were referred from centre and north of Basra hospitals from August to October 2020 after agreement of the Arabic board council in Iraq were taken. All confirmed cases had more than two clinical manifestations, approved by Swab and blood chemistry positive for SARS-CoV-2 by real-time reverse transcription polymerase chain reaction (RT – PCR) assay and chest computed tomography according to the Novel Coronavirus Pneumonia Diagnosis and Treatment Guideline (6th edn.) published by the National Health Commission of China [15]. Severity of lung injury were calculated by special radiological formula depending on the counting of the involved lung lobes and the percentage by which each lobe is affected assessed visually by CT scan (reported by expert radiologist) as shown in (Table I).

Percentages and scoring above are done and include each lobe of both lungs alone and the sum of 5 is taken [16]. According to the formula of severity of lung injury above, the table can assess the severity accordingly:

- A total score of (0-8): Mild lung involvement.
- A total score of (9-15): Moderate lung involvement.
- A total score of (16-25): Severe lung involvement.

EXCLUSION CRITERIA

- Patients with pre-existing lung disorders, to exclude primary lung disease because that will need more sophisticated study to determine whether lung injury is solely due to COVID-19 or pre-existing lung disease.
- Patients on anticoagulation therapy before COVID-19 infection.
- Patients diagnosed with high Risk factors for thrombosis.
- Patients with known cause of high D-Dimer before COVID-19 infection.
- «Patients with BMI over 35, because obesity is considered as a major cause for elevated D-Dimer levels and coagulation activator.

All research tools including, demographical data, swabs, blood chemistry, D-Dimer and chest CT imaging were collected at the same time using a date collection form.

STATISTICAL ANALYSIS

Statistical calculations were done using Statistical Package for the Social Sciences version 25 (SPSS Inc.) in which categorical data expressed as numbers and percentages, and the differences between the groups were analysed using Chi-square test (χ^2). Continuous data expressed as medians or mean ± SD and the differences between the groups were analysed by non-parametric Mann-Whitney U test for abnormally distributed data and independent t-test for normally distributed data. Shapiro-Wilk test used to test the normality of the data, and outliers were detected using Box plot methods. Linearity was assessed visually by simple histogram chart; nonlinear related data was transformed to Log10 to be fit with the test assumptions. Independence of observations was checked using the Durbin-Watson statistic. The prediction ability of the significant variables was assessed using Binomial logistic regression test in which odds ratio and coefficient value was calculated. Hosmer and Leme show test used to assess the data fitness for the test. Non-parametric spearman correlation test was used to calculate the correlation between the variables 95% confidence interval were applied as the dependent interval in statistics and P-values <0.05 were accepted as statistically significant.

RESULTS AND DISCUSSION

A total of 74 COVID-19 patients were enrolled in the study. The mean age was (54.36 ±14.26 year), and 52.7% males to 47.3% females. Regarding the D-Dimer results the mean was (0.524 ±0.431), while 70.3% of the enrolled patient's lies within moderate severity of lung injury assessed by CT scan in comparison to 29.7% severe cases only (Table II).

Table IV. Prediction of the D-Dimer levels for the severity of lung injury.

Variables	Coefficient (B)	p value	Lower bound	Upper bound
D-Dimer levels * severity of lung injury	3.016	<0.05	3.679	113.314

Table V. Correlations of CT result and D-Dimer variables in COVID-19 patients.

Variables		Percentages of lung involvement
D-Dimer	Correlation Coefficient	0.582
	p value	<0.05
	N	74

Mann-Whitney U test was used to assess the difference between the medians in which D-Dimer levels (for moderate 0.235, and 0.645 for severe) significantly differs between the groups. Age and gender showed no statistical significance (Table III).

Binary logistic regression showed that elevated levels of D-Dimer can be considered as an independent predictors of severe lung injuries (r, 3.016, p value<0.05), which mean every increase in one unit in D-Dimer levels is associated with increase in the risk of having severe lung injury by 3 folds (Table IV).

Non- parametric spearman correlation analyses revealed the relationships between percentages of lung involvement assessed by CT scan and D-Dimer levels in COVID-19 patients (Table V). It showed that D-Dimer levels were strongly correlated with percentages of lung involvement (r = 0.582, P = <0.05) (Fig. 4-5).

COVID-19 syndrome caused by a novel type of corona viruses of genus b type of single strand RNA strain, its round or oval in shape, lined by special envelope, with a diameter of 60-140 nm. the virus invade the alveolar lining by binding with the ACE II receptors that are responsible for synthesis and production of surfactant which is the main cause of alveolar damage and collapse [17], the consequences of vascular endothelial cells damages and hypoxia [18] with activation of inflammatory cascades, which in turn enhance the activation of coagulative pathway and then followed by systemic micro-thrombotic changes may give a hint of the future multi-organ failure in severe COVID-19 patients [19]. Although D- Dimer is considered as indirect marker of active thrombotic events [20], it is still a powerful predictive way of consequent coagulative cascades alteration especially when excluding the possible contributors of high D-Dimer levels such as pregnancy, cancer, chronic liver diseases, post trauma and surgery status, and vasculitis [21]. This alteration of D-Dimer levels during SARS-CoV-2 infection and its relation to the severity of the cases is well encountered and established in this study. In 2019, seventy-one diagnosed case with COVID-19 enrolled in a study conducted by Jin Zhu at al. in China to investigate the correlation between severity of lung injury assessed by CT scan and levels of D-Dimer, the study concluded that D-Dimer levels is positively correlated with lung severity assessed by CT scan (correlation coefficient= 0.237, odd ratio=2.460) [22].

GROUND-GLASS APPEARANCE (GGO)

It is defined as a pathogneumonic picture of severe inflammatory reaction that can be showed by chest x-ray and CT scan which is characterised by a defined area of hazy opaci-

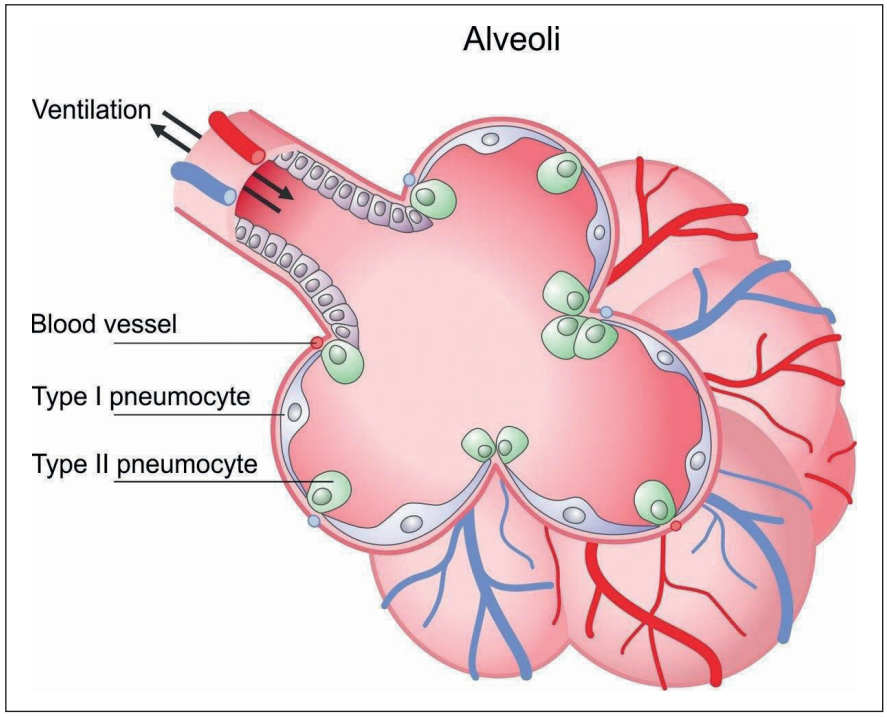


Fig. 3. Simple diagram demonstrates pneumocyte types.
Source: https://www.mpg.de/14673057/using-alveolar-epithelia-as-a-model-for-corona-infection

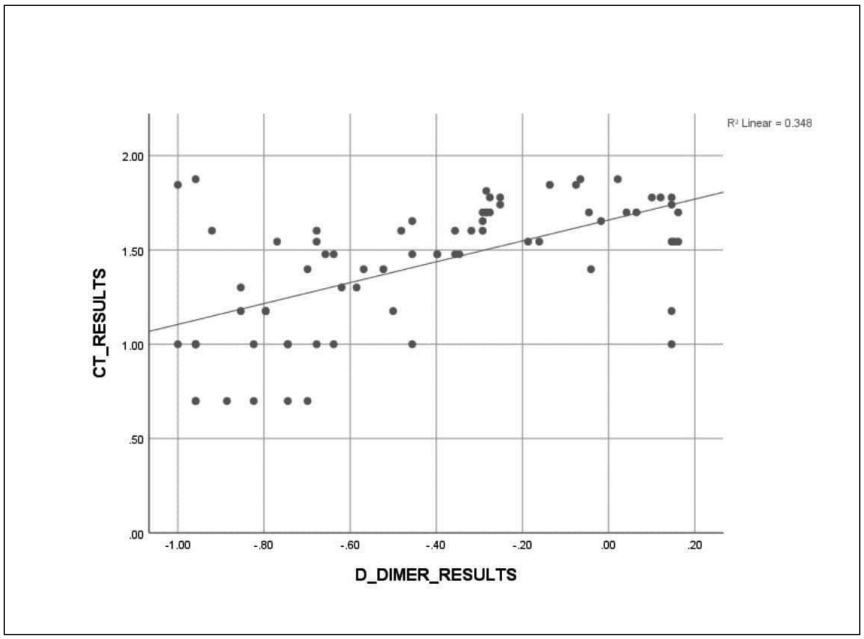


Fig. 4. Scattered dots showing the correlation between D-Dimer values and CT results among COVID-19 patients.

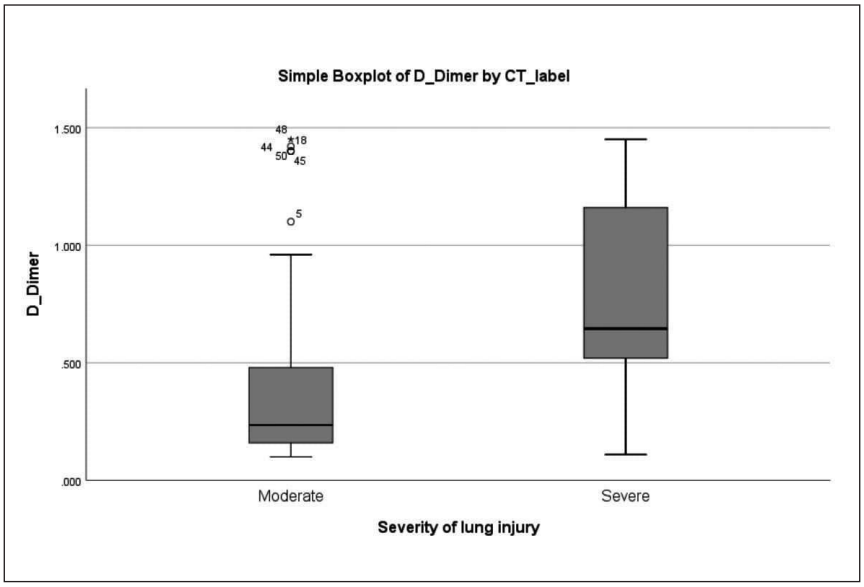


Fig. 5. Box plot of D-Dimer values distribution among severity of lung injury assessed by CT among COVID-19 patients.

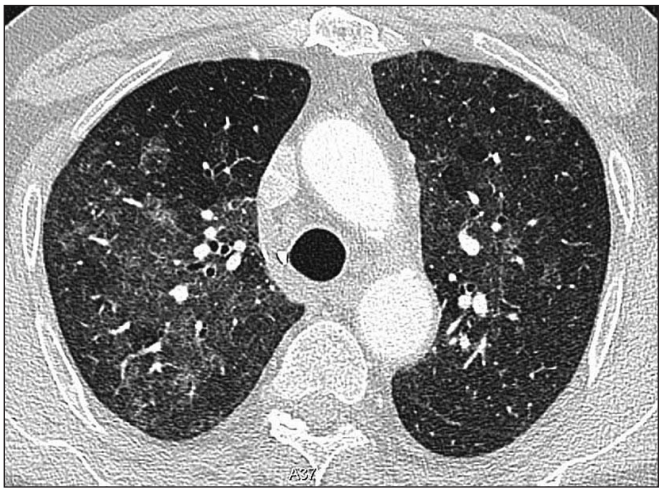


Fig. 6. Chest CT shows Ground Glass appearance with diffuse consolidations in severe acute inflammatory process in CAP (blue arrow).
Source: https://en.wikipedia.org/wiki/Ground-glass_opacity

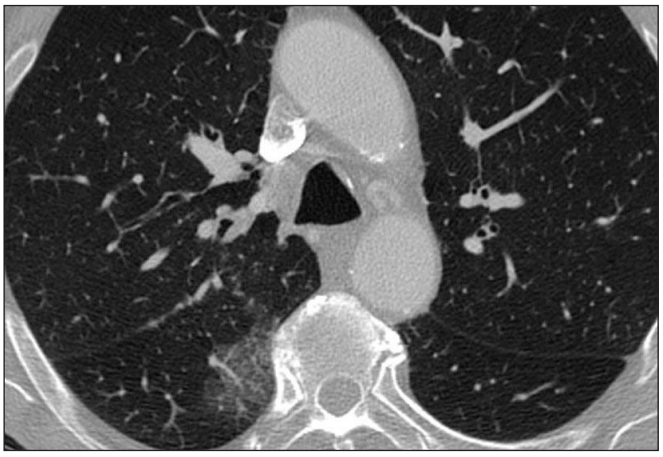


Fig. 7. Axial Section of high-resolution Chest CT that shows a ground glass opacity (blue arrow), which is persistent, rounded, and about 3 cm in diameter. Focal interstitial Fibrosis found by post mortem biopsy.
Source: https://commons.wikimedia.org/wiki/File:Fibrosis_focal_intersticial.jpg

ties in x-ray and dense attenuation in CT scan. This can happen as a result of presence of fluid that shifts air and diminish lung tissue markers, and can happen as well in airway collapse, pulmonary fibrosis and very rarely in normal lung, but eventually it is a good picture of Interstitial Lung Disease and Infections [23]. Although, GGO manifestation in chest CT is non-specific for SARS-CoV-2 infection, as several related lung disease tend to present such appearances, commonly seen in community acquired pneumonia (CAP) especially in viral pneumonia rather than non-viral type [24], and in spite of the simple GGO with different sizes and the density changes very slowly in contrast to CAP which characterized by its rapid consolidation [25], PCR is highly recommended in such inconclusive vague radiological appearance, as it considered the gold standard investigative [26-27] in some circumstances when the diagnosis is very urgent CT findings should be combined with the epidemiological history, clinical characteristics and haematological examination to improve the accuracy of acovid-19 diagnosis (Fig. 6).

Pulmonary lesions manifestation in CT imaging graded from light density with appearance of cloud, ground nodules with limited scopes in outer parts of the lungs among mild cases to completely white lung appearance in sever critical cases [28]. This alteration in CT imaging is highly associated with D-Dimer levels and some other inflammatory markers, which is seen obviously among patients with severe lung injury, as the disease progress and the number of dense lobes expand and increase, the obvious pulmonary fibrosis remained even after treatments (Fig. 7) [29].

In 2020, Yumeng Yao conducted a study on 248 confirmed cases withSARS-Cov2 infection, in Wuhan, China. The study reviewed all the involved cases retrospectively to demonstrate the impact and the relationship between D-Dimer levels and severity of lung injury assessed clinically, by chest CT, and oxygenation index, regarding chest CT, it concluded that D-Dimer levels are significantly elevated 5-folds in patients with severe COVID-19 infection (>50%) assessed by CT scan compared to (<30%) in non-COVID severe lung infections and can be considered as a prognostic factor for patient's mortality (Kendall'stau-b = 0.378, odds ratio= 3.93 p = 0.000) [30]. Wang J C et al. in 2019 discovered that healthy victims even with aggressive lung damage with fibrosis, the damaged lung tissue thrived significantly improving pulmonary fibrosis and enhance its absorption after initiation of treatment and close monitoring, and then the levels of D-Dimers are dramatically reduced even fall to the normal range [31]. A wide range of studies established to explore the exact relationship between D-Dimer levels and severity of lung injury, and all of these are consistent with these study findings, although some studies differ in design and population [32-34].

CONCLUSION

In COVID-19 infected patients, the Severity of lung injury assessed by chest computed tomography is positively correlated with D-Dimer levels, and it can be considered as an independent predictor of severe cases.

RECOMMENDATIONS

Early administration of anticoagulants and tPA (tissue plasminogen activator) as a prophylactic therapy can improve the outcome and reduce morbidity and mortality in COVID-19 patients.

REFERENCES

1. Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention . [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. Zhonghua Liu Xing Bing Xue Za Zhi 2020;41:145.
2. WHO. Coronavirus disease 2019 (COVID-19): situation report-79. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200408-sitrep-79-covid-19.pdf?sfvrsn=4796b143_6 [Access: 28.02 2020]
3. WHO Director-General's Opening Remarks at the Media Briefing on COVID-19 – 11 March 2020. https://www.who.int/dg/speeches/detail/who-director- general-s-opening-remarks-at-themedia-briefing-on-covid-19—11-March-2020 [Access: 18.03.2020].
4. Vu HT, Leitmeyer KC, Le DH, et al. Clinical description of a completed outbreak of SARS in Vietnam, February-May 2003. Emerg Infect Dis. 2004;10:334-338.
5. Donnelly CA, Malik MR, Elkholy A, Cauchemez S, Van Kerkhove M.D. Worldwide reduction in MERS cases and deaths since 2016. Emerg Infect Dis. 2019;25:1758-1760.
6. Fang Y, Zhang H, Xie J, Lin M, Ying L, Pang P, Ji W. Sensitivity of chest CT for COVID- 19: comparison to RT-PCR. Radiology 2020 Apr;295(1):208-209, doi: 10.1148/radiol.2020200280
7. Yaun M, Yin W, Tao Z, Tan W, Hu Y Association of radiological findings with mortality of patients infected with 2019 novel coronavirus in Wuhan, China. PLoS One. 2020 Mar 19;15(3):e0230548, doi: 10.1371/journal.pone.0230548. eCollection 2020
8. Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020;20:425-434.
9. Suzuki Y, Yasui H, Brzoska T, Mogami H, Urano T. Surface-retained tPA is essential for effective fibrinolysis on vascular endothelial cells. Blood 2011;118:3182-3185, doi: 10. 1182/blood-2011-05-353912
10. Cohen J, Normile D. New SARS-like virus in China triggers alarm. Science 2020;367:234-235, doi: 10.1126/science. 367.6475.234
11. Huang C, Wang Y, Li X, et al Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet Lond Engl 2020;395:497-506, doi: 10.1016/S0140-6736(20)30183-5
12. Zhou F, Yu T, Du R et al (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395:1054-1062, doi: 10.1016/S0140-6736(20)30566-3
13. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. N Engl J Med. 2020;9;383(2):120-128. doi: 10.1056/NEJMoa2015432.
14. Herrmann J, Mori V, Bates JHT, Suki B. Modeling lung perfusion abnormalities to explain early COVID-19 hypoxemia. Nat Commun. 2020 Sep 28;11(1):4883, doi: 10.1038/s41467-020-18672-6.
15. Novel Coronavirus Pneumonia Diagnosis and Treatment Guideline (6th edn). [Access: 03.03 2020].
16. Saeed GA, Gaba W, Shah A, et al. Correlation between Chest CT Severity Scores and the Clinical Parameters of Adult Patients with COVID-19 pneumonia. Radiol Res Pract. 2021 Jan 6;2021:6697677, doi: 10.1155/2021/6697677. eCollection 2021

17. Zhang H, Penninger JM, Li Y, et al. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46:586-590.
18. Poterucha TJ, Libby P, Goldhaber SZ. More than an anticoagulant: do heparins have direct anti-inflammatory effects? Thromb Haemost 2017;117:437-444.]
19. Liu PP, Blet A, Smyth D, et al. The science underlying COVID-19: implications for the cardiovascular system. Circulation 2020;142:68-78
20. Linkins LA, Takach Lapner S. Review of D-dimer testing: good, bad, and ugly. Int J Lab Hematol. 2017;39(Suppl. 1):98-103.
21. Lippi G, Bonfanti L, Saccenti C, Cervellin G. Causes of elevated D-dimer in patients admitted to a large urban emergency department. Eur J Intern Med. 2014;25(1):45-8.
22. Zhu J, Chen C, Shi R, Li B. Correlations of CT scan with high-sensitivity C-reactive protein and D-dimer in patients with coronavirus disease 2019. Pak J Med Sci. 2020;36(6):1397-1401.
23. Rohrich S, Prayer F, Prosch H. Differential Diagnosis of Diffuse Ground Glass Opacifications Made Easy. Poster: ECR 2019/ C-2055, doi: 10.26044/ecr2019/C-2055
24. Kim JE, Kim UJ, Kim HK, et al. Predictors of viral pneumonia in patients with community-acquired pneumonia. PLoS One. 2014 Dec 22;9(12):e114710.
25. Liu KC, Xu P, Lv WF, et al. Differential diagnosis of coronavirus disease 2019 from community-acquired-pneumonia by computed tomography scan and follow-up. Infect Dis Poverty. 2020;9:118.
26. Zhang NR, Wang LL, Deng XQ, et al. Recent advances in the detection of respiratory virus infection in humans. J Med Virol. 2020;92(4):408-17.
27. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-74.
28. Zhu J, Chen C, Shi R, Li B. Correlations of CT scan with high-sensitivity C-reactive protein and D-dimer in patients with coronavirus disease 2019. Pak J Med Sci. 2020 Sep-Oct;36(6):1397-1401, doi: 10.13929/j.issn.1003 3289.2020.02.01
29. Liu FM, Ding HL, Gong XM, Song L, Li H, Lu ZY. [Chest CT manifestations and clinical features of novel coronavirus 2019 (COVID-19) pneumonia]. Radiol Pract. 2020;35(3):266- 268.
30. Yao Y, Cao J, Wang, Q, et al. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. J Intensive Care 2020;8:49.
31. Wang JC, Liu JP, Wang YY, et al. [Chest CT imaging dynamic changes of patients with novel coronavirus 2019 (COVID-19) pneumonia]. J Zhejiang Univ (Med Sci). 2020;49(2):191-197.
32. Tang N, Li D, Wang X, et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18: 844-847.
33. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-1069.
34. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy 2020 Jul;75(7):1730-1741.

ORCID and contributionship:
Mustafa Abdul Ridha Alnowfal: 0000-0001-6690-5687 [A,E-F]
Nawfal Almubarak: 0000-0002-4736-8625 [B-C]
Murtadha A. Jeber: 0000-0002-5356-0496 [D-E]

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

CORRESPONDING AUTHOR
Nawfal Almubarak
Head of Arab Board Council, College of Medicine,
University of Basra, Iraq
e-mail: nawfalmubarak@yahoo.com

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 CONTENT OF ZINC AND CADMIUM IN BLOOD AND ORAL FLUID IN GENERALIZED PERIODONTITIS IN PEOPLE EXPOSED TO ADVERSE ENVIRONMENTAL FACTORS

DOI: 10.36740/WLek202110108

Zoryana B. Popovych, Mykola M. Rozhko, Iryna Z. Ostapyak, Oleksandra M. Ilnytska, Iryna Z. Chubiy, Yulia B. Bodnaruk

IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

The aim: To study the content of trace elements (cadmium and zinc) in the blood and oral fluid in people with generalized periodontitis and work and live permanently in adverse environmental conditions.

Materials and methods: In order to study the prevalence of periodontal diseases in adults living in areas with high level of soil contamination with heavy metal salts and working in the workplace with occupational hazards, there were studied 163 people who did not have somatic diseases, namely: 133 employees of Burshtyn Thermal Power Plant (TPP) and 30 persons who do not work at Burshtyn TPP.

Results: The results of biochemical examination of blood and oral fluid in persons with generalized periodontitis of the I, II degree of severity and being exposed to adverse environmental factors, show changes in the trace element spectrum of blood and oral fluid, namely: a decrease in amount of zinc and an increase in amount of cadmium, which may indicate the disorder of microelement metabolism under conditions of chronic influence of small doses of salts of heavy metals.

Conclusions: As a result of the performed study, a violation of micronutrient metabolism in biological fluids (blood and oral fluid) was found in persons exposed to adverse environmental factors.

KEY WORDS: ecology, dental morbidity, biochemical studies

Wiad Lek. 2021;74(10 p.I):2407-2411

INTRODUCTION

Public health – is one of the important indicators of the environmental condition in the country. There is the depletion of adaptive capacity and the associated steady growth of diseases, anthropo-ecological deformation of health, especially in children with the lowest threshold of sensitivity to xenobiotics in the intensive and long-term impact of adverse environmental factors on the human body. One of the most dangerous consequences of environmental pollution is an increase in the level of genetic disorders, which is manifested, in particular, in the growth of congenital anomalies in children.

Scientific studies [1-7] have confirmed that the pollution of the biosphere has led to changes in the natural spectrum of trace elements in tissues and organs, and this contributes to the development of anthropogenically dependent conditions, syndromes. Children are the most sensitive to the occurrence of metabolic-induced chronic pathologies. According to the studies [5], most people with various chronic diseases have a lack of magnesium, zinc, copper, silicon, chromium, mangan with an excess of aluminum and cadmium.

Scientists [8- 13] point at a positive correlation between environmental pollution, including heavy metals (Pb, Sr, Zr, As, Cd), and diseases such as allergic dermatitis, infectious skin lesions, numerical increase in lymph nodes of subcutaneous tissue, hyperplasia of the lymphoid ring of the pharynx, enlargement of the tonsils, chronic diseases of the ENT-organs.

It has been determined [12-14] that heavy metals, in particular lead and cadmium, bind sulfhydryl groups of glutathione and proteins, resulting in activation of lipid peroxidation of cell membranes, which causes a violation of their function. The final stage is the violation of the morphofunctional state of membranes and metabolic processes in them, the release of acid hydrolases, the general strengthening of hydrolytic processes in tissues, disintegration of membranes, destruction of their structure [5]. One of the options for the influence of cadmium on regulatory processes in the cell is its competition with calcium ions. Cadmium belongs to the group of toxic heavy metals, which even in microdoses can cause the development of pathological processes [5]; that's why the determination of its content in food is recognized by the Joint Committee of WHO as a mandatory control parameter. Dangerous xenobiotics form geochemical provinces with their increased content in the environment, from where they enter the body in

combination with water, air, food and form the so-called total daily dose [5, 8, 10, 13, 15].

In recent decades, there has been an increase in the number of eco-dependent diseases, including dental ones. Children are especially sensitive to the effects of adverse environmental factors [1-14], which is due to the functional immaturity of the adaptive and protective mechanisms of the child's organism. Dental diseases are among the most common, particularly in Ukraine. The reasons for the high prevalence of the dental diseases and their relationship with environmental living conditions have been studied at the Department of Postgraduate Dentistry of Ivano-Frankivsk National Medical University for a long time, some of research results have been already published [8, 9].

Ivano-Frankivsk region is located in the west of Ukraine, and, taking into consideration its natural conditions, it is the territory combining areas with almost no industrial enterprises' harmful emissions and areas where these emissions significantly exceed the allowable level. At the same time, the largest industrial potential of the western region of Ukraine is concentrated in the Ivano-Frankivsk region. These ones are mainly energy (Burshtyn Thermal Power Plant (TPP)), chemical (Kalush, concern "Oriana", JSC "Barva"), petrochemical ("Petrochemist of Prykarpattia") industry, timber industry enterprises and others creating a significant man-made burden on the environment. The third place in Ukraine among the top of 100 companies- pollutants of the ennvironment in terms of air emissions for several years is a separate division of "Burshtyn TPP" PJSC "DTEK Zakhidenergo" (Burshtyn, Halytskyy district), in particular, according to the Ministry of Energy and Environmental Protection of Ukraine in 2018 emissions of harmful substances of this Thermal Power Plant into the air amounted up to 182.922 tons, which is more than in 2017 – 158.556 tons, in 2019 – 169.888 tons. The results for 2020 have not been published yet. According to the results of the study of the Center for Bioelementology of Ivano-Frankivsk National Medical University in the area near the Burshtyn Thermal Power Plant – villages Zadnistriansk, Bovshiv, Kinashiv, Bilshivtsi, the cadmium content in soils exceeds the maximum allowable concentration (0.7 mg/kg) and ranges (from 1.2 mmg/kg up to 2.2 mg/kg).

THE AIM

The aim of our work was to study the content of trace elements (cadmium and zinc) in the blood and oral fluid in people with generalized periodontitis and work and live permanently in adverse environmental conditions.

MATERIALS AND METHODS

We've studied the prevalence and peculiarities of the clinical course of dental diseases in the population of the Western region of Ukraine, and won a competition for scientific-research work "Clinical effectiveness of comprehensive treatment of the diseases of hard tissues of the teeth and periodontal diseases in the population of environmentally unfavorable regions", funded by the Ministry of Health of Ukraine from the state budget.

The study has been conducted for several years.

In order to study the prevalence of periodontal diseases in adults living in areas with high level of soil contamination with heavy metal salts and working in the workplace with occupational hazards, there were studied 163 people who did not have somatic diseases, namely: 133 employees of Burshtyn Thermal Power Plant (TPP) and 30 persons who do not work at Burshtyn TPP. A comprehensive examination using clinical, biochemical, radiological methods of study was performed in 125 patients with generalized periodontitis (GP) of the I and II degrees of severity (64 patients with generalized periodontitis of the I degree and 61 – with generalized periodontitis of the II degree) and 30 people with intact periodontium.

Clinical examination and treatment of patients was performed on the basis of the clinic of the Burshtyn Thermal Power Plant and the clinic of the Department of Dentistry of the Educational-Scientific Institute of Postgraduate Education of Ivano-Frankivsk National Medical University.

All patients were performed a comprehensive examination with the inclusion of biochemical research methods. The obtained results were put in the patient's record and the developed examination card.

Biochemical studies of blood and oral fluid were performed at the Center for Bioelementology of Ivano-Frankivsk National Medical University (Director of the Center – Dr. habil. in Biological Sciences, Full Professor – Erstenyuk H.M.).

QUANTITATIVE DETERMINATION OF MICRO- AND MACROELEMENTS (CADMIUM, ZINC) IN BIOLOGICAL MATERIAL USING THE SPECTRAL METHOD

Biological fluids (blood and oral fluid) of patients were dried at a temperature of 70-80°C. After drying, it was calcined in a muffle furnace at a temperature of 450-500°C. Mineralization was carried out until the ash was free of coal impurities. Determination of the content of micro- and macroelements (cadmium, zinc) was performed in ash solutions on a C-115 atomic adsorption spectrophotometer in accordance with the requirements of GOST (government standard) 30178-96 and GOST (government standard) 26570-85.

RESULTS

The examined employees of Burshtyn Thermal Power Plant (TPP) (133 people) were diagnosed with generalized periodontitis of the I, II degrees of development, 125 of them – were determined chronic course of the disease and 8 patients – were determined the periodontal abscess, it was 93.98% and 6.02%, respectively. During the process of work, 125 employees of the Burshtyn Thermal Power Plant were diagnosed with generalized periodontitis of the chronic

Table I. Distribution of patients with generalized periodontitis of the I and II degree according to age

Age of patients (years)	The degree of development of generalized periodontitis			
	Generalized periodontitis of the I degree		Generalized periodontitis of the II degree	
	Abs. amount	%	Abs. amount	%
20-29	30	24.0	1	0.8
30-39	23	18.4	25	20.0
40-49	11	8.8	35	28.0
Totally	64	51.2	61	48.8

Table II. The content of trace elements in the blood and oral fluid of patients with generalized periodontitis (M±m)

Indices		Groups of patients		
		Healthy	Patients with generalized periodontitis of the I degree	Patients with generalized periodontitis of the II degree
		n=27	n=56	n=54
Cadmium, μg % /raw material	blood	0.92±0.07	1.44±0.05 p<0.05	1.87±0.067 p<0.001 p_1<0.001
	oral fluid	0.08±0.006	0.13±0.006 p<0.001	0.19±0.008 p<0.001 p_1<0.001
Zinc, μg% / raw material	blood	72.80±2.46	61.67±2.54 p<0.001	59.90±2.48 p<0.001 p_1<0.05
	oral fluid	64.60±2.42	52.95±2.46 p<0.001	44.10±2.44 p<0.001 p_1<0.01

Note: p – the probability of the difference between the indices of healthy individuals with generalized periodontitis of the I and II degrees,
p1 – the probability of the difference between the indices of generalized periodontitis of the I and II degrees.

course of the I, II degrees of severity, among them 64 patients were diagnosed generalized periodontitis of the I degree and 61 – were diagnosed the II degree of the disease (Table I).

The examined patients with generalized periodontitis had different work experience at the Burshtyn Thermal Power Plant (TPP). The vast majority of patients with generalized periodontitis of the I degree worked at the Thermal Power Plant for 1-10 years, namely: 57.81% – from 1 to 5 years and 37.50% – from 6 to 10 years and 4.69% – from 11 to 15 years.

In patients with generalized periodontitis of the II degree, the distribution according to the length of work was different. Thus, only in 6.56% of these patients the work experience was 6-10 years, in 27.87% – from 11 to 15 years and in 65.57% – more than 15 years.

The results of our research on the level of trace elements in the blood and oral fluid of patients employed at the Burshtyn Thermal Power Plant are represented in Table II.

Changes in the content of trace elements clearly depend on the severity of generalized periodontitis (see Table II).

In the spectrogram of trace elements, among those we have determined, a special position is occupied by zinc, which plays an important role in the metabolic processes of cells and tissues. The level of zinc in the blood of patients with generalized periodontitis decreased and was (61.67 ± 2.54) μg% / raw material in generalized periodontitis of the I degree, and of the II degree – (59.90±2.51) μg % /raw material, exceeding the rate of healthy individuals – (72.80±2.46) mcg% /raw material at 15.29% and 17.72%, respectively, p<0.001 (Fig. 1).

With the progression of the disease, there were deeper changes in the content of this element in the oral fluid, namely: its significant decrease at 18.04% in generalized periodontitis of the I degree and at 31.74% in generalized periodontitis of the II degree, compared with the corresponding indicator in healthy people (p<0.001).

DISCUSSION

An important role in the regulation of metabolic processes belongs to trace elements, which are cofactors of enzymes, as well as structural components of the inorganic matrix of bone tissue.

There is a correlation between the work experience and the degree of generalized periodontitis.

The obtained data indicate a violation of the trace element status, which is expressed by the changes in the content of essential trace elements against the background of increasing concentration of the toxic element cadmium [5, 10].

In employees of Burshtyn TPP, who were diagnosed with generalized periodontitis of varying severity, the cadmium content in the studied biosubstrates was significantly increased. Thus, in patients with generalized periodontitis of

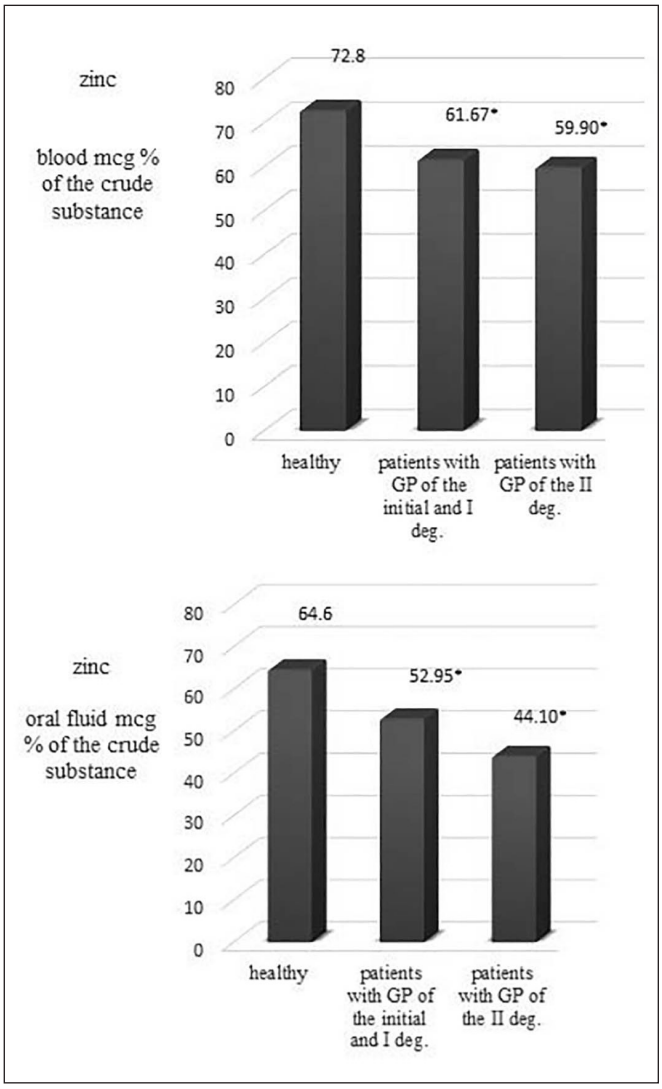


Fig. 1. The content of zinc (μg% /raw material) in the blood (A) and oral fluid (B) of patients with generalized periodontitis.
Note. * – the difference between healthy individuals and patients with generalized periodontitis of the I degree and generalized periodontitis of the II degree is statistically significant.

the I degree of severity, the content of cadmium in the blood exceeded these indices in healthy individuals at 56.52% ($p<0.05$), and in oral fluid – at 103.26% ($p<0.001$). In patients with generalized periodontitis of the II degree, we've observed even greater increase in the level of cadmium in the blood – at 62.5% ($p<0.01$), in the oral fluid – at 137.5% ($p<0.001$).

In such circumstances, it is important to study the level of essential trace elements that are antagonists to cadmium ions, zinc in particular.

The results of the performed study indicate a violation of the content of zinc in the blood and oral fluid of patients with generalized periodontitis, who are employees of Burshtyn TPP.

Thus, the results of the study show the changes in the microelement spectrum of blood and oral fluid, namely: a decrease in zinc, which may indicate a violation of micronutrient metabolism under chronic exposure to low doses of heavy metal salts [12-15]. Therefore, we can conclude that the severity of generalized periodontitis is due to the changes in the content of trace elements that affect the permeability of cell membranes, the activity of enzymes that determine the compensatory mechanisms in tissues [5, 8, 12, 13], including periodontal tissues.

This is confirmed by more significant violations of the microelement spectrum of blood and oral fluid in patients with disease progression (generalized periodontitis of the II degree), which were accompanied by zinc content decrease at 66.67% and at 31.74%, ($p<0.001$). At the same time, the level of heavy metal – cadmium – has increased 1.56-fold ($p<0.05$), in the blood and 1.62-fold ($p<0.001$) – in the oral fluid of patients with generalized periodontitis of the I degree, and in patients with generalized periodontitis of the II degree of severity – 2.02-fold and 2.37-fold ($p<0.001$), respectively.

CONCLUSIONS

As a result of the performed study in people with generalized periodontitis and exposed to small doses of heavy metal salts for a long time, there was revealed a violation of biometals in blood and oral fluid – an increase in cadmium and a decrease in zinc levels, which can affect overall resistance of the body.

Taking this into consideration, we believe that the development of prevention schemes should be differentiated, with due regard for the peculiarities of every territory and region. When developing an optimal, regionally oriented scheme of preventive measures for the population that is permanently living in environmentally unfavorable conditions, it is necessary to combine in a certain sequence of factors that increase nonspecific resistance and normalize the functional abilities of the organism, and specific therapy, which is used for the prevention and treatment of dental diseases. The choice of prevention and treatment should take into account the specific peculiarities of each territory, which determines the amount of trace elements in water, food products, and the level of negative human-induced burden that have a total impact on the human body.

REFERENCES

1. Serdyuk A.M. Profilaktychne spryamuvannya medytsyny yak stratehiya reform okhorony zdorov'ya [Preventive medicine as a strategy for health care reform.]. Journal of the National Academy of Medical Sciences of Ukraine. 2011; 17 (1): 39-43. (In Ukrainian).
2. Ertec S., Cicero A.F.G., Caglar O. et.al. Relationship between serum zinc levels, thyroid hormones and thyroid volume following successful iodine supplementation. Hormones. 2010; 9(3): 263-268.
3. Korzun V.N. Vplyv ekolohichnykh i tekhnohennykh chynnykiv na funktsiyu shchytopodibnoyi zalozy. In: Korzun VN. ,Vorontsova TO., eds. Ekolohiya i zakhvoryuvannya shchytopodibnoyi zalozy. Kyiv., Medinform 2018, 327 p.
4. Moiseyenko R.O., Dudina O.O., Hoyda N.H. Analiz stanu zakhvoryuvanosti ta poshyrenosti zakhvoryuvan' u ditey v Ukrayini za period 2011-2015 roky [Analysis of the incidence and prevalence of diseases in children in Ukraine for the period of 2011-2015]. Modern Pediatrics. 2017; 2(82): 17-27. (In Ukrainian).

5. Dmytrukha N.M. Imunotoksychna diya svyntsyu i kadmiyu yak hihiyenichna problema (do patohenezu, diahnostyky ta profilaktyky intoksykatsiy vazhkymy metalamy): avtoref. dys. na zdobuttya nauk stupenya d-ra med. nauk: spets.[Immunotoxic effects of lead and cadmium as a hygienic problem (to the pathogenesis, diagnosis and prevention of heavy metal intoxication] Author's abstract. Doctor of Medical Sciences. Kyiv, 2011. 32 p. (In Ukrainian).
6. Antypkin Y.H., Volosovets' O.P., Maydannyk V.H. et al. Stan zdorovya dytyachoho naselennya – maybutnye krayiny (chastyna 1). [The state of health of children – the future of the country (part 1)] For cite: Zdorov'e Rebenka. 2018; 13(1):1-11. doi: 10.22141/2224-0551.13.1.2018.127059.
7. Ostapko O.I. Naukove obgruntuvannya shlyakhiv ta metodiv profilaktyky osnovnykh stomatolohichnykh zakhvoryuvan' u ditey v rehionakh z riznym rivnem zabrudnennya dovkillya [Scientific substantiation of ways and methods of prevention of major dental diseases in children in regions with different levels of environmental pollution]. Author's abstract. Doctor of Medical Sciences. 2011; 41p. (In Ukrainian).
8. Popovych Z.B., Rozhko M.M. Osoblyvosti profilaktyky stomatolohichnykh zakhvoryuvan' u ditey, yaki prozhyvayut' na terytoriyakh z nyz'kym vmistom deyakykh mikroelementiv [Peculiarities of prevention of dental diseases in children living in areas with low content of some trace elements]. Dentistry news. 2018; 4: 22-25. (In Ukrainian).
9. Popovych Z., Rozhko M., Ostapyak I., Oktysyuk Y. Biochemical indexes of mineral metabolismin patients from the polluted regionaffected by chronic periodontitis. Pharmacia. 2020; 67(1): 23–28. doi:10.3897/pharmacia.67.e36150.
10. Khomenko L.O., Ostapko O.I., Timofeeva O.O. Stan stomatolohichnoho zdorovya ditey, yaki prozhyvayut' v umovakh ekolohichnoyi kryzy [The level of dental health of children living in an ecological crisis]. Scientific Bulletin of the Bogomolets National Medical University. 2006; 2: 110-114. (In Ukrainian).
11. Jyothirmayi B., Vasantha M. Study of zinc and glycated Hb levels in diabetic complications. Int J Pharm Clin Res. 2015; 7(5):360–363.
12. Hara T., Takeda T.A., Takagishi T. et al. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. J Physiol Sci. 2017; 67(2):283–301.
13. Rotter I., Kosik-Bogacka D., Dolegowska B. et al. Relationship between the concentrations of heavy metals and bioelements in aging men with metabolic syndrome. Int J Environ Res Public Health. 2015;12:3944–3961.
14. Stelmakhivska V.P., Berzin V.I. Zdorov"ya ditey ta pidlitkiv ta dovkillya [Children's and adolescents' health and the environment]. Problems of ecology and medicine. 2008; 12 (1-2): 33-36. (In Ukrainian).
15. Ghasemi A., Zahediasl S., Hosseini-Esfahani F., Azizi F. Gender differences in the relationship between serum zinc concentration and metabolic syndrome. Ann Hum Biol. 2014;41(5):436–442.

ORCID and contributionship:
Zoryana B. Popovych: 0000-0003-4472-3748 [A,B,D]
Mykola M. Rozhko: 0000-0002-6876-2533 [F]
Iryna Z. Ostapyak: 0000-0002-3042-7019 [B,C]
Oleksandra M. Ilnytska: 0000-0002-9294-4783 [E]
Iryna Z. Chubiy: 0000-0001-9368-0874 [E]
Yulia B. Bodnaruk: 0000-0001-9723-6279 [E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Zoryana B. Popovych
Ivano-Frankivsk National Medical University
2 Halytska St., 76018 Ivano-Frankivsk, Ukraine
tel: +380501545865
e-mail: zpopovych6@gmail.com

Received: 05.02.2020
Accepted: 08.08.2021

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

EVALUATION OF HORMONAL FUNCTION IN WOMEN WITH CERVICAL INSUFFICIENCY AND INFERTILITY IN THE HISTORY

DOI: 10.36740/WLek202110109

Oksana M. Perkhulyn[1], Lyudmyla V. Pakharenko[1], Vladyslav S. Sukhin[2], Oleksiy V. Saltovskiy[3], Viktoriia M. Kovalchuk[4], Hanna I. Hranovska[2], Olha V. Kravchenko[5]

[1]IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE
[2]INSTITUTE OF MEDICAL RADIOLOGY OF S. P. GRIGORIEV NATIONAL ACADEMY OF MEDICAL SCIENCES OF UKRAINE, UKRAINE
[3]KHARKIV NATIONAL MEDICAL UNIVERSITY, UKRAINE
[4]MEDICAL CENTER "EVVIVA", UKRAINE
[5]POLTAVA REGIONAL CLINICAL ONCOLOGICAL DISPENSARY OF POLTAVA REGIONAL COUNCIL, UKRAINE

ABSTRACT

The aim: To assess the levels of hormones in women with cervical insufficiency and infertility in the history in the II trimester of gestation.

Materials and methods: 120 pregnant women with cervical insufficiency and anovulatory infertility in the history were examined in the II trimester of gestation: in the I group (60 persons) pregnancy occurred after hormonal treatment of infertility, in the II group (60 individuals) – after in vitro fertilization. 30 pregnant women without cervical insufficiency and a history of infertility were controls. The levels of estradiol, progesterone, placental lactogen, prolactin and cortisol were determined in the blood serum.

Results: The concentration of maternal progesterone was lower in the persons in the I group on 12.36 %, in the II group – on the 15.37 % ($p=0.03$) compared to the healthy women. Cortisol and prolactin amounts were statistically higher in I and II groups ($p<0.001$) than in controls. While the levels of estradiol and placental lactogen were slightly less in the subjects with cervical insufficiency and a history of anovulatory infertility compared to the healthy women.

Conclusions: In pregnant women with cervical insufficiency and a history of anovulatory infertility in the II trimester of gestation there are decrease progesterone level and high prolactin and cortisol concentrations in blood serum. The changes in estradiol and placental lactogen amounts are not significant compared to healthy women.

KEY WORDS: cervical insufficiency, anovulatory infertility, hormones

Wiad Lek. 2021;74(10 p.I):2412-2416

INTRODUCTION

Physiological pregnancy and labor are one of the main priority aspects of modern obstetrics. Nowadays the percent of the complications during pregnancy growths up that leads to the negative obstetrical and perinatal outcomes. According to the data of the World Health Organization 15 million newborns are born before 37 completed weeks of gestation, and the frequency of preterm birth varieties from 5 % to 18 % of babies born [1]. There are different risk factors of prematurity – gestational complications such as placental disorders, diabetes, hypertension, cervical insufficiency (CI), treatment of infertility with additional reproductive technology (ART), such as in vitro fertilization (IVF), donor oocytes and/or thawed embryos [2]. Thus, especially difficulties with the gestational period have the women with a history of infertility. In Ukraine, more than 15 % of married couples are infertile [3]. Such women usually need specific and complex medical treatment for pregnancy occurs. So, the correction of these hormonal problems can lead to pregnancy, but such gestation is usually complicated. Pregnancy loss, missed abortion, miscarriage, premature labor, CI, negative perinatal outcomes are closely related to pregnancies after ART [4, 5].

Cervical insufficiency is one of the reasons for preterm labor and its rate is approximately 1 % [6] but its frequency is much higher in the patients after IVF – up to 9.7-14.4 % [7]. Generally, most of the cases of CI development are connected with organic pathology – cervical trauma during previous labor, gynecological manipulations on the cervix. But also hormonal dysbalance, which is present in the women with anovulatory infertility, can play an important role in the genesis of functional CI.

THE AIM

The aim of the study was to assess the levels of hormones in women with cervical insufficiency and infertility in the history in the II trimester of gestation.

MATERIALS AND METHODS

We examined 120 pregnant women with cervical insufficiency in the term of 19-22 weeks of gestation. The diagnosis of CI was based according to the transvaginal ultrasound criteria: the length of the cervix is 25 mm and less, V-shaped transformation of the cervical canal

Table I. Reproductive characteristics of observed groups (abs., (%))

Parameter	I group (n=60)	II group (n=60)	Control group (n=30)
Age:			
till 19 years	-	-	1 (3.33)
19-34 years	55 (91.67)	50 (83.33)	26 (86.67)
35 and more years	5 (8.33)	10 (16.67)	3 (10.00)
Pregnancy:			
the first	29 (48.33)	39 (65.00)	17 (56.67)
the second	26 (43.34)	10 (16.67)	8 (26.67)
the third and more	5 (8.33)	11 (18.33)	5 (16.66)
Labor:			
null	41 (68.33)	55 (91.67)	19 (63.33)
one	17 (28.34)	5 (8.33)	8 (26.67)
two and more	2 (3.33)	-	3 (10.00)
Miscarriage	7 (11.67)	9 (15.00) •	2 (6.67)
Missed abortion	3 (5.00)	3 (5.00)	-
Molar pregnancy	1 (1.67)	1 (1.67)	-
Induced abortion	3 (5.00)	5 (8.33) °	2 (6.67)
Ectopic pregnancy	1 (1.67)	5 (8.33)	-

Notes: • - three of nine persons had two miscarriages; ° - one from five women had two induced abortions.

Table II. The levels of hormones in the blood serum in examined patients

Hormone	I group (n=60)	II group (n=60)	Control group (n=30)
Estradiol, pg/ml	9414.18±182.39	9243.12±199.64	9826.43±286.38
Progesterone, ng/ml	46.29±1.38	44.70±1.93*	52.82±3.18
Placental lactogen, mg/l	2.64±0.09	2.53±0.11	2.86±0.19
Prolactin, ng/ml	231.02±7.91*	269.07±10.39*	162.33±10.76
Cortisol, nmol/L	534.57±18.22*	583.56±17.59*	409.04±25.09

Note: * – the statistical significance of differences of indicator relative to the control group ($p<0.05$).

on 40 % and more [8]. All these persons in anamnesis had infertility associated with anovulation. According to the type of the treatment of infertility the women with CI were divided into two groups. Thus, the I group consisted of 60 patients with CI and infertility in whom the pregnancy occurred after hormonal treatment (ovarian stimulation with clomiphene citrate, gonadotropin-releasing hormone agonists). 60 women with CI and infertility who became pregnant after the use of ART – in vitro fertilization – formed the II group. In the I trimester of pregnancy persons in the I group received vaginal micronized progesterone 200 mg ones a day, in the II group – 400 mg. Infertility was diagnosed according to the recommendations of the World Health Organization [9]. The control group involved 30 women with physiological pregnancy and without a history of infertility. Inclusion criteria: singleton pregnancy, CI, infertility associated with anovulation, written consent of the patient. Exclusion criteria: multiple pregnancy, antiphospholipid syndrome, thrombophilia, pregnancy complicated with ovarian hyperstimulation syndrome, cytogenetic causes of pregnancy loss induced by IVF, male infertility, connective tissue dysplasia, increased risk of chromosomal fetal abnormalities according to first or second genetic screening. The study was carried in City Clinical Perinatal Centre (Ivano-Frankivsk, Ukraine) and approved by the Ethics Commission at Ivano-Frankivsk National Medical University (protocol 97/17, 19.10.2017).

ELISA method was used to determine hormones in the serum blood in pregnant women. The levels of hormones were studied in the term of the 19-22 weeks of pregnancy after a confirmed diagnosis of CI. The concentrations of estradiol, progesterone, placental lactogen, prolactin, and cortisol were determined with reagents "IMMULITE 2000 Estradiol", "IMMULITE 2000 Progesterone", "IMMULITE 2000 Placental lactogen", "IMMULITE 2000 Prolactin" and "IMMULITE 2000 Cortisol" respectively.

Statistical data were analyzed by the program Statistica 6.0. We calculated arithmetic mean value, average standard error, criterion χ^2 (Yates corrected Chi-square), the nonparametric Mann-Whitney test was used to compare two independent groups by a single feature. The difference between the values was considered significant by $p\leq0.05$.

RESULTS

Our data demonstrated that the average age of women with the history of infertility after IVF (31.42±0.56 years,

p<0.001) was significantly higher compared to control persons (27.30±0.92 years). While in the II group there was no considerable difference in average age of examined patients (29.07±0.59 years) compared to controls. Also, there was no distinction in the age structure between individuals of all groups (table I). Persons of active reproductive age (20-34 years old) predominated in all groups. The number of primigravida subjects over multigravida ones was more in the II and control groups, multigravida women were in majority in the I group, but the difference between individuals with the first pregnancy and the second or more pregnancies was not significant. At the same time, 55 (91.67 %) pregnant women with CI after IVF were going to deliver at the first time, that was in 1.45 and 1.34 times more than in control group (63.33 %; χ^2=9.13, p=0.003) and in the I group (68.33 %; χ^2=8.80, p=0.003) respectively. In the I group primary infertility was diagnosed in 29 (48.33 %) individuals, secondary one – in 31 (51.67 %), in the II group – 39 (65.00 %) and 21 (35.00 %) women respectively.

Endometriosis was the most spread gynecological pathology among the patients with the history of infertility – 22 (36.67 %) women in the I group and 29 (48.33 %) subjects in the II. In the I group hyperprolactinemia was in the second place among gynecological diseases – 19 (31.67 %) individuals, 10 (16.67 %) women had diminished ovarian reserve, 9 (15.00 %) – thyroid diseases, 3 (5.00 %) – uterine myoma. In the II group besides endometriosis, 23 (38.33 %) patients were diagnosed polycystic ovary syndrome, 16 (26.67 %) – diminished ovarian reserve, 7 (11.67 %) – hyperprolactinemia, 5 (8.33 %) – uterine myoma and 2 (3.33 %) – pathology of the thyroid gland. Only 2 (6.67 %) controls persons had endometriosis.

It was found some variations in the concentrations of hormones between control persons and women in the I and II groups (table II). The level of estrogen and placental lactogen in the blood serum in the patients with CI and infertility in both groups was slightly less than in healthy subjects. The amount of progesterone was lower in individuals in the I group on 12.36 %, in the II – on the 15.37 % (p=0.03) compared to the healthy persons. The most significant changes related to the levels of cortisol and prolactin. So, the concentration of prolactin was higher in the women in the I group on 42.32 % (p<0.001), in the II group – on the 65.75 % (p<0.001) compared to the control persons. A similar trend observed regarding the amount of cortisol. Its level was on the 30.69 % (p<0,001) and 42.67 % (p<0.001) more in the I and II group respectively compared to the control individuals.

DISCUSSION

Numerous researches indicate the hormonal changes in the women after ART in the I trimester of pregnancy compared to spontaneous pregnancy. Thus, Vygivska LM and Nykoniuk TR found that the rate of pregnancy loss in the patients with endocrine infertility was determined in 4.5 and 5.8 times more often compared with the subjects with tubal infertility and male infertility respectively [10]. They estimated that in the women with endocrine infertility and ART the concentration of estradiol in blood serum in the I trimester of gestation was in 2 times higher compared to the women without infertility and use of ART, in individuals with tubal infertility – in 1.6, male infertility – 1.3 times more than in controls. The authors associated such hyperestrogenism with the use of gonadotropin-releasing hormone agonists and human menopause gonadotropins for ovarian stimulation. At the same time, the level of progesterone was slightly less in subjects with infertility that can be explained that the pregnant women with ART take progesterone drugs in the I trimester of gestation [10]. However, individuals after ART have higher cortisol level at the beginning of gestation compared with healthy women with spontaneous conceived [11]. Furthermore, according to the data of Vygivska LM et al. the amount of cortisol was significantly greater in the I, II, III trimester of gestation in the pregnant persons after ART compared to control individuals (p<0.05), as well as prolactin concentration, which was higher in such patients during the whole gestational period (p<0.05). The scientists believe that such changes in the amount of theses hormones are connected with increased level of state and trail anxiety. Grossi E. et al. studied the concentrations of 17β-estradiol and progesterone in venous blood of women with spontaneous singleton pregnancy between 5^{+0} and 13^{+6} weeks of gestation. Their results indicate the presence of specific week variations of 17β-estradiol in the I trimester that can be helpful for assessment of the course of twin gestation and pregnancy after ART [12]. It is known that there is a higher concentrations of β-chorionic gonadotropin and estradiol in maternal blood samples by twin pregnancy compared to singleton pregnancy after the use of ART in the first trimester of gestation [13].

Cervical insufficiency is mostly diagnosed in the II trimester of gestation, so, the results of hormonal variations in patients with CI commonly regard the second part of pregnancy (II or the III trimester). Impairment of the cervix obstructive function is also relative to the variations of estradiol and progesterone in the blood serum in pregnant women [14]. Estradiol level in the persons with CI in the II and III trimesters was correlated to the control indices of the physiological pregnancy. However, the progesterone concentration was almost in 2 times less compared to the individuals with normal cervical obstructive function. Such differences in the concentrations of the hormones lead to relative hypoprogesteronemia [14, 15].

According to the research of Patil AS et al. the maternal amount of progesterone and its metabolites, especially 11-deoxycorticosterone, may have meaning in the development of spontaneous preterm delivery and increases its risk. It was found that the concentration of deoxycorticosterone at the end of the I trimester and the beginning of the II trimester was associated with spontaneous delivery until 32 weeks

of gestation. The ratio of 11-deoxycorticosterone / 16-alpha-hydroxyprogesterone was higher in women with preterm labor until 32 weeks [16].

We did not find scientific publications about estradiol, progesterone, placental lactogen, prolactin and cortisol concentrations in blood serum in pregnant women in the II trimester of gestation, who conceived after anovulatory infertility and were diagnosed cervical insufficiency. So, the results of our research demonstrated that in the II trimester of gestation the there is a lower concentration of progesterone in the pregnant women with CI and infertility in the history, especially in patients conceived after IVF ($p<0.05$), compared to controls, that corresponds to the data of other scientists. But it's worth mentioning that such results of progesterone concentration were obtained despite the fact that patients in the II group received progesterone drugs. Also, we found the tendency to decrease of placental lactogen amount in women with CI and infertility in the history. The higher levels of cortisol and prolactin in blood serum in women with CI and infertility are consistent with other studies that demonstrate the similar increased parameters in patients who have CI, as well in pregnant persons after the use of ART. According to the results of this research it is worth to discuss the possibility to prolong to use vaginal progesterone in the II trimester of gestation in women, who conceived after anovulatory infertility, as well as about additional psychological support for decrease of stress-related hormones such as prolactin and cortisol.

CONCLUSIONS

In the II trimester of gestation the concentration of progesterone in blood serum of pregnant women with cervical insufficiency and anovulatory infertility in the history, expressly in persons after IVF, is significantly lower compared to persons without cervical incompetence and infertility and amounts of prolactin and cortisol are significantly higher. At the same time, there was no pronounced difference between levels of estradiol and placental lactogen in women with cervical insufficiency and anovulatory infertility and controls, but there is a trend to decrease of these hormones in the II trimester.

REFERENCES

1. World Health Organization. Preterm birth. https://www.who.int/news-room/fact-sheets/detail/preterm-birth. [Accessed September 8, 2019].
2. Luke B., Brown M.B., Wantman E. et al. Risk of prematurity and infant morbidity and mortality by maternal fertility status and plurality. J Assist Reprod Genet. 2019;36(1):121-138. doi: 10.1007/s10815-018-1333-z.
3. Vspomogatel'nye reproduktivnye tekhnologii. Opyt ispol'zovaniya VRT v SSHA, Evrope, Izraile i Ukraine [Assisted reproductive technologies. Experience of the use of ART in USA, Europe, Israel and Ukraine]. Slovo o zdorov'e. 2017; 2 (8): 6–10. (In Russian).
4. Luke B. Pregnancy and birth outcomes in couples with infertility with and without assisted reproductive technology: with an emphasis on US population-based studies. Am J Obstet Gynecol. 2017;217(3):270-281. doi: 10.1016/j.ajog.2017.03.012.
5. Stern J.E., Liu C.L., Cabral H.J. et al. Birth outcomes of singleton vaginal deliveries to ART-treated, subfertile, and fertile primiparous women. J Assist Reprod Genet. 2018;35(9):1585-1593. doi: 10.1007/s10815-018-1238-x.
6. Brown R., Gagnon R., Delisle M.F. Cervical insufficiency and cervical cerclage. J Obstet Gynaecol Can. 2019;41(2):248-263. doi: 10.1016/j.jogc.2018.11.028.
7. Trifonova N.S., Zhukova E.V., Grineva A.M. et al. Klinicheskie osobennosti techeniya beremennosti, rodov i perinatal'nye iskhody u zhenshchin posle ekstrakorporal'nogo oplodotvoreniya s primeneniem donorskih ovocitov [Clinical features of the course of pregnancy, labor, delivery, and perinatal outcomes in women after in vitro fertilization using donor oocytes]. Rossijskij vestnik akushera-ginekologa. 2017; 1:46-52. (In Russian).
8. Klinichnyi protokol «Nevynoshuvannia vahitnosti». Nakaz №624 Ministerstva okhorony zdorovia Ukrainy vid 03.11.2008. [Clinical protocol «Miscarriage». Order №624 of the Ministry of Health of Ukraine dated 03.11.2008.]. https://zakon.rada.gov.ua/rada/show/v0624282-08#Text. (In Ukrainian).
9. Zegers-Hochschild F., Adamson G.D., Mouzon de J. et al. on behalf of ICMART and WHO. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Human Reproduction. 2009; 24(11): 2683–2687.
10. Vyhivska L.M., Nykoniuk T.R. Etiopatohenetychni aspekty perebihu I trymestra vahitnosti u zhinok pislia zastosuvannia dopomizhnykh reproduktyvnykh tekhnolohii [Ethiopathogenetic aspects of the I trimester pregnancy course in the women after the use of assisted reproductive technologies]. Zdorov'ia zhinky. 2017; 4 (120): 98-101. (In Ukrainian).
11. Vyhivska L.M., Usevych I.A., Maidannyk I.V. et al. Osoblyvosti psykhoemotsiinoho stanu ta bioprodukuvannia stres-asotsiiovanykh hormoniv u vahitnykh pislia zastosuvannia dopomizhnykh reproduktyvnykh tekhnolohii [Features of psycho-emotional state and bioproduction of stress-associated hormones in the pregnant women after the use of assisted reproductive technologies.]. Zdorov'ia zhinky. 2018; 6 (132): 118-121. (In Ukrainian).
12. Grossi E., Parisi F., Duca P. et al. Maternal Estradiol and Progesterone Concentrations Among Singleton Spontaneous Pregnancies During the First Trimester. J Endocrinol Invest. 2019;42(6):633-638. doi: 10.1007/s40618-018-0961-6.
13. Póvoa A., Xavier P., Matias A. et al. First Trimester β-hCG and Estradiol Levels in Singleton and Twin Pregnancies After Assisted Reproduction. J Perinat Med. 2018;46(8):853-856. doi: 10.1515/jpm-2017-0132.
14. Zhabchenko I.A., Oleshko V.F. Rol hormonalnoho ta obminnoho dysbalansu v rozvytku porushen obturatsiinoi funktsii shyiky matky ta sposoby yoho korektsii [The role of hormonal and metabolic imbalance in the development of disorders of the cervical obstruction function and methods of its correction]. Medychni aspekty zdorov'ia zhinky. 2017; 2 (107): 5-14. (In Ukrainian).
15. Zhabchenko I.A., Oleshko V.F., Bondarenko O.M. et al. Osoblyvosti hormonalnoho homeostazu vahitnykh iz funktsionalnym porushenniam obturatsiinoi funktsii shyiky matky [Features of hormonal homeostasis in pregnant women with functional disorders of the cervix obstructive function]. Reproduktyvna endokrynolohiia. 2016; 5(31): 85-89. (In Ukrainian).
16. Patil A.S., Gaikwad N.W., Grotegut C.A. et al. Alterations in Endogenous Progesterone Metabolism Associated With Spontaneous Very Preterm Delivery. Hum Reprod Open. 2020;2:007. doi: 10.1093/hropen/hoaa007.

ORCID and contributionship:
Oksana M. Perkhulyn: 0000-0002-0033-5156 [B-D]
Lyudmyla V. Pakharenko: 0000-0003-4774-8326 [A, D-F]
Vladyslav S. Sukhin: 0000-0002-4403-3707 [A, F]
Oleksiy V. Saltovskiy: 0000-0002-1597-7493 [A, D, F]
Viktoriia M. Kovalchuk: 0000-0002-6597-5154 [C, E]
Hanna I. Hranovska: 0000-0002-1238-5855 [C, F]
Olha V. Kravchenko: 0000-0002-2562-7497 [C, E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Lyudmyla V. Pakharenko
Ivano-Frankivsk National Medical University
2 Halytska st., 76018 Ivano-Frankivsk, Ukraine
tel: +380974306921
e-mail: ludapak@ukr.net

Received: 16.09.2020
Accepted: 15.08.2021

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

ASSOCIATION BETWEEN LEUKOCYTES COUNT AND THE SEVERITY OF COVID-19 INFECTION

DOI: 10.36740/WLek202110110

Ahmed Hamza Ajmi[1], Wassan Abdul-Kareem Abbas[2], Dalya Basil Hanna[2], Maysaa Ali Abdul Khaleq[3]
[1]REPUBLIC OF IRAQ HEALTH, ENVIRONMENT THE STATE COMPANY FOR MARKETING DRUGS AND MEDICAL APPLIANCES, BAGHDAD, IRAQ
[2]DEPARTMENT OF CLINICAL LABORATORY SCIENCES, COLLEGE OF PHARMACY, MUSTANSIRIYAH UNIVERSITY, BAGHDAD, IRAQ
[3]COLLEGE OF PHARMACY, UNIVERSITY OF MASHREQ, BAGDAD, IRAQ

ABSTRACT

The aim: To find an association of COVID-19 on different types of leukocytes either count increasing or decreasing.

Materials and methods: A cross sectional study conducted from October /2020 to January /2021. Eighty patients out of 170 were enrolled in this study, who were attended a private clinic for clinical investigation and a private laboratory for laboratory diagnosis of COVID-19 who were divided into two groups, asymptomatic or mild (group 1), and moderate or severe (group 2). Five milliliter of blood samples were collected from patients with COVD-19 by venipuncture using a syringe for evaluation of different cells.

Results: The current study revealed a significant difference in white blood cells count, neutrophils count, monocytes count, basophils count, and neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio between group 1 and group 2. While lymphocytes, and eosinophil showed no significant difference.

Conclusion: The current study concluded that COVID-19 may affect the count of some leukocytes in patient with severe infection.

KEY WORDS: COVID-19, neutrophil, lymphocyte

Wiad Lek. 2021;74(10 p.I):2417-2422

INTRODUCTION

Coronaviruses (CoVs) have been associated with important disease outbreaks in the Middle East and East Asia. In 2002 and 2012, respectively, sever acute respiratory syndrome and Middle East respiratory syndrome (MERS) started to appear [1]. At present time, a novel coronavirus (SARS-CoV-2), causing the Coronavirus Disease 2019 (COVID-19), had emerged in late 2019, which had posed a global health threat with its ongoing pandemic in many countries and territories [2]. The crown-like spikes on the surface of these viruses were named coronavirus. Coronaviruses are from order Nidovirales, family Coronaviridae, and subfamily Coronavirindae. Coronaviruse are divided up into four main subgroups which is alpha, beta, gamma, and delta. The studies, research found seven types of human coronaviruses cause infection in humans alpha coronavirus including HCoV-229E , HCoV-NL63, and beta coronavirus including HCoV-OC43, HCoV-HKU1, Middle East Respiratory Syndrome (MERS-CoV), Severe Acute Respiratory Syndrome (SARS-CoV), and the newly identified 2019 Novel Coronavirus, defined as SARS-CoV-2, causing the Coronavirus Virus Disease of 2019 (COVID-19) [3]. The most affect of COVID-19 on lymphocyte and Neutrophil either lymphopenia neutrophilia. Lymphopenia is often associated with leukopenia after coronavirus infection, and white blood cell count persists within normal limits [4]. Within one week of initiation, the first occurrence of SARS-COVID-19 infection in the United States showed a slight reduction in white cell count [5]. A number of COVID-19 studies indicated that intensive care unit (ICU) cases were more likely to develop neutrophilia, which is a predictor associated with disease progression [6-8]. Since lymphocytes are virus-attacking effector cells, most viruses infect humans and cause lymphocytosis [9]. SARS-CoV, MERS-CoV, and SARS-CoV-2 all induced lymphocytic depletion in infected patients [10, 11], with the mechanism of processes being either direct coronavirus attack on lymphocytes or immune-mediated lymphocyte apoptosis [12-14].

THE AIM

To find an association of COVID-19 on different types of leukocytes either count increasing or decreasing.

MATERIALS AND METHODS

This cross sectional study included 80 patients with COVID-19, 31were females, and 49 were males. Five milliliter of blood samples were collected from patients with COVD-19 by venipuncture using a syringe. The blood samples were obtained from the superficial vein of the upper limb, by the tourniquet which was applied in 3-4 inches above the site of collection, then syringe was inserted

Table I. Association of COVID-19 infection with cell differentiation.

Leukocytes	Groups	N	Mean ± SD	*p-value*
White Blood Cells (10^9/L)	Asymptomatic or mild (group 1)	39	7.814±3.233	0.000*
	Moderate or severe (group 2)	41	12.972±4.430	
Neutophil (10^9/L)	Asymptomatic or mild (group 1)	39	4.658±2.141	0.000*
	Moderate or severe (group 2)	41	10.048±4.080	
Lymphcyte (10^9/L)	Asymptomatic or mild (group 1)	39	2.491±1.112	0.031*
	Moderate or severe (group 2)	41	2.000±0.871	
Monocyte (10^9/L)	Asymptomatic or mild (group 1)	39	0.584±0.346	0.020*
	Moderate or severe (group 2)	41	0.988±0.419	
Eosinophil (10^9/L)	Asymptomatic or mild (group 1)	39	0.082±0.091	0.310*
	Moderate or severe (group 2)	41	0.100±0.070	
Basophil (10^9/L)	Asymptomatic or mild (group 1)	39	0.057±0.036	0.001*
	Moderate or severe (group 2)	41	0.087±0.040	
Neurophil-Lymphocyte Ratio (10^9/L)	Asymptomatic or mild (group 1)	39	1.861±0.583	0.002*
	Moderate or severe (group 2)	41	7.158±10.275	
Lymphocyte –Monocyte Ratio (10^9/L)	Asymptomatic or mild (group 1)	39	5.566±3.660	0.000*
	Moderate or severe (group 2)	41	2.200±0.874	

* $p < 0.05$
Abbreviations
M – mean
SD – standard deviation

in to the vein to collect the blood sample. Blood sample was kept in ethylene di amine tetra acetic acid (EDTA) tube to isolate blood serum in a gel tube for measuring of leukocytes count.

Ruby analyzer was used to evaluate the cell differentiation (lymphocyte, neutrophil, basophile, and eosinophil), and the Neutrophil-to-Lymphocyte Ratio.

RESULTS

The results of the current study showed that the range of age of patients with COVID -19 in group 1 was (18-85) years old, and in group 2 was (34-85) years old. The infection with COVID-19 was higher in males rather than females.

White blood cell count was done on all blood samples of patients with COVID-19 that enrolled in the current study (n=80), in group 1 of asymptomatic or mild signs of COVID-19, the mean of white blood cells was (7.814±3.233) (10^9/L) and predominantly, neutrophilia with a mean of (4.658±2.141) (10^9/L) *vs* (2.491±1.112 (10^9/L) for lymphocytes). The mean of monocytes, eosinophil, and basophil counts was 0.584±0.346 (10^9/L), 0.082±0.091 (10^9/L), and 0.057±0.036 (10^9/L), respectively. In moderate or severe group (group 2), the mean of WBCs count was 12.972±4.430 (10^9/L), mean of neutrophil count was 10.048±4.080 (10^9/L), mean of lymphocytes count was 2.000±0.871 (10^9/L). The mean counts of monocytes, eosinophil, and basophil were 0.988±0.419 (10^9/L), 0.100±0.070 (10^9/L), and 0.087±0.040 (10^9/L) respectively.

Interestingly, patients in group 2 were with lower lymphocyte counts and higher neutrophil count than group 1. Also the ratios of cell populations were analyzed in this study, including the neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR). The results showed that the patients with COVID-19 in group 2 had higher mean of NLR ratio which was 7.158±10.275 *vs* 1.861±0.583 in group 1, while the LMR was 2.200±0.874 *vs* 5.566±3.660 in group 1. The results also showed a significant difference regarding the white blood cells count (p=0.000), neutrophil (p=0.000), monocytes (p=0.020), basophil (p=0.001), NLR (p=0.002), and LMR (p=0.000), between group 1 and group 2. While there was no significant difference of lymphocytes (p=0.031), and eosinophil count (p=0.310) between the two patient studied groups (Table I).

To find the association between cell differentiation (WBCs, neutrophil, lymphocytes, monocytes, and basophil) with the severity of COVID-19 infection, analysis by ROC was done to evaluate the sensitivity and specificity of the cut off for patients with severe COVID-19 is equal or higher than the specified cut off value. For WBCs, neutrophil, monocytes, basophil, NLR, and MLR the area under the curve was 0.829, 0.892, 0.796, 0.724, 0.964, and 0.872 respectively, the sensitivity was 56.1%, 90%, 90%, 73%, 95%, and 95% respectively and the specificity was 12.8%, 33%, 34%, 48%, 20%, and 28% respectively. The cut off value for WBCs, neutrophil, lymphocytes, monocytes, and basophil, was 12.350, 5.525, 475, 0.55, 2.450, and 3.500 (10^9/L) respectively. The lymphocytes count was not significant as a tool in predicting the severity of COVID-19 infection (p=0.143) with the area under the curve equal to 0.405 (Table II, Fig. 1-3).

Table II. Association of COVID-19 with cell differentiation.

Test variable(s)	Accuracy	Area (AUC)	Asymptomatic Significance	Asymptomatic 95% Confidence Interval	
				Lower Bound	Upper Bound
White Blood Cell	Very good	0.829	0.000*	0.741	0.916
Neutophil	Very good	0.892	0.000*	0.825	0.960
Lymphcyte	Bad	0.405	0.143*	0.278	0.532
Monocyte	Good	0.796	0.000*	0.700	0.893
Basophil	Good	0.724	0.001*	0.614	0.835
NLR	Excellent	0.964	0.000*	0.920	1.000
LMR	Very good	0.872	0.000*	0.790	0.954

* $p < 0.05$

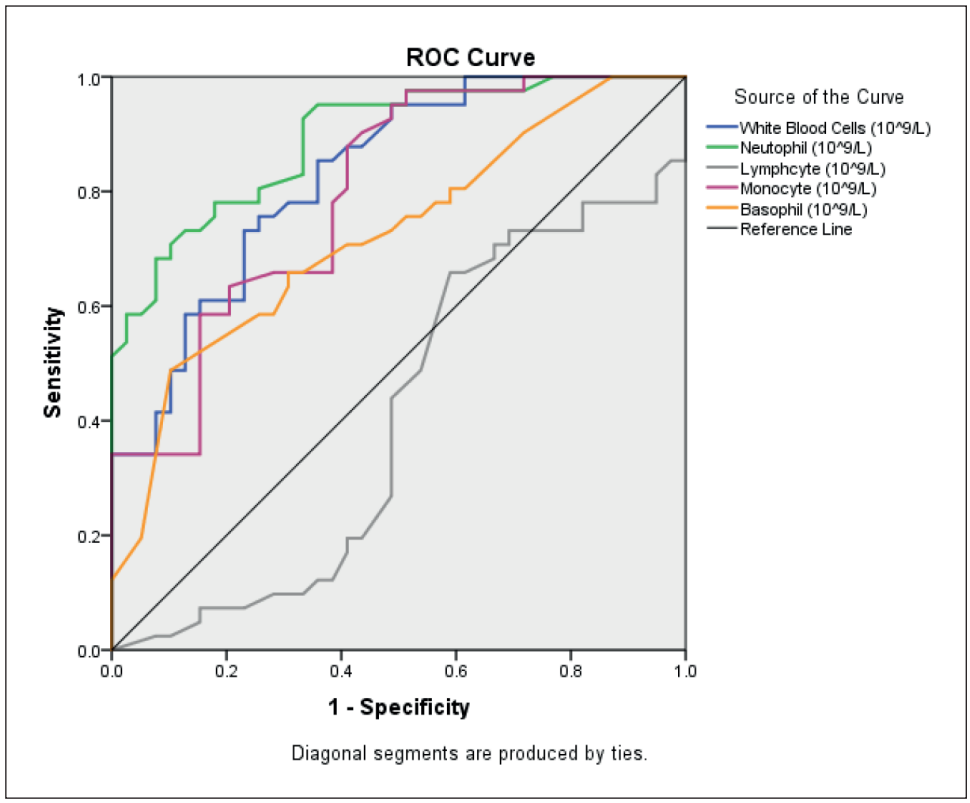


Fig. 1. Receiver operating characteristic curve for measuring the area under curve for cell differentiation in patients with COVID-19.

DISCUSSION

The moderate and severe infection was found in elderly patients rather than the asymptomatic or mild infection which included the younger patients. Elderly patients may develop severe infection due to the presence of other diseases or condition which affect the immune state of the body or increase the susceptibility to show more severe symptoms [15].

The statistical analysis of the results of this study confirmed no significant difference between male and female regarding the severity of COVID-19 infection.

Men have about twice the likelihood of dying from COVID-19, according to popular belief, despite the fact that the number of male cases is similar to the number of female cases. This has led to a range of ideas, ranging from lifestyles to chromosomal structure differences [16].

COVID-19 has the potential to affect a variety of organ systems in its host. According to studies, hematological profiles vary during SARS-CoV-2 infection. So the present study was designed to evaluate different cells in patients who were included in this study.

Patients with COVID-19 pneumonia may have a normal leukocyte count (4–11x10^9/L), a low leukocyte count, or a high leukocyte count. This could make it easier to track the disease's course and make treatment decisions. Physicians should be mindful of the cytokine storm and avoid using granulocyte colony-stimulating factor for leukopenia caused by SARS CoV-2 because it can worsen the illness and lead to acute respiratory disease syndrome. Some studies on COVID-19 patients approved that the patients had higher white blood cell and neutrophil counts, as well as a lower lymphocyte count, while other studies demonstrated that assessing the neutrophil-to-lymphocyte ratio can be used as a biomarker to predict the fate of an infection [17].

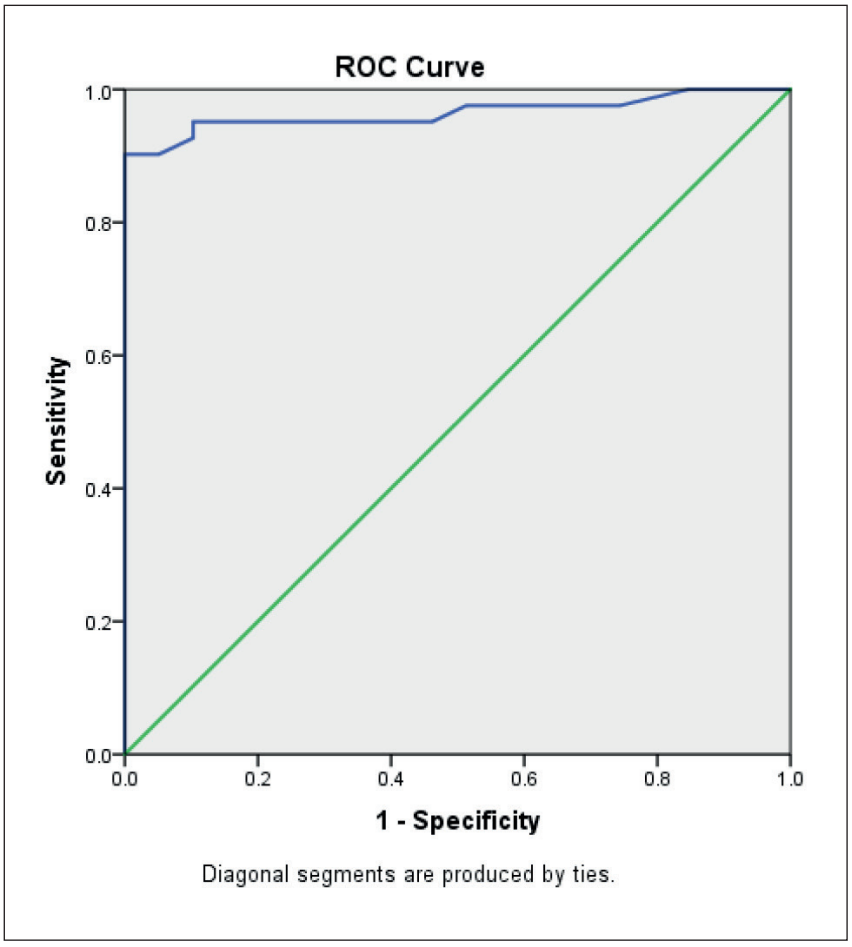


Fig. 2. Receiver operating characteristic curve for measuring the area under curve for Lymphocyte/ Monocyte Ratio in patients with COVID-19.

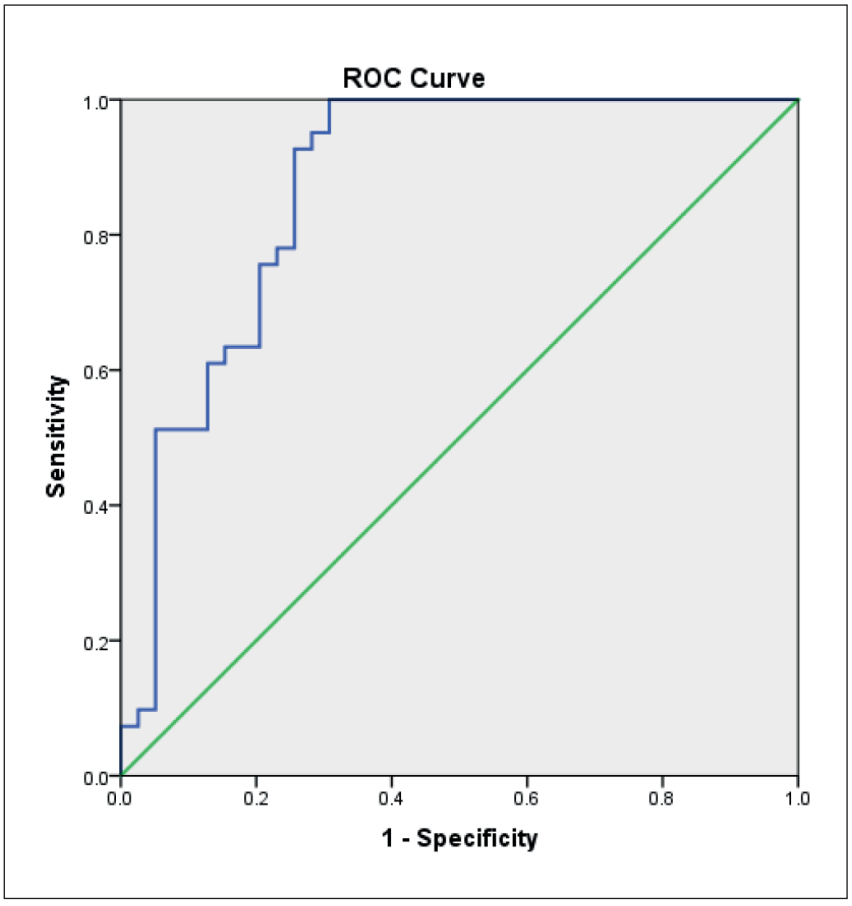


Fig. 3. Receiver operating characteristic curve for measuring the area under curve for Lymphocyte/ Monocyte Ratio in patients with COVID-19.

White blood cells (WBC) count and peripheral blood lymphocytes are normal or slightly reduced in the early stages of COVID-19 disease when patients have no specific symptoms, according to studies, but these indications may change as the disease progresses [18].

In contrast to prior research, Hong-Yi Zheng et al., found that the absolute count of leukocytes in COVID-19 patients with mild and severe disease stayed within acceptable limits [19].

Neutrophils play a role in the early stages of antiviral defense, so that in the presence of pathogenic agents and tissue injury, neutrophil numbers normally rise. Neutrophils, on the other hand, become cytotoxic during severe pneumonia due to degranulation and lysis [20]. Neutrophil recruitment has been suggested to enhance COVID-19 immunopathology in some studies [21].

Lympopenia is a common symptom in COVID-19 patients, as it is with other coronaviruses and viral infections. The overall number of lymphocytes, TCD4+, TCD8+, B cells, and natural killer cells (NK cells) declines in these patients, with the drop of CD8+ T cells being the most dramatic. Lymphocytopenia is a reliable sign of early SARS CoV-2 infection, and it aids in contact tracing as well as illness development during COVID-19 pneumonia [17]. A research by Qin et al., also discovered that lower T helper levels are linked to the severity of COVID-19 [22]. Because lymphocytes include ACE2 and CD147 on their membranes, it's thought that SARS-CoV-2 could infect them directly, causing lymphocyte lysis and lymphopenia. A concept of a second hypothesis is that increased cytokine activity first causes atrophy of secondary lymphatic organs, such as the spleen, and disrupts lymphocyte turnover, and then enhances the expression of death receptor FAS and Apoptosis, resulting in lymphopenia [23]. Lymphopenia is not specific for prognosis of the disease or as a predictor for severity [24].

The results of neutrophil-to-lymphocyte ratio was agreed with the results of previous study which indicated that the NLR ratio was significantly higher in severe COVID-19 patients [25]. While another study of 93 laboratory-confirmed COVID-19 cases found that the NLRis an independent predictor of poor clinical outcome in COVID-19 patients [26].

Several researchers found that severe cases of COVID-19 pneumonia included neutrophilia (absolute neutrophil count above the normal range; 3–7.5 109/L) and/or lymphocytopenia (lymphocyte count 1.5 109/L) that were associated with poor prognosis. The ratio of neutrophils to lymphocytes (NLR) has also been demonstrated to be a good predictor of illness severity. Small studies, on the other hand, found a considerable decline in granulocytes in severe patients when compared to non-severe individuals [27].

A study of Yuan et al., agreed with the results of the present study which revealed had a higher total leukocyte count in patients with severe COVID-19 [27].

The current study was agreed with the study of Sun *et al*., regarding the association between monocyte count and the progression or severity of COVID-19 illness. The researchers in a study done by Sun et al., on COVID-19 patients found that there was a significant difference between monocytes count and the severity of the disease, and they found that the monocyte-lymphocyte ratio (MLR) was higher than in the control group, particularly in those with severe COVID-19 [28].

CONCLUSIONS

Patients with COVID-19 may have a normal leukocyte count, a low leukocyte count, or a high leukocyte count, so the physicians should estimate the count of different cells of leukocytes during the infection to predict the prognosis of the disease.

REFERENCES

1. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020 Mar;579(7798):270-273, doi: 10.1038/s41586-020-2012
2. Rodriguez-Morales AJ, Bonilla-Aldana DK, et al. History is repeating itself: Probable zoonotic spillover as the cause of the 2019 novel Coronavirus Epidemic. Infez Med. 2020 Mar 1;28(1):3-5.
3. Centre for Disease Control and Prevention (CDC). Human Coronavirus Types. National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases. https://www.cdc.gov/coronavirus/types.html [Access: 10.01.2020].
4. Lee N, Hui D, Wu A, Chan P, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med. 2003 May 15;348(20):1986-94.
5. Holshue ML, DeBolt C, Lindquist S, et al. First Case of 2019 Novel Coronavirus in the United States. N Engl J Med. 2020 Mar 5;382(10):929-36.
6. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020 Feb 15;395(10223):497-506.
7. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Mar 17;323(11):1061-1069.
8. Liu J, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 novel coronavirus in the early stage. Transl Med. 2020 May 20;18(1):206, doi: 10.1186/s12967-020-02374-0.
9. Zhu Y, Cao X, Tao G, Xie W, Hu Z, Xu D. The lymph index: a potential hematological parameter for viral infection. Int J Infect Dis. 2013;17(7):e490–e493493.
10. Rabaan AA, Al-Ahmed SH, Haque S, et al. SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview. Infez Med. 2020 Jun 1;28(2):174-84. PMID: 32275259 [ahead of print].
11. Al-Tawfiq JA, Hinedi K, Abbasi S, Babiker M, Sunji A, Eltigani M. Hematologic, hepatic, and renal function changes in hospitalized patients with Middle East respiratory syndrome coronavirus. Int J Lab Hematol. 2017 Jun;39(3):272-78.
12. Panesar NS. What caused lymphopenia in SARS and how reliable is the lymphokine status in glucocorticoid-treated patients? Med Hypotheses. 2008 Aug;71(2):298-301.
13. He Z, Zhao C, Dong Q, Zhuang H, Song S, Peng G, Dwyer DE. Effects of severe acute respiratory syndrome (SARS) coronavirus infection on peripheral blood lymphocytes and their subsets. Int J Infect Dis. 2005 Nov;9(6):323-30.

14. Chu H, Zhou J, Wong BH, et al. Middle East Respiratory Syndrome Coronavirus Efficiently Infects Human Primary T Lymphocytes and Activates the Extrinsic and Intrinsic Apoptosis Pathways. J Infect Dis. 2016 Mar 15;213(6):904-14.
15. Guo T, Shen Q, Guo W, et al. Clinical characteristics of elderly patients with COVID-19 in Hunan province, China: A multicenter, retrospective study. Gerontology. 2020;66:467-475.
16. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, Liu S, Yang JK. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. Front Public Health. 2020 Apr 29;8:152, doi: 10.3389/fpubh.2020.00152.
17. Selim S. Leukocyte count in COVID-19: an important consideration. Egypt J Bronchol. 2020;14(43):1-2.
18. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. Am J Hematol. 2020;95(6):E131- E134.
19. Zheng HY, Zhang M, Yang CX, et al. Elevated exhaustion levels and reduced functional diversity of T cells in peripheral blood may predict severe progression in COVID-19 patients. Cell Mol Immunol. 2020;17(5):541-543.
20. Haick AK, Rzepka JP, Brandon E, Balemba OB, Miura TA. Neutrophils are needed for an effective immune response against pulmonary rat coronavirus infection, but also contribute to pathology. J Gen Virol. 2014;95:578-590.
21. Didangelos A. COVID-19 hyperinflammation: what about neutrophils? mSphere. 2020;5(3):e00367-20, doi: 10.1128/mSphere.00367-20.
22. Qin C, Ziwei MP, Tao SY, Ke PC, Shang MM. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020 Jul 28;71(15):762-768, doi: 10.1093/cid/ciaa248
23. Watanabe A, Yoneda M, Ikeda F, Terao-Muto Y, Sato H, Kai C. CD147/EMMPRIN acts as a functional entry receptor for measles virus on epithelial cells. J Virol. 2010;84(9):4183-4193.
24. Wiersinga WJ, Rhodes A. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA. 2019.
25. Kong J, Wang T, Di Z, et al. Analysis of hematological indexes of COVID-19 patients from fever clinics in Suzhou, China. Int J Lab Hem. 2020;42:e204-e206.
26. Liu Y. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect. 2020;81:e6-e12.
27. Khartabil TA, Russcher H, van der Ven A, de Rijke YB. A summary of the diagnostic and prognostic value of hemocytometry markers in COVID19 patients. Int J Lab Hematol. 2021 Feb;43(1):29-39. doi: 10.1111/ijlh.13339.
28. Sun S, Cai X, Wang H, et al. ClinicaChimicaActa Abnormalities of peripheral blood system in patients with COVID-19 in. Clin Chim Acta. 2020;507:174-180.

Acknowledgments:
The Authors would like to thank the laboratory staff of Department of Clinical Laboratory Sciences/ Mustansiriyah University for the technical assistance. This work was private financially supported.

ORCID and contributionship:
Ahmed Hamza Ajmi – 0000-0002-9913-0539 [B-C,]
Wassan Abdul-Kareem Abbas – 0000-0001-9084-591X [A-B,F]
Dalya Basil Hanna – 0000-0003-3201-4303 [C-D]
Maysaa Ali Abdul Khaleq – 0000-0003-3548-7835 [E-F]

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

CORRESPONDING AUTHOR
Ahmed Hamza Ajmi
Republic of Iraq Health,
Environment the State Company for Marketing
Drugs and Medical Appliances, Baghdad, Iraq
e-mail: ahmed_ajmi10@yahoo.com

Received: 28.07.2021
Accepted: 02.10.2021

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

CHARACTERISTICS OF METAL ALLOYS PROPERTIES FOR DENTAL CASTING AFTER THEIR REPEATED REMELTING

DOI: 10.36740/WLek202110111

Petro A. Hasiuk[1], Viktor Kindiy[2], Volodymyr Radchuk[1], Dmytro Kindiy[2], Tetiana Dzetsiukh[1], Dmytro Korol`[2]
[1]I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE
[2]POLTAVA STATE MEDICAL UNIVERSITY, POLTAVA, UKRAINE

ABSTRACT

The aim: To study clinical and experimental substantiation of the possibility of using cobalt-chromium and nickel-chromium alloys after repeated remelting for non-removable one-piece prosthetics.

Materials and methods: Experimental studies of Remanium GM 700 and Remanium CSe dental alloys have been carried out. To study the physical and mechanical properties of alloys, samples were obtained by sequential six-fold remelting in a Tiegelschleuder TS casting dental unit manufactured by Degussa. All samples were subjected to chemical analysis and metallographic studies by methods generally known in metallurgy, which included studies of microhardness, elasticity, tensile deformation, bending deformation and alloy structure after repeated remelting.

Results: Thus, the obtained results of the chemical, physicomechanical and structural properties of the remelts indicate that the Remanium GM 700 and Remanium CSe alloys can be reused many times for the manufacture of one-piece orthopedic structures in that they are identical with certified alloys.

Conclusions: In terms of structure, qualitative and quantitative composition of elements, physical and technological properties, the Remanium GM 700 and Remanium CSe alloys have been repeatedly remelted meet the requirements of materials used in biologically active environments of the patient's body. High-quality and cost-effective remelted multiple times Remanium GM 700 and Remanium CSe alloys can be recommended for use in practical prosthetic dentistry six times.

KEY WORDS: metal alloy, casting, cobalt-chromium alloy, nickel-chromium alloy

Wiad Lek. 2021;74(10 p.I):2423-2427

INTRODUCTION

At the present stage of its development, dental materials science has a wide range of various materials, the properties of which allow their use in certain clinical cases. Undoubtedly, orthopedic constructions made of the latest materials using advanced technologies actively compete with previously known materials which are due to the requirements for modern orthopedic treatment, taking into account the functional properties of prostheses, their durability, aesthetics, biocompatibility and cost effectiveness. However, certain clinical cases dictate the choice of material for the manufacture of orthopedic structures, based on its properties, which cannot always be achieved using modern materials. One of such constructions is one-piece prostheses [1-4].

The demand of the population for prosthetics is quite high, and the demand for the manufacture of fixed structures of dentures reaches 80% of the total number of patients who have applied for prosthetics [5-7].

Changes in the main indicators of the physical and mechanical properties of metal alloys during their operation can lead to galvanic disorders, toxic effects, allergic reactions, and changes in the reactivity of the body. In addition, the quality of alloys also depends on the conditions of their melting and casting – in open induction or vacuum furnaces [8].

Due to thelarge number of cobalt-chromium and nickel-chromium alloys names most of which, as a rule, have a high cost as well as a large a mount of non-recyclable waste from the production oforthopedic structures and based on the principles of economy and rational use of resources it is advisable to use such alloys repeatedly.

Research focused on solving this issue showed that the double vacuum casting method provides increased purity of alloys and allows the use of up to 50% remelting [9, 10].

In the available literary sources, there is a small amount of research on the physicochemical and technological characteristics of these alloys by their multiple remelting [11]. Thus, this issue is relevant and requires a comprehensive study.

THE AIM

The aim of the study is a clinical and experimental substantiation of the possibility of using cobalt-chromium and nickel-chromium alloys after repeated remelting for non-removable one-piece prosthetics.

MATERIALS AND METHODS

Experimental studies of two dental alloys used for the manufacture of fixed solid structures of dentures have been

carried out. All standardized metal alloys are conditionally divided into two groups. Samples of alloys Remanium GM 700 (group I) and Remanium CSe (group II) served as controls. The cobalt-chromium alloy Remanium GM 700 was introduced into the III experimental group, which was successively remelted six times by the method of vacuum remelting in the mode specified by the manufacturer "Dentaurum" (Germany), in our modification. In the IV experimental group was introduced alloy Remanium CSe, which passed a similar path of study.

To study the physical and mechanical properties of alloys, samples were obtained by sequential six-fold remelting in a dental casting unit Tiegelschleuder TS from Degussa. The remelting took place according to the instructions for these alloys in ceramic crucibles. Melting point 1410°C and holding time 10 minutes. After melting and holding at an appropriate temperature for 1 min. the melt was poured into a casting flask made of Castorit-Super C material and cooled naturally to room temperature.

For each subsequent remelting, the remnants of the re-alloy were carefully cleaned from the molding sand by the traditional method and an equal part of the alloys of the certificate supply was added.

The next remeltswere carried out at temperatures of 1500°, 1600°, 1700°, 1800°, 1030°, 870°, and 36 heats were performed in 110 seconds. and 36 swimming trunks – for 220 sec. each one. The temperature drop from the furnace crucible to the mold was 100°C.

Then all samples were subjected to heat treatment under the following conditions: 36 samples of Remanium GM 700 and Remanium CSe, cast in 110 seconds; chilled gradually. The same number of samples was cooled in 10% saline solution at room temperature. Samples cast in 220 seconds were cooled by a similar method. The temperature was determined with a thermoelectric thermometer.

The cast metal was processed in a sandblasting machine and the first heat treatment was carried out at t=1000°C for 5 minutes, then the oxide film was removed with a sandblasting machine, again subjected to heat treatment at t=1000°C for 5 minutes and so 2-3 times to the formation of a high-quality oxidizing gray films.

Three types of samples were prepared from each remelting: 1 – for the study of micro hardness – 10 samples in the form of a 10x10x2 mm plate; 2 – for measuring the modulus of elasticity – 10 samples in the form of a cylinder 36 mm long and 3 mm in diameter; 3-10 samples for stretching in the form of double-sided blades with the size of the working part 25x5x0. 4 mm.

For the study, the samples were obtained in the form of plates with a thickness of 0.4 mm, the surface of which was subjected to sandblasting with alumina with a dispersion of 50 μm and treated with sandpaper on a glass plate with abrasive powder, degreased with steam at 160°C.

All samples were subjected to chemical analysis and metallographic research according to well-known methods in metallurgy.

To study the microhardness (in MPa), the samples of the studied alloys were additionally ground and polished on suede with diamond paste to a mirror shine. The microhardness was investigated on a PMT-3 device at various loads on the indenter (10, 20, 50, 100 g). On each of the test samples, 100 prints were obtained and the average value was found.

The modulus of elasticity was measured by the acoustic method of a double vibrator at a resonant frequency of 73 kHz, at the amplitude of the sound wave $\Sigma=10^{-7}$.

Tensile deformation was performed in a bursting installation MRC-1 with a strain rate of 0,2 mm/min on samples of a dumbbell-shaped form with the sizes of a working part of 25x5x0,4 mm. In this case, 10 samples were examined in the initial state after casting, 10 samples of the alloy after each remelting.

The obtained deformation curves were used to determine the conditional boundary of yield $\delta_{0,2}$, ultimate strength $\delta_{elasticity}$ and the maximum deformation before failure Σ_{max}.

Bending deformation was also performed in the installation of MRC-1 with a prefix for four-point bending. For this purpose, samples of alloys with a size of 40x5x0,5 mm from different remelting groups were used.

The structure of the alloy was studied on a JSM-820 scanning electron microscope with a Link AN 10/85 S X-ray microanalysis system by plotting element distribution maps using the corresponding X-ray lines. The studies were carried out on cross-sectional sections of the samples, in this case, both the chemical and phase composition were studied, and fractographic studies of the fracture surfaces of the samples after tensile deformation were carried out. X-ray structural studies were performed on a diffractometer DRON-3 M.

The surface morphology of the samples destroyed after deformation by tension was also investigated in a JSM-820 scanning microscope in both absorbed and reflected electrons.

Quantitative indicators of physical and mechanical properties of metal alloys were processed by the method of variation statistics according to Student-Fisher. Statistical processing of the obtained results was performed using an integrated application package "Microsoft Excel" [12, 13].

RESULTS

To study the microhardness index, the mechanical properties and structure of the dental alloys Remanium GM 700 and Remanium CSe manufactured by the Dentaurum Company were studied after six successive remelts.

Table I shows the average data of measurements of the microhardness of the investigated alloy Remanium GM 700 and Remanium CSe at 20 points both in the state of delivery and after each of six successive remelts at various loads on the indenter.

An analysis of microhardness measurements of the Remanium CSe alloy shows that two tendencies are well traced – a decrease in microhardness for an increase in the load on the indenter and a decrease in microhardness during successive remeltings. Both of these tendencies took place in the study of the Remanium GM 700 alloy

Table I. The value of microhardness (Hv, in GPa) of samples (n=10) of the alloy Remanium GM 700 and Remanium CSe at different loads

	Remanium GM 700				Remanium CSe			
Load Sample	**10 gr**	**20 g**	**50 g**	**100 g**	**10 g**	**20 g**	**50 g**	**100 g**
Primarysample	7,9	7,9	6,8	6,4	4,5	5,02	4,89	4,65
I remelting	6,7	5,5	5,8	5,6	3,22	3,05	2,98	2,75
II remelting	6,6	5,8	5,2	5,7	2,93	2,92	2,74	2,52
III remelting	5,9	5,1	5,4	4,9	2,58	2,87	2,8	2,82
IV remelting	4,8	5,4	4,9	4,8	3,0	3,08	3,0	2,79
V remelting	4,9	5,1	4,6	4,6	2,72	2,63	2,54	2,45
VI remelting	4,7	4,7	4,3	4,5	2,6	2,20	2,45	2,39

Table II. The modulus of elasticity of samples of alloys Remanium GM 700 and Remanium CSe (E, GPa)

	Remanium GM 700	Remanium CSe
Passport data	230	170
I remelting	231	188
II remelting	234	195
III remelting	220	190
IV remelting	215	196
V remelting	220	193
VI remelting	223	192

samples, moreover, because the first of them is typical for all materials.

According to the second tendency, for the samples of the RemaniumC Se alloy, a decrease in the microhardness is observed already after the first remelting, while for the samples of the Remanium GM 700 alloy it is less pronounced.

Table II shows the values of the modulus of elasticity for samples of the alloy Remanium GM 700 and Remanium CSe after each remelting in comparison with the passport data.

Within the experimental error, it is clearly seen that the value of the elastic modulus remains constant after all six remelts of the Remanium GM 700 alloy and corresponds to the passport data. In remelted samples of Remanium CSe alloy, the modulus of elasticity is higher than in the passport data, which cannot be explained by accidental error.

The most significant difference in comparison with the passport data was observed in the value of the maximum deformation before damage: in the samples studied by us, Σ_{max} did not exceed 1%, while the manufacturer guarantees this value at the level of 4%. Regarding the values of $\delta_{0,2}$, and $\delta_{elasticity}$, for the initial remelting there is a good coincidence of measured and passport data.

At the same time, after repeated remelting, another type of deformation curves is observed, which is characterized by a much earlier damage, mainly in the area of elastic deformation, which indicates the brittle nature of the damage. The latter is also confirmed by the appearance of the damaged surface. In this case, the ultimate strength of such samples $\delta_{elasticity}$ can be several times lower in comparison with the ultimate strength for curves of the first type $\delta_{elasticity}$.

Of particular interest is the relationship between the indicated types of deformation curves and the nature of damage – if only the first type of curves is characteristic of the initial remelting, then starting from the third remelting, the second type appears, and it begins to prevail before the sixth remelting.

The data of the conditional yield point of $\delta_{0,2}$ forsampels having deformation curves of the first type within the experimental data does not depend on the number of remelts and quite accurately coincides with the passport value of 740 MPa.

Ultimate strength $\delta_{elasticity}$ tends to decrease as the number of remelts increases and does not reach the passport value of 960 MPa, which is primarily associated with the above-mentioned marked decrease in the maximum deformation to damage. These values of $\delta_{elasticity}$ (for curves of the second type) indicate a catastrophic decrease in the strength of these samples.

Thus, the presented results of a comprehensive study of the mechanical characteristics of the dental alloy Remanium GM 700 indicate a tendency for these characteristics to decrease as the number of remelts increases. At the same time, some samples after the third remelting and further have a brittle behavior with a catastrophic decrease in strength characteristics, and its probability increases significantly as the number of remelts increases.

The deformation curves of the Remanium CSe alloy sampels after each remelting have a standard parabolic form. Three parameters were studied on these curves: the conditional yield strength of $\delta_{0,2}$, the yield strength of δ_{max}, the maximum deformation to damage Σ_{max}.

In terms of the Σ_{max} value, as in the Remanium GM 700 alloy, there was a significant decrease in this value in comparison with the passport data (15%). According to our data, Σ_{max} does not exceed 3%. The conditional yield strength systematically exceeds the passport data, while the yield strength is partially inferior to the passport value.

Thus, the mechanical properties of the alloy Remanium CSe at six consecutive remelts practically do not show degradation. There is only a slight decrease in the indi-

vidual studied characteristics (Hv, δ_{max}, Σ_{max}) after the first remelting, while other characteristics (E, $\delta_{0,2}$) even exceed the passport data after all remelts.

The data obtained using X-ray microanalysis averaged over an area of 500x500 μm^2 data on the content of the three main alloy elements after each of the successive remelts suggests that the composition of the Remanium GM 700 alloy has not changed and corresponds to its passport data after all remelts.

However, the analysis of local data indicates a significant inhomogeneity of the composition in the remelted samples, which also grows as the number of remelts increases. The indicated inhomogeneity, on the one hand, is due to the fact that the alloy is two-phase. At the same time, if in the initial samples the second phase is so small that it was possible to recognize it only with the help of X-ray structural analysis, then in the remelted samples the sizes of the inclusions of the second phase are higher, and it was possible to find it on the electron microscopic images of the surface of thin sections of all remelted samples.

In our opinion, the structural differences between the remelted alloy samples and the primary ones found by us cannot make significant changes in their mechanical properties which were proved in the course of the study.

A comparative study of the fracture surface of the samples after plastic and brittle damage has shown that for brittle damage rather large (~ 50 μm) grains are formed on the fracture surface, embedded in the amorphized matrix. The composition of these inclusions, determined by X-ray microanalysis, corresponds to silicon oxide with carbon impurities.

It is known that silicon and carbon in small amounts are included in the alloy. In addition, silicon and carbon are contained both in the material of the crucibles, where the initial alloy is melted, and in the material from which the molds are made, where the remelt is poured for solidification.

All this allows us to assume that silicon and carbon, which are part of the alloy, and obtained additionally from the outside, are localized in certain places of remelting, making these places very low-strength due to the high brittleness of oxides and insufficient connection of inclusions with the matrix.

The number of samples with a high local concentration of these inclusions increases with successive remelting, which leads to an increase in the number of brittle damage to the samples during stretching.

Changing the remelting mode (holding time in the molten state, additional movement of the melt, vacuum remelting, addition of a certified alloy to the remainders during remelting) made it possible to avoid these negative phenomena, which made it possible to more efficiently reuse the Remanium GM 700 alloy in orthopedic structures.

The results obtained by X-ray microanalysis for the content of the three main elements (Ni, Cr, Mo) of the alloy after each remelting showed that repeated remelting does not lead to significant changes in the content of the main elements. However, in the Remanium CSe alloy, a rather high compositional inhomogeneity is observed. As evidenced by scanning electron microscopic observations and X-ray diffraction analysis, the research alloy is two-phase, and the dimensions of the second phase are extremely small, although they increase with the number of remelts.

X-ray microanalysis of the composition of the alloy on the surfaces of the damage showed that in contrast to the alloy Remanium GM 700 in the experimental alloy Remanium CSe found no oxides and carbides, which lead to the fragility of this alloy. The revealed circumstance fully explains the fact that in the process of six-fold remelting of the Remanium CSe alloy, a catastrophic decrease in its strength characteristics is not observed.

Obtained results indicate that Remanium CSe alloy can be reused repeatedly for the manufacture of one-piece orthopedic structures, and for Remanium GM 700 alloy can also be recycled, but for this the alloy does not need to be overheated.

Due to the fact that there are a large number of alloys of different types and with different numbers of components, as well as different manufacturers, we consider it appropriate to sort the residues of alloys by type and manufacturer in order to carry out full recycling.

Thus, the obtained results of the chemical, physic-mechanical and structural properties of the remelts indicate that the Remanium GM 700 and Remanium CSe alloys can be reused many times for the manufacture of one-piece orthopedic structures in that they are identical with certified alloys.

DISCUSSION

To solve these problems, laboratory and experimental studies were conducted, which consisted in a comparative assessment of the physical and mechanical properties and structure of the experimental alloys after their repeated remelting.

The analysis of local data indicates a significant inhomogeneity of the composition in the remelted samples, which increases with an increase in the number of remelts. This inhomogeneity, on the one hand, is due to the fact that the investigated alloys are two-phase.

Studying the physical and mechanical properties of research metal alloys, a structural analysis of prototypes on the fracture surface was carried out. The results of the study showed that the Co-based alloy (Remanium GM 700) has a higher microhardness in the cast and heat-treated state, and, in contrast to the Ni-based alloy (Remanium CSe), heat treatment of this alloy increases its microhardness. This can be explained by the fact that after casting Remanium GM 700 we found large (5-10 μm) inclusions of the second phase, enriched with chromium and molybdenum, and the microhardness of this phase is much higher than the microhardness of the matrix [14-16]. According to our data, heat treatment partially dissolves the second phase in the matrix, which causes the transition of Cr and Mo atoms into it and increases the microhardness.

The structural differences of the remelted samples from the initial ones revealed by us cannot make significant changes in their mechanical properties, which have been proved.

The selection of the optimal alloying degree of alloys by using microadditives from standardized alloys made it possible to obtain alloys with sufficient strength, ductility, and fluidity that meet international standards and provide the functional properties of non-removable solid cast structures of dentures, and reduce the consumption of alloys.

CONCLUSIONS

The study shows the possibilities of using the alloys Remanium GM 700 and Remanium CSe after repeated remelting for the manufacture of non-removable one-piece structures of dentures.

Based on laboratory and experimental research data, a method of alloy recirculation has been developed. It can be argued that the method of six-fold vacuum remelting provides an increased purity of the Remanium GM 700 and Remanium CSe alloys and allows the use of remelting with the addition of alloying components many times.

In terms of structure, qualitative and quantitative composition of elements, physical and technological properties of repeatedly remelted alloys Remanium GM 700 and Remanium CSe meet the requirements for materials used in biologically active environments of patients.

High-quality and cost-effective reusable vacuum remelted alloys Remanium GM 700 and Remanium CSe can be recommended for use in practical orthopedic dentistry six times.

REFERENCES

1. Dhima M., Paulusova V., Carr A.B. et al. Practice-based clinical evaluation of ceramic single crowns after at least five years. J Prosthet Dent. 2014;111(2):124-130.
2. Euán R., Figueras-Álvarez O., Cabratosa-Termes J. et al. Marginal adaptation of zirconium dioxide copings: influence of the CAD/CAM system and the finish line design. J Prosthet Dent. 2014;112(2):155-162.
3. Güth J.F., Wallbach J., Stimmelmayr M. et al. Computer-aided evaluation of preparations for CAD/CAM-fabricated all-ceramic crowns. Clinical oral investigations. 2013;17(5):1389-1395.
4. Nesse H., Ulstein D.M., Vaage M.M. et al. Internal and marginal fit of cobalt-chromium fixed dental prostheses fabricated with 3 different techniques. J Prosthet Dent. 2015;114(5):686-692.
5. Kalashnikov D., Hasiuk P., Vorobets A. et al. Features of the course of enamel biomineralization processes in various anatomical areas of the tooth. Wiad Lek. 2020;73(15):864-867.
6. Amaral F.R., De Andrade M.F. Saad JRC et al. Functional and esthetics prosthetic rehabilitation in the elderly patient with metal ceramic crowns. Scientific Journal of Dentistry. 2015;2:42-45.
7. Fastovets O., Masheiko I., Lukash A. Evaluation of bone resorptive potential in the treatment of generalized periodontitis. Wiad Lek.2020;73(11):2396-2402.
8. Demkovych A., Bondarenko Yu., Hasiuk P. Effects of quercetin on antioxidant potential in the experimental periodontitis development. Interventional Medicine & Applied Science. 2019;11(1):60-64.
9. Hasiuk P.A., Vorobets A.B., Demkovych A.Ye. et al. Features of occlusal correlations of molars in the dental clinic. Wiad Lek. 2021;74(5):1130-1133.
10. Hasiuk P., Vorobets A., Hasiuk N. et al. Sex differences of odontometrical indexes crowns of molars. Interventional Medicine and Applied Science. 2017;9(3):160-163.
11. Kindiy V., Kindiy D., Maliuchenko N. et al. Mechanical properties and structure of Co-Cr-Mo alloy Remanium GM 700 at multiple successive refinings. Dental Science and Practice. 2014;4(4):10-14.
12. Hasiuk P., Hasiuk N., Kindiy D. et al. Characteristics of cellular composition of periodontal pockets. Interventional Medicine & Applied Science. 2016;8(4):172-177.
13. Yanishen I., Tkachenko I., Skrypnikov P. et al. Wear resistance of dental materials which are used for anterior teeth restorations. Wiad Lek. 2020;73(8):1677-1680.
14. Doddy L., Reddy S., Reddy S. et al. Comparative evaluation of the marginal adaptation of cast Ni-Cr copings, direct metal laser sintering Co-Cr copings, and computer-aided design and computer-aided manufacturing Zr copings: An In-vitro Study. Cureus. 2019;11(11):e6091. doi: 10.7759/cureus.6091.
15. Mercieca S., Caligari Conti M., Buhagiar J. et al. Assessment of corrosion resistance of cast cobalt- and nickel-chromium dental alloys in acidic environments. J ApplBiomaterFunct Mater. 2018;16(1):47-54.
16. Galo R., Rocha L.A., Faria A.C. et al. Influence of the casting processing route on the corrosion behavior of dental alloys. Mater SciEng C Mater Biol Appl. 2014;45:519-523.

ORCID and contributionship:

Petro A. Hasiuk: 0000-0002-2915-0526 [A]
Viktor Kindiy: 0000-0003-0665-0269 [C]
Volodymyr Radchuk: 0000-0001-9019-6008 [B]
Dmytro Kindiy: 0000-0003-2312-4981 [D]
Tetiana Dzetsiukh: 0000-0001-7163-1844 [F]
Dmytro Korol`: 0000-0002-8331-0500 [E]

Conflict of interest:

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Petro A. Hasiuk
I. Horbachevsky Ternopil National Medical University
1 Maidan Voli st., 46002 Ternopil, Ukraine
tel: +380961445444
e-mail: gasiukpa@tdmu.edu.ua

Received: 27.09.2020
Accepted: 15.08.2021

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 THE MICROBIAL CONTENT OF PERIODONTAL POCKETS IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS AND CORONARY ARTERY DISEASE

DOI: 10.36740/WLek202110112

Olesya I. Hodovana, Oksana V. Skybchyk, Tetiana M. Solomenchuk, Tetiana M. Rumynska
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

The aim: To study the rate of detection of specific periodontopathogenic microbiota in patients with chronic generalized periodontitis (CGP) and coronary artery disease (CAD) and assessment of the risk of periodontal pathogens in the development of CAD.

Materials and methods: A microbiological study of the content of periodontal pockets was carried out in 64 patients with CGP and CAD of the study group (mean age – 56.9±7.9 years) and 20 patients of the comparison group (mean age – 45.2±11,8 years) who were not burdened with CAD.

Results: It was established that in patients with CGP and CAD the following periodontal pathogens were found more frequently than in the comparison group: *Aggregatibacter actinomycetemcomitans* (56.3±6.20% vs 25.0±9.68%; p=0.01), *Prevotella intermedia* (54.7±6.22% vs. 20.0±8.94%; p=0.01), and *Fusobacterium spp.* (34.4±5.94 vs. 10.0±6.71%; p=0.04). The increase in the percentage of the association of the periodontal pathogens was revealed in patients with CAD, which increased with the severity of the pathological process in periodontal tissues. The results of the study indicate the association of *A. actinomycetemcomitans, P. intermedia, Fusobacterium spp.* with CAD: *A. actinomycetemcomitans*: OR=3.86 (95% CI: 1.25-11.90), p=0.015; *P. intermedia*: OR=4.83 (95% CI: 1.45-16.05), p=0.007; *Fusobacterium spp.:* OR=4.71 (95% CI: 1.00-22.20), p=0.035.

Conclusions: Analysis of the microbiological study indicates a high rate of detection of specific periodontal pathogens in patients with CGP and CAD. It can be assumed that the presence of such periodontal pathogens as *A. actinomycetemcomitans*, *P. intermedia, Fusobacterium spp.*, significantly increases the risk of CAD.

KEY WORDS: periodontitis, periodontal pathogens, coronary artery disease

Wiad Lek. 2021;74(10 p.I):2428-2432

INTRODUCTION

Periodontal diseases invariably remain among the most common dental diseases. According to the epidemiological data from international studies, the prevalence of periodontal diseases is 80-100% of the world population [1]. The leading place in the structure of clinical forms of generalized lesions of periodontal tissue belongs to chronic generalized periodontitis (CGP) as the main disease that leads to premature tooth loss.

Currently, there is a great number of scientific papers devoted to the in-depth study of the relationship of CGP with general somatic pathology, in particular with cardiovascular diseases (CVD) [2-4], which remains the most common cause of death worldwide [5].

Coronary artery disease (CAD), which results from the atherosclerotic vascular lesion, is diagnosed in 15-20% of the adult population of different countries [5]. An active discussion of the etiopathogenetic links and the impact of CGP on the development and course of CVD, including CAD, received a response from cardiologists. The European guidelines on cardiovascular disease prevention in clinical practice (ESC, 2016), which aim to identify and control the main risk factors for diseases of the circulatory system, indicate the important role of CGP in the development of CAD [6]. CGP is considered an independent risk factor for cardiac diseases associated with atherosclerotic vascular lesions; in particular, special emphasis is placed on the presence of periodontal pathogenic microflora. The existence of a close relationship between disorders of lipid metabolism and hypercholesterolemia with CGP is evidenced by the INVEST study (Oral Infections and Vascular Disease Epidemiology Study), which found that improving the clinical and microbiological parameters of periodontal tissues reduces the rate of progression of thickening of carotid intima-media complex, i.e. the progression of atherosclerosis [7].

In recent years, the increase in the number of scientific studies has been observed to raise the questions of direct or indirect pathogenetic effects of specific microbiota of periodontal pockets on the process of atherogenesis [4,7]. The main factor in the virulence of periodontal gram-negative microorganisms is endotoxins, which are similar to lipopolysaccharides by their chemical nature that are a part of cell membranes. The very presence of lipopolysaccharides leads to the activation of the immune system, increase in plasma concentrations of proinflammatory cytokines (IL-6, TNF-α), biochemical parameters such as C-reactive protein, fibrinogen, total cholesterol, triglycerides, etc., which in turn cause systemic inflammation and then atherosclerosis [8].

On the other hand, the relationship between periodontal microbiota and cardiovascular pathology, including CAD, endocarditis, etc., is associated with transient bacteremia that occurs after such dental manipulations as professional hygiene, tooth extraction, and other surgical interventions in the oral cavity [8-10]. According to Mazur I.P. (2018) and Kharchenko N.L. (2012), the presence of pathogenic microorganisms – *Staphylococcus aureus* (16.3%), *Staphylococcus pyogenes* (11.6%), and *Streptococcus viridans* (14.0%) was revealed in the bloodstream at the 15th minute in 44.1% of patients who underwent dental interventions. These microorganisms also remained in the blood at the 30th minute after manipulation and were detected in 16.2% of patients [10]. The increased risk of transient bacteremia is more typical for people with periodontal diseases because periodontal pockets contain a large number of pathogenic microflora [9,10]. Short-term or long-term episodes of bacteremia lead to the spread of microorganisms in the bloodstream and their attachment to the walls of blood vessels, endocardium, heart valves, and atherosclerotic plaques [9]. The results of polymerase chain reaction studies indicate the presence of rDNA of such periodontal pathogens as *Porphyromonas gingivalis*, *Aggregatibacter Actinomycetemcomitans*, *Bacteroides forsythus*, *Treponema denticola* in atherosclerotic plaques of human carotid arteries [8, 12, 13]. Despite the significant amount of data that indicate the presence of periodontal pathogens in atherosclerotic lesions, there are also studies that contain conflicting results of similar studies [14, 15].

Thus, determining the types of periodontal pathogens that increase the risk of CAD, as well as determining the impact of their quantitative composition and the role of virulence in this process remain incompletely studied and require further research.

THE AIM

To analyze the rate of detection of specific periodontopathogenic microbiota in patients with CGP and CAD, in particular, to study the risk of the association of these periodontal pathogens with the development of CAD.

MATERIALS AND METHODS

A microbiological study of the contents of periodontal pockets of 64 patients with CGP and CAD of the study group (mean age – 56.9±7.9 years), who were admitted to the Cardiology department and 20 persons with CGP not burdened with CAD or other systemic diseases, who were in the comparison group (mean age – 45.2±11,8 years). Among 64 patients of the study group, CGP initial-I degree was diagnosed in 24 patients (37.5%), CGP II degree – in 26 patients (40.6%), and CGP III degree – in 14 patients (21.9%). In the comparison group, CGP initial-I degree was diagnosed in 15 patients (75.0%), CGP II degree – in 4 subjects (20.0%) and CGP III degree was observed in one examined patient (5.0%).

Diagnosis of periodontal status of patients was performed according to the classification by Danilevsky M.F. (1994) on the basis of history taking, filling the periodontal examination card, which included assessment of periodontal tissue, in particular determining the degree of inflammation of gum tissue using PMA periodontal index (papillary-marginal-alveolar index by M. Massler modified by C. Parma, 1960).

The diagnosis of CAD and the results of laboratory tests were obtained by analyzing the medical records of the inpatients. The exclusion criteria were patients with a history of other systemic diseases and patients with CAD and complete secondary adentia.

Material for microbiological examination (the content of periodontal pockets) was collected with sterile paper points and immediately introduced into the semi-liquid Thioglycolate broth medium to detect the anaerobic bacteria. Culture from Thioglycolate medium was plated in basic agar for anaerobes, Schaedler agar with 5% Sheep Blood and Schaedler neomycin vancomycin agar. The system GENbox anaer (BioMerieux, France) was used to cultivate anaerobes. Studies of anaerobic microflora were continued after 3-4 days. Further identification of the group and the type of microorganisms was performed by morphotinctorial, cultural, and biochemical properties. Biochemical characteristics of the microorganisms were determined using test kits API 20E (BioMerieux, France). The identification of microorganisms was performed according to Bergey's classification.

According to the requirements of the basic bioethical provisions of the European Convention on Human Rights and Biomedicine from 04.04.1997 and the Helsinki Declaration of the World Medical Association on ethical principles for medical research involving human subjects (1964-2008), patients signed consent for examination and research (protocol No.3 from 25.03.2019, discussed and approved by the Committee on ethics of scientific research, experimental development and scientific works of Danylo Halytsky Lviv National Medical University).

Statistical analysis of the results was performed using Excel and IBM SPSS Statistics 20. The results of the study are presented as mean±standard deviations (M±SD) (PMA index) and relative values: fractions with error ($P \pm m_p$). Pearson's chi-squared test ($\chi 2$) was used to estimate the probable difference in the results in the compared groups. The constructed tables 2×2 and the criterion of conditional independence (Cochran) were used to compare the odds ratio (OR) with 95% confidence intervals (95% CI). The difference between the groups was considered significant at $p<0.05$.

RESULTS

The analysis of the obtained results of the microbiological study of the contents of periodontal pockets of the study group and the comparison group did not reveal significant differences in the species composition of periodontopathogenic microbiota, but the proportion of detected individual anaerobic microorganisms differed in the comparison group. According to the prevalence of periodontal pathogens, the following species were more common in

Table I. Rate of detection of periodontal pathogens in periodontal pockets (P±m_p, %)

Periodontal pathogens	Study group (n=64)		Comparison group (n=20)		χ2	p
	n	P±m_p, %	n	P±m_p, %		
Porphyromonas gingivalis	57	89.1±3.90	18	90.0±6.71	0.01	0.91
Aggregatibacter actinomycetemcomitans	36	56.3±6.20	5	25.0±9.68	5.96	0.01
Prevotella intermedia	35	54.7±6.22	4	20.0±8.94	7.37	0.01
Fusobacterium spp.	22	34.4±5.94	2	10.0±6.71	4.44	0.04
Peptostreptococcus anaerobius	33	51.6±6.25	11	55.0±11.12	0.07	0.79

Table II. Rate of detection of periodontal pathogens in periodontal pockets of patients with CAD depending on the severity of CGP (P±mp, %)

Periodontal pathogens	CGP Initial-I degree (n=24)		CGP II degree (n=26)		CGP III degree (n=14)		χ2	p
	N	P±m_p, %	n	P±m_p, %	n	P±m_p, %		
Porphyromonas gingivalis	21	87.5±6.75	22	84.6±7.08	14	100±0	1χ2=0.09	0.77
							2χ2=1.90	0.17
							3χ2=2.39	0.12
Aggregatibacter actinomycetemcomitans	10	41.7±10.06	13	50.0±9.81	13	92.9±6.88	1χ2=0.35	0.55
							2χ2=9.70	0.002
							3χ2=7.35	0.01
Prevotella intermedia	15	62.5±9.88	15	57.7±9.69	5	35.7±12.81	1χ2=0.12	0.73
							2χ2=2.54	0.11
							3χ2=1.76	0.18
Fusobacterium spp.	5	20.8±8.29	8	30.8±9.05	9	64.3±12.81	1χ2=0.64	0.42
							2χ2=7.17	0.01
							3χ2=4.18	0.04
Peptostreptococcus anaerobius	8	33.3±9.62	17	65.4±9.33	8	57.1±13.23	1χ2=5.13	0.02
							2χ2=2.06	0.15
							3χ2=0.26	0.61

Notes:
1χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP initial-I degree and CGP II degree;
2χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP initial-I degree and CGP III degree;
3χ2 – the value of Pearson's criterion of the probability of difference between the indicators of CGP II degree and CGP III degree;

patients with CGP and CAD: *A. actinomycetemcomitans*, *P. intermedia*, and *Fusobacterium spp*. In particular, *A. actinomycetemcomitans* were detected in 56.3±6.20% of the patients in the study group against 25.0±9.68% of those in the comparison group (p=0.01). *P. intermedia* and *Fusobacterium spp.* detected in 54.7±6.22% and 34.4±5.94% of patients in the study group and 20.0±8.94% (p=0.01) and 10.0±6.71% (p=0.04) in the comparison group, respectively (Table I). The study did not find a statistically significant difference in the rate of detection of *P. gingivalis* and *P. anaerobius* in both groups (p>0.05).

Analyzing the data on the rate of detection of periodontal pathogens in patients with CAD, depending on the degree of CGP (Table II), it was found that *P. gingivalis* (87.5±6.75%), *P. intermedia* (62.5±9.88%), and *A. actinomycetemcomitans* (41.7±10.06%) were detected the most frequently in patients with CGP initial-I degree in the settings of the inflammatory process of moderate severity (PMA index was 50.3±12.6%).

There was a slight percentage increase in the rate of detection of gram-negative anaerobic microflora: *A. actinomycetemcomitans* (50.0±9.81%) and *Fusobacterium spp.* (30.8±9.05) in patients with CAD with CGP II degree and severe inflammatory process (PMA – 76.8±12.5%). The percentage of detection of *P. anaerobius* gram-positive anaerobic microorganism also increased (65.4±9.33%; p=0.02).

There was a significant increase of gram-negative anaerobic microorganisms in patients with CAD and CGP III degree with the PMA indicator of 86.9±8.3%, which corresponds to the severe degree of inflammation in gums, compared with groups of patients with CAD and

Table III. Association between periodontal pathogens and the risk of CAD

Periodontal pathogens	Study group		Comparison group		Odds ratio		Criterion of conditional independence (Cochran)	
	R*	95% CI	R	95% CI	OR**	95% CI***	χ2	P
Porphyromonas gingivalis	0.99	0.84-1.17	1.09	0.25-4.85	0.91	0.17-4.75	0.014	0.91
Aggregatibacter actinomycetemcomitans	2.25	1.02-4.95	0.58	0.40-0.85	3.86	1.25-11.90	5.956	0.015
Prevotella intermedia	2.73	1.11-7.76	0.57	0.40-0.80	4.83	1.45-16.05	7.372	0.007
Fusobacterium spp.	3.44	0.88-13.36	0.73	0.58-0.92	4.71	1.00-22.20	4.436	0.035
Peptostreptococcus anaerobius	0.94	0.59-1.49	1.08	0.62-1.86	0.87	0.32-2.39	0.07	0.79

Note. * – ratio; ** – odds ratio; *** – confidence interval.

CGP initial-I and II degree. Thus, the detection rate of *A. actinomycetemcomitans* increased to 92.9±6.88% (p=0.01), and *Fusobacterium spp.* – 64.3±12.81% (p=0.04). It should be noted that *P. gingivalis* was detected in 100% of the examined patients with CAD and CGP III degree.

Analysis of the results of the odds ratio indicates that the following periodontal pathogens were significantly associated with CAD: *A. actinomycetemcomitans*: OR=3.86 (95% CI: 1.25-11.90), p=0.015; *P. intermedia*: OR=4.83 (95% CI: 1.45-16.05), p=0.007; *Fusobacterium spp .:* OR=4.71 (95% CI: 1.00-22.20), p=0.035 (Table III).

Thus, the probability of detection of *A. actinomycetemcomitans* in patients with CAD is 3.86 times higher than in patients with CGP without CAD. A similar situation is also characteristic of *P. intermedia* and *Fusobacterium spp*. – the probability of detection of these microorganisms in patients with CGP and CAD is 4.83 and 4.71 times higher than in the comparison group, respectively.

DISCUSSION

Reviewing the researches that studied the relationship between periodontal pathogens and CVD, including CAD, it may be assumed that most authors confirm the hypothesis of the association of periodontal pathogens with CAD [4,7,8,16,17]. Therefore, in the study of the contents of periodontal pockets of patients with CAD, the most common periodontal pathogens were: *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans, Fusobacterium nucleatum, T. forsythensis (Tannerella forsythensis)*, *T. denticola (Treponema denticola)*, and others [16,17,18,19]. However, the results of studies of the species composition namely, the association of individual periodontal pathogens with CAD, are ambiguous. For example, a study by Spahr A. et al. CORODONT (The Coronary Event and Periodontal Disease) did not find a significant statistical difference in the prevalence of periodontal pathogens in patients with CAD and in subjects who have not been diagnosed with CAD [19]. At the same time, the total periodontal pathogenic infection in periodontal pockets was significantly higher in patients with CAD, with the main portion of *A. actinomycetemcomitans* and, to a lesser extent, *P. intermedia*.

The high rate of detection and association of *A. actinomycetemcomitans* and *P. intermedia* with CAD are indicated by the results of studies by Mantyla P. et al. and Nonnemacher C. et al., respectively [17,18]. It should be noted that the periodontal pathogen *P. intermedia*, which is associated with CAD, has also been frequently detected in patients who suffered from myocardial infarction, as confirmed by a study by Andriankaja O. et al. [16].

According to the analyzed literature data, which is also confirmed by the results of our study, there is no reliable association between CAD and *P. gingivalis*, which is considered one of the most common pathogens of periodontal disease, in particular, CGP. There was no significant difference in the prevalence and total amount of *P. gingivalis* in the content of periodontal pockets of patients with CAD compared with those who were not burdened with CAD.

Moreover, our study found an association between CAD and *Fusobacterium spp*. However, the available literature has insufficiently covered information about the role of this periodontal pathogen.

Thus, the results of the study complemented and confirmed the scientific data about the importance of the relationship between periodontal pathogens and the risk of CAD. Further study of the quantitative and qualitative properties of periodontal pathogens and their probable impact on the development of CAD will improve approaches to the comprehensive treatment of patients with mutually aggravating diseases – CGP and CAD.

CONCLUSIONS

Analysis of the microbiological studies of the contents of periodontal pockets of patients with CGP and CAD indicates a high rate of detection of specific periodontal pathogens, the percentage of which increases with the severity of the pathological process in the periodontal tissues. It can be assumed that the presence of such periodontal pathogens as *A. actinomycetemcomitans*, *P. intermedia*, *Fusobacterium spp.* significantly increases the risk of CAD by 3.86-4.71 times.

REFERENCES

1. Maliy D.Y., Antonenko M.Y. Epidemiologiya zahvoryuvan parodonta: vikovyyi aspect. Klinichna medycyna. 2013;4:41-43. (in Ukrainian)

2. Skochko O.V., Mamontova T.V., Vesnina L.E., Kaidashev I.P. Vzaimosvyaz zabolevanii parodonta s faktoramy riska razvitiya ishemicheskoi bolyezni serdtsa. Ukrayinskyi kardiolohichnyi zhurnal. 2015;2:87-94. (in Russian)
3. Sukhovolets I.O., Matsko N.V. Vplyv sertsevo-sudynnoyi patologiyi na perebih zapalno-dystrofichnyh zahvoryuvan tkanyn parodontu. Klinichna stomatologiya. 2014;4:18-21. (in Ukrainian)
4. Haynes W.G., Stanford C. Periodontal Disease and Atherosclerosis: From Dental to Arterial Plaque. Arterioscler Thromb Vasc Biol. 2003;23(8):1309-1311. doi: 10.1161/01.ATV.0000087144.24654.71.
5. Gandzyuk V.A. Analiz zahvoryuvanosti na ishemichnu khvorobu sertsya v Ukrayini. Ukrayinskyi kardiolohichnyi zhurnal. 2014;3:45-52. (in Ukrainian).
6. Piepoli M.F., Hoes A.W., Agewall S., Albus C. et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2016;37(29):2315–81. doi: 10.1093/eurheartj/ehw106.
7. Desvarieux M., Demmer R.T., Jacobs D.R. et al. Changes in clinical and microbiological periodontal profiles relate to progression of carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology study. J Am Heart Assoc. 2013; 2(6): e000254. doi: 10.1161/JAHA.113.000254.
8. Pietiäinen M., Liljestrand J.M., Kopra E., Pussinen P.J. Mediators between oral dysbiosis and cardiovascular diseases. Eur Journal of Oral Sci. 2018;126(1):26-36. doi: 10.1111/eos.12423.
9. Mazur I.P., Vitovsky R.V., Slobodyanyk M.V. Poshyrenist parodontopatogennoyi mikroflory v patsiyentiv iz klapannoyu patologiyeyu sertsevo-sudynnoyi systemy. Suchasna stomatologiya. 2018;2:24-29. (in Ukrainian)
10. Harchenko N.L. Mikrobiologichnyi monitoring tranzytornoyi bacteriyeemiyi u stomatologichnyh khvoryh. Sovryemennaya stomatologiya. 2012;1:144. (in Ukrainian).
11. Atarbashi-Moghadam F., Havaei S.R., Havaei S.A. et al. Periopathogens in atherosclerotic plaques of patients with both cardiovascular disease and chronic Periodontitis. ARYA Atheroscler. 2018;14(2):53–7. doi: 10.22122/arya.v14i2.1504.
12. Fiehn N-E., Larsen T., Christiansen N. et al. Identification of Periodontal Pathogens in Atherosclerotic Vessels. J Periodontol. 2005; 76(5):731-6. doi: 10.1902/jop.2005.76.5.731.
13. Haraszthy V.I., Zambon J.J., Trevisan M. et al. Identification of periodontal pathogens in atheromatous plaques. J Periodontol. 2000; 71(10):1554-1560. doi:10.1902/jop.2000.71.10.1554.
14. Aimetti M., Romano F., Nessi F. Microbiologic Analysis of Periodontal Pockets and Carotid Atheromatous Plaques in Advanced Chronic Periodontitis Patients. J Periodontol. 2007;78(9):1718-23. doi:10.1902/jop.2007.060473.
15. Cairo F., Gaeta C., Dorigo W. et al. Periodontal pathogens in atheromatous plaques. A controlled clinical and laboratory trial. J Periodontal Res. 2004; 39(6): 442-6. doi:10.1111/j.1600–0765.2004.00761.x.
16. Andriankaja O., Trevisan M., Falkner K. et al. Association between periodontal pathogens and risk of nonfatal myocardial infarction. Community Dent Oral Epidemiol. 2011;39(2):177–85. doi: 10.1111/j.1600-0528.2010.00582.x.
17. Mäntylä P., Buhlin K., Paju S. et al. Subgingival Aggregatibacter actinomycetemcomitans associates with the risk of coronary artery disease. J Clin Periodontol [Internet]. 2013;40(6):583–90. doi:10.1111/jcpe.12098.
18. Nonnenmacher C., Stelzel M., Susin C. et al. Periodontal microbiota in patients with coronary artery disease measured by real-time polymerase chain reaction: a case–control study. J Periodontol. 2007;78(9):1724-30. doi:10.1902/jop.2007.060345.
19. Spahr A., Klein E., Khuseyinova N. et al. Periodontal infections and coronary heart disease: role of periodontal bacteria and importance of total pathogen burden in the Coronary Event and Periodontal Disease (CORODONT) study. Arch Intern Med. 2006;166(5):554-9. doi: 10.1001/archinte.166.5.554.

The work is a fragment of scientific research work of Department of Therapeutic Dentistry FPGE, Danylo Halytsky Lviv National Medical University: "Metabolic disorder and its impact on the development of combined dental and somatic pathology", state registration number 0120U002131.

ORCID and contributionship:
Olesya I. Hodovana : 0000-0003-4833-8935 [A,E,F]
Oksana V. Skybchyk: 0000-0002-0279-1323 [A-D]
Tetiana M. Solomenchuk : 0000-0002-6153-0457 [A,E,F]
Tetiana M. Rumynska: 0000-0002-0669-5865 [B,C]

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

CORRESPONDING AUTHOR
Oksana V. Skybchyk
Danylo Halytsky Lviv National Medical University
69 Pekarska st., 79010 Lviv, Ukraine
tel: +380985543320
e-mail: oksana.skybchyk@gmail.com

Received: 04.10.2020
Accepted: 15.08.2021

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

COMORBIDITIES AT THE TUBERCULOSIS AMONG CHILDREN

DOI: 10.36740/WLek202110113

Zoriana I. Piskur, Lidiia I. Mykolyshyn
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

The aim: To study the structure of clinical forms of tuberculosis (TB) which are combined with the comorbidities, to prevent the development, and to improve the diagnostics of TB among children with nonspecific diseases.

Materials and methods: A retrospective, selective research of 330 cards of children for the age group from 0 to 15 years old who were treated on local forms of pulmonary and extrapulmonary TB in a specialized pediatric department for the last 30 years was conducted.

Results: Among 92.9 % children with comorbidities, the specific process of respiratory system was detected. Every seventh child has developed generalized forms of pulmonary TB. 43.8 % of children had extrapulmonary TB. In the structure of comorbidities among children with extrapulmonary TB were observed iron deficiency anemia, the diseases of digestive and endocrine systems, malnutrition, cachexia and rickets. The iron deficiency anemia was accompanied by TB of the peripheral LN and TB of the CNS. TB of the rare localization and TB of the CNS were combined with diseases of the digestive system. At pulmonary TB were detected infectious and parasitic diseases. The concomitant pathology of the respiratory and cardiovascular systems was often detected with the TB of intrathoracic LN, and concomitant pathology of the eyes, ears and CNS – with primary tuberculosis complex. The variety of comorbidities and extrapulmonary TB has led to the diagnostic errors and prolonged stay of children in several somatic hospitals.

Conclusions: To prevent the development of TB among children with non-specific diseases, it is necessary to strengthen anti-TB measures among them.

KEY WORDS: extrapulmonary tuberculosis, pulmonary tuberculosis, concomitant pathology, children

Wiad Lek. 2021;74(10 p.I):2433-2438

INTRODUCTION

Tuberculosis (TB) is a known infectious disease and one of the top 10 causes of death worldwide. In 2019, an estimate of 10.0 million new cases of TB occurred all over the globe, and 11% of these cases were represented by children under 15 years old [1]. Epidemiological factor is one of main factors at the tuberculosis. The risk of developing TB after contracting infection is variable. Some studies [2, 3] presented that 28.7-45.5% of children who were in contact with a patient with TB for 3 years or more, developed specific process. In the foci of TB infection, children acquire a disease caused by Mycobacterium TB derived from a source of infection [4, 5]. The social factor [6] and the absent of the BCG vaccination are also important in the development of the TB among children. The clinical case of pulmonary TB combined with specific destructive spondylitis in a 2.5-year-old BCG unvaccinated child showed a severe course of the process and difficulties in diagnosing. Child`s lack of antituberculosis immunity caused a rapid progressive lesion of the spine and lungs which was rapidly complicated by a nonspecific pathology [7]. Equally important is the presence among children of the comorbid pathology [8], which weakens the child`s body, reduces immunity, what contributes to the accession of the infection. The research shows that among children with urogenital disorders developed extrapulmonary forms of TB or combinations of pulmonary and extrapulmonary forms [9]. The clinical manifestations of the accompanying diseases, in combination with a specific process, complicate the diagnostics of tuberculosis [10, 11]. It is important to assess which forms of tuberculosis are combined with comorbidities and what variants of comorbidities are typical.

THE AIM

The aim is to study the structure of clinical forms of tuberculosis which are combined with comorbidities, to prevent the development and to improve the diagnostics of tuberculosis among children with nonspecific diseases.

MATERIALS AND METHODS

This documental and retrospective research used medical files to investigate the characteristics associated with TB among total number of hospitalized children. All medical files from children aged zero to fifteen years that were hospitalized to receive treatment for any local forms of TB from January 1988 to December 2017 available during data collection period were included. No medical file was excluded from data collection. The selective, retrospective data was collected and analyzed on the medical histories of 330 children, who were hospitalized in the pediatric department of the Lviv Anti-TB Dispensary for pulmonary (169 children) and extrapulmonary (161) tuberculosis (TB). The children were divided into the following groups: the first (1st) group – TB with comorbidities (n=112); the

second (2nd) group – TB without comorbidities (n=218). It was analyzed the following information: age, sex, place of residence, social factors, the presence of BCG vaccination, clinical forms of pulmonary and extrapulmonary TB, comorbidities, the presence of the contact with a patient with TB, the amount of non-specialized facilities in which the children were before the admission to a specialized hospital, the method of the detection and the duration of the specific process before the diagnosing. Children, whose postvaccination scar was absent or 1-3 mm in size, were considered ineffectively vaccinated with BCG. The same group included unvaccinated, and those children who did not have vaccination data.

The statistical processing of materials was performed using the computer program Statistica 8.0. The reliability of the difference between the two relative indicators was assessed using the Fisher test with the Metropolis algorithm [12]. The difference in $p<0.05$ was considered statistically significant.

RESULTS

Young children were more often observed in the 1st group (40.2%), children aged 8-14 years – in the 2nd group (43.1%), preschoolers were in the same amount in both groups (27.7% and 29.4% respectively). The number of boys (55.4% and 49.5% respectively) and girls (44.6% and 50.5% respectively) in both groups did not differ significantly. In both groups, urban children predominated (54.5% and 56.0% respectively). The adverse social factors were found in 53.6% of children of the 1st group and 47.7% of children of the 2nd one. In the 1st group there were significantly more orphans (6.3% vs. 1.4%; $p<0.05$). The bad habits of parents were more often observed in the 1st group (4.5% vs. 1.8%; $p>0.05$); in the same group there were children who were living in poor conditions (28.6% vs. 26.1%; $p>0.05$). The children from the 2nd group were more often half-orphans (20.2% vs. 15.2%; $p>0.05$), and from large families (25.7% vs. 23.2%; $p>0.05$). The vast majority of children in both the1st (77.7%) and 2nd (71.6%) groups were ineffectively vaccinated with BCG ($p>0.05$).

The study established that among 92.9% of children from the 1st and 85.3% of children from 2nd groups was observed pulmonary TB ($p<0.05$). At the same time, in 56.3% and 48.6% (respectively) cases, the pulmonary TB was as an independent form ($p>0.05$), and in 36.6% and 36.7% children ($p>0.05$), it was combined with an extrapulmonary TB. In the structure of the TB of the respiratory organs in the 1st group the primary tuberculosis complex was observed more often (44.2% vs. 38.2%; $p>0.05$), and significantly less often the TB of intrathoracic lymphatic nodes (LN) (43.3% vs. 58.6%; $p<0.05$). It should be noted that the miliary pulmonary TB was diagnosed in both research groups, but in the 1st one it was occurred significantly more often (11.5% vs. 2.7%; $p<0.01$). At the same time, there was a significantly ($p<0.05$) higher percentage of children with the generalized forms of the pulmonary TB in the 1st group in comparison to the 2nd one (14.3% vs. 3.6%). It was established that the proportion of the pulmonary TB detected in the phase of infiltration, seeding and decay among children of the 1st group was significantly higher (26.0% vs. 13.4%; $p<0.01$), but detected in the infiltration phase (59.6% vs. 67.2%; $p>0.05$), in the phase of calcification and calcination was slightly lower (5.7% vs. 10.8%; $p>0.05$) (Figure 1). The phase of compaction of children of the studied groups did not differ significantly (8.7% vs. 8.6%; $p>0.05$). Among children of the 1st group bilateral lesions were noticed more frequently (31.7% vs. 22.0%; $p>0.05$). At the same time, in the 1st group of children, compared to the 2nd one, the complications of the pulmonary TB were found significantly often (19.2% vs. 1.6%; $p<0.001$). It should be noted that three children from the 1st group had the complex of complications of the pulmonary and the extrapulmonary TB.

It was found that among 49 (43.8 %) children from the 1st group and 112 (51.4%) children from the 2nd one had extrapulmonary TB. The comparative analysis of the structure of clinical forms of the extrapulmonary TB (Figure 2) showed that 32.7% (16 of 49) of children from the 1st group and 33.0% (37 of 112) of children from the 2nd one were found with the TB of the peripheral LN. The tuberculous meningitis and the TB of the central nervous system (CNS) (18.4% vs. 23.2%), the TB of the bones and joints (14.3% vs. 25.0%), and the TB of the eyes (6.1% vs. 8.0%) were observed somewhat less frequently ($p>0.05$) among children from the 1st group. At the same time, the proportion of the TB of other localization was slightly higher in the 1st group (14.3% vs. 7.1%; $p>0.05$). In the structure of the TB of other localizations among children from the 1st group the TB of intestines was observed in 4 cases, the TB of spleen – in two cases, one child had the TB of skin; in the 2nd group it was found the TB of kidneys and skin in three cases, the TB of intestines and spleen – in one. The complications of the extrapulmonary tuberculosis were developed among 24.5% (12 of 49) children from the 1st group and 27.7% (31 of 112) children from the 2nd one.

Thus, among children with comorbidities, the pulmonary TB was more often found, the primary tuberculosis complex and the miliary pulmonary TB were prevailed. The specific process of the respiratory system was often bilateral, in the phase of infiltration, seeding and decay. The extrapulmonary TB was observed among children with comorbidities and without them.

In order to analyze the comorbidities (Table I) the children from the 1st group were divided into 2 subgroups: a subgroup of children with the pulmonary TB (63 people) and a subgroup of children with the extrapulmonary TB (49). It was found that children with the pulmonary TB were significantly more likely to have infectious and parasitic diseases (41.3% vs. 22.4%; $p<0.05$), and respiratory diseases (14.3% vs. 2.0%; $p<0.05$). In addition, only this group was diagnosed with diseases of the eyes (6.3 %) and ears (1.6%). Among infectious and parasitic diseases among children with the pulmonary TB most often were found ascariasis (42.3%), enterobiosis (23.1%), in one case – a combination of them, and in 7.7% – trichofacillosis;

Table I. Distribution of the comorbidities among children with extrapulmonary and pulmonary tuberculosis

Presence of the infectious or somatic diseases or conditions of the various organs and systems	Pulmonary tuberculosis (n=63)		Extrapulmonary tuberculosis (n=49)	
	number	%	number	%
Infectious and parasitic diseases	26	41.3	11	22.4
Protein and mineral metabolism disorders	1	1.6	5	10.2
Diseases of the respiratory system	9	14.3	1	2.0
Diseases of the hematopoietic system	4	6.3	8	16.3
Diseases of the digestive system	3	4.8	5	10.2
Diseases of the endocrine system	2	3.2	4	8.2
Diseases of the cardiovascular system	5	7.9	4	8.2
Diseases of the immune system	2	3.2	3	6.1
Diseases of the central nervous system	2	3.2	3	6.1
Diseases of the urinary system	2	3.2	3	6.1
Diseases of the muscular system	1	1.6	2	4.1
Diseases of the eyes	4	6.3	-	-
Diseases of the ears	1	1.6	-	-
Hand skin burn	1	1.6	-	

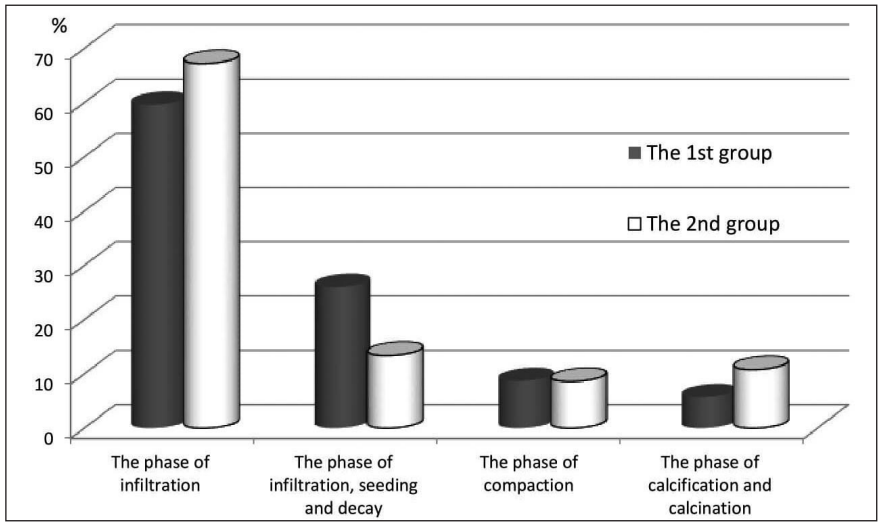


Fig. 1. The phases of the pulmonary tuberculosis among children of the 1st and the 2nd groups.

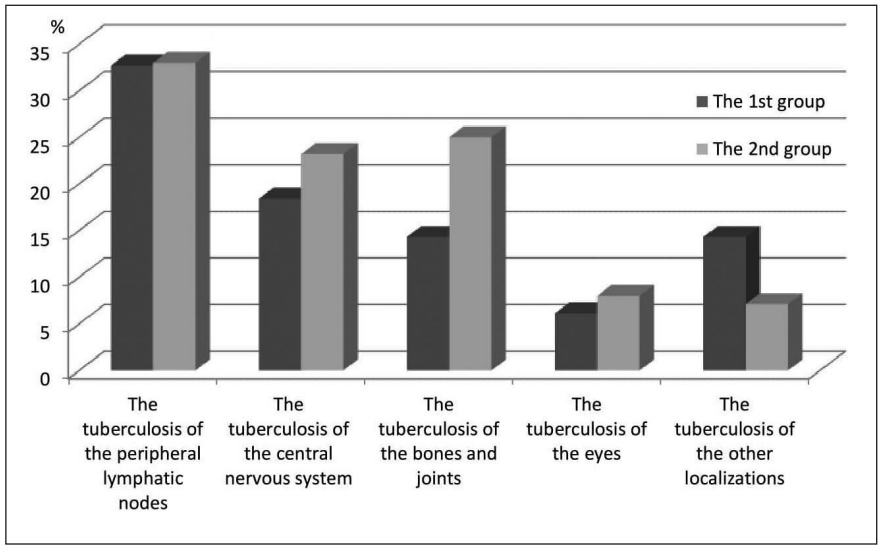


Fig. 2. The structure of clinical forms of the extrapulmonary tuberculosis among children of the 1st and the 2nd groups.

among diseases of the respiratory system it was found bronchitis (55.6%). The infectious and parasitic diseases were combined with the TB of intrathoracic LN and the primary tuberculosis complex in equal amounts (50.0%). The diseases of the respiratory (55.6% vs. 44.4%; p>0.05) and cardiovascular (80.0% vs. 20.0%; p>0.05) systems were more common among children with the TB of intrathoracic LN. At the same time, diseases of the eyes (75.0% vs. 25.0%; p>0.05), diseases of the CNS and ears were accompanied by the primary tuberculosis complex.

The children with the extrapulmonary TB were significantly more likely to have diseases of the hematopoietic system (16.3% vs. 6.3%; p>0.05), the vast majority of iron deficiency anemia (87.5%), as well as diseases of the digestive system (10.2% vs. 4.8%; p>0.05): gastritis, gastroduodenitis, cholecystitis, enterocolitis. The iron deficiency anemia was observed in 50.0% of children with the TB of the peripheral LN, in 25.0% – with the TB of the CNS, in one case – with the TB of the bones and joints and with the TB of intestinal organs. The diseases of the digestive system were detected among children with the TB of the intestinal organs, the TB of the spleen, the TB of the mesenteric LN, and in 40.0% of the cases – with the TB of the CNS. Among children with the extrapulmonary TB, protein and mineral metabolism disorders were observed somewhat more often (10.2% vs. 1.6%; p>0.05), namely it was found in 3 (60.0%) children the malnutrition of the second stage and in one case – cachexia and rickets. The pathology of the endocrine system (growth disorders, thyroid and adrenal function) was also more common among children with the extrapulmonary TB (8.2% vs. 3.2%; p>0.05), at the same time in 50.0% of the cases – with the TB of the bones and joints. It should be noted that two children with the TB of the peripheral LN and one child with the TB of intrathoracic LN the pathology of the immune system was represented by HIV infection. Thus, bronchitis, infectious and parasitic diseases prevailed among children with the pulmonary TB, anemia, diseases of the digestive and endocrine systems were more often observed among children with the extrapulmonary TB.

It is important that the larger amount of children in the researched groups had a contact with a patient with TB (56.3% vs. 51.4%; p>0.05), more often with family member (68.3% vs. 69.6%; p>0.05). Both children from the 1st and 2nd groups were in the contact with person with discharging bacteria (34.9% vs. 37.5%; p>0.05), and were from foci of death (11.1% vs. 13.4%; p>0.05). Children from the 1st group who were in contact with person with discharging bacteria were less often under the supervision of the Anti-TB dispensary (63.6% vs. 81.0%; p>0.05). Chemoprophylaxis in both study groups was performed rarely (7.9% vs. 17.9%; p>0.05). It is important to note that 45.5% of children from the 1st and 16.7% children from the 2nd group were in contact with person with discharging bacteria who had resistant forms of the TB (p<0.05). Thus, 36.4% of children with comorbidities, who were into contact with patients, including those with resistant TB, were not under the supervision of the Anti-TB dispensary.

It was established that mostly children of these groups were found during the application for the medical care (72.3% vs. 72.0%; p>0.05), more seldom during the initial examination the children who were in the contact with TB patients (17.9% vs. 20.2%; p>0.05) and during the preventive examination (9.8% vs. 7.8%; p>0.05). It should be noted that children from the 1st group during delivering medical care were more often detected within 2 weeks (33.3% vs. 30.6%; p>0.05) and one month (44.4% vs. 31.8%; p>0.05), and children from the 2nd group – within 6 months (13.8% vs. 19.7%; p>0.05), one year (3.7% vs.12.7%; p<0.05), and more than one year (4.9% vs. 5.1%; p<0.01). At the same time, it was investigated that during the examination of the contacts of children from the 1st group, compared with children from the 2nd group, the diagnosis was significantly less often established within 2 weeks (30.0% vs. 70.5%; p<0.01), slightly often within one month (55.0% vs. 29.5%; p>0.05). It should be noted that only among children with TB and concomitant pathology before diagnosing, the specific process lasted 6 months in one case and one year – in two cases. Thus, the comorbidities contributed to the londer-term detection among children who were in the contact with a patient with TB.

It was found that the vast majority of the children were diagnosed with the TB in the clinic (39.3% vs. 40.8%; p>0.05), and during their stay in one of the non-specialized medical facilities (37.5% vs. 35.8%; p>0.05). It should be noted that before the diagnosing, the children from the 1st and 2nd groups were in two (17.0% vs. 17.0%; p>0.05) and three or more (6.2% vs. 6.4%; p>0.05) somatic hospitals. Among children from the 1st group, the clinical manifestations of the concomitant pathology made the diagnosis of TB complicated, among children from the 2nd group, the symptoms of extrapulmonary TB led to an erroneous diagnosis.

DISCUSSION

Identification of the best strategies for prevention and control of concomitant diseases is vital for progress towards the fulfillment of the Sustainable Development and the End TB Strategy Goals. This can only be possible with a thorough knowledge of how these diseases develop. The analysis of literature sources of scientometric databases revealed single publications of the combination of TB of different localizations and other pathological conditions. The publication of studies of the urogenital disorders among ill children with TB partially reveals the impact of comorbidities on the course of TB. The study showed that children with concomitant pathology were more often diagnosed with TB of the respiratory organs (70.1%), 12.1% had generalized TB, and 17.8% of children had TB of the extrapulmonary localization. [9]. However, in our study we analyzed a number of indicators among children with specific lesions of the pulmonary and extrapulmonary localizations and the concomitant pathology and identified significant differences. The vast majority of children with comorbidities had adverse social factors and was ineffectively vaccinated with BCG, what contributed to

the accession of a specific infection and the development of TB. It was found that the TB of the respiratory system mainly developed as the primary tuberculosis complex (44.2%) and the miliary pulmonary TB (11.5%). Among children with the concomitant pathology, the pulmonary TB significantly more often was observed in the phase of infiltration, seeding and decay (26.0%) with bilateral lesions (31.7%), and the subsequent development of complications of a specific process (19.2%). At the same time, the extrapulmonary TB was more often represented by a specific process in the peripheral LN (32.7%) and the rare localization in intestines, spleen and skin (14.3%).

A systematic review of TB-endemic areas of Africa has emphasized the potential importance of Mycobacterium tuberculosis in acute severe pneumonia in children as a primary cause or underlying comorbidity. Was systematically reviewed clinical and autopsy studies done in tuberculosis-endemic settings that enrolled 100 children aged younger than 5 years with severe pneumonia. Of the 3644 patients who had culture of respiratory specimens for *M. tuberculosis* undertaken, 275 (7-5%) were culture positive, and an acute presentation was common. Inpatient case-fatality rate for pneumonia associated with tuberculosis ranged from 4% to 21% in the four clinical studies that reported pathogen-related outcomes [13]. M.A. Romanova et al. [14] described the structure of clinical forms of TB and its concurrent conditions among children of different ages depending on the degree of social disadaptation of their families. According to the authors, concomitant pathology was more often observed among children with active TB from socially disadaptation families 82.7% vs. 76% (χ^2-1.051, p-0.305), namely pathology of the cardiovascular system. In our study, the analysis of comorbidities showed that the extrapulmonary TB was more often combined with the diseases of the hematopoietic (16.3%), digestive (10.2%) and endocrine (8.2%) systems, as well as with the disorders of protein and mineral metabolism. The iron deficiency anemia in half of the cases was accompanied by the TB of the peripheral LN, in 25.0% – the TB of the CNS. The TB of the rare localization and the TB of the CNS were combined with diseases of the digestive system. The infectious and parasitic diseases were more often observed with the pulmonary TB (41.3%), equal amounts (50.0%) with the TB of the intrathoracic LN and the primary tuberculosis complex. The concomitant pathology of the respiratory (55.6%) and cardiovascular (80.0%) systems was often detected with the TB of the intrathoracic LN, and the concomitant pathology of the eyes, ears and CNS – with the primary tuberculosis complex. HIV infection was noted in both subgroups.

It was established that more than the half of the children in this group were in a contact with a patient with TB, almost 70.0% had a family contact, 35.0% – with a person discharging the bacteria, and 45.5% – with exudates that had resistant forms of the TB. The stay of the children in two or more non-specialized medical hospitals before the establishment of the TB indicates the difficulties in the detecting and the diagnosing the disease. Most children were found during administering medical care and less often during the examination of the source of the infection and during the preventive examination. It was proved that during the examination of the contact with a patient with TB among children with comorbidities till the diagnosing, the specific process lasted longer, which led to the development of the complications and the generalization in various organs. Thus, all of the above proves the difficulty in the differential diagnostics and setting the diagnosis of TB, due to the masking of the symptoms of the comorbidity, due to a combination of specific and nonspecific processes, and symptoms of the extrapulmonary TB, which was assessed as nonspecific processes.

CONCLUSIONS

1. Pulmonary TB was found among 92.9% children with comorbidities. The specific process of the respiratory system was often bilateral and in the phase of infiltration, seeding and decay. Every seventh child has developed generalized forms of the pulmonary TB. 43.8% of children with comorbidities had extrapulmonary TB, which became complicated in 24.5% cases.
2. In the structure of the comorbidities among children with the extrapulmonary TB more often were observed iron deficiency anemia, the diseases of the digestive and endocrine systems, malnutrition of second stage, cachexia and rickets. The iron deficiency anemia was accompanied by the TB of the peripheral LN and the TB of the CNS. The TB of the rare localization and the TB of the CNS were combined with diseases of the digestive system.
3. Among children with the pulmonary TB were detected infectious and parasitic diseases. The concomitant pathology of the respiratory and cardiovascular systems was often detected with the TB of the intrathoracic LN, and the concomitant pathology of the eyes, ears and CNS – with the primary tuberculosis complex.
4. The variety of symptoms of the concomitant pathologies and the extrapulmonary TB has led to the diagnostic errors, and prolonged stay of children in several somatic hospitals.
5. To prevent the development of the TB among children with non-specific diseases, it is necessary to strengthen anti-TB measures among them.

REFERENCES

1. World Health Organization. Global tuberculosis report 2019. Geneva: WHO; 2019. https://www.who.int/teams/glob....
2. Aksenova V.A., Klevno N.I., Kavtarashvili S.M. et al. Ochah tuberkuleznoi ynfektsyy kak rysk razvytyia u detei tuberkuleza s mnozhestvennoi lekarstvennoi ustoichyvostiu [The nidus of tuberculous infection as a risk factor of multiple drug resistant tuberculosis in children]. Tuberculosis and Lung Diseases. 2018; 96(1): 11-17. doi: 10.21292/2075-1230-2018-96-1-11-17. (in Russian)
3. Gubkina M.F., Khokhlova YU.YU., Yukhimenko N.V. et al. Kharakterystyka tuberkuleznыkh protsessov u ystochnykov ynfektsyy y u zabolevshykh detei pry razlychnoi prodolzhytelnosty kontakta [Description of the course of tuberculosis in the sources of infection and children who developed tuberculosis after exposure to the infection for different periods of time]. Tuberculosis and Lung Diseases. 2018; 96(9): 17-22. doi: 10.21292/2075-1230-2018-96-9-17-22. (in Russian)

4. Barmina N.A., Baryshnikova L.A. Vozmozhnosty povышenyia эffektyvnosty profylaktyky zabolevanyia u detei v ochahakh tuberkuleznoi ynfektsyy na prymere Permskoho kraia [Ways to enhance the efficiency of tuberculosis prevention in the children exposed to tuberculous infection illustrated by the example of Perm kray]. Tuberculosis and Lung Diseases. 2018; 96(9): 50-56. doi: 10.21292/2075-1230-2018-96-9-50-56. (in Russian)
5. Lozovskaya M.E., Kurova A.S., Vasilieva E.B. et al. Osobennosty klynycheskoho techenyia y khymyoterapyy tuberkuleza u detei yz ochahov s razlychnыm spektrom lekarstvennoi chuvstvytelnosty mykobakteryi [Specific features of the clinical course and chemotherapy of tuberculosis in children exposed to tuberculosis with different drug resistance patterns]. Tuberculosis and Lung Diseases. 2019; 97(9): 22-27. doi: 10.21292/2075-1230-2019-97-9-22-27. (in Russian)
6. Zolotova N.V., Akhtyamova A.A., Ovsyankina E.S. et al. Sotsyalnыi portret semei detei y podrostkov, bolnыkh tuberkulezom orhanov dыkhanyia [Social features of the families of children and adolescents suffering from respiratory tuberculosis]. Tuberculosis and Lung Diseases. 2018; 96(10): 23-29. doi: 10.21292/2075-1230-2018-96-10-23-29. (in Russian)
7. Raznatovska O.M., Myronchuk YU.V., Pushnova O.O. et al. Osoblyvosti perebihu tuberkulozu v ditei, yaki ne shchepleni vaktsynoiu BTsZh [Features of tuberculosis course in BCG unvaccinated children]. Zaporozhye medical journal. 2018; 20(4): 593-602. doi: 10.14739/2310-1210.2018.4.135754. (in Russian)
8. Polyakova A.S., Bagdasaryan T.R., Stepanyan I.E. et al. Sluchai dyssemynyrovannoho porazhenyia lehkykh pry sochetannom zabolevanyy [A clinical case of disseminated pulmonary lesions with a concurrent condition]. Tuberculosis and Lung Diseases. 2018; 96(7): 55-59. doi: 10.21292/2075-1230-2018-96-7-55-59. (in Russian)
9. Romanova M.A., Mordyk A.V., Ivanova O.G. et al. Bolezni mochepolovoy sistemy u bol'nykh tuberkulezom detey [Urogenital disorders in children ill with tuberculosis]. Tuberculosis and Lung Diseases. 2018; 96(2): 36-40. doi: 10.21292/2075-1230-2018-96-2-36-40. (in Russian)
10. Kulchavenya E.V., Zhukova I.I. Vnelegochnyy tuberkulez – voprosov bol'she, chem otvetov [Extrapulmonary tuberculosis – more questions than answers]. Tuberculosis and Lung Diseases. 2017; 95(2): 59-63. doi:10.21292/2075-.... (in Russian).
11. Dasho M.B., Lyshenyuk C.A., Chulovska U.B. et al. Trudnoshchi i pomylky dyferentsialnoi diahnostyky pry tuberkuloznomu meninhoentsefaliti u dytyny 6,5 misiatsiv [The difficulties and mistakes in the differential diagnostics of tuberculous meningoencephalitis of child aged 6,5 months]. Infection Diseases. 2016; 2(84): 87-91. doi: 10.11603/1681-2727.2016.2.6529. (in Russian)
12. Miller M. P. RxC program for the analysis of contingency tables (Department of Biological Sciences – Box 5640 Northern Arizona University: 1997). http://www.marksgeneticsoftwar....
13. Oliwa J.N., Karumbi J.M., Marais B.J. et al. Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review. The Lancet Respiratory Medicine. 2015; 3(3): 235-243. doi: 10.1016/S2213-....
14. Romanova M.A., Mordyk A.V., Tsygankova E.A. Strukturы klynycheskykh form tuberkuleza y sopustvuiushchei emu patolohyy u detei v zavysymosty ot stepeny sotsyalnoi dezadaptatsyy ykh semei [Structure of clinical forms of tuberculosis and its concurrent conditions in children depending on the degree of social disadaptation of their families]. Tuberculosis and Lung Diseases. 2015; 7: 120-121. https://www.tibl-journal.com/j.... (in Russian).

ORCID and contributionship:
Zoriana I. Piskur: 0000-0001-9920-2291 [A-D,F]
Lidiia I. Mykolyshyn: 0000-0003-1893-8446 [E,F]

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

CORRESPONDING AUTHOR
Zoriana I. Piskur
Danylo Halytsky Lviv National Medical University
8/24 Brand Vovchok st., 79016 Lviv, Ukraine
tel: +380505072124
e-mail: zorjanapiskur@gmail.com

Received: 09.10.2020
Accepted: 15.08.2021

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

COMPARATIVE PSYCHOMETRIC ANALYSIS OF COGNITIVE FUNCTIONS IN PATIENTS WITH HYPERTENSIVE DISEASE AND HYPOTHYROIDISM

DOI: 10.36740/WLek202110114

Mariana I. Lesiv, Victoriia A. Hryb
IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

The aim: To determine whether certain cognitive domains exist in the assessment of cognitive functions in HD patients, patients with hypothyroidism and HD patients with concomitant hypothyroidism.

Materials and methods: The patients were divided into 3 groups according to nosology: Group I – 21 patients with hypertensive disease (HD); Group II – 18 patients with hypothyroidism, Group III – 19 hypertensive patients with concomitant hypothyroidism.

Results: It was revealed that patients with HD had a decrease in memory according to the test proposed by A.R. Luria for learning 10 words, ($p<0.05$), as well as Digit span from Mattisse scale, ($p<0.05$). In patients with hypothyroidism, a short span of attention was revealed, according to the method of "Selectivity of attention" (G. Munsterberg test), ($p<0.05$). The analysis of the results showed that considering the interaction of factors (HD and hypothyroidism), the most affected cognitive domains are memory, executive functions and optical-spatial functions, respectively, ($p<0.05$).

Conclusions: To diagnose CI in patients with HD who have problems with the domain of cognitive function memory, it is advisable to use a test for learning 10 words according to the method proposed by A.R. Luria and Digit span from Mattisse scale. In patients with hypothyroidism, attention and executive functions should be determined using the Schulte Tables and the "Selectivity of Attention" method (G. Munsterberg test). With the combined pathology, HD patients with a concomitant hypothyroidism should use Schulte Tables, test for learning 10 words by A.R. Luria and Clock Drawing Test.

KEY WORDS: cognitive functions, hypertensive disease, hypothyroidism, neuropsychological testing

Wiad Lek. 2021;74(10 p.I):2439-2443

INTRODUCTION

The issue of early diagnosis of cognitive disorders is extremely relevant in modern medicine, especially in patients with several diseases that may affect the state of cognitive functions, which directly or indirectly adversely affect blood supply to the brain and/or metabolism, in particular hypertension and hypothyroidism.

Studies have shown that one of the most common pathogenetic factors in the formation and development of cognitive impairment (CI) is HD, especially its long course which leads to CI of varying degrees; cerebrovascular pathology on the background of HD is one of the main causes of cognitive decline [1]. Studies of Systolic Hypertension in Europe trials, PROGRESS, LIFE, SCOPE, MOSES have shown that normalization of blood pressure (BP) significantly reduces the risk of developing and progressing of CI [2]. However, according to the analysis of studies, there is no convincing evidence that a decrease in blood pressure in patients with HD without cerebrovascular disease in the anamnesis prevents the development of CI [3]. Despite the widespread world's reports about the need to control blood pressure, the XVIII European Cardiac Congress in Birmingham presented disappointing data on the detection of HD, the attitude of patients to their condition and the quality of health care [4]. It was found that among all patients who suffer from HD, only about 50% know they are sick. Among those aware, only half receive antihypertensive treatment. And of this half, only one in two is treated adequately, which is 12.5% of the cohort of people with HD [5]. On the basis of the Kharkiv city polyclinic, a long-term prospective study was conducted with the aim to study the course of HD, the adequacy of antihypertensive drugs and complications of HD in patients with diseases of the internal organs [6]. The results of the study (described in 2010) revealed the presence of polypragmatism in the treatment of high blood pressure, frequent use of second-line drugs (25.1%), lack of practice of titrating doses of antihypertensive drugs to optimal, underestimation by doctors of more careful blood pressure control and lack of timely dose adjustment of antihypertensive drugs for HD patients who take drugs for the treatment of comorbidities that can increase blood pressure and as a result – a fairly low percentage of achieving the target blood pressure in patients (36.6%), i.e. only a third of cases.

Among all causes of cognitive impairment, metabolic disorders account for about 5%, in particular, thyroid dysfunction with the development of hypothyroidism. Moreover, given its duration and severity, the prevalence

of CI in this pathology varies, according to various authors, from 1.3% to 10.3% (Niafar M. et al., 2009; Catherine M. Otto, et al., 2012). Some authors [7] hold the view that cognitive functions are restored by compensating for hypothyroidism, but there is another opinion: CI that developed in thyroid hormone deficiency is irreversible or partially reversible [8]. According to the results of study of thyroid pathology in Ukraine and Poltava region in recent years, the prevalence of hypothyroidism in 2005-2013 has been studied; it was found that doctors underestimate the need for more thorough diagnosis and lack of timely dose adjustment [9]. It should be noted that prolonged deficiency of thyroid hormones causes cognitive deficits, which leads to disability and reduced quality of life of patients. Our previous studies identified insufficient control of blood pressure in patients with hypertension and thyroid-stimulating hormone (TSH) in patients with hypothyroidism. According to the retrospective analysis of medical documentation of patients with HD, we found insufficient blood pressure control; the average level of systolic/diastolic blood pressure (SBP/DBP) was 159.20±8.36/98.00±8.54; 162.50±6.34/101.04±10.01 mm Hg, respectively, for patients with hypertension and HD patients with concomitant hypothyroidism. Analyzing TSH level, it was found that at the time of examination the patients were at the stage of compensation (TSH=3.96±1.09 mIU/l; 4.12±0.78 mIU/l, respectively, for patients with hypothyroidism and HD patients with concomitant hypothyroidism). We also assessed TSH levels in patients with a confirmed history of hypothyroidism. A retrospective analysis of the data was performed and it was found that in the examined patients the average level of TSH was insufficiently corrected, TSH=7.14±2.37 mIU/l; 8.03±6.77 mIU/l, for patients with hypothyroidism, HD patients with concomitant hypothyroidism, respectively. Given that HD and hypothyroidism are important risk factors for CI, moreover, these diseases can be mutually burdensome, it is especially important to study cognitive function in people with combined pathology to determine whether these two pathologies have an additive effect on CI. Thus, based on our results, which show that HD patients had a significantly lower score in the memory domain, patients with hypothyroidism had a significantly lower score in the domain of attention and executive functions, and analysis of the results showed that taking into account the interaction factors (HD and hypothyroidism) it was found that 40% of the most affected cognitive domains are memory and speech, and 60% of patients have impaired executive functions, as well as optical-spatial functions, respectively. Based on the results, the following psychometric methods were used to screen for cognitive-mnestic disorders: a test for learning 10 words according to the method proposed by A.R. Luria and Digit span from Mattisse scale for patients with HD; for patients with hypothyroidism – the method of "Selectivity of attention" (G. Munsterberg test), Schulte Tables; for HD patients with concomitant hypothyroidism – a test for learning 10 words according to the method proposed by A.R. Luria, Schulte Tables and Clock Drawing Test. The set of neuropsychological techniques should be simple enough to diagnose CI regardless of the medical diagnosis of the test subject, but at the same time it should be sensitive to relatively minor CI, which in the absence of timely correction can lead to more severe disorders with dementia.

THE AIM

The aim of the study was to determine whether certain cognitive domains exist in the assessment of cognitive functions in HD patients, patients with hypothyroidism and HD patients with concomitant hypothyroidism.

MATERIALS AND METHODS

To achieve the goal, we used the results of our published work on the study of neuropsychological structure of patients with HD, patients with hypothyroidism and HD patients with concomitant hypothyroidism. Retrospective analysis of medical records revealed insufficient long-term control of blood pressure and TSH, which in the future became the cause of CI in this group of patients [10]. A comprehensive clinical and neurological examination of 58 patients (27 men and 31 women), mean age – 47.84±0.36 years, disease duration – 13.56±0.79 years was carried out. The CG involved 18 people representative of age and sex ratio without a history of HD, hypothyroidism. Patients were divided into 3 groups by nosology: Group I – 21 patients with HD who received antihypertensive therapy; Group II – 18 patients with hypothyroidism who received L-thyroxine at a dose of 100-150 mg, Group III – 19 HD patients with concomitant hypothyroidism who received appropriate treatment. Data used to select patients with HD were the following: stage of the disease, the degree of increase in blood pressure, disease duration, the level of SBP and DBP. Patients with HD received basic therapy (antihypertensive). The diagnosis of hypothyroidism was made by an endocrinologist according to the recommendations of the American Association of Clinical Endocrinologists and the American Thyroid Association for the Diagnosis and Treatment of Hypothyroidism in Adults [11]. Hypothyroidism caused by autoimmune thyroiditis was revealed in 9 (50%) individuals, as a result of thyroid surgery – in 5 (27.7%) patients, and in 4 (22.3%) individuals hypothyroidism occurred spontaneously. At the time of examination, the stage of compensation of hypothyroidism was diagnosed in 5 (27.8%) patients, subcompensation – in 13 (72.2%) patients. Exclusion criteria: history of traumatic brain injury and stroke, mental illness, diseases of the blood system, cancer, persistent atrial fibrillation, chronic obstructive pulmonary disease (respiratory failure of the I-II stages), Diabetes mellitus, renal failure and hepatic failure in the stage of decompensation.

The study was conducted on the basis of the Endocrinology and Neurology departments of the Ivano-Frankivsk Regional Clinical Hospital, as well as on the basis of the Hypertension department of the Ivano-Frankivsk Regional Clinical Cardiology Dispensary. All patients gave informed

Table I. Frequency of subjective complaints of patients (M ± m)

Subjective complaints of patients	Control Group, n=18	Group I, n=21	Group II, n=18	Group III, n=19
Memory				
"I cannot remember new information"	11.11±7.41	14.29±7.64	44.44±11.71*•	57.89±11.33×
" I can't do mental arithmetic "	5.56±5.40	19.05±8.57	22.22±9.80	31.58±10.66
Attention				
"I lose my mind"	16.7±8.78	14.29±7.64	33.33±11.11	42.11±11.33×
" I don't listen after 10 minutes of conversation "	5.56±5.40	14.29±7.64	44.44±11.71*•	36.84±11.07
"Psychomotor speed"				
"It's hard to concentrate"	11.11±7.41	9.52±6.41	44.44±11.71*•	52.63±11.45×
"I think slowly"	11.11±7.41	9.52±6.41	61.11±11.49*•	68.42±10.66×
"Brain fog"	0.00	9.52±6.41	50.00±11.79*•	52.63±11.45×
Executive functions				
"I can't perform several tasks simultaneously"	11.11±7.41	19.05±8.57	27.78±10.56	31.58±10.66
"I've lost confidence in the right choice"	11.11±7.41	9.52±6.41	27.78±10.56	26,32±10.66
"I postpone decisions"	11.11±7.41	9.52±6.41	27.78±10.56	26.32±10.66

Notes:* reliability of the difference between the data compared to CG, ($p<0.05$);
• reliability of the difference between the data of Groups I and II, ($p<0.05$);
× reliability of the difference between the data of Groups I and III, ($p<0.05$).

consent before enrollment in the study. Assessment of patients' condition was performed on the basis of an algorithmic standard thematic map, which included sections of clinical and neuropsychological testing. General clinical examination included examination by a physician, endocrinologist, ECG, laboratory tests. Neuropsychological research included: Schulte Tables to assess executive functions, the rate of sensorimotor responses, the amount of active attention, training status and fatigue of patients, "Learning 10 words" – the method of A.R. Luria and digit span from Mattisse scale, which allowed to study the processes of memorization, preservation and reproduction of information [12]; attention studies were performed using the "Munsterberg Technique" [13]. Disorders of spatial functions were most clearly manifested when performing Clock Drawing Test [13]. Statistical processing of the obtained data was performed using the statistical data analysis package Statistica 6.0. The non-parametric Kolmogorov-Smirnov criterion was used to assess the statistical significance of the difference between the groups. Changes in indicators under $p<0.05$ were considered probable.

RESULTS

The main complaints of patients during the examination were the following: "I cannot remember new information", "I can't do mental arithmetic", "I lose my mind", "I don't listen after 10 minutes of conversation", "It's hard to concentrate", "I think slowly", "Brain fog", "I can't perform several tasks simultaneously", "I have lost confidence in the right choice", "I postpone decisions" (Table I). A decrease in memory, impaired attention and psychomotor slowing were registered in Group III.

A detailed neuropsychological examination was performed, which makes it possible to compare cognitive functions according to individual domains (Table II).

Having selected batteries of tests for rapid assessment of cognitive functions with the consideration of the specific clinical situation, we have registered in HD patients a decrease in memory according to the test for learning 10 words according to the method proposed by A.R. Luria ($p<0.05$), as well as Digit span from Mattisse scale ($p<0.05$). In patients with hypothyroidism, according to the method of "Selectivity of attention" (G. Munsterberg test), a short span of attention was noted ($p<0.05$). For an extended assessment, Schulte Tables were chosen, the result of which demonstrates the state of the domain of the patient's executive functions and proves a short span of attention. Taking into account the interaction of factors (HD and hypothyroidism) the analysis of results showed that the most affected cognitive domains are memory, executive functions and optical-spatial functions, respectively ($p<0.05$). Analyzing the data, it should be noted that patients of Group II spent more time for searching words, they made more errors and omissions while searching and underlining words according to the method of "Selectivity of attention" in comparison with

Table II. Indicators of cognitive functions according to neuropsychological testing

	Control Group, n=12	I, n=21	II, n=18	III, n=19
Memory				
Luria's Test-1, words	9 [9; 10]	7 [5; 8] *	9 [8; 10]	6 [5; 7] ******
Luria's Test-6, words	8 [7; 9]	8 [7; 10]	8 [7; 9]	4 [3; 6] ******
Digit span from Mattisse scale	17 [16; 17]	13[12; 15] *	16 [14; 17]	15 [13; 17] ******
Attention				
Method "Selectivity of attention", points	14 [12; 16]	11 [9; 13]*	7 [5; 9] ***	7 [4; 9] ******
Executive functions / Schulte Tables /				
Work efficiency, sec	39 [33; 44]	40 [39; 41]	65 [53; 78] ***	72 [59; 86] ******
Optical-spatial functions				
Clock Drawing Test	9 [9; 10]	9 [9; 10]	9 [8; 9]	8 [7; 9] ******

Notes: * reliability of the difference between the data compared to CG ($p<0.05$);
** reliability of the difference between the data compared to Group I ($p<0,05$);
*** reliability of the difference between the data compared to Group II ($p<0.05$).

Group I ($p<0.05$). In addition, they spent more time filling Schulte Tables, which also confirms a short span of attention and instability of executive functions. The results of studies revealed moderate CI in patients of Group III in comparison with Group I ($p<0.05$). Thus, patients with a combination of HD and hypothyroidism showed a significant decrease in memory, executive functions, impaired optical-spatial functions in comparison with Group II ($p<0.05$).

DISCUSSION

Therefore, all patients, according to neuropsychological testing, had a decrease in cognitive functions, but the most pronounced disorders were observed in patients with a combination of HD and hypothyroidism. Testing analysis showed that these patients had a decrease in memory, executive functions and impaired optical-spatial functions. The obtained data are consistent with the results of similar studies. For example, according to Yakovlev O.O. (2013), hypertensive patients with stage II have a decrease in short-term memory with a relative preservation of a long-term one. However, there are other data in the literature: in many cases the decrease in concentration, memory impairment, as well as increased fatigue are observed [14]. There are controversial data on the development of CI in subclinical hypothyroidism. It should be noted that CI in patients with hypothyroidism is characterized primarily by slowed thinking and processing of information – bradyphrenia: the patient needs more than normal time and effort to solve intellectual tasks. Many studies have found a positive association of cognitive decline in subclinical hypothyroidism (Jensovsky J., et al., 2002; Boxtel M.P., et al., 2004). Regarding the denial of such a correlation, a number of authors argue that subclinical hypothyroidism does not cause global cognitive dysfunction. But in specific cognitive domains (memory and executive functions) there may be a slight deficit. Patients with subclinical hypothyroidism and significant cognitive dysfunction may have independent diagnoses that should be evaluated and treated separately (Samuels M.H., 2010; Roberts R.O., 2014; Kim J.M., 2010; Kramer C.K., et al., 2009). It is known that a long-term combination of HD with concomitant hypothyroidism leads to varying degrees of CI [12] and is a common pathogenetic factor in the development of dementia. The relevance of the study of this problem is also justified by the fact that today there are no clear criteria for the diagnosis of CI in the combination of HD with concomitant hypothyroidism. This comparative analysis of conventional scales for determining CI can be used in planning a diagnostic search to ensure early effective verification of changes in cognitive-mnestic functions.

CONCLUSIONS

1. To diagnose CI in hypertensive patients who have cognitive function memory loss, it is advisable to use "10 words learning test" according to A.R. Luria and Digit span from Mattisse scale. In patients with hypothyroidism, attention and executive functions should be determined using the method of "Selectivity of attention" (G. Munsterberg test) and Schulte Tables. Hypertensive patients with a concomitant hypothyroidism should undergo "10 words" method of A.R. Luria, Schulte Tables and Clock Drawing Test.
2. The study proved that patients, who suffer from the mentioned disorders for a long time, have unstable levels of TSH and blood pressure, which affects cognitive

function: 3.6% of hypertensive patients and 7.2% of patients with hypothyroidism have cognitive disorders. In the future, this will make it possible to identify patients with CI among patients with HD and hypothyroidism and to treat the disorders. Therefore, the results of the research prove that the choice of neuropsychological techniques should be made with the consideration of the specific clinical situation. This comparative analysis of generally accepted scales for determining CI can be used in planning a diagnostic search to ensure early effective verification of changes in intellectual and mnestic functions. All this necessitates the use in practice of simple and reliable psychometric tools for early diagnosis of cognitive disorders.

REFERENCES

1. Koval S.M. Peculiarities of the clinical course and differentiated approaches to the treatment of arterial hypertension in patients with concomitant diseases of the internal organs. Journal "Arterial hypertension". 2010;6(14):36-41.
2. Lesiv M.I. Cognitive-Mnestic Functions in Patients Suffering from Hypertensive Disease with Hypothyroidism Comorbidity. NTH Pulse. 2017; 7(43): 56-61.
3. Zakharov V.V. Neuropsychological tests. Necessity and possibility of application/ Consilium-medicum. 2011; 13(2): 98 –106.
4. O'Brien J.T. Clinical practice with anti-dementiadrugs: A revised (third) consensusst at ement from the British Association for Psychopharmacology. Journal of Psychopharmacology. 2017; 31(2): 147–68.
5. Syst-Eurstudy F. (Systolic hypertension in Europe Investigators. The prevention of dementia with antihypertensive treatment: new evidence from the Systolic Hypertension in Europe (Syst-Eur). Arch. Intern.Med. 2002;162:661-3.
6. McGuinness B., Todd S., Passmore P., Bullock R. Blood pressure lowering in patients without prior cerebrovascular disease for prevention of cognitive impairment and dementia. Cochrane Database of Systematic Reviews. 2009;4: CD004034.
7. Trenkwalder P. The study on Cognition and Prognosis in the Elderly (SCOPE) recent analyses. J.Hypertension. 2006; 24: 107-14.
8. Siegmund W. Replacement therapy with T4 plus T3. Blackwell Publishing Ltd, Clinical Endocrinology. 2004; 60: 750–7.
9. Persania L., Brabant G., Dattani M., Bonomi M. Guidelines on the Diagnosis and Management of Central Hypothyroidism: European Thyroid Association. Thyroid. 2018;(7):225–37.
10. Petrova M.M., Schneider N.A., Eremina O.V. Characteristics of cognitive disorders in patients with arterial hypertension. Cardiovascular therapy and prevention. 2008;(2):23–6.
11. Kovalenko O.E., Litvin O.V. Peculiarities of hypertensive encephalopathy in patients with hypothyroidism. Ukrainian Medical Almanac. 2011;14(6):103.
12. Vinall M. Digest of the Congress of the European Society of Cardiology. Arterial hypertension. 2016;6(50):81-98.
13. Britov A.N., Bystrova M.M. Resistant arterial hypertension: modern approaches to diagnosis and treatment. Rational Pharmacotherapy in Cardiology. 2010;6(2):206-11.
14. Lesiv M.I. Cognitive Functions in Patients Suffering from Hypertension and Hypothyroidism with Retrospective Evaluation of the Control of Disease Compensation. Medicni perspektivi. 2020;25(1):51-8.

ORCID and contributionship:

Maryana I. Lesiv: 0000-0001-8687-3344 [B-D]
Victoriia A. Gryb: 0000-0001-6111-7921 [A,E,F]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Maryana I. Lesiv
Ivano-Frankivsk National Medical University
2 Halytska St., 76000 Ivano-Frankivsk, Ukraine
tel: +38095856995
e-mail: maryanalesiv2222@gmail.com

Received: 13.10.2020
Accepted: 15.08.2021

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

COGNITIVE FUNCTIONS IN MULTIPLE SCLEROSIS PATIENTS DEPENDING ON THE DIFFERENT RISK FACTORS PRESENCE

DOI: 10.36740/WLek202110115

Oksana O. Kopchak[1], Tetiana A. Odintsova[1], Oleksandr R. Pulyk[2]
[1]DEPARTMENT OF NEUROLOGY, PSYCHIATRY AND PHYSICAL REHABILITATION, KYIV MEDICAL UNIVERSITY, KYIV, UKRAINE
[2]DEPARTMENT OF NEUROREHABILITATION, UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim: To clarify the influence of different risk factors on cognitive impairment (CI) in general and in separate domains and their association with MRI findings in patients with relapsing-remitting (RRMS) and progressive forms of multiple sclerosis (SPMS and PPMS).

Materials and methods: One hundred and thirty-seven participants with MS (102 females and 35 males) aged from 22 to 69 years were enrolled into this study. All patients completed the Montreal Cognitive Assessment (MoCA), Beck Depression Inventory (BDI), Hamilton Anxiety Rating Scale (HAM-A) and undergone MRI.

Results: According to MS phenotypes all participants were divided into two groups: patients with RRMS (n= 106) and with progressive phenotypes (n= 31). A significant positive correlation was present between MoCA and BDI scores in all participants (p= 0,0015). Presence of anxiety did not demonstrate any valid influence on cognitive performance, although severe anxiety was significantly more often found in subjects with progressive phenotypes in presence of brain atrophy (p= 0,0028). Patients with higher education had no CI more frequently compare to those without it (p= 0,0019), whereas, participants smoking cigarettes had higher prevalence of severe CI than non-smokers (p= 0,0061).

Conclusions: Among cognitive domains memory, visual-spatial and executive functions, abstract thinking were impaired the most in MS patients, though abstract thinking was more often affected in progressive forms. The results demonstrated that physical disability, depression and smoking negatively impacted cognitive performance, meanwhile presence of higher education demonstrated a favorable influence on cognition in MS patients.

KEY WORDS: multiple sclerosis, cognitive impairment, depression

Wiad Lek. 2021;74(10 p.I):2444-2451

INTRODUCTION

Multiple sclerosis (MS) is an insidious chronic inflammatory autoimmune neurodegenerative disease of the central nervous system (CNS), which eventually leads to a severe long-term disability of a significant number of able-bodied population (2.8 million people worldwide) as a result of the neurological and motor deficit along with cognitive impairment (CI) and psycho-emotional disorders [1; 2; 3]. CI is a common socio-economically disabling feature of MS, prevailing in 40-65% of those patients, furthermore, CI can be present already on the early stages of the disease as well as predict the shift to the motor phase and future motor impairment [4; 5]. The factors influencing cognition still are not fully comprehended due to variety of controversial results of multiple studies. In particular, severity of CI is frequently correlated with age and physical disability [6], it also depends on the MS phenotype, age of the disease debut, education level, although one of the most debatable factors is the disease duration (DD) on account of the difficulty in determining the onset [7; 8]. Apart from those, some comorbidities, like anxiety or depression, can impact the severity of CI [4; 9]. While depression's impact on CI proves to be solid [10; 11], anxiety's influence according to various sources is still controversial [9; 12; 13]. Among the environmental factors, cognitive reserve (high IQ prior to the disease onset, presence of higher education, stimulating hobbies and speaking foreign language) demonstrates positive influence on cognitive performance in MS patients [14; 15], whereas, smoking cigarettes not only increases the frequency of relapses and hastens progression, but also contributes into CI deterioration [14; 16].

Conventional magnetic resonance imaging (MRI) of the brain and the spinal cord plays the crucial role for diagnostic (according to the 2017 reviewed McDonald criteria) and disease-modifying purposes since it corresponds to the observed clinical picture [17].

Nevertheless, the factors contributing to development and deterioration of CI in people suffering from MS are disputable and, therefore, still being studied in order to improve methods of treatment and rehabilitation.

THE AIM

To clarify the influence of different risk factors (smoking, physical disability, disease duration, anxiety, depression and level of education) on cognitive impairment (CI) in general and in separate domains and their association with MRI findings in patients with relapsing-remitting (RRMS) and progressive forms of multiple sclerosis (SPMS and PPMS).

MATERIALS AND METHODS

This study included one hundred and thirty-seven participants with MS (102 females and 35 males) aged from 22 to 69 years. Thus, all participants were divided into two groups: A – patients with RRMS (n= 106; 81 females and 25 males aged from 22 to 67 years, mean age: 41.8±10.7, disease duration (DD): 10.3±8.5 years) and B – the study subjects with progressive phenotypes (n= 31; 21 females and 10 males aged from 28 to 69 years, mean age: 47,2±13,6, DD: 16,6±12,5 years). The mean EDSS score in group A was 3,5±1,6, whereas in the group B it was 5,1±1,3. The study subjects were diagnosed RRMS, SPMS and PPMS according to McDonald's Criteria 2017 [17].

A medical history was obtained from each participant. The examination consisted of a standard clinical evaluation, neurological examination, the application of neuropsychological questionnaires, laboratory tests (complete blood count, biochemical parameters, TSH) and polymerase chain reaction test for Covid-19 (everyone had negative results). Every patient underwent MRI of the brain. The disability level in MS patients was evaluated by means of Kurtzke's Expanded Disability Status Scale (EDSS). Mild disability equals 1-3,5 points, moderate – 4-6 points and 6,5-8 stand for severe disability [18]. The Montreal Cognitive Assessment (MoCA) was applied to evaluate presence and severity of CI. The MoCA includes six subcategories according to the domains: memory (M), language (L), attention (A), abstract thinking (AT), visual-spatial and executive functions (VS/EF). The scale score was interpreted as: 30-26 points – no CI; 25-18 points – mild CI; <18 points – severe CI [19]. Beck Depression Inventory (BDI) was applied to screen for the presence and asses the severity of depression. This scale consists of 21 items that cover major depression symptoms according to diagnostic criteria listed in the Diagnostic and Statistical Manual for Mental Disorders. Each answer is scored from 0 to 3 points. Mean score 0–9 indicates absence of depression, 10–18 – mild depression, 19–29 – moderate depression and 30–63 – severe depression [20]. To measure the severity of perceived anxiety symptoms we used Hamilton Anxiety Rating Scale (HAM-A). The scale consists of 14 items, each defined by a series of symptoms, and measures both psychic anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety). Each item is scored on a scale of 0 (not present) to 4 (severe), with a total score range of 0–56, where 0-13 – absence of anxiety, 14-17 indicates mild severity, 18–24 moderate severity and ≥ 25 severe anxiety [21].

The patients were excluded from the study in case they were younger than 18 years, had progressive forms of MS, exacerbation stage of MS, severe depression, pelvic disorders, pregnancy, also including participants treated with corticosteroids and INF-β, that could alter the study's parameters.

All study subjects provided written informed consent and the study was approved by the Institutional Ethics Committee.

The statistical data was processed by means of Graph Pad Prism version 9 and STATISTICA 12.5. 192.5. Student's t-test (t) was applied for evaluating credibility between mean quantitative positions of two samples. Proportions were compared using $\chi 2$. Relationships between different indicators were assessed using the Pearson's correlation coefficient (r) according to statistical distribution. A $p<0.05$ value was considered statistically significant.

RESULTS

Our patients had the following complaints: decreased memory, difficulties in verbalization (vocabulary), inability to concentrate, decreased occupational performance, fatigue, lack of energy during usual daily activities, presence of disturbing thoughts, mood swings, general weakness, fatigue and anxiety. Meanwhile, the neurological examination revealed pyramidal signs, presence of pathological reflexes, increased muscle tone (spastic type), coordination impairment (intention tremor, gait and truncal ataxia, missing the mark), brainstem disorders (vertigo, nausea, slight dysarthria) and sensory disorders (in particular, impaired vibration and proprioception sense, paresthesia, Lhermitte's sign) were revealed. Pyramidal disturbances (p= 0,0018), cerebellar dysfunction (p= 0,0439) and brainstem disorders (p= 0,0054) were observed more frequently in the group B.

In accordance with the results of the brain MRI, the majority of the study subjects had multifocal lesions in the white and gray matter, especially in periventricular, corpus callosum (CC), cortical (FL, TL, PL) areas, cerebellum and brainstem, presence of brain atrophy. Parietal (p= 0,0109) and occipital lobe (p= 0,0055) lesions, brain atrophy (p= 0,0008), combined lesions of frontal lobe with brain atrophy corpus callosum (p= 0,0021), combination of parietal lobe and corpus callosum lesions (p=0,0279) and simultaneously affected parietal lobe with brain atrophy (p= 0,0138) were found in the group B more often than in the group A.

The mean MoCA score in group A was 23,4±4,1, while in the group B – 22,67±3,67. Based on MoCA score, participants in these groups were divided into three subgroups: 1 – without CI (A1 – 40; B1 – 10), 2 – with moderate CI (A2 – 53; B2 – 19), 3 – with severe CI (A3 – 13; B3 – 2). There was no significant difference between the main groups. Moderate CI were found more frequently comparing to severe CI and absence of CI in the group A (p= <0,0001). And similarly, in the group B moderate CI were observed more often in comparison to severe and absence of CI (p= <0,0001) (Fig. 1).

In the group A the most frequently affected domain compare to the rest was M (69%) (p <0,0001), followed by VS/EF (58%) and AT (48%). As for the group B, the most common deteriorated cognitive domains were the same: M (77%) (p <0,0001), VS/EF and AT (both 68%). AT was present substantially more often in the group B compare to group A in case of TL lesion (p= 0,0024), CC lesion (p= 0,0219), simultaneous FL, TL and PL lesions (p= 0,0302). There were no difference concerning other cognitive domains between the two groups (Fig. 2).

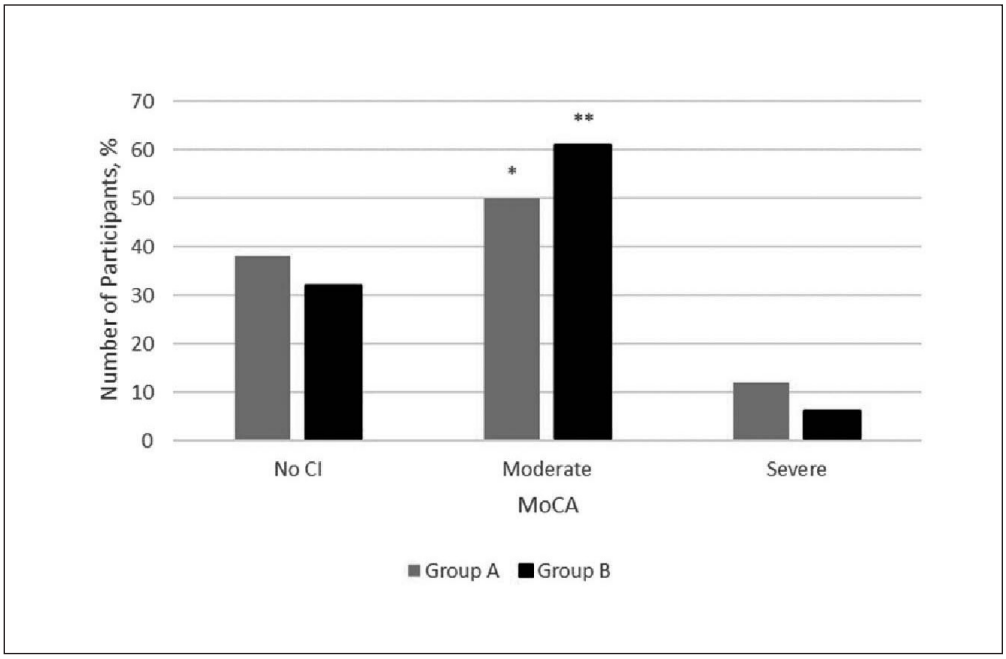


Fig. 1. Distribution of the Levels of CI in Both Study Groups.
* p= <0,0001
** p= <0,0001

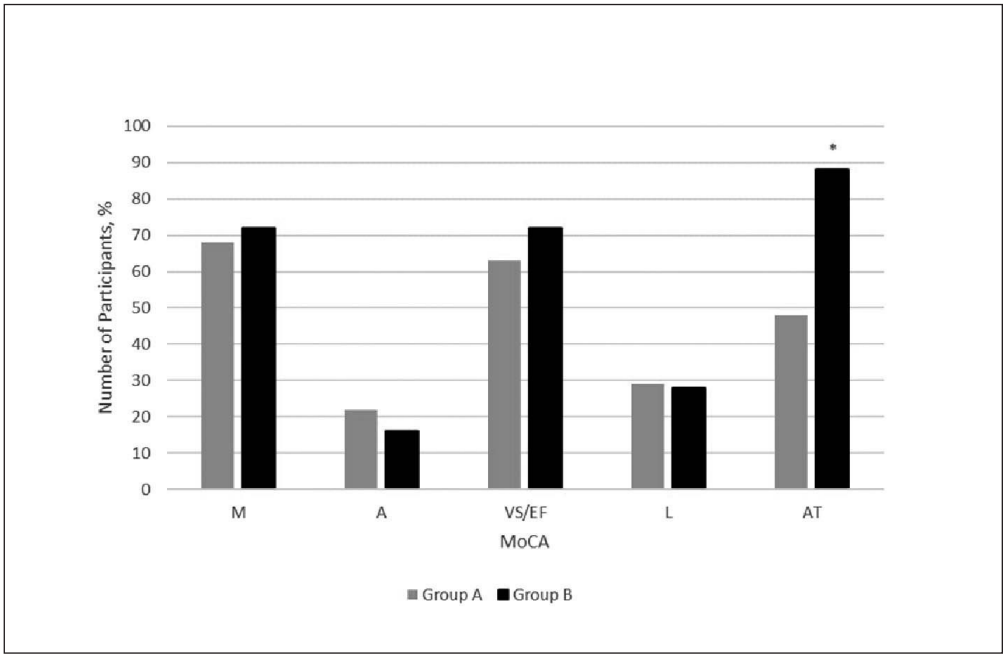


Fig. 2. Distribution of Affected Domains in Participants of Both Groups in Case of Lesion of Temporal Lobe.
*p= 0,0024

None of the groups demonstrated a connection between the severity of CI and DD (group A: p= 0,8210; group B: p= 0,4503).

The current study did not demonstrate a correlation between age and CI in both study groups (p= 0,3747). No connection between age and CI was observed in participants with RRMS (p= 0,7999), as well as in patients with progressive types of MS (p= 0,0639).

There was a significant correlation between CI and EDSS score (r= 0,2433, p= 0,0042) in all participants. A significant positive correlation between CI and EDSS score (r= 0,1999, p< 0,01) was detected in the group A (Fig. 3). The study subjects of the group B also show the strong positive correlation between CI and EDSS (r= 0,3941; p< 0,05) (Fig. 4).

The results of BDI revealed that, the participants had either no signs of depression or its presence of mild and moderate severity, as the severe level was not detected. In group A 52 (49%) patients had no signs of depression, 38 (36%) had mild depression, 16 (15%) had moderate. As for the group B: 11 (35%) had no depression, 16 (52%) – mild depression, 4 (13%) – moderate one.

A substantial positive correlation between severity of CI and depression's degree of manifestation was observed in all study subjects (r= 0,3038; p= 0,0015) (Fig.5). Significant positive correlation between CI and depression severity was found in patients of the group A (r= 0.3717, p= 0.0093), as well as in patients of the group B (r=0,4125; p=0,0211), indicating that CI can influence the depressive disorder and vice versa in all MS phenotypes.

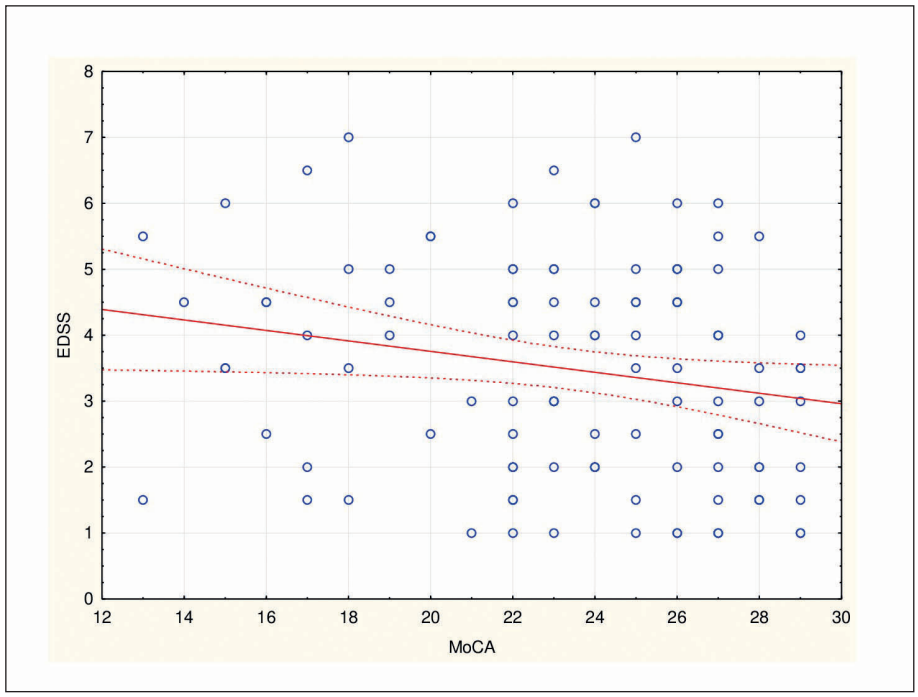


Fig. 3. The Correlation between CI and EDSS Scores in the group A.

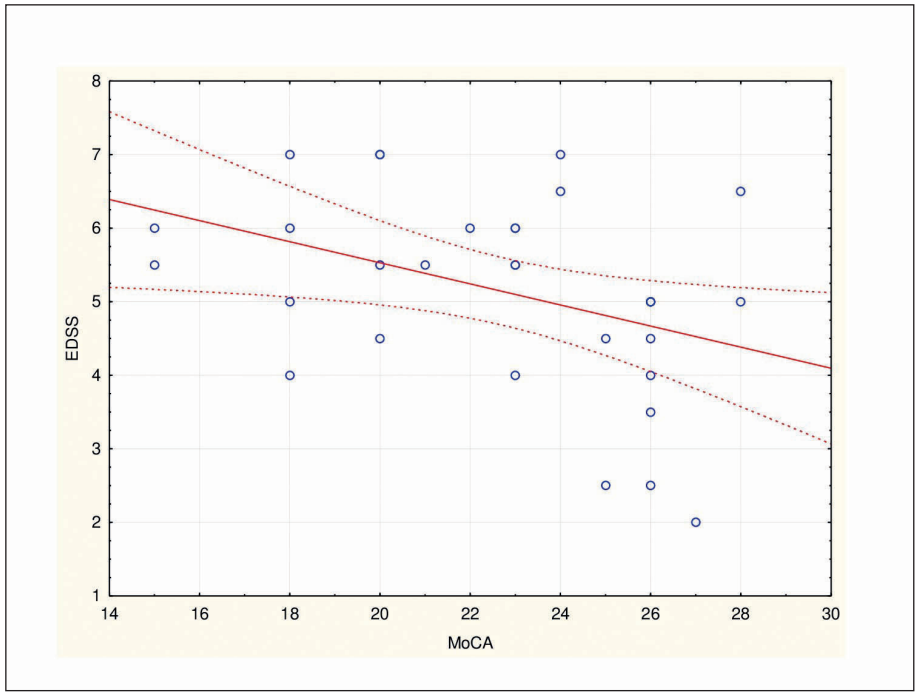


Fig. 4. The Correlation between CI and EDSS Scores in the group B.

In the group A mild depression was associated with lesions of CC (73%), FL (63%) and PL (52%), whereas, moderate was connected with lesions of FL, CC (both 75%) and BA (62%). Meanwhile, in the group B mild depression was detected most frequently in case of CC (87%), FL (75%), TL (69%) lesions and BA (69%), moderate depression was observed more often in patients with PL (75%) and CC (75%). Presence of BA in case of mild depression was significantly more frequent in the group B compare to the group A (p= 0,0035).

Among all study subjects 97 demonstrated the presence of anxiety. In 22 (23%) mild level of anxiety was observed, in 32 (33%) moderate and in 43 (44%) severe. FL was more frequently affected in cases of mild and moderate anxiety in comparison to severe (p= 0,0119) in all patients.

There was no correlation found between CI severity and the level of anxiety in the group A (p= 0,02537). In the group A 33 (31%) participants had no signs of anxiety, 16 (15%) – had mild, 24 (23%) – moderate and 33 (31%) had severe level of anxiety. Absence and severe level of anxiety appeared more often compare to mild and moderate (p= 0,0176) in the patients of the group A. Mild level of anxiety was associated with lesions of CC (81%), FL (75%), TL (62%) and PL (50%); moderate – with FL (79%), PL (67%), TL (50%) and CC (58%); severe anxiety – with lesions in

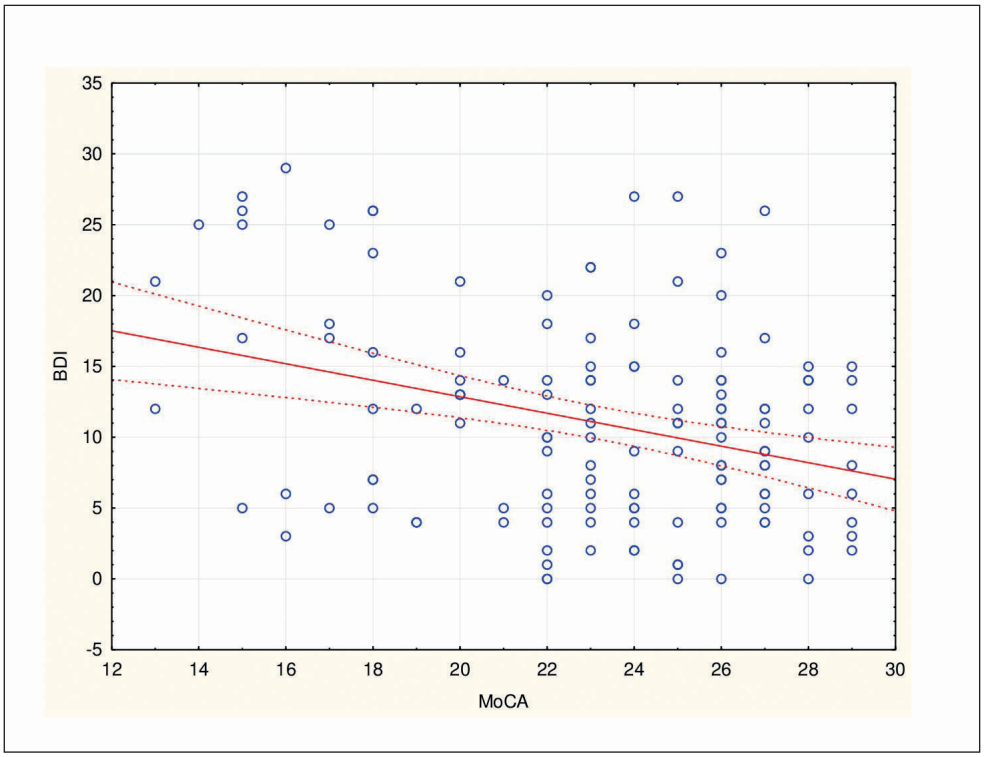


Fig. 5. The Correlation between MoCA and BDI Scores in All Participants.

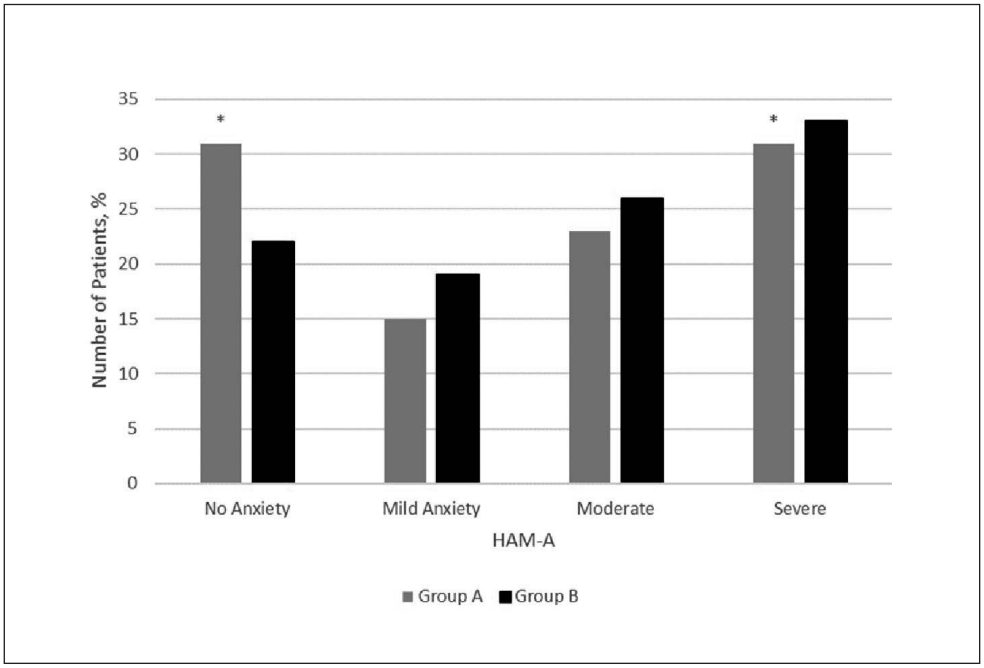


Fig. 6. The Severity of Anxiety in All Study Groups.

CC (72%), FL (51%), PL (48%), FL (45%). The group B did not demonstrate a correlation between the level of CI and anxiety severity (p= 0,9405) as well. Among the study subjects of this group 7 (22%) had no anxiety, 6 (19%) had mild, 8 (26%) – moderate and 10 (33%) – severe; there was no significant difference in frequency of axiety severity levels (p= 0,6810) (Fig. 6). Concerning this group, mild level was connected with lesions of FL (100%), CC (80%); moderate was predominantly due to lesions of FL (93%), CC (67%) and PL (67%); severe was associated with CC (80%), BA (80%), TL (60%) and FL (50%). BA was detected more frequently in the group B compare to the group A in case of severe anxiety (p= 0,0028).

The strong positive correlation between EDSS score and BDI score was observed in the group A (r= 0,4396; p= <0,0001). No such connection was observed in the group B (p= 0,2813; r= 0,1252).

Among all participants 72 had received a higher education, among which 35 did not demonstrate signs of a CI, 32 had moderate and 5 had severe. 65 of our study subjects did not have a higher education, 15 of whom had no CI, 32 had moderate and 18 had severe. Patients with MS and

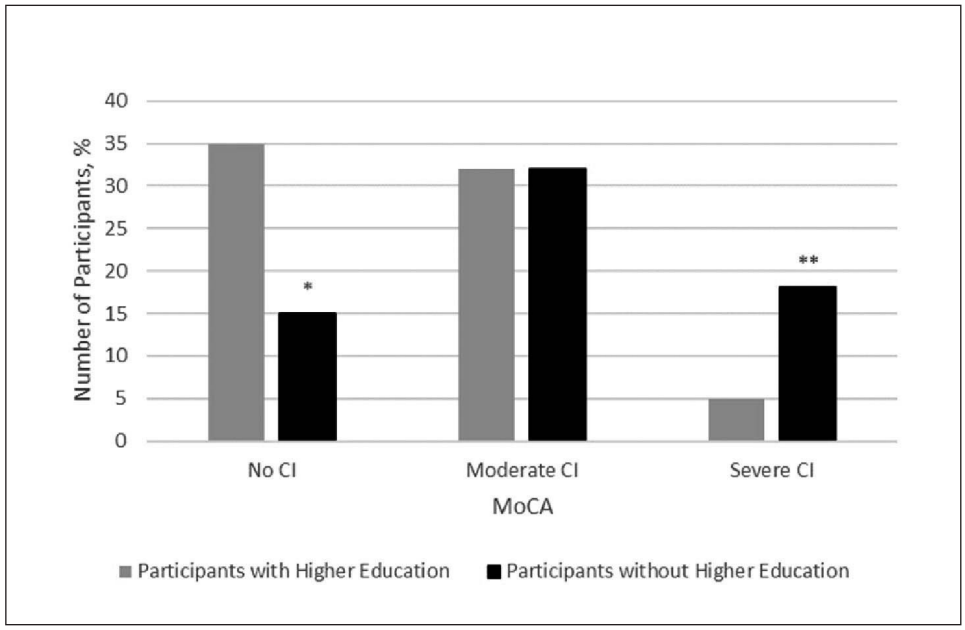


Fig. 7. The Distribution of Levels of Cognitive Impairment in All Participants Depending on the Presence of Higher Education.
* p= 0,0019
** p= 0,0012

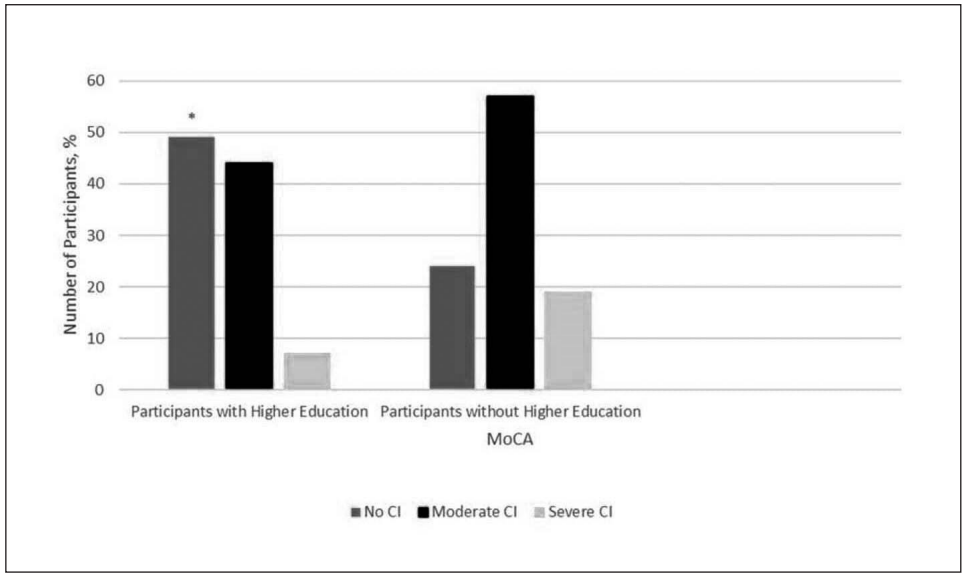


Fig. 8. Distribution of the CI Severity in the Group A Depending on the Presence of Higher Education
* p= 0,0066

higher education more frequently had no CI comparing to those without higher education (p= 0,0019), meanwhile higher frequency severe CI was observed in patients without higher education (p= 0,0012) (Fig.7).

In the group A 59 participants had higher education (29 did not have CI, 26 had moderate and 4 had severe) and 47 did not (11 had no signs of CI, 27 had moderate and 9 had severe). No CI was more frequently diagnosed in those patients of the group A with high education (p= 0,0066) (Fig. 8). 13 study subjects from the group B were with higher education (6 without CI, 7 with moderate, severe were not detected) and 18 were without (4 were without CI, 12 with moderate and 2 with severe). In the group B no connection was detected between CI severity and level of education.

30 of all patients were smoking cigarettes at the time of the study, 107 did not have the habit. Among smokers 26% had no CI, 40% had moderate CI and 34% had severe. In the non-smoking group 39% of the study subjects showed no signs of CI, 49 had moderate CI and 12% had severe. The severe CI prevailed in smokers compare to the non-smoking participants (p= 0,0061).

DISCUSSION

According to Brochet B. and Ruet A. preclinical forms and RRMS are characterized by the slowness of information processing speed and episodic verbal and visual-spatial memory deficits, that are more prominent than executive functions and verbal fluency impairment, meanwhile the progressive forms are characterized by information processing speed, attention, working memory, executive functions, and verbal episodic memory deterioration to the greater extent compare to the RRMS [7]. And similarly our study demonstrated that RRMS patients had impairment of memory, visual-spatial and executive functions, abstract thinking,

the same domains were the most frequently impaired in RRMS and progressive MS. Abstract thinking was affected more prominently in progressive forms than in RRMS in case of lesions of temporal lobe, corpus callosum and the simultaneously affected frontal, temporal and parietal lobes.

Several researches claimed that the duration of the disease impacts the cognitive functions [8], while others stated that it had no influence on cognitive performance [22]. In this study such connection was revealed only in RRMS phenotype, progressive forms did not demonstrate this kind of relation. Multiple studies proved that cognitive dysfunction is linked in direct ratio to a patient's age [6], however, the current research did not demonstrate any correlation between MoCA score and age. This could be explained by immense incidence of higher education (44%) and EDSS score lower than 6,5 (90%) in the study subjects, which would have a favorable influence on cognitive performance.

The physical disability, according to various researches, always had a strong influence on patients' mental status followed further deterioration in direct ratio, with seldom exceptions, and cognitive deficit was the credible predictor of the disability's onset [8; 22; 23] and vice versa, especially concerning the primary progressive phenotype [24], therefore, our study confirmed it demonstrating a strong correlation between severity of cognitive dysfunction and level of physical disability in both relapsing-remitting and progressive types of MS.

Depression, as the most widespread psychiatric disorder to be present in MS, was associated with lesions and atrophy in fronto-temporal and frontal lobes separately [25]. It was established that depression was impacting information processing speed, executive function, attention, motor functions and memory, but potentially could affect all cognitive domains [4; 26], although Whitehouse C. E. et al. [10] stated that MS participants of their study with depression had reduced cognitive performance, but the working memory was intact. Our study similarly confirmed that appearance of depression was connected to deterioration of the cognitive performance in relapsing-remitting and progressive forms, accordingly; also, it was more prominently associated with developed brain atrophy in participants of the group B.

According to various sources, anxiety in case of MS either was directly its symptom [27], or a comorbidity, or an adverse effect of some medications [3], ergo, it influenced cognitive and social performance [28; 29], mainly episodic memory and executive functions, but generally it was hard to separate anxiety's and depression's impact on cognition [4]. In this study anxiety's impact on cognition was not observed. Severe level of anxiety in patients with progressive phenotypes of MS was associated with presence of brain atrophy, as it was present significantly more frequently comparing to relapsing-remitting phenotype.

Concerning the environmental risk factors, several studies stated that smoking is a susceptible and prognostic risk factor for both physical disability and cognitive impairment as it had a great contribution to the processes of demyelination [14; 16], so our study confirmed smoking's impact on cognition, as it demonstrated higher incidence of severe cognitive deficit. Previous researches demonstrated a solid proof that cognitive reserve (high IQ score prior to MS debut, presence of higher education, speaking foreign language and stimulating hobbies) had favorable effect on MS patients' cognitive skills [14; 30], ergo, our study revealed higher prevalence of cases with intact cognition and substantially lower number of severe cognitive impairment in participants with higher education.

CONCLUSIONS

Cognitive impairment is the one of the common, yet disabling and socially disrupting, manifestations of MS. At the same time, it can be further deteriorated by progression of physical disability, presence of neuropsychological disorders and environmental factors, like lack of higher education and smoking cigarettes. Quite frequently complaints of poor cognitive performance are dismissed on the background of neurological deficit. Results of our study stress on the importance of a mandatory thorough screening of cognitive impairment, since cognitive performance can be reliably assessed, and management of risk factors in all MS patients from early stages in order to prevent further socio-economical maladjustment and disability of such patients and enroll them in adequate disease-modifying therapies.

REFERENCES

1. Walton V., King R., Rechtman L., Kaye W., et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Multiple Sclerosis Journal. 2020;14:1816-1821. doi:10.1177/1352458520970841
2. Kobelt G., Thompson A., Berg J., Gannedahl M., Eriksson J. MSCOI Study Group. European Multiple Sclerosis Platform. New insights into the burden and costs of multiple sclerosis in Europe. Mult Scler. 2017; 23(8):1123-1136. doi: 10.1177/1352458517694432
3. Bass A. D., Van Wijmeersch B., Mayer L., Mäurer M., et al. Effect of Multiple Sclerosis on Daily Activities, Emotional Well-being, and Relationships: The Global vsMS Survey. International journal of MS care. 2020;22(4):158–164. doi: 10.7224/1537-2073.2018-087
4. Oset M., Stasiolek M., Matysiak M. Cognitive Dysfunction in the Early Stages of Multiple Sclerosis-How Much and How Important? Current neurology and neuroscience reports. 2020; 20(7):22. doi: 10.1007/s11910-020-01045-3
5. Migliore S., Ghazaryan A., Simonelli I., Pasqualetti P., et al. Cognitive Impairment in Relapsing-Remitting Multiple Sclerosis Patients with Very Mild Clinical Disability. Behavioural neurology. 2017;7404289. doi: 10.1155/2017/7404289
6. Ruano L., Portaccio E., Goretti B., Niccolai C., et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. Mult Scler. 2017;23(9):1258-1267. doi: 10.1177/1352458516674367
7. Brochet B., Ruet A. Cognitive Impairment in Multiple Sclerosis With Regards to Disease Duration and Clinical Phenotypes. Front Neurol. 2019;20(10):261. doi: 10.3389/fneur.2019.00261
8. Sadigh-Eteghad S., Abbasi Garravnd N., Feizollahi M., Talebi M. The Expanded Disability Status Scale Score and Demographic Indexes Are Correlated with the Severity of Cognitive Impairment in Multiple Sclerosis Patients. Journal of clinical neurology (Seoul, Korea). 2021;17(1):113–120. doi: 10.3988/jcn.2021.17.1.113

9. Oreja-Guevara C., Ayuso Blanco T., Brieva Ruiz L., Hernández Pérez M. Á., Cognitive Dysfunctions and Assessments in Multiple Sclerosis. Frontiers in neurology. 2019;10: 581. doi: 10.3389/fneur.2019.00581
10. Whitehouse C. E., Fisk J. D., Bernstein C. N., Berrigan L. I., et al. CIHR Team in Defining the Burden and Managing the Effects of Psychiatric Comorbidity in Chronic Immunoinflammatory Disease. Comorbid anxiety, depression, and cognition in MS and other immune-mediated disorders. Neurology. 2019;92(5):406–417. doi: 10.1212/WNL.0000000000006854
11. Marrie R.A., Patel R., Bernstein C.N., Bolton J.M., et al. Anxiety and depression affect performance on the symbol digit modalities test over time in MS and other immune disorders. Mult Scler. 2020;25:1352458520961534. doi: 10.1177/1352458520961534
12. Marrie R.A., Patel R., Figley C.R., Kornelsen J., et al. Diabetes and anxiety adversely affect cognition in multiple sclerosis. Multiple Sclerosis and Related Disorders. 2019;27:164-170. doi: 10.1016/j.msard.2018.10.018
13. Turner A.P., Hartoonian N., Hughes A.J., Arewasikporn A., et al. Physical activity and depression in MS: The mediating role of behavioral activation. Disability and Health Journal. 2019;12(4):635-640. doi: 10.1016/j.dhjo.2019.04.004
14. Amato M. P., Prestipino E., Bellinvia A., Niccolai C., et al. Cognitive impairment in multiple sclerosis: An exploratory analysis of environmental and lifestyle risk factors. PloS one. 2019;14 (10):e0222929. doi: 10.1371/journal.pone.0222929
15. Clare L., Wu Y. T., Teale J. C., MacLeod C., et al. CFAS-Wales study team. Potentially modifiable lifestyle factors, cognitive reserve, and cognitive function in later life: A cross-sectional study. PLoS medicine. 2017;14(3):e1002259. doi: 10.1371/journal.pmed.1002259
16. Ozcan M. E., Ince B., Bingöl A., Ertürk S., et al. Association between smoking and cognitive impairment in multiple sclerosis. Neuropsychiatric disease and treatment. 2014;10:1715–1719. doi: 10.2147/NDT.S68389
17. Thompson A.J., Banwell B.L., Barkhof F., Carroll W.M., et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology. 2018;7:162-173. doi: 10.1016/S1474-4422(17)30470-2
18. Kurtzke J.F. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;3:1444-1452. doi: 10.1212/WNL.33.11.1444
19. Nasreddine Z.S., Phillips N.A., Bédirian V.,et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. Journal of American Geriatric Society. 2005;53:695–699. doi: 10.1111/j.1532-5415.2005.53221.x
20. Beck A., Ward S.T., Mendelson M., Mock J., et al. An inventory for measuring depression. Archives of General Psychiatry. 1961;4:561-571. doi: 10.1001/archpsyc.1961.01710120031004
21. Hamilton M. The assessment of anxiety states by rating. Br J Med Psychol. 1959;32:50-55.
22. Lynch S.G., Parmenter B.A., Denney D.R. The association between cognitive impairment and physical disability in multiple sclerosis. Multiple Sclerosis Journal. 2005;1(4):469-476. doi: 10.1191/1352458505ms1182oa
23. Harel Y., Kalron A., Menascu S., Magalashvili D., et al. Cognitive function in multiple sclerosis: A long-term look on the bright side. PLOS One. 2019. doi: 10.1371/journal.pone.0221784
24. Pérez-Miralles F., Prefasi D., García-Merino A., Ara J.R., et al. Short-term data on disease activity, cognition, mood, stigma and employment outcomes in a cohort of patients with primary progressive multiple sclerosis (UPPMS study). Multiple Sclerosis and Related Disorders. 2021. doi: 10.1016/j.msard.2021.102860
25. Schneider R., Genç E., Ahlborn C., Gold R., et al. Temporal Dynamics of Diffusion Metrics in Early Multiple Sclerosis and Clinically Isolated Syndrome: A 2-Year Follow-Up Tract-Based Spatial Statistics Study. Frontiers in Neurology. 2019;05. doi: 10.3389/fneur.2019.01165
26. Golan D., Doniger G.M., Wissemann K., Zarif M., et al. The impact of subjective cognitive fatigue and depression on cognitive function in patients with multiple sclerosis. Mult Scler. 2018;24(2):196–204. doi: 10.1177/1352458517695470
27. Văcăraș Y., Văcăraș V., Nistor C., Văcăraș D., et al. The Influence of Depression and Anxiety on Neurological Disability in Multiple Sclerosis Patients. Behavioral Neurology. 2020. doi: 10.1155/2020/6738645
28. Carotenuto A., Arcara G., Orefice G., Cerillo I., et al. Communication in Multiple Sclerosis: Pragmatic Deficit and its Relation with Cognition and Social Cognition. Arch Clin Neuropsychol. 2018;33(2):194-205. doi: 10.1093/arclin/acx061
29. Vissicchio N.A., Altaras C., Parker A., Schneider S. et al. Relationship Between Anxiety and Cognition in Multiple Sclerosis: Implications for Treatment. Int J MS Care. 2019;21(4):151–156. doi: 10.7224/1537-2073.2018-027
30. Elshebawy H., Fahmy E.M., Elfayoumy N.M., Abdelalim A.M., Ismail R.I. Clinical predictors to cognitive impairment in multiple sclerosis patients. Egypt J Neurol Psychiatry Neurosurg. 2021;57:38. doi: 10.1186/s41983-021-00292-6

ORCID and contributionship:

Oksana O. Kopchak: 0000-0003-2666-0616 [A-D,F]
Tetiana A. Odintsova: 0000-0003-2455-6778 [B,D,F]
Oleksandr R. Pulyk: 0000-0002-8717-047X [E,F]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Oksana O. Kopchak
Department of Neurology, Psychiatry and
Physical Rehabilitation, Kyiv Medical University, Kyiv, Ukraine
e-mail: dr.kopchak@kmu.edu.ua

Received: 10.05.2021
Accepted: 15.09.2021

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

OPINIONS OF PARENTS OF CHILDREN WITH AUTISM SPECTRUM DISORDERS ON ART THERAPY IN THE IMPROVEMENT OF THEIR FUNCTIONING

DOI: 10.36740/WLek202110116

Julia Wypyszyńska[1], Natalia Zaboklicka[1], Maria Stachura[1], Zuzanna Sito[1], Tomasz Męcik-Kronenberg[2]
[1]STUDENT RESEARCH GROUP AT THE CHAIR AND DEPARTMENT OF PATHOMORPHOLOGY FACULTY OF MEDICAL SCIENCES IN ZABRZE, ZABRZE, POLAND
[2]CHAIR AND DEPARTMENT OF PATHOMORPHOLOGY, FACULTY OF MEDICAL SCIENCES IN ZABRZE MEDICAL UNIVERSITY OF SILESIA, ZABRZE, POLAND

ABSTRACT

The aim: Our work aims to investigate the therapeutic effect of art on children and adolescents with autism spectrum disorder (ASD) up to the age of 18 and to disseminate knowledge about its use as a part of therapy.

Materials and methods: The authors created an anonymous online questionnaire addressed to parents and legal guardians of children and adolescents with and without ASD. The studied population comprised 190 subjects, of which 108 were a control group (children and adolescents without ASD) and 82 – a study group (children and adolescents with ASD).

Results: Most respondents did not hear about the therapeutic use of art (59.5%). 20.73% of respondents with ASD participated in therapy using artistic activities. 82.4% participated in music therapy, 23.5% – in theater therapy, and 70.6% – in art therapy. These forms of therapy effectively improved disorders related to social relationships, speech, and atypical behavior.

Conclusions: There is a positive therapeutic effect of art on children and adolescents with ASD. The therapeutic use of art among the respondents is not very widespread. Artistic activities have a positive impact on social relations, speech, and unusual behavior. Music and visual arts activities seem to positively impact social relationship disorders, while theater activities seem to impact speech disorders positively. Combining several arts in therapy is more beneficial than using just one. Art therapy is most often used at the age of 13 and older.

KEY WORDS: music therapy, autism spectrum disorder, art therapy

Wiad Lek. 2021;74(10 p.I):2452-2459

INTRODUCTION

Autism spectrum disorder (ASD) is a comprehensive developmental disorder that includes abnormalities in social interaction, communication, and restricted and repetitive behavior patterns. It typically appears in early childhood but can also be diagnosed at later stages of development [1, 2]. The World Health Organization estimates the prevalence of ASD among children and adolescents at 1: 160 (0.63%) worldwide [3]. In Poland, the number of disabled people up to 16 years of age with diagnosed ASD is 44,302, which stands for 1: 115 (0.87%) [4]. Autism spectrum disorders are more common in boys than girls [1, 5-7]. Currently, the ratio is 3: 1 [5].

Already in antiquity, people were aware of the beneficial effects of contact with art on human health and psyche. Aristotle acknowledged this phenomenon by defining catharsis (Greek for purification) in his work Poetics [8]. However, it was not until the 20th century that the painter Adrian Hill introduced the concept of art therapy. He noticed the positive influence of art on the healing process and human psyche on himself and other sanatorium patients where he was struggling with tuberculosis. In the following years, he was employed as the first art therapist in one of the British psychiatric hospitals and became the president of the British Society of Art Therapists (BAAT). He is also considered one of the pioneers of this type of therapy [9].

Artistic activities can be a complementary form of treatment in children and adolescents with autism spectrum disorders. They are based on non-verbal communication, which makes them appropriate for them [10]. However, despite the increasing number of reports on the positive impact of art on patients, its use is not widespread [11-15].

One of the forms of art therapy is music therapy. It takes the form of improvising, singing, vocalizing, or listening to music. It is carried out by the therapist individually or in groups, often in the presence of the family [11]. It involves using music to improve communication, social skills, sharing, and recognizing emotions [16, 17]. In addition, it increases the sense of security and self-esteem [18]. Many studies show a clearly beneficial effect of music therapy on children and adolescents with ASD [11, 16, 19, 20].

Another form of art therapy is theater therapy. It deals with using elements of the performing arts, such as role-playing, pantomime, puppetry, and theatrical performances for psychotherapeutic purposes [12]. It is successfully used in children and adolescents with ASD by influencing their imagination [13]. In addition, it allows for the development of social skills, reducing anxiety, limiting

Table I. Characteristics of the study population.*

		Respondents n (%)	Control group n (%)	Study group n (%)
Sex	girl	74 (38.9)	53 (71.6)	21 (28.4)
	boy	116 (61.1)	55 (47.4)	61 (52.6)
Age	0 - 3 yrs	2 (1.1)	2 (1.1)	0 (0)
	4 - 6 yrs	5 (2.6)	0 (0)	5 (2.6)
	7 - 9 yrs	49 (25.8)	33 (17.4)	16 (8.4)
	10 -13 yrs	75 (39.5)	45 (23.7)	30 (15.8)
	> 13 yrs	59 (31.1)	28 (14.7)	31 (16.3)
Population in the inhabited town	up to 50,000	106 (55.8)	76 (40)	30 (15.8)
	up to 100,000	18 (9.5)	8 (4.2)	10 (5.3)
	up to 250,000	46 (24.2)	21 (11.05)	25 (13.15)
	above 250,000	20 (10.5)	3 (1.6)	17 (8.9)

* data obtained from parents and legal guardians of children and adolescents up to 18 years of age.

problem behaviors, and establishing interpersonal relationships, especially family relationships [21, 22].

Another form of art therapy is artetherapy. It is a type of psychotherapy in which art materials are the main form of communication. Thanks to the mediation of an image or other object, it is easier for people who have problems with communication and verbal expression of their feelings to communicate with the therapist [15, 23]. In addition, the analysis of the colors and shapes used by the child allows for the analysis of the progress of therapy [24]. Artetherapy in children with ASD aims to alleviate symptoms related to difficulties with social communication and limited, repetitive behavior patterns [15].

Every year we observe a 20% increase in the ASD frequency among children and adolescents in Poland [4]. This is related to the increased demand for the introduction of additional, effective methods supporting the psychosocial development of children and adolescents with ASD. One of them may be the use of art, which is why we focus on comparing the effectiveness of various forms of artistic activity in our work.

THE AIM

The study aimed to assess the impact of broadly understood art on children and adolescents with ASD. Unfortunately, there are still many inaccuracies regarding its documented impact and influence on improving health in children with ASD. Therapy with the use of elements of art is also little known by many specialists, and our activities were associated with little dissemination of knowledge about it among interested parties.

MATERIALS AND METHODS

The voluntary study involved parents and legal guardians of children and adolescents up to 18 years of age with diagnosed ASD and children and adolescents up to 18 years of age without such disorders. Each respondent represented one family. The respondents came from all Polish voivodships.

We obtained 190 responses, of which 108 were the control group (children and adolescents with no diagnosed ASD), and 82 were the study group (children and adolescents with diagnosed ASD). The number of boys was 116 (61.1%), and the number of girls was 74 (38.9%), of which 55 boys and 53 girls were in the control group, and 61 boys and 21 girls were in the study group. The analysis distinguished the following age ranges: 0 to 3 years old, 4 to 6 years old, 7 to 9 years old, 10 to 13 years old, and more than 13 years old. The respondents also provided information on the population in the inhabited town, based on which we distinguished ranges: up to 50,000, up to 100,000, up to 250,000, and over 250,000 inhabitants. The characteristics of the respondents are presented in Table I.

The study group distinguished children and adolescents in whom artistic activities were an element of essential therapy (17 subjects). To analyze the effects of various fields of art, we distinguished three groups of artistic activities based on: music, theater, and art classes, hereinafter referred to as music therapy, theater therapy, and artetherapy. Their numbers were 14, 4, 12 subjects, respectively.

We conducted the study in the form of a voluntary, anonymous, online survey addressed to parents and legal guardians of children and adolescents with ASD (study group) and without such disorders (control group). The questionnaire was widely available on internet forums and in educational institutions throughout Poland. The questionnaire consisted of a demographic part, general questions about the interests of children and adolescents in the field of art and the knowledge of the concept of its therapeutic application, questions about the type and scope of disorders, and a part about therapy with the use of art elements.

RESULTS

Parents and legal guardians completed an electronic questionnaire entitled: *Therapeutic impact of art on children and adolescents with comprehensive development disorders (autism spectrum disorders) and children and adolescents without diagnosed developmental disorders.*

One hundred ninety parents and legal guardians of children and adolescents participated in the study, of which 43% (82 subjects) were parents and legal guardians of children and adolescents with ASD.

Table II. The impact of artistic activities on the well-being and behavior of children and adolescents with diagnosed disorders from the autism spectrum and without such disorders.

	Type of impact	n (%)
Children and adolescents without autism spectrum disorders	positive	82 (76)
	negative	1 (1)
	no impact	25 (23)
Children and adolescents with autism spectrum disorders	positive	63 (77)
	negative	0 (0)
	no impact	19 (23)

Table III. Have you experienced the therapeutic application of art?

	Respondents	Control group	Study group
Yes	77 people (40.5%)	36 people (33.3%)	41 people (50%)
No	113 people (59.5%)	72 people (66.7%)	41 people (50%)

Table IV. When was your child diagnosed?

The age of diagnosis	n (%)
below the age of 3	20 (24.4)
3 - 6 years old	33 (40.2)
7 - 8 years old	17 (20.7)
after the age of 10	12 (14.6)

RESULTS CONCERNING THE INTERESTS OF CHILDREN AND ADOLESCENTS IN THE FIELD OF ART AND THE KNOWLEDGE OF THE CONCEPT OF ITS THERAPEUTIC APPLICATION

Respondents described the impact of artistic activities on the well-being and behavior of their children. We divided the answers into positive, negative, and no impact. The results are presented in Table II. In both groups, we noticed a clear predominance of responses proving the positive impact of artistic activities on the well-being and behavior of children and adolescents with diagnosed ASD (the study group – 77%) and without such disorders (the control group – 76%). As examples of the positive impact, parents and legal guardians mentioned, among others, calming and reassuring children, improving concentration, and increasing self-esteem.

For the respondents from the control group, artistic classes were most often a form of additional classes in which they participated to develop their interests and passions and improve relations with their peers. Thus, within the study group, we could distinguish children and adolescents

Table V. Symptoms in terms of social relations, speech disorders, and atypical behavior in children and adolescents with ASD.

	Symptoms	n (%)
in terms of social relations	difficulties in establishing relationships with peers	64 (78)
	not making eye contact	41 (50)
	no spontaneous need to share emotions	30 (36.6)
	impaired or different reaction to other people's emotions	40 (48.8)
	none of the above	4 (4.9)
	other	5 (6)
Speech disorders	delayed or complete absence of speech development	41 (50)
	lack of willingness to start and sustain a conversation	29 (35.4)
	stereotypical and repetitive use of words and phrases	26 (31.7)
	lack of spontaneous variety in pretend play or social role play	42 (51.2)
	none of the above	12 (14.6)
	other	4 (4.8)
Atypical behavior	absorbing one or more stereotypical interests, e.g., a form of play is to arrange objects (blocks, cars) in a row, in a specific order	44 (53.7)
	attachment to specific, non-functional routines	38 (46.3)
	stereotypical and repetitive movement patterns, including tapping or twirling your fingers, or complex whole-body movements	36 (43.9)
	none of the above	12 (14.6)
	other	6 (7.2)

Table VI. Reasons for not using the elements of art in the therapy of children and adolescents with ASD.

Reasons for not using the elements of art in the therapy of children and adolescents with ASD	n (%)
I haven't heard about art therapy	38 (58.5)
It is not possible to participate in this form of therapy in the immediate vicinity	10 (15.4)
I believe that the effectiveness of art therapy is low	3 (4.6)
Financial issues	4 (6.15)
Nobody recommended this form of therapy	2 (3.1)
Child disinterest	4 (6.15)
This form of therapy is not suitable for my child	1 (1.5)
Others	3 (4.6)

Table VII. The relationship between the child's age and the use of art therapy.

The child's age	Children taking part in art therapy n (%)
0-3 years old	0 (0)
4-6 years old	2 (11.8)
7-9 years old	2 (11.8)
10-13 years old	3 (17.6)
above the age of 13	10 (58.8)

with ASD who received therapy using elements of art and those who had contact with artistic activities that were not elements of therapy in the home and school environment.

Then we asked the respondents if they had encountered a therapeutic application of art. The results are presented in Table III. 40.5% (77 subjects) of the respondents experienced its therapeutic use, of which 33.3% (36 subjects) were in the control group, and 50% (41 subjects) were in the study group.

RESULTS ON THE TYPE AND EXTENT OF DISORDERS

Parents and guardians of children and adolescents with ASD provided information on the age at which their children were diagnosed with ASD. The results are presented in Table IV. Among the respondents, the largest group of children was diagnosed between the ages of 3 and 6 (40.2%).

The next questions concerned the symptoms of children and adolescents diagnosed with ASD. We asked the respondents to list the symptoms occurring in their charges regarding social relations, speech disorders, and unusual behavior. The obtained results are presented in Table V. The most common symptoms in children and adolescents with ASD regarding social relations were difficulties in establishing relationships with peers (78%). In terms of speech disorders, it was the lack of spontaneous variety of playing pretend or playing imitating social roles (51.2%) and delayed or complete lack of speech development (50%). However, in terms of atypical behaviors, it was absorbed in one or more stereotypical interests (53.7%).

RESULTS OF THERAPY WITH THE USE OF ART ELEMENTS

Then we asked the study participants about the use of elements of art in the therapy of their charges. Some children combined more than one type of artistic activity. The results are presented in Figure 1. Art therapy was used in 20.7% (n = 17) of children and adolescents with ASD, of which 14 subjects (82.4%) received music therapy, four subjects (23.5%) received theater therapy, and 12 subjects (70.6%) – artetherapy.

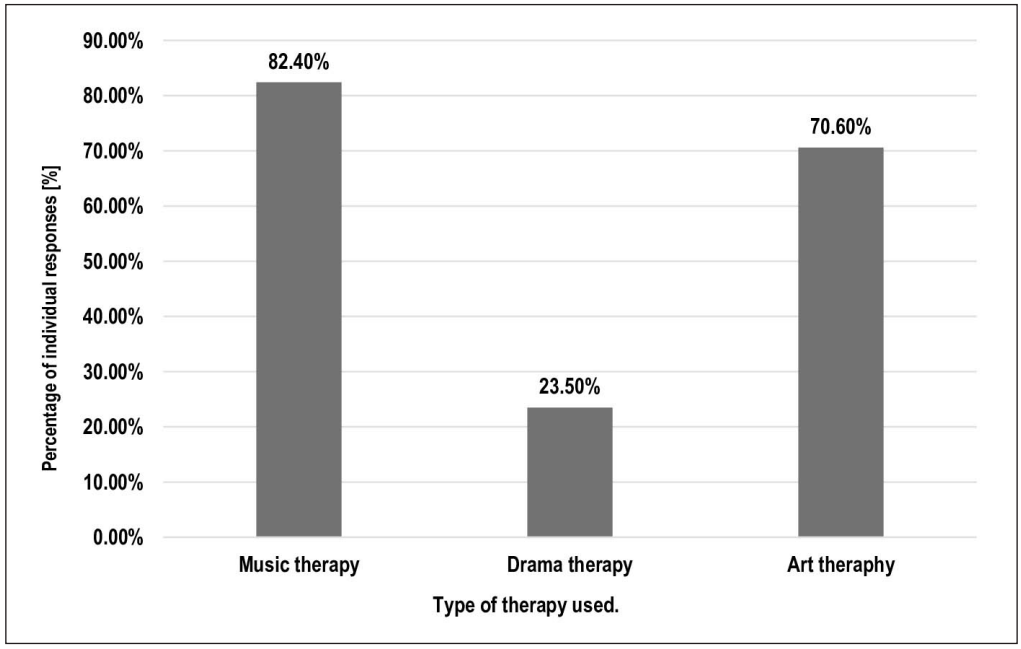


Fig. 1. Percentage of each form of art therapy used with children and adolescents with ASD.

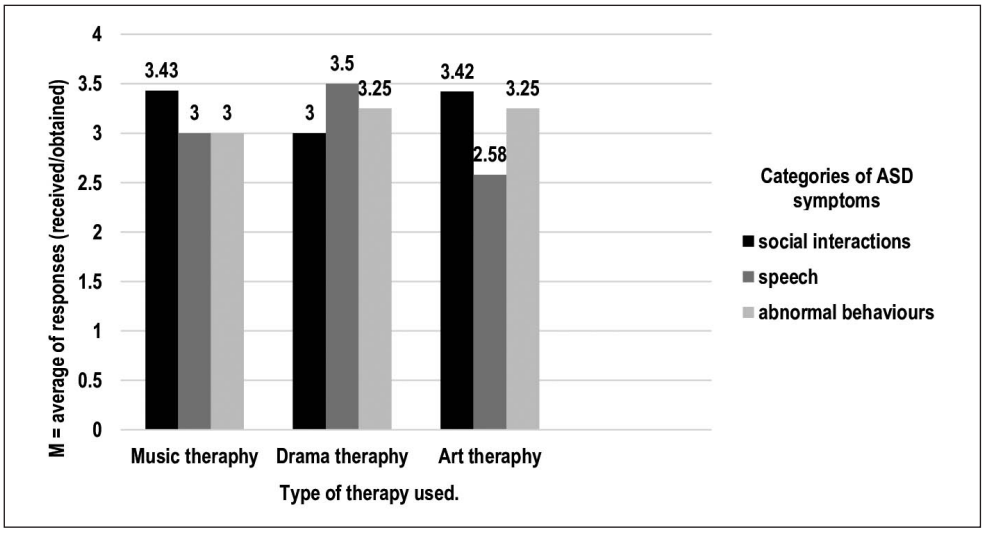


Fig. 2. The correlation between the type of art therapy used and the improvement achieved, by ASD symptoms category.

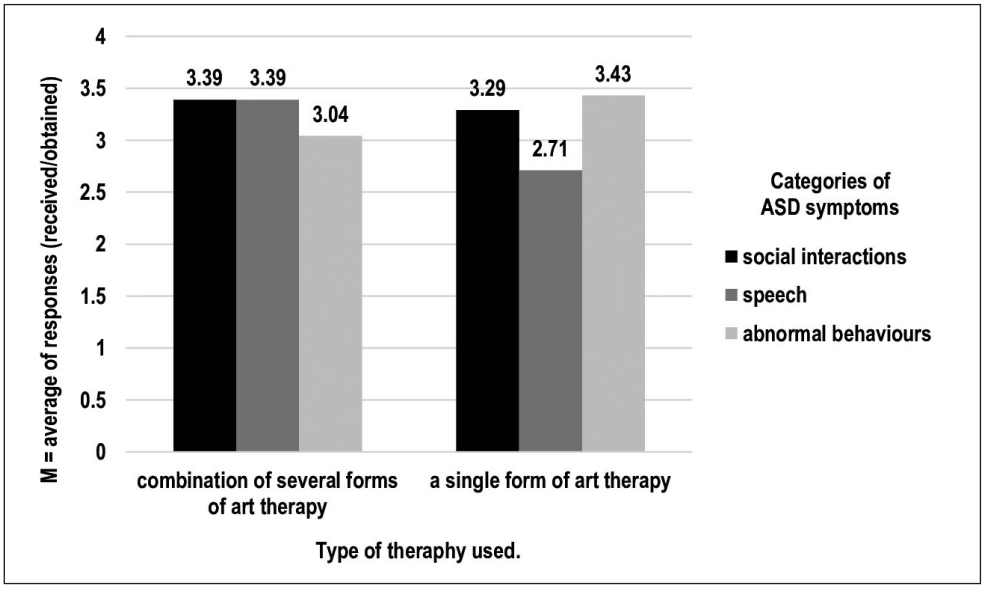


Fig. 3. The correlation between the use of a single form of art therapy and a combination of several forms of art therapy and the improvement obtained, taking into account each category of ASD symptoms.

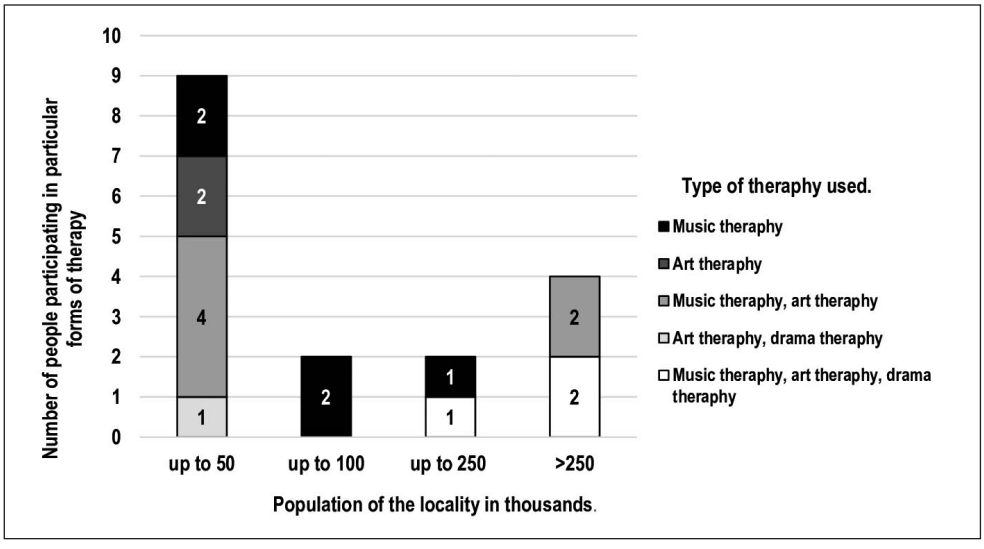


Fig. 4. The relationship between the therapy used and the population of the respondents locality.

Parents and legal guardians of children and adolescents with ASD who underwent art therapy assessed the effectiveness of this type of therapy on the improvement of symptoms (social relations, speech, atypical behavior) occurring in their charges. For this purpose, the respondents marked the scope of improvement on the axis consisting

of a 5-point scale, where 1 was no visible effects, and 5 was the improvement to a large extent. Based on the obtained results, we calculated the means (M), considering the type of therapy (music therapy, theater therapy, artetherapy) and the category of ASD symptoms (social relations, speech, unusual behavior). Detailed results are presented in Figure 2. Among the respondents, music therapy (M = 3.43) and artetherapy (M = 3.42) had the most positive effect on disorders in social relations. In contrast, theater therapy had the most positive effect on speech disorders (M = 3.5).

We also analyzed the effects of using only one form of art therapy and the effects of combining several of their forms with each other. 58.8% (10 subjects) received more than one form of therapy, while 41.2% (7 subjects) received single therapy. In the analysis, we considered the average of the obtained data in the field of disorders in social relations, speech, and atypical behavior. The obtained results are presented in Figure 3.

We asked the parents and legal guardians of children and adolescents with ASD who did not use the elements of art in therapy why this form of treatment was not used in their charges. Detailed results are presented in Table VI. Among the respondents, the most common reason for not using the elements of art in the therapy of children and adolescents with ASD was the lack of knowledge about the existence of such a form of therapy (58.5%).

We also analyzed the relationship between the child's age and the type of art therapy used. The obtained results are presented in Table VII. Children participating in art therapy were most often over 13 (58.8%).

We also analyzed the relationship between the therapy used and the size of the town inhabited by the respondents. The results are presented in Figure 4. Most of the respondents who received art therapy came from towns of up to 50,000 inhabitants.

DISCUSSION

THE PART CONCERNING THE INTERESTS OF CHILDREN AND ADOLESCENTS IN THE FIELD OF ART AND THE KNOWLEDGE OF THE CONCEPT OF ITS THERAPEUTIC APPLICATION

Based on the respondents' answers, we concluded that in our population, activities related to art showed a positive impact on the well-being and development of children and adolescents, both without and with diagnosed ASD. Within the control group, 76% of parents and guardians noticed the positive impact of artistic activities on their children, and in the study group, it was 77%. The most frequently reported positive effects were silence, calming down, relaxation, joy, improvement of manual dexterity under the influence of art, and in children and adolescents with ASD, additionally improvement of concentration and speech.

Thanks to the positive influence of art on the development of children and adolescents, it was used as a complementary therapy, among others, in ASD [13, 14, 18, 20]. 40.5% (77 subjects) of respondents heard about the therapeutic use of art. It was 50% (41 subjects) within the study group, and in the control group, it was only 33.3% (36 subjects). This proves that art therapy is not very widespread among the respondents. Still, there is a noticeable slightly greater knowledge of this form of therapy among parents and guardians of children and adolescents with ASD.

THE PART ON THE TYPE AND SCOPE OF DISORDERS

Children and adolescents with ASD struggle with problems related to various spheres of life. They often have a higher level of anxiety [25, 26]. Moreover, there are gastrointestinal symptoms, sleep problems, and seizures [1, 27]. We categorized the basic symptoms of children and adolescents with ASD into three main groups. Within disorders of social relations, there are, among others, difficulties in understanding roles and social situations [28]. In terms of speech, disorders refer to a delay or complete lack of development [29]. They are most likely one of the causes of irregularities in functional play [30]. Repetitive atypical behaviors such as clapping hands, spinning in circles, and swaying belong to the last category of symptoms [31].

The questionnaire showed that the most common symptoms in terms of social relationships are difficulties in establishing relationships (78%), lack of making eye contact, and impaired or different response to other people's emotions. Regarding speech disorders, 51.2% of the respondents pointed to the lack of spontaneous variety of play in their charges and half of the delayed or complete lack of speech development. Finally, among atypical behaviors, the most common (52.7%) mentioning was a preoccupation with one or more stereotypical interests.

THE PART ON THERAPY WITH THE USE OF ART ELEMENTS

Based on the questionnaire, we compared music therapy, artetherapy, and theater therapy. The groups of subjects who received music therapy and artetherapy were comparable (14 and 12 subjects), while the participants of theater therapy were definitely fewer (4 subjects). Moreover, theater therapy was in each case combined with a different form of art therapy (Fig. 4).

The most frequently chosen form of art therapy among the surveyed parents and legal guardians (82.4%) was music therapy. Available data indicates its impact on the improvement of social relations [32]. Our study confirms this, and in addition, its impact on this category of ASD symptoms turned out to be the greatest (M = 3.43). To a lesser extent, it positively affects the improvement of speech disorders (M = 3) and unusual behavior (M = 3).

Theatre therapy was the least frequently used form of therapy among children and adolescents with ASD. Our survey showed its most significant impact on speech disorders (M = 3.5), slightly less on atypical behavior (M = 3.25), and the least on social relations (M = 3). The respondents indicated that artetherapy had the most significant

impact on social relationship disorders (M = 3.42), less on atypical behavior (M = 3.25), and the least on speech disorders (M = 2.58).

The above data showed that music therapy and artetherapy have a comparable and the most significant impact on disorders in the field of social relations. However, in terms of speech disorders, theater therapy brought the most significant improvement. On the other hand, artetherapy had the most positive impact on atypical behavior.

More than one form of art therapy was used among our respondents more often (58.8%). Combining several forms had a much more significant impact on improving speech than using single therapy. The ratio of means (M:M) expressing improvement was 3.39:2.71. In terms of social relations, the effect of the therapy was similar (M:M = 3.39:3.29). On the other hand, the use of an independent form of art therapy (M = 3.43) seems to be more beneficial in improving atypical behaviors than combining several forms with each other (M = 3.04).

The respondents said that the main reason for not using art therapy was that they had not heard about this form of treatment (58.5%). In addition, 15.4% of the respondents indicated the inability to participate in this form of therapy in the immediate vicinity as the main reason. Other responses that emerged were financial reasons and the lack of recommendation of such a form of therapy by specialists. Other reasons for not using this form of treatment were its low effectiveness (4.6%) and the child's lack of interest in art (6.15%).

There was also a response pointing to the negative impact of art therapy on the child's well-being (problems with keeping up with peers, high stress related to performances). It was noted that: "this form of therapy was not recommended for every case." The analysis of the relationship between age and the applied art therapy showed that people over 13 years of age (58.8%) took part in it most often. This may be due to increased self-determination, i.e., the ability to make life decisions, including those related to therapy. It is a necessary factor facilitating entry into adulthood [33].

The correlations between the size of the place of residence of children and adolescents with ASD and the use of art therapy indicated that it was most often used in children and adolescents with ASD living in towns of up to 50,000 inhabitants (52.9%). Additionally, music therapy was the only form of art therapy used in children and adolescents living in towns with up to 100,000 inhabitants. It is also the most frequently used form of art therapy, regardless of the size of the town or city.

CONCLUSIONS

1. Art has a positive impact on the well-being and development of children and adolescents, which was confirmed by most people participating in the study.
2. The therapeutic application of art among the people involved in the study is not very widespread.
3. Artistic activities positively impact disorders in the field of social relations, speech, and atypical behavior, which is confirmed by the average of the obtained results on the effects of therapy.
4. Music and visual arts activities seem to positively impact social relationship disorders, while drama activities seem to impact speech disorders positively.
5. Combining several arts in therapy is more beneficial than using just one.
6. Therapy with elements of art is most often used in children and adolescents over 13 years of age.

We plan to continue the research thanks to the promising results and the profound interest of both the authors and families of subjects with ASD. Soon, we plan to significantly increase the number of subjects surveyed by establishing cooperation with foundations supporting people with autism spectrum disorders. This will allow for detailed analysis and increasing the credibility of the results. We hope that our work will help to broaden the knowledge on the use of art in therapy and contribute to its dissemination.

The authors declare that they have no ties or financial dependence on any organization or anyone with a direct financial contribution to the research subject or materials studied in this work (through employment, consulting, shareholding, fees). At the same time, they declare that they do not show any conflict of interest.

REFERENCES

1. Szulc A, Gałecki P, Pilecki M, Rymaszewska J, Sidorowicz S, Wciórka J. Kryteria diagnostyczne zaburzeń psychicznych DSM-5. Wrocław: Urban & Partner, 2018.
2. 6A02 Autism spectrum disorder. https://www.findacode.com/icd-11/code-437815624.html [Access: 1.03.2021]
3. Autism spectrum disorders. https://www.who.int/news-room/q-a-detail/autism-spectrum-disorders-(asd) [Access: 1.03.2021]
4. NIK o wsparciu osób z autyzmem i zespołem Aspergera w przygotowaniu do samodzielnego funkcjonowania. https://www.nik.gov.pl/aktualnosci/wsparcie-osob-z-autyzmem-i-zespolem-aspergera.html [Access: 1.03.2021]
5. Loomes R, Hull, Mandy W P L. What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. J Am Acad Child Adolesc Psychiatry 2017; 56 (6):466-474.
6. Rynkiewicz A, Janas-Kozik M, Słopień A. Girls and women with autism. Psychiatr Pol 2019; 53(4):737-739.
7. Bitsika V, Sharpley C F, Mills R. Sex differences in Sensory Features between boys and girls with Autism Spectrum Disorder. Res Autism Spectr Disord. 2018;51: 49-55.
8. Linek A. Arteterapia – poznanie i wyrażanie siebie. Zeszyty Naukowe Towarzystwa Doktorantów UJ Nauki Humanistyczne 2012;1(4): 84.
9. Bitonte R. A, De Santo M. Art therapy: an underutilized, yet effective tool. Mental Illness 2014;6(1):18-19.
10. D'Amico M, Lalonde C. The Effectiveness of Art Therapy for Teaching Social Skills to Children With Autism Spectrum Disorder. Art Therapy 2017; 34 (4):176-182.
11. Geretsegger M, Elefant C, Mössler KA, Gold C. Music therapy for people with autism spectrum disorder. Cochrane Database Syst Rev. 2014;6:5, 18-21.
12. Kurtz LA. Understanding Controversial Therapies for Children with Autism, Attention Deficit Disorder, and Other Learning Disabilities: A Guide to Complementary and Alternative Medicine. London and Philadelphia: Jessica Kingsley Publishers, 2008.

13. Wu J, Chen K, Ma Y, Vomočilová J. Early intervention for children with intellectual and developmental disability using drama therapy techniques. Children Youth Servic Rev. 2020;109(104689):1-7.
14. Van Lith T, Woolhiser Stallings J, Harris C E. Discovering good practice for art therapy with children who have Autism Spectrum Disorder: The results of a small scale survey. Arts Psychother. 2017;54:78-79, 83-84.
15. Schweizer C, Knorth EJ, Spreen M. Art therapy with children with Autism Spectrum Disorders: A review of clinical case descriptions on 'what works'. The Arts in Psychotherapy 2014;41(5):577-578, 591.
16. Rabeyron T, Robledo del Canto JP, et al. A randomized controlled trial of 25 sessions comparing music therapy and music listening for children with autism spectrum disorder. Psychiatry Res 2020; 293(113377):1-6.
17. Knapik-Szweda SM. Zastosowanie muzyki w terapii dzieci i młodzieży z autyzmem – przegląd badań. Psychiatr Psychoter. 2017;13(2):23-27, 31-32.
18. Kokowska M. Psychodynamiczne spojrzenie na praktykę muzykoterapii w psychiatrii. Psychiatr Psychoter. 2017;13(3):31-33.
19. Galińska E. Znaczenie muzykoterapii w rehabilitacji neurologicznej. Psychiatr Pol. 2015; 49(4): 836-837.
20. Boster JB, McCarthy JW, Benigno JP. Music therapy is beneficial for children with autism spectrum disorders, but more specific clinical guidance is a future need. Evidence Based Communication Assessment and Intervention 2017; 11(1-2): 21.
21. Haythorne D, Seymour A. Dramatherapy and autism. New York, Routledge: 2016.
22. Ioannou S, Key AP, Muscatello RA, Klemencic M, Corbett BA. Peer Actors and theater Techniques Play Pivotal Roles in Improving Social Play and Anxiety for Children With Autism. Frontiers Psychol. 2020;11: 1-9.
23. Case C, Dalley T. The handbook of art therapy. East Sussex, New York: Routledge, 2014.
24. Schweizer C, Knorth EJ, Yperen T, Spreen M. Evaluating art therapy processes with children diagnosed with Autism Spectrum Disorders: Development and testing of two observation instruments for evaluating children's and therapists' behaviour. Arts Psychother. 2019;66:1.
25. van Steensel FJA, Heeman EJ. Anxiety Levels in Children with Autism Spectrum Disorde: A Meta-Analysis. J Child Family Stud. 2017;26:1753-1767.
26. Magiati I, Ong C, Lim XY, et al. Anxiety symptoms in young people with autism spectrum disorder attending special school: Associations with gender, adaptive functioning and autism symptomatology. Autism 2016;20(3):306-320.
27. Mahajan R, Bernal MP, Panzer R, et al. Clinical Practice Pathways for Evaluation and Medication Choice for Attention Deficit/Hyperactivity Disorder Symptoms in Autism Spectrum Disorders. Pediatrics 2012;130(2):125-138.
28. Davidson C, O'Hare A, Mactaggart F, Green J, Young D, Gillberg C, Minnis H. Social relationship difficulties in autism and reactive attachment disorder: Improving diagnostic validity through structured assessment. Res Dev Disabil. 2015;40:63-72.
29. Hagberg KW, Jick SS. Validation of autism spectrum disorder diagnoses recorded in the Clinical Practice Research Datalink. Clinic Epidemiol. 2017;9:475-482.
30. Lee SY, Lo Y, Lo Y. Teaching functional play skills to a young child with autism spectrum disorder through video selfmodeling. J Autism Development Disord. 2017;47(8):2295-2306.
31. Olin SS, Mcfadden BA, Golem DL, Pellegrino JK, Walker AJ, Sanders DJ, Arent SM. The effects of exercise dose on stereotypical behavior in children with autism. Med Sci Sports Exerc. 2017; 49(5): 983-990.
32. Ghasemtabar SN, Hosseini M, Fayyaz I, Arab S, Naghashian H, Poudineh Z. Music therapy: An effective approach in improving social skills of children with autism. Adv Biomed Res. 2015;4:157.
33. Hodgetts S, Park E. Preparing for the future: review of tools and strategies to suport autonomous goal setting for children and youth with autism spectrum disorders. Disabil Rehabil. 2017;39(6): 535.

ORCID and contributionship:
Julia Wypyszyńska: 0000-0001-5402-492X [A-D,F]
Natalia Zaboklicka: 0000-0002-1529-000X [A-D,F]
Maria Stachura: 0000-0002-2755-1924 [A-D,F]
Zuzanna Sito – 0000-0002-2911-4453 [A-D,F]
Tomasz Męcik-Kronenberg: 0000-0002-0618-8265 [D-F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Julia Wypyszyńska
Student Research Group at The Chair and Department
of Pathomorphology Faculty of Medical Sciences in Zabrze
Zabrze, Poland
e-mail: jwypyszynska@interia.pl

Received: 07.05.2021
Accepted: 30.08.2021

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 EFFECT OF SPIRONOLACTONE ON SERUM ELECTROLYTES AND RENAL FUNCTION TESTS IN PATIENTS WITH SEVERE CHRONIC HEART FAILURE

DOI: 10.36740/WLek202110117

Seher Abdurasool Almedeny[1], Jabbar Yasir AL- Mayah[1], Mohanmed S. Abdulzahra[2], Najah R. Hadi[1]
[1]DEPARTMENT OF PHARMACOLOGY, FACULTY OF PHARMACY, UNIVERSITY OF KUFA, KUFA, IRAQ
[2]DEPARTMENT OF MEDICINE, FACULTY OF MEDICINE, UNIVERSITY OF KUFA, KUFA, IRAQ

ABSTRACT

The aim: To evaluate the effect of single daily 25 mg of spironolactone on serum electrolytes and kidney function tests in patients with severe chronic left sided heart failure.

Materials and methods: 60 patients with severe chronic left sided heart failure were enrolled in this study and they were divided in to 2 equal groups' one group with standard therapy of HF and the other with spironolactone in a dose of 25 mg / day, as an additive therapy to the standard one. Serum electrolytes and kidney function tests were assessed at the beginning of the study and after 3 months.

Results: A significant increment in serum potassium ($p<0.05$) was observed in the spironolactone group after 3 months treatment, while no significant reduction in serum sodium ($p>0.05$) and no significant increase in serum creatinine and blood urea ($p>0.05$) was noticed in the same group, control group showed no significant changes ($p>0.05$), in both serum electrolytes (S.K and S.Na) and renal function tests (S.C and B.U).

Conclusions: Spironolactone caused a significant elevation of serum potassium level but this elevation is still with the clinically accepted ranges when low dose of spironolactone is used and with intact renal function. Serum creatinine level was not significantly increased with 25 mgl day of spironolactone**.** We conclude that Renal function tests namely blood urea and serum Creatinine, and serum potassium should be closely monitored in patients on spironolactone therapy especially those patients who use ACEI and ARBs in addition.

KEY WORDS: HF, spironolactone, S.K, S. Na, S.C, B.U.

Wiad Lek. 2021;74(10 p.I):2460-2462

INTRODUCTION

Spironolactone is related to the low efficacy diuretic group; this group is also called potassium sparing diuretics. Spironolactone has a chemical structure similar to other steroids but with a last one substitute at c-17. It has an excellent bioavailability of 90% with a limited first pass effect, and has a half-life of approximately 14 hours. Its active metabolites can renone (with a half-life of 9 hours) and can renoate are excreted primarily in urine and secondarily in bile. Spironolactone is available as tablet, usually taken once or twice daily; it should be taken with food or milk to increase absorption [1]. Spironolactone acts not only to competitively inhibit aldosterone but also react with testosterone and progesterone receptors causing side effects as impotence, gynecomastia and menstrual irregularities [2]. Recent studies on this drug have concentrated on its effect in patients with left side H F as an aldosterone antagonist [3]. It is postulated to work synergistically with ACEIs to provide more thorough blocking of the of the renin-angiotensin system.

PRECAUTIONS AND CONTRAINDICATIONS OF SPIRONOLACTONE

Precautions [2]:
1- If patient is allergic to Spironolactone.
2- Presence of hepatic or renal disease.
3- Pregnancy and breast feeding.
4- If patient is taking K supplements.

Contraindications [4]:
1- Hyperkalemia when S.K 5 mmole /dl.
2- In patients with impaired renal function.

Side effects of Spironolactone:
1- Nausea, vomiting and abdominal cramping [2].
2-Sore throat and skin rash.
3-Menstrual irregularity in females, and gynecomastia and impotence in males [5].
4- Hyperkalemia and Hypernatremia can occur.
5-Mental confusion.

Drug interaction:
1- Spironolactone increases the half-life of digoxin thus may increase the risk of digoxin toxicity [6].
2- Risk of hyperkalemia increases with the concomitant use of ACEIs [7].
3- Its effectiveness would decrease if used with salicylate [8].

THE AIM

To evaluate the effect of single daily 25 mg of spironolactone on serum electrolytes and kidney function tests in patients with severe chronic left sided heart failure.

Table I. Effect of 25 mg /day of spironolactone on serum electrolytes and renal function tests.

	Before Treatment	After 3 months Treatment	p-value
Serum Potassium, mmol/L	4.27 ± 0.102	4.57 ± 0.118	< 0.05
Serum Sodium, mmol/L	137.87 ± 1.04	137.2 ± 1.4	> 0.05
Blood Urea mg/dl	33.37 ± 2.93	34.27 ± 2.2	> 0.05
Serum Creatinin mg/dl	0.76 ± 0.040	0.787 ± 0.052	> 0.05

Data are expressed as Mean ± SEM, n=15

Table II. Serum electrolytes and renal function tests in control group.

	At base line	After 3 months	p-value
Serum Potassium, mmol/L	4.34 ± 0.084	4.24 ± 0.081	> 0.05
Serum Sodium, mmol/L	137.36 ± 1.49	137.64 ± 1.29	> 0.05
Blood Urea mg/dl	36.14 ± 2.60	35.36 ± 2.5	> 0.05
Serum Creatinin mg/dl	0.714 ± 0.035	0.721 ± 0.037	>0.05

Data are expressed as Mean ± SEM, n=14

Table III. Mean difference in serum electrolytes and renal function tests between controls and spironolactone treated group.

	Mean change±SEM	MD±SEM	p-value
Serum Potassium, mmol/L	Spironolactone 0.307 ± 0.078 Control -0.093 ± 0.109	0.330 ± 0.146*	< 0.05
Serum Sodium, mmol/L	Spironolactone -0.667 ± 1.95 Control 0.286 ± 0.934	-0.443 ± 1.95	> 0.05
Blood Urea mg/dl	Spironolactone 0.553 ± 3.36 Control -0.786 ± 1.86	0.196 ± 3.12	> 0.05
Serum Creatinin mg/dl	Spironolactone 0.027 ± 0.051 Control 0.007 ± 0.037	0.041 ± 0.061	> 0.05

*Mean difference is significant at 0.05 level

MATERIALS AND METHODS

60 patients (15 females, 45 males) with chronic left sided systolic HF and with an average age of 61 ±5 years were enrolled in the study successively in Najaf teaching hospital, the study continued for one and a half year. All patients were treated with ACEIs and loop diuretics, 63.3% patients were on nitro vasodilators, 28.3% patients were on digoxin (this represent the standard therapy). Potassium sparing diuretics were not permitted in this study. Patients were randomly distributed in to 2 equal groups:

Control group: remain on their standard therapy

Treated group: received spironolactone in a dose of 25 mg/day in addition to their standard treatment.

Follow up continued for 3 months. Laboratory tests including serum electrolytes, renal function tests were investigated for the 2 groups before and after receiving treatment. From those 60 patients only 29 patients continued the study (14 patients in the control group and 15 patients in the spironolactone group).

LABORATORY TESTS

Serum electrolytes including serum potassium (S.K) [9] and serum sodium [10], renal function tests including blood urea (B.U) and serum creatinine (S.C) [9] were performed for each patient at initial visit, repeated after 4 weeks and after 3 months.

STATISTICAL ANALYSIS

Within group changes from base line to 3months were analyzed by the paired t-test and p-values less than 0.05 were considered to be statistically significant. Comparison between the 2 groups of mean changes from base line to 3 months were performed by ANOVA

RESULTS AND DISCUSSION

Effect of spironolactone on serum electrolytes and renal function tests

A significant increase in serum potassium ($p<0.05$) was observed in the spironolactone group after 3 months treatment, while no significant reduction in serum sodium ($p>0.05$) and no significant increase in serum creatinine and blood urea ($p>0.05$) was noticed in the same group (Table I). Control group showed no significant changes ($p>0.05$), in serum electrolytes (S.K and S.Na) and renal function tests (S.C and B.U) (Table II).

Comparison between the two groups revealed a significant difference in the mean level of serum potassium ($p<0.05$), whereas no significant difference in the mean levels of serum sodium, serum creatinine and blood urea were obtained ($p>0.05$) (Table III).

Patients treated with spironolactone showed a significant increase in the mean serum level of potassium this increase although was significant but still with the accepted clinical limits. This result was also obtained by Pitt et al. and Han [11-12]. Others as Svensson, and Wrenger et al., stated that serious hyperkalemia can be resulted from combination of spironolactone and ACEIs in patients with heart failure especially with advanced age due to physiological regression of renal function with advanced age, higher dose of spironolactone and impaired renal function [13-14]. Serum sodium exhibits no significant reduction in the spironolactone treated patients this may be attributed to the low dose of spironolactone that used in this study and this was consistent with results that obtained by Yasky et al. [15]. Renal function tests showed no significant increase in blood urea and serum creatinine, this result was similar to that obtained by Yasky et al. [15], but others like Pitt, et al., and Svensson et al., assured that spironolactone can cause significant but still accepted increase in serum creatinine levels in patients with heart failure and the chance for raised level of serum creatinine will increase with elderly patients due to increasing risk of renal impairment with advanced age and higher dose of spironolactone [11-15].

CONCLUSIONS

Spironolactone caused a significant elevation of serum potassium level but this elevation is still with the clinically accepted ranges when low dose of spironolactone is used and with intact renal function. Serum creatinine level was not significantly increased with 25mgl day of spironolactone**.** We conclude that Renal function tests namely blood urea and serum Creatinine, and serum potassium should be closely monitored in patients on spironolactone therapy especially those patients who use ACEI and ARBs in addition.

REFERENCES

1. Whalen K, Finkel R, Panavelil T. Potassium sparing diuretics. Lippincott illustrated Reviews: Pharmacology, 6th edn. Amsterdam: Elservier, 2015, pp. 248-250.
2. Rang HP, Ritter JM, Flower RG, Henderson G. RANG and DALES Pharmacology.8th edn, Amsterdam: Elservier, 2016, pp., 363-364,
3. Nappi J, Sieg A. Aldosterone and aldosterone receptor antagonists in patients with chronic heart failure. Vasc Health Risk Manag. 2011;7:353-363
4. Yancy CW, Jessup M, Bozkurt B, et al. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. J Card Fail. 2017 Aug;23(8):628-651.
5. Lainscak M, Pelliccia F, Rosano G, Vitale C, Schiariti M, Greco C, Speziale G, Gaudio C. Safety profile of mineral corticoid receptor antagonists: Spironolactone and eplerenone. Int J Cardiol. 2015 Dec 01;200:25-9.
6. Gwendolyn A. Millin Mc, Kamisha L. Johnson D. Chapter 13 – Issues of Interferences in Therapeutic Drug Monitoring. Accurate Results in the Clinical Laboratory, A Guide to Error Detection and Correction 2013:195-211
7. Charny JW, Choi JK, James WD. Spironolactone for the treatment of acne in women, a retrospective study of 110 patients. Int J Womens Dermatol. 2017 Jun;3(2):111-115.
8. Tsutamoto T, Weda A, Maeda K, et al. Spironolactone inhibit the transcardiac extraction of aldosterone. JAM Coll Cardiol. 2003;6(3);838-844.
9. Dolovich L, Gavura S, Pottie K. Hyperkalemia associated with spironolactone therapy. Can FAM Physician. 2005 Mar 10;51(3):357-360.
10. Worth HGJ. Plasma sodium concentration bearer of false prophecies. Br Med J. 1983;287:567-568.
11. Pitt B, Zannad F, Remme W, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. NEJM 1999;341:709-717
12. Han YL, Tong M, Jing QM, Hu XL, Lin Q. Combined therapy of Captopril and spironolactone for refractory congestive Hf. Clin Med J (Engl). 1994;107(9):688-692
13. Svensson M, Gustafsson F, et al. Hypokaelemia and impaired renal function in patients taking spironolactone for congestive HF. BMJ 2003; 327:1141-1142
14. Wrenger E. Muller R, et al .Interaction of spironolactone with ACEIs or ARBS. BMJ 2003; 327:147-149
15. Yasky J, Ledesma GA, Tutera A, Collia LF. A Fixed dose combination of Furosemide and spironolactone in digitalized congestive Hf patients. Pharmatherapeutica.1986;4(8):473-479

ORCID and contributionship:

Seher Abdulrasool Almedeny: 0000-0001-8367-5975 [D-E]
Jabbar Yasir AL-Mayah: 0000-0002-0193-0643 [C]
Mohammed Saeed Abdulzahra: 0000-0002-9377-7876 [C]
Najah R. Hadi: 0000-0002-8415-5311 [A,E-F]

Conflict of interest:

The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Najah R. Hadi
Department of Pharmacology & Therapeutics,
University of Kufa, 54003, Kufa, Iraq
e-mail: drnajahhadi@yahoo.com

Received: 30.06.2021
Accepted: 21.09.2021

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

CURRENT ASPECTS OF DELIVERY IN HEALTHY WOMEN IN ACCORDANCE WITH THE DATA OF RETROSPECTIVE ANALYSIS

DOI: 10.36740/WLek202110118

Natalia Ya. Skripchenko, Yuliia V. Nevyshna, Liliia A. Lozova, Olena M. Pavlova, Nadiya V. Gerevich
STATE INSTITUTION «INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY NAMED AFTER ACADEMICIAN O.M. LUKYANOVA ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KYIV, UKRAINE

ABSTRACT

The aim: to study the current aspects of the course of labor in healthy women using retrospective indicators.

Materials and methods: To study this topic, an analysis of 1,078 births on the basis of the maternity ward for pregnant women with obstetric pathology of the State Institution «IPAG. acad. O.M. Lukyanova National Academy of Medical Sciences of Ukraine». It was found that among all births, the share of first-borns was 602 (55.8 %) women, of whom 451 (41.8 %) were pregnant for the first time, and only 86 (8 %) were healthy pregnant women.

Results: It was found that among 86 births the frequency of physiological births was 64%, of which in 47.7% of cases the birth was complicated, and pathological – 36%. The most common complications during childbirth were: premature rupture of membranes (PRPO), episio- and perineotomy, trauma to the birth canal. The main causes of pathological childbirth: abnormalities of labor, fetal distress, defect of the placenta and membranes, clinically narrow pelvis, malposition of the fetus and early postpartum hemorrhage. All children were born alive. It should be noted that all births where the Apgar score was ≤ 6 had no partner support, and the women themselves did not receive any preparation for childbirth.

Conclusions: According to our data, in almost healthy women who gave birth for the first time and had no perinatal loss in the anamnesis, did not undergo prenatal training and did not have partner support during childbirth, the number of complications during childbirth is increasing. Therefore, this group of healthy pregnant women needs more detailed study and analysis, development of prenatal training algorithms to improve perinatal indicators.

KEY WORDS: pregnancy, childbirth, complications in childbirth, partner childbirth, psychophysical training

Wiad Lek. 2021;74(10 p.I):2463-2465

INTRODUCTION

The management of pregnancy and childbirth, prevention and prediction of complications during childbirth in the mother and fetus remain today a pressing problem of modern obstetrics [1-4]. According to the literature, it is established that in women with extragenital pathology childbirth will not always be pathological or complicated, and in almost healthy – physiological [5]. In modern conditions, against the background of the development of market relations and socio-economic transformations, which ambiguously affected various spheres of life, and especially the social and medical aspects of the family, the demographic situation in Ukraine has deteriorated. This is evidenced by fertility, maternal and perinatal mortality, women's reproductive health – an increase in the problem of infertility in marriage, gynecological diseases, miscarriage, complications during pregnancy and labor, the postpartum period and the progressive growth of somatic pathology in pregnant women [6]. To date, there is much debate about the generally accepted concept of «normal labor» [1]. However, over the past 20 years, obstetric aggression has increased, which is expressed in a decrease in the frequency of physiological childbirth and an increase in the frequency of operative childbirth not only in Ukraine but also in other more developed countries [7]. Psychophysical preparation for childbirth and partner involvement counteract this phenomenon when the partner is a close person, usually a man, who is the best mediator between the health worker and the mother [8]. Unfortunately, over time, interest in the use of psychoprophylactic preparation for childbirth and in general for pregnant women as individuals has decreased [9]. Most pregnant women approach childbirth without proper training, relying solely on their knowledge and health (almost healthy). According to the literature, of those women who gave birth with a partner, only half attended the «School of Family Preparation for Childbirth» [10]. Which indicates the relevance, the need for careful study and further research in this direction.

THE AIM

To study current aspects of childbirth in healthy women according to retrospective data.

MATERIALS AND METODS

To study this topic, we analyzed 1,078 births on the basis of the department for pregnant women with obstetric pathology of the State Institution «Institute of Pediatrics, Obstetrics and Gynecology named after Academician

OM Lukyanova of the National Academy of Sciences of Ukraine.» There were 602 first-borns (55.8%), of whom 451 (41.8%) were pregnant for the first time, and only 86 (8%) were healthy pregnant women, whom we will study. A group of healthy pregnant women believed that such a large number of women, and because they are healthy, the course of their pregnancy and childbirth should automatically be physiological and will not require intervention. This myth has recently come into force among women who consider themselves the healthiest and do not see the need to spend time training, as well as doctors themselves, who have lost interest in the personality of a pregnant woman and her psychophysical mood. Even in many maternity hospitals and women's clinics there is no preparation for childbirth. Criteria for inclusion in the study were: first pregnancy, chronic diseases in remission, one fetus, compensated fetal condition. Exclusion criteria: gynecological diseases and gynecological operations in the anamnesis, infertility, pregnancy after in vitro fertilization, polyhydramnios, fetoplacental dysfunction, isthmic-cervical insufficiency, severe anemia, preeclampsia, inflammatory diseases in early pregnancy, severe extragenital pathology.

RESULTS

Clinical evaluation of labor was performed according to the history of pregnancy and childbirth in healthy women who are pregnant for the first time. The mean age of the surveyed women was 25.9 ± 3.4 years. Distribution of women by age: up to and including 19 years of age – 6 (7 %) women; 20-24 years – 19 (22.1 %) persons; 25–29 – the largest group – 48 (55.8 %) women, 30–34 years – 11 (12.8 %) women, after 35 years – 2 (2.3 %) women. Among the 86 patients, women engaged in mental activity predominated, their share was 48.8 %, 19.9 % – had physical activity, 20.9 % – unemployed, 9.3 % of women studied. All women regularly attended women's clinics, and only 8 (9.3 %) attended their parents' school. Childbirth with the support of a partner amounted to – 17.4 %. It was found that among 86 births the frequency of physiological births was 64 %, of which in 47.7 % of cases the birth was complicated, and pathological – 36 %. Despite the fact that all births took place in almost healthy women and had to be timely, still 3.5 % of them were premature, but not earlier than 34 weeks of pregnancy, all children survived. The tendency to prolonged pregnancy (at 41–42 weeks) was observed in 4 (4.7 %) cases with all births with pathological course, in 2 cases – cesarean section, 1 – vacuum extraction of the fetus, in one case by manual examination of the uterine wall. The most common complications during childbirth were: premature rupture of membranes, episio- and perineotomy, injuries of the birth canal (rupture of the cervix, rupture of the vagina and perineum). Among these complications, the largest share was episio- and perineotomies – 35 (40.7 %), and the smallest – 17 (19.9 %) injuries of the genital tract.

Premature rupture of membranes during childbirth occurred in 20 (23.3%) women. The causes of abnormal labor in the examined women were: abnormalities of labor, fetal distress, defect of the placenta and membranes, clinically narrow pelvis, abnormal position of the fetus and early postpartum hemorrhage. 11 (12.8%) – cesarean section, 7 (8.1%) of which the indication for surgery was fetal distress, 2 (2.3%) – incorrect fetal position, 2 (2.3%) – clinically narrow pelvis. The largest share of surgical interventions was instrumental and manual revision of the walls of the uterine cavity 13 (15.1%) during childbirth, indications were defects of the membranes and lobes of the placenta and 11 (12.8%) – during cesarean section. Abnormalities of labor were recorded in 4 cases, in one case the labor was rapid, in two – the weakness of labor in the second period of labor, in the other case – the weakness of labor in the second period of labor and fetal distress, which led to additional intervention: episiotomy with surgical extractor . Early postpartum hemorrhage occurred in some labor in women 25 years old, large fetus – 4240 g, which could be the cause of postpartum hypotonic bleeding. In the structure of pathological labor there is a dependence on the age of the woman. There fore, only 12.9% of women with pathological course were in the age group up to 24 years, and 87.1% – women aged 25 years and older.

DISCUSSION

According to the analysis, it was found that anesthesia during labor was used in 66 (76.7 %) pregnant women, of whom in 6 (6.9 %) cases used two methods of analgesia – a combination of antispasmolitics and narcotic analgetics. Epidural anesthesia was given to 12 (14 %) women, narcotic analgetics to 24 (27.9 %) mothers, and antispasmolitics to 15 (17.4 %). All children were born alive, of which 6 (7 %) children received 6 points or less in the first minute, 26 (30.2 %) children received 7 points, and 54 (62.8 %) children received 8 or more points. The birth of children with a score of 6 or less is due to labor: 2 children were born prematurely, 2 – cesarean section in acute distress, 2 – vacuum extraction due to the weakness of the powerful period on the background with epidural anesthesia. It should be noted that all labor where the Apgar score was ≤ 6 had no partner support, and the women themselves did not receive any preparation for labor.

CONCLUSIONS

According to our data, in almost healthy women who gave birth for the first time and had no perinatal loss in the anamnesis, did not undergo prenatal training and did not have partner support during childbirth, the number of complications during childbirth is increasing. Therefore, this group of healthy pregnant women needs more detailed study.

REFERENCES

1. WHO recommendations: intrapartumcarefor a positivechildbirthexperience. WHO, 2018; 210 p.
2. Nakaz MOZ Ukraïni vid 31.12.2004 № 676 «Pro zatverdzhennja klinichnih protokoliv z akushers'koï ta ginekologichnoï dopomogi». Dostup na: https://zakon.rada.gov.ua /rada/show/v0624282-08. (in Ukrainian).

3. Vdovichenko SJu. Profilaktika akushers'koï ta prenatal'noï patologiï pri vikoristanni rodinno-orientovanih tehnologij pid chas vagitnosti ta pologiv. [Prevention of obstetric and prenatal pathology using family-oriented technologies during pregnancy and childbirth]. Zdorov'e zhenshhiny. 2017;2:78–82. (in Ukrainian).
4. Zhabchenko IA. Suchasni pidhodi do profilaktiki akushers'kogo travmatizmu ta jogo naslidkiv. [Modern approaches to the prevention of obstetric injuries and its consequences]. Medichni aspekti zdorov'ja zhinki. 2020;1:5–10. (in Ukrainian).
5. Medvedev MV, Davydova JuV. Profilaktika perinatal'nyh poter': na puti k personalizirovannoj medicine. [Prevention of perinatal losses: on the way to personalized medicine]. Perinatologija i pediatrija. 2016;1(65):59–65. (in Russian).
6. Vdovichenko SJu. Vpliv partners'kih pologiv na harakter pologovoï dijal'nosti zhinok, jaki narodzhujut' upershe i povtorno. [The impact of partner childbirth on the nature of labor of women who give birth for the first and second time]. Zdorov'e zhenshhiny. 2015;7(103):32–33. (in Ukrainian).
7. Ishhenko GI, Demenina NK. Dinamika operativnogo rozrodzhennja v suchasnomu akusherstvi (ogljad literaturi). [Dynamics of operative delivery in modern obstetrics (literature review)]. Perinatologija i pediatrija. 2019;2(78):54–57. (in Ukrainian).
8. Vdovichenko SJu. Rol' psihologichnoï ocinki podruzhnih par pri provedenni partners'kih pologiv. [The role of psychological assessment of married couples in partner childbirth]. Zdorov'e zhenshhiny. 2015;2(98):87–89. (in Ukrainian).
9. Manuhin IB, Silaev KA. Rol' psihoprofilakticheskoj podgotovki beremennyh k rodam. [The role of psychoprophylactic preparation of pregnant women for childbirth]. Rossijskij vestnik akushera-ginekologa. 2016;16(6):9–13. (in Russian).
10. Sjusjuka VG. Ocinka vplivu programi mediko-psihologichnoï korekciï psihoemojcijnoï dezadaptaciï u vagitnih na perinatal'ni naslidki ïh rozrodzhennja. [Assessment of the impact of the program of medical and psychological correction of psychoemotional maladaptation in pregnant women on the perinatal consequences of their birth]. Perinatologija i pediatrija. 2016;3:43–48. (in Ukrainian).

The work is carried out within the framework of the research work "Investigate the reproductive health of women who have suffered from chronic inflammatory diseases of the genital organs and the risks of perinatal disorders" (state registration number 01.11.U002.055).

ORCID and contributionship:
Yuliia V. Nevyshna: 0000-0001-5492-6392 [A, E, F, E]
Natalia Ya. Skrypchenko: 0000-0003-2849-8499 [B, C, D, F]
Liliia A. Lozova: 0000-0002-7118-6899 [B, D, F]
Olena M. Pavlova: 0000-0002-5500-4653 [D, E]
Nadiya V. Gerevich: 0000-0002-5984-3307 [C, E]

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

CORRESPONDING AUTHOR
Yuliia V. Nevyshna
State Institution «Institute of pediatrics, obstetrics and gynecology, named after academician O.M. Lukyanova National academy of medical sciences of Ukraine»
St. P. Mayboroda, 8, 04050 Kyiv, Ukraine
tel: +380976000007
e-mail: Likar_nevushna@ukr.net

Received: 23.05.2021
Accepted: 15.09.2021

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

MEDICAL AND ECONOMIC ANALYSIS OF THE CHOICE OF THERAPEUTIC PLASMAPHERESIS METHOD TO OPTIMIZE THE TRANSFUSION CARE QUALITY

DOI: 10.36740/WLek202110119

Vladislav V. Liubchak, Liliia M. Khomenko, Michael P. Kovalishyn, Viktoriia V. Ilyina, Vladyslav A. Smiianov, Volodymyr V. Sikora, Tetiana V. Babar
SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

The aim: To analyze the medical and economic aspects of the manual and different types of automatic plasmapheresis (manual, automatic centrifugal, automatic membrane, plasmapheresis with plasma therapy and mixed) used for therapeutic purposes.

Materials and methods: The Baxter Auto-C, Haemonetics PCS2, Haemophenics, Baxter CPDA anticoagulant and saline, Baxter 16GA needles were used. Total protein was examined by the biuret method, hemoglobin by the Sally method, total bilirubin by the colorimetric photometric method, cell fragments by the Goryaev camera microscopy method; patient comfort – with a 10-point scale. Healthy blood donors participated in the study. Manual plasmapheresis was performed in 31 people, automatic plasmapheresis with centrifugal technology – 36 people, with membrane technology – 21 people, mixed technology – 36 people.

Results: An analysis of the different technologies impact on hematological, psychological and medical and economic indicators was performed. Native hemoglobin was absent in the bloodstream and in the final plasma with all technologies. Bilirubin index was within normal limits. There were no cell fragments. It was proved the absence of significant differences in various technologies on hematological parameters. The lower level of patient comfort by manual plasmapheresis was established.

Conclusion: All therapeutic plasmapheresis technologies have the same effect on the patient's blood hematological parameters and did not have a negative impact on the body by the indicators: hemolysis, the presence of cell fragments, patient discomfort and citrate reactions during the standard procedure of sampling 800 ml of plasma. The most effective are plasmapheresis machines with centrifugal technology by medical and economic parametres.

KEY WORDS: automatic plasmapheresis, manual plasmapheresis, membrane plasmapheresis, centrifugal plasmapheresis, economic effect

Wiad Lek. 2021;74(10 p.I):2466-2470

INTRODUCTION

Therapeutic plasmapheresis is one of the most modern techniques that can be used by physicians in almost all specialties [1-5]. According to the standards, plasmapheresis is used for pathological conditions resulting from autoimmune pathology, the presence of endo- and exopathology and infectious diseases, especially of viral etiology. According to studies conducted in Ukraine, the most common pathologies for therapeutic plasmapheresis are bronchial asthma, acne, viral hepatitis, psoriasis and alcohol intoxication, which indicates a narrower use of this method [6]. Plasmapheresis is divided into two types: therapeutic and donor [7]. There are five types of plasmapheresis: manual, automatic membrane, automatic centrifugal, plasmapheresis with plasma therapy and with mixed technology.

Manual plasmapheresis is carried aut as discrete collection of whole blood, its further separation and return of cells. Up to 400 ml of blood is taken at a time, in double or triple bags, using stirrer scales. The final blood is separated by centrifugation in special centrifuges. This high-cost and big equipment must be installed in a specially prepared room. In total, it takes about 2-3 hours and at least 4 sets of consumables to obtain 800 ml of plasma [7]. The advantage of this method is the availability of performance in clinics that have such equipment in blood transfusion departments. The disadvantage is not only the high cost of centrifuges, but also significant requirements for the room and the foundation of the building due to the potential danger of long-term vibrations with a large amplitude. Four sets of consumables, and the employment of the production unit with working hours of employees for up to 3 hours, can also be attributed to the disadvantages of significant costs. Also, the human factor is not excluded during this procedure, which, according to the GCP standard, is unacceptable.

Membrane plasmapheresis is a logical development of plasmapheresis. This procedure uses a filter that passes only blood plasma, trapping cells. Such filters are produced in the city of Dubno (Russia) using an elementary particle accelerator, that punches holes in the membrane of the appropriate size. Thanks to the static or pressurized pump, a transmembrane pressure is created, it pushes plasma through the filter element. The advantages of this method are the low volume of blood taken outside the body (this allows you to perform low-volume plasmapheresis without additional

effort) and the mobility of the device. The disadvantages are the need to create transmembrane pressure, which could theoretically lead to cell destruction, as well as the relatively high cost of the membranes and filters in general. It should also be kept in mind that some of these machines do not automatically control dilution with anticoagulant.

Centrifugal machines have a slightly larger volume of blood to be processed. Due to the fundamental difference in technology and the lack of high-cost materials in cost systems, the cost of one procedure is much lower. According to some authors, the use of a centrifuge can also lead to cell destruction, but we have not found confirmed data on this. In addition, from our personal experience, this was not observed. Among the advantages of this method, the high speed of the procedure, low cost, and the ability to use on the road are significant. These devices were primarily designed for donor plasmapheresis, so safety and reliability are at extremely high level; the normal load of these machines is up to 6 procedures per day [6]. The basis of this technique is the use of a centrifugal bell, which in a completely closed system, with single-needle access, that is periodically filled with whole blood, with roller pumps that have no contact with the blood itself. The rate of collection and return, as in membrane plasmapheresis is clearly controlled, a separate pump is used for dosing anticoagulant. Air sensors, are responsible for safety. Reliability, simplicity and mobility of a design made these automatic machines one of the market leaders.

Mixed type devices have a rotating membrane. In general, this system corresponds to the principle of operation of centrifugal devices, but is more expensive and immobile (can be moved only within the room).

Plasma therapy devices for plasmapheresis in Ukraine are beyong compare for the above methods, due to the complex use and incomparably higher price.

Nowadays in Ukraine the following plasmapheresis machines are used:

1. Scinomed
2. Nigale
3. Haemonetic
4. Fenwall (Baxter)
5. Haemophenix
6. AmPlTT (as Haemophenix)
7. AC-B-02
8. Phemos-PF [6].

There are plasma therapy machines and centrifuges presentsd on the market. However, the cost of the procedure with using these systems is much higher.

According to the research, the procedure of plasmapheresis is performed according to conventional methods, but the method of the course is very different, which allows to obtain different results for the same pathology on the same equipment.

Plasmapheresis is a modern technique in donation and therapy [8]. More and more blood centers in the world use the automatic method instead of the manual one [6]. And this despite the fact that the method is used for almost 100 years [7].

The procedure according to the analysis of many sources, does not differ depending on the type of equipment used. Any single-needle plasmapheresis is performed intermittently. Plasma is removed and the cells are returned to the patient. This method works on all methods of plasmapheresis, including manual, using the centrifuge (collecting whole blood in a plastic container, followed by centrifugation at a speed sufficient to separate the blood into components and separation on the device for squeezing). Plasma is moved to an additional package that is removed at manual plasmapheresis whole blood sampling of some volume (from 30 to 400 ml) then its separation on plasma on cells is carried out repeatedly.

Plasmapheresis is a complex procedure that requires highly qualified doctors. Thus, despite the fact that the history of the blood service of Ukraine dates back to 1918-1919 [9-10], the first plasmapheresis was performed only in the 1970s. It should be noted that in some clinical cases, plasmapheresis in combination therapy gives much better effect than conventional therapy [11]. Also, therapeutic plasmapheresis is often used to promote donor plasmapheresis as a useful procedure [12-20].

THE AIM

The aim of the work is to analyze the medical and economic aspects of the manual and different types of automatic plasmapheresis (manual, automatic centrifugal, automatic membrane, plasmapheresis with plasma therapy and mixed) used for therapeutic purposes.

To achieve this goal it is necessary to perform the following tasks:

1. To establish the difference between the influence of different plasmapheresis technologies on hematological blood parameters.
2. To highlight the negative impact of the studied technologies on the following indicators: hemolysis, the presence of cell fragments, discomfort and citrate reactions during the collection of 800 ml of plasma.
3. To establish the most effective technology for therapeutic plasmapheresis among the studied ones.

MATERIALS AND METHODS

The Baxter Auto-C machines with mixed technology (it includes rotating membrane technology), Haemonetics PCS2 machines (using centrifugal technology, blood separates in the bell during its rotation), Haemophenics machines (using filters «Rosa», separation occurs in them) for the study) were used. The Baxter CPDA anticoagulant and saline to compensate for hypovolemia were used. Vascular access was performed with 16GA needles.

Total protein was examined by the biuret method, hemoglobin by the Sali method, total bilirubin by the colorimetric photometric method, and cell fragments by the Goryaev camera microscopy method. The patient's comfort was assessed with a 10-point scale, where 10 is a state of complete comfort, 1 – a state of complete discomfort.

Table I. Comparison of methods

Type of technology	Mixed		Centrifugal		Membrane		Manual plasmapheresis	
	before	after	before	after	before	after	before	after
Access	1 needle 16GA		1 needle 16GA		1 needle 16GA		1 needle 16GA	
Procedure time	58±5		58±4		62±10		120±30	
Plasma volume, ml	800		800		800		800	
Total protein, g/l	75±4	73±5	79±6	74±2	70±3	68±8	72±1	70±6
Haemoglobin	145±7	146±6	140±10	142±6	135±7	136±8	148±9	145±4
Native haemoglobin in plasma	Missing		Missing		Missing		Missing	
Native haemoglobin in the channel	Missing		Missing		Missing		Missing	
Bilirubin	Norm		Norm		Norm		Norm	
Cell fragments	Missing		Missing		Missing		Missing	
Hematocrit	Norm	±5%	Norm	±5%	Norm	±5%	Norm	±5%
Patient comfort	9,2±0,8		9,6±0,4		8,9±1,1		7±2	
Used ml of anticoagulant	255ml±30		250 ml ±25		355 ml ±80		290 ml ±60	
Set cost, $ *	38		20		60		20	
Other expenses**	10		10		10		30	
The cost of the procedure	48		30		70		50	

* According to data obtained from open sources.
** According to the assessment of the blood center.

Blood donors admitted to the donation as healthy (participated) in this study. This eliminated the effects of pathological conditions in the result.

In total, manual plasmapheresis was performed in 31 persons, including 15 women and 16 men. Automatic plasmapheresis with centrifugal technology was performed in 36 persons, including 16 women and 20 men. Automatic plasmapheresis with membrane technology was performed in 21 persons, including 11 women and 10 men. Automatic plasmapheresis by mixed technology was performed in 36 person, including 18 women and 18 men.

RESULTS AND DISCUSSION

According to a survey in patients with plasmapheresis on a Baxter device, the level of comfort during the procedure according to a 10-point scale was 9.2 ± 0.8. We used anticoagulant CPD in an amount of 255 ml ± 30 ml ($p < 0,05$). At the same time the possibility of carrying out procedure without use of citrates at their replacement by heparin, in the corresponding dose for ensuring decrease in level of blood coagulation within to 1 hour remains. The cost of consumables was $ 38, and other costs were $ 10.

When performing the procedure on a Haemonetic device, according to a survey of patients, the level of comfort during the procedure according to a 10-point scale was 9.6 ± 0.4. We used anticoagulant CPD in an amount of 250 ml ± 25 ml ($p < 0,05$). At the same time, it is possible to perform the procedure without the use of citrates and replace them with heparin in the appropriate dose to reduce blood clotting for up to 1 hour. The cost of consumables was $ 20, and other costs were $ 10.

During using the Heamophenix device, the level of comfort during the procedure according to a 10-point scale was 8.9 ± 1.1. We used anticoagulant SPD in the amount of 285 ml ± 80 ml ($p < 0,05$). At the same time, it is possible to perform the procedure without the use of citrates and replace them with heparin in the appropriate dose to reduce the level of blood clotting for up to 1 hour. The cost of consumables was $ 60, and other costs were $ 10.

During manual plasmapheresis the level of comfort at procedure on a 10-point scale made 7 ± 2. We used anticoagulant GPD in the amount of 290 ml ± 60 ml ($p < 0,05$). At the same time, it is possible to perform the procedure without the use of citrates and replace them with heparin in the appropriate dose to reduce the level of blood clotting for up to 1 hour. The cost of consumables was $ 20, and other costs were $ 30.

As a result of comparing the effect of plasmapheresis on hematological parameters, no significant changes were shown, that indicates the same impact of this procedure on the body, regardless of the method by which it was performed (Table I).

Thus, the reduction of total protein occurs in all types of procedures without significant difference. In the analysis of the possible negative impact of different types of plasmapheresis, no significant differences were found. Thus, in no case did plasmapheresis lead to hemolysis, the presence of cell fragments that could be found in the methods we used.

Patient comfort is slightly reduced in manual plasmapheresis due to increased procedure time and accentuation of the patient, long cycles of collection and return, as well as significantly longer procedure time, according to the method associated with multiple blood sampling of 400-450 ml. To obtain plasma, this method requires from 4 to 6 cycles, which also include the preparation of containers with blood and its centrifugation. The use of high-cost centrifuges leads to increasing of depreciation costs and a significant increase in time of the procedure leads to a significant levels of cost of this procedure. In addition, this is indicated by the need for each cycle to use a new cost set and the involvement at this time of both nurses and doctors. Thus, the considerable time of the procedure with manual method, which also leads to an increase in its cost, the need to use special centrifuges that have special requirements for installation, make this method not competitive with automatic plasmapheresis. However, the greatest value in this case is the cost of the procedure, as shown by its effectiveness, there are no significant differences depending on the type of technique.

It should be noted that the procedure on a plasma therapy machine is very expensive due to the price, consumables, that are designed for more complex and technological procedures: hemofiltration, immunosorption, hemodialysis and others.

The cost of sets for machines such as Haemophenix is quite high due to the use of filtration technology, which according to some data is less traumatic to blood cells, but we did not find this effect in the study. Such filters are manufactured, using particle accelerators and other state-of-the-art technologies, but this makes them high-value.

Haemonetic machines (Scinomed iPCM, etc.) are designed primarily for donor plasmapheresis. That is why, in addition to high technology and quality, the manufacturer has another priority – the price, which is the lowest among the technologies we study. These machines are not inferior in quality and efficiency of the procedure for therapeutic purposes to other technologies. They also have European certification according to the standard of medical equipment. In the process of plasmapheresis, the patient, donating plasma, feels just as comfortable as when using other technologies. It should be noted that modern plasmapheresis machines are available in a large number of plasma centers, and can be used by doctors of medical institutions. This allows one to use these machines in a flexible mode and achieve high results in the treatment of most diseases.

Unfortunately, during the study it was not possible to compare the effect on the patient of plasma therapy machines on the studied technologies. This needs further study.

CONCLUSIONS

Based on the study, it was found that all 4 studied therapeutic plasmapheresis technologies have the same effect on the hematological parameters of the patients' blood in this study.

It was found that the study did not have a negative impact on the body by the following indicators: hemolysis, the presence of cell fragments, patient discomfort and citrate reactions during the standard procedure of sampling 800 ml of plasma in patients without predisposition to reactions.

It was found that the most perfect are plasmapheresis machines with centrifugal technology according to the result of medical and economic justification. They lead to increased efficiency by reducing the price with the same quality of procedures. It helps to improved quality of medical care. This is because efficiency is an inherent part of quality.

The results of this study can be used in clinical work when choosing a method of plasmapheresis, if the procedure on plasma therapy machines is impractical or unavailable.

REFERENCES

1. Padmanabhan A., Connelly-Smith L., Aqu N., Balogun R.A., Klingel R., Meyer E., Pham H.P., Schneiderman J., Witt V., Wu Y., Zantek N.D., Dunbar N.M., Schwartz G.E.J. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice – Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Eighth Special Issue. J Clin Apher. 2019;34(3):171-354.
2. Williams M.E., Balogun R.A. Principles of separation: indicators and therapeutic targets of plasma exchange. Clin J Am Soc Nephrol. 2014;9:181-90.
3. Bobati S.S., Naik K.R. Therapeutic Plasma Exchange – An Emerging Treatment Modality in Patients with Neurologic and Non-Neurologic Diseases. Journal of Clinical and Diagnostic Research. 2017;11(8):EC35-EC37.
4. Balagholi S., Dabbaghi R., Mohammadi S. et al. Potential of therapeutic plasmapheresis in treatment of COVID-19 patients: Immunopathogenesis and coagulopathy. Transfus Apher Sci. 2020;59(6):102993.
5. Ataca P., Adsiz O., Ayyildiz E. et al. Therapeutic plasmapheresis in geriatric patients: Favorable results. Transfusion and Apheresis Science. 2014;51(3):64-67.
6. Liubchak V.P., Zahrebelna A.O., Liubchak V.V. Pidvyshchennya yakosti propozytsiyi transfuziolohichnoyi dopomohy pid chas vykorystannya novykh metodyk provedennya plazmaferezu pry psoriazi [Improving the quality of transfusion care when using new methods of plasmapheresis for psoriasis]. Likarska sprava. 2018;5-6:156-162. (In Ukrainian).
7. Liubchak V.V. Vyrobnytstvo preparativ plazmy krovi [Production of blood plasma preparations]. In: Liubchak V.V., Liubchak V.P., Tymchenko A.S. et al. Vyrobnycha transfuziolohiya [Industrial transfusiology]. Sumy: SumDU; 2017, p.246-250. (In Ukrainian).
8. Khomenko L.M., Saher L.Y, Polcyn Y. Analysis of the marketing activities in the blood service: bibliometric analysis. Health Economics and Management Review. 2020;1(1):20-36.
9. Liubchak V.V. Istoriya sluzhby krovi Ukrayiny [History of the blood service of Ukraine]. In: Liubchak V.V., Liubchak V.P., Tymchenko A.S. et al. Istoriya sluzhby krovi [History of blood service]. Sumy: SumDU; 2020, p. 20-23. (In Ukrainian).
10. Liubchak V.V., Plaksa V.M., Maligon O.I. et al. Zhurnal «Likarska sprava»: u dzherel 100-richnoi istorii ukrainskoi medychnoi publitsystyky [Journal "Medical business": the sources of 100-year history of Ukrainian medical journalism]. Likarska sprava. 2018;7-8(26):164-169. DOI: 10.31640/JVD.7-8.2018(26). (In Ukrainian).

11. Lyubchak V.V., Plaksa V.M., Pelo I.P. et al. Therapeutic Plasmapheresis in a Complex Treatment of Patients with Chronic Hepatitis. Wiad Lek. 2020;7(73):1454-1458.
12. Khomenko L.M, Saher L.Yu. & Lyubchak V.V. Analiz reklamy sluzhby krovi v drukovanykh ZMI (na prykladi Sumskoho oblasnoho tsentru sluzhby krovi) [Analysis of blood supply service advertisements in print media (on the example of Sumy regional blood supply service center)]. Galician economic journal. 2020;66(5):170-179. (In Ukrainian).
13. Letunovska N.Ye, Internet marketing. In: Letunovska N.Ye, Khomenko L.M., Liulov O.V. Marketynh u tsyfrovomu seredovyshchi [Marketing in the digital environment]. Sumy: SumDU; 2021, p. 130-162. (In Ukrainian).
14. Aravindakshan A., Rubel O., Rutz O. Managing blood donations with marketing. Marketing Science. 2015;34(2):269- 280.
15. Barboza S. I. S., Costa F. J. D. Social marketing of blood donation: an analysis of new donors' predisposition. Cadernos de Saúde Pública. 2014;30(7):1463-1474.
16. Carver A., Chell K., Davison T. E. et al. What motivates men to donate blood? A systematic review of the evidence. Vox sanguinis. 2018;113(3):205-219.
17. Chell K., Davison T. E., Masser B. et al. A systematic review of incentives in blood donation. Transfusion. 2018;58(1):242-254.
18. Kranenburg F. J., Kreuger A. L., Arbous M. S. et al. The effect of World Blood Donor Day on digital information seeking and donor recruitment. Transfusion. 2017;57(10):2458-2462.
19. Lucena T.F.R., Negri L.Q., Marcon D. et al. Is WhatsApp Effective at Increasing the Return Rate of Blood Donors? Telemedicine and e-Health. 2019;3(26):304-309.
20. Martín-Santana J.D., Reinares-Lara E., Reinares-Lara P. Using radio advertising to promote blood donation. Journal of Nonprofit & Public Sector Marketing. 2018;30(1):52-73.

ORCID and contributionship:
Vladislav V. Liubchak: 0000-0003-0352-4355 [A,B,D,F]
Liliia M. Khomenko: 0000-0001-5690-1105 [A,C,E,F]
Michael P. Kovalishyn: 0000-0002-7120-0304 [B,D]
Viktoriia V. Ilyina: 0000-0002-3972-4327 [B,D]
Vladislav A. Smiianov: 0000-0002-4240-5968 [E,F]
Volodymyr V. Sikora: 0000-0003-4021-2334 [B,E]
Tetiana V. Babar: 0000-0002-5889-2429 [B,E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Vladislav V. Liubchak
Medical Institute, Sumy State University
1 Sanatorna str, 40018 Sumy, Ukraine
tel: +380958000333
e-mal: ur3abm@i.ua

Received: 12.05.2021
Accepted: 27.09.2021

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

COVID-19 AS A STRESS TEST OF HEALTHCARE ESTABLISHMENTS EFFECTIVENESS AND RELIABILITY MEASURED NATIONALLY AND GLOBALLY

DOI: 10.36740/WLek202110120

Oleksandr P. Yavorovskyi[1], Oleksandr M. Naumenko[1], Yurii M. Skaletsky[1,2], Roman P. Brukhno[1], Mykhailo M. Rigan[3], Sergii T. Omelchuk[1], Taisiya V. Loboda[4], Oleksandr V. Ivan'ko[5], Tetyana O. Zinchenko[1]

[1]BOGOMOLETS NATIONAL MEDICAL UNIVERSITY, KYIV, UKRAINE

[2]SI «O.M. MARZEIEV INSTITUTE FOR PUBLIC HEALTH OF THE NAMS OF UKRAINE», KYIV, UKRAINE

[3]NATIONAL UNIVERSITY OF UKRAINE ON PHYSICAL EDUCATION AND SPORT, KYIV, UKRAINE

[4]KNP «KYIV CITY CLINICAL HOSPITAL №12 OF THE EXECUTIVE BODY OF THE KYIV CITY COUNCIL (KYIV CITY STATE ADMINISTRATION)», KYIV, UKRAINE

[5]KNP «KYIV CITY CLINICAL HOSPITAL №1 OF THE EXECUTIVE BODY OF THE KYIV CITY COUNCIL (KYIV CITY STATE ADMINISTRATION)», KYIV, UKRAINE

ABSTRACT

The aim: The authors aimed to estimate the healthcare environment risks and safety problems of the medical staff and patients, methods of neutralizing the negative public health effects and to suggest the new approaches to improved effectiveness and reliability of the healthcare establishments functioning under the emergencies.

Materials and methods: The study includes data of questioning of 163 healthcare workers of certain institutions in Ukraine using the questionnaire of the Agency for Healthcare Research and Quality (the USA) on adherence to the patients' safety culture. In this study only the data on the patients' safety culture "response to mistakes" are represented. The more positive answers the respondents gave, the less they are aware that their mistakes and reports do not influence them negatively.

Results: Hospital environment reprsents a complex multi-component system, in which specific medical and social tasks are executed, with their fulfillment accompanied with hazardous and unsafe biological, psycho-physiological, chemical, physical and social effects on the staff, patients and the environment. The joined effect of the hospital environment negative factors on the staff is stipulated for the stress and functional tiredness accumulation; it leads to increase in medical mistakes occurrence, which, in its turn, increases probability of occupational catching COVID-19, thus, raising the hospital environment risks under the COVID-19 circumstances both for the medical staff and the patients.

Conclusions: The COVID-19 pandemics turned to be a helpful factor to define critical issues in the hospital environment safety, proving the necessity of further studies, aimed at transforming the safe hospital environment notion from its theoretical meaning into the working paradigm, minimizing practical risk in hospital establishments.

KEY WORDS: COVID-19, hospital environment safety, safety culture, hospital environment risks

Wiad Lek. 2021;74(10 p.I):2471-2476

INTRODUCTION

Healthcare institutions, in which human lives and health are saved, are not as safe as they may seem. Healthcare branch is one of the most hazardous in the USA by non-lethal opportunistic injury rate [1]. The healthcare workers life duration due to dangerous, hard and stressful working conditions is on average shorter than that of other citizens [2-3]. It is only in single economically developed countries that the medical staff average life duration is the same as that of the population [4].

Each tenth patient in the in-patient department has been accidentally endangered during his medical servicing. Due to the medical staff mistakes and other preventable incidents, two million six hundred thousand patients die annually in countries with low and average income level. Among the other factors, fear of reporting mistakes in hospital institutions as a consequence of unfair depressive culture inhibitt the progress and training aimed at staff actualization and prevention of mistakes [5].

Risks, severity and stressfulness of the healthcare work increases abruptly under the conditions of medical and biological emergencies. Medical workers were the first to face the COVID-19 pandemics danger [6]. In case of infectious epidemics not only the staff of certain medical departments, but of whole hospitals may be affected, which brings considerable difficulties in providing healthcare to the local societies [7-8].

These tragedies are represented not only with the human, financial and other losses, but as the turning points in certain branches progress as well as human progress at all. For example, analysis of the atomic nuclear power station "Three-mile island"(the USA) 1979 accident causes led to introduction of such important safety principle as the human factor, and the unbiased analysis of the 1986 Chornobyl atomic nuclear power station provided humanity with the global safety principle – safety culture. Nowadays these principles underlie the safety basis not only in the nuclear sphere [9], but almost in all human activity spheres.

The stress-tests, developed and held in the atomic power stations after the "Fucushima-1" accident, resulted in positive consequences for the power stations safety, including the Ukrainian ones [10].

On the other hand, evidences show that informational silence and superficial approach to the accident results in the new similar ones [11].

Nowadays, under the COVID-19 pandemics, despite tremendous human and economic losses, caused by it, the priority attention is paid to the re-interpretation of the political directions aimed at prevention of similar pandemics in the future [12].

The COVID-19 pandemics should be regarded as a stress-test for the global healthcare and humanity, concentrating not on the missed opportunities, but the new impulses [13]. The consequence of such position among the others should be new approaches to the hospital environment safety.

THE AIM

The authors aim to estimate the healthcare safety problems manifested during the COVID-19 pandemics and to suggest new approaches to improved safety and reliability of the healthcare institutions.

MATERIALS AND METHODS

The study includes data of questioning of 163 healthcare workers of certain institutions in Ukraine using the questionnaire of the Agency for Healthcare Research and Quality (the USA) on adherence to the patients' safety culture [14]. In this study only the data on the patients' safety culture "response to mistakes" are represented. The more positive answers the respondents gave, the less they are aware that their mistakes and reports do not influence them negatively.

The authors have reviewed and analyzed Ukrainian standard legislative documents on the healthcare service provision and control, as well as the papers of Ukrainian and foreign authors dedicated to the patients' safety. The annual reports on the healthcare in Ukraine by State Institution "Center of Medical Statistics of Ministry of Health of Ukraine" and the statistical reports of the State Statistical Service of Ukraine, dedicated to occupational injury rates, have been analyzed; as well as the WHO data and operational information of the State Service of Labour Protection.

The authors used the following methods: bibliosemantic, questionnaire, hygienical, statistical and mathematical methods.

RESULTS AND DISCUSSION

Even in 2019, under the COVID-19 pandemics emergence (fig.1), the occupational injury rate in Ukrainian healthcare and Social service branch was rising. Compared to 2019, when 286 official employees were injured performing their professional duties, the value increased in 2020 by more than 10 times, reaching 3288 people. The same changes are observed with the mortal injury rate: from 7 people in 2019 to 79 people in 2020. Here the rise occurs mostly due to increase in the healthcare branch victims, where the mentioned values in 2020 made up 3238 and 68 people respectively.

The hazardous medical staff labour conditions reflected negatively on their professional performance. Figure 2 demonstrates not only increase in the in-patient adult mortality rate (from 1.72% to 2.90%), which turned out to be the most susceptible to the SARS-CoV-2. Similar dramatic increase trends in 2020 compared to 2019 were characteristic for the general children in-patient mortality rate (from 0.18% to 0.29%) and mortality rate of the children under 1 year (from 0.93% to 1,28%). Postoperative mortality in Ukrainian in-patient departments has also significantly increased (from 0.58% to 0.75 %).

This generally confirms the WHO statement that one cannot provide for the patients' safety without safe medical personnel labour conditions.

This proves that the COVID-19 pandemic has become a peculiar stress-test, which detected problems in the healthcare safety branch, the problems requiring for the thorough studies with further elimination of the problems causes.

Nowadays, no accurate statistical data on the 2020 occupational injury rate in various branches of Ukraine, including the healthcare, are available. As for the previous years (2006-2018), the prevailing were accidents due to poor organization and psychophysiological causes, i.e. human factor (Fig.3).

All the described above calls for attention paid to the organization culture and safety culture in Ukrainian healthcare establishments as effective instruments of the safety-centered human values mobilization. Unfortunately, the country experiences difficulties with safety culture in the healthcare branch.

The results of questioning on the safety culture adherence in Ukrainian healthcare institutions regarding the "Response to mistakes" are provided as follows (Fig. 4): the Ukrainian medical staff, like their colleagues in the other countries of the CIS [15] and the USA [16], gave less than 50% positive answers regarding the characteristics "Response to mistakes", with this parameter characterizing weakness of the safety culture. The only exception is the medical personnel of Sweden healthcare institutions [17], as for the respondents the "Response to mistakes" was their strong safety culture side. In Ukraine the value was only 30%.

I.e., the majority of medical staff, particularly in Ukraine, is afraid of disclosing mistakes and faulty actions due to possible, sometimes unjustified disciplinary punishment and negative effect of such information on the career ladder growth.

This requires for special research.

At the same time, high values of positive answers regarding the characteristic "Response to mistakes" obtained from the Ukrainian atomic nuclear power stations staff [15-16] is

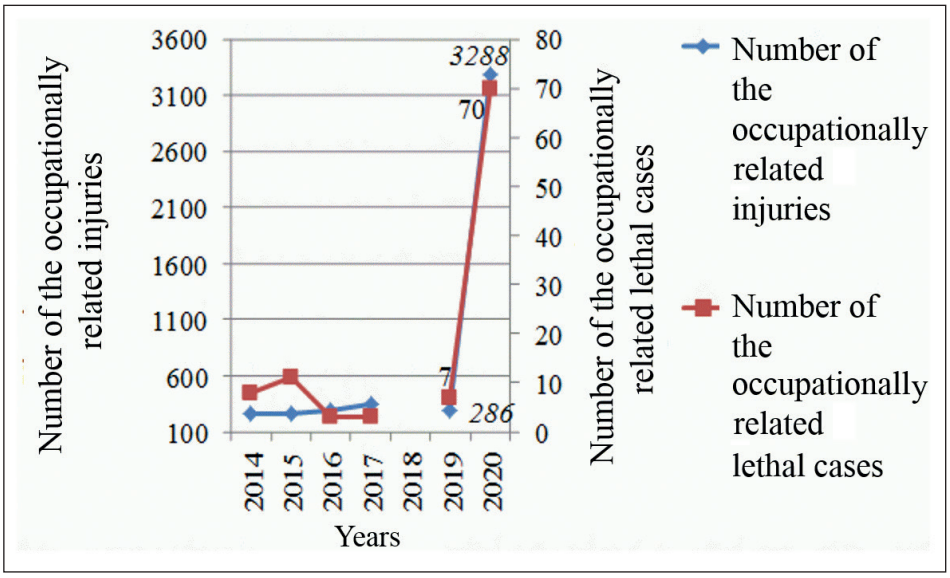


Fig. 1. Changes in healthcare and social services occupational injury rate (Ukraine, 2014 – 2020).

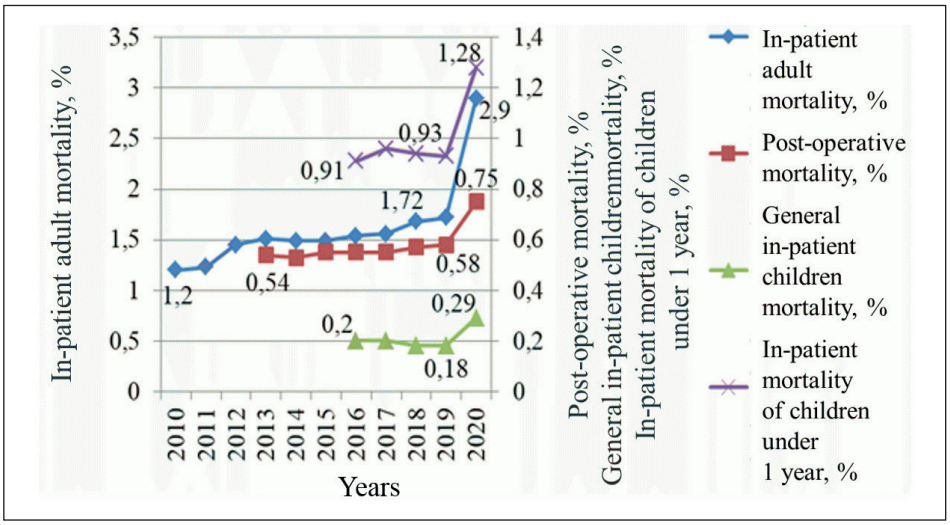


Fig. 2. Changes in the values of in-patient and post-operative adult and children mortality in healthcare institutions of Ukraine.

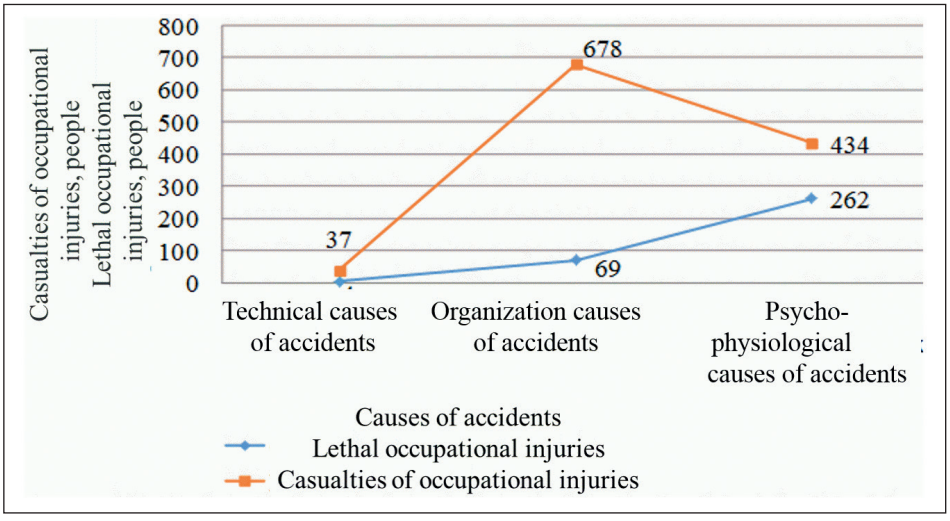


Fig. 3. Relation of the occupation injury rate to their causes in Ukrainian healthcare during the 2006-2018 period.

encouraging the thought that even in the Ukrainian healthcare system it is possible to shape appropriate attitude to the patients and medical staff safety. There are convincing evidences [18-19] that an appropriate safety culture in a medical establishment cannot be shaped if various contingent layers (patients, staff) safety is provided via different programs.

Another healthcare safety problem, observed both in Ukraine and in the world, is occupational injury rate, in-

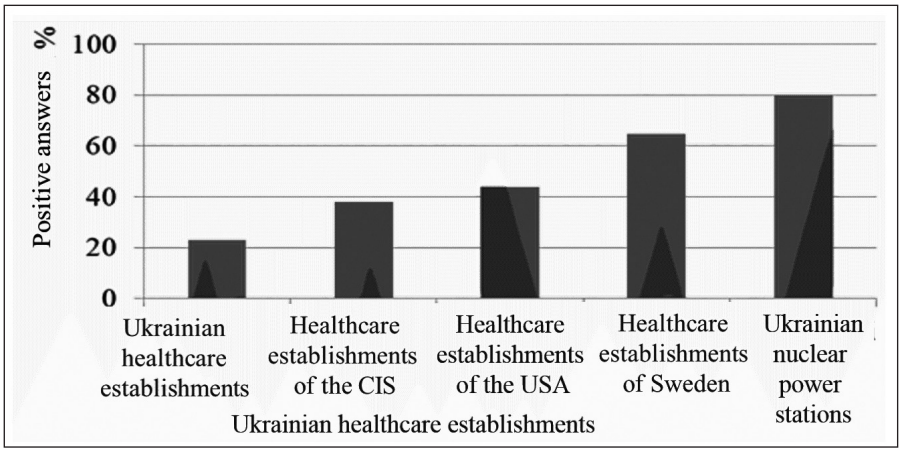


Fig. 4. Share of positive answers regarding the "response to mistakes" obtained from the Ukrainian and foreign healthcare establishments staff as well as the Ukrainian atomic nuclear power station personnel.

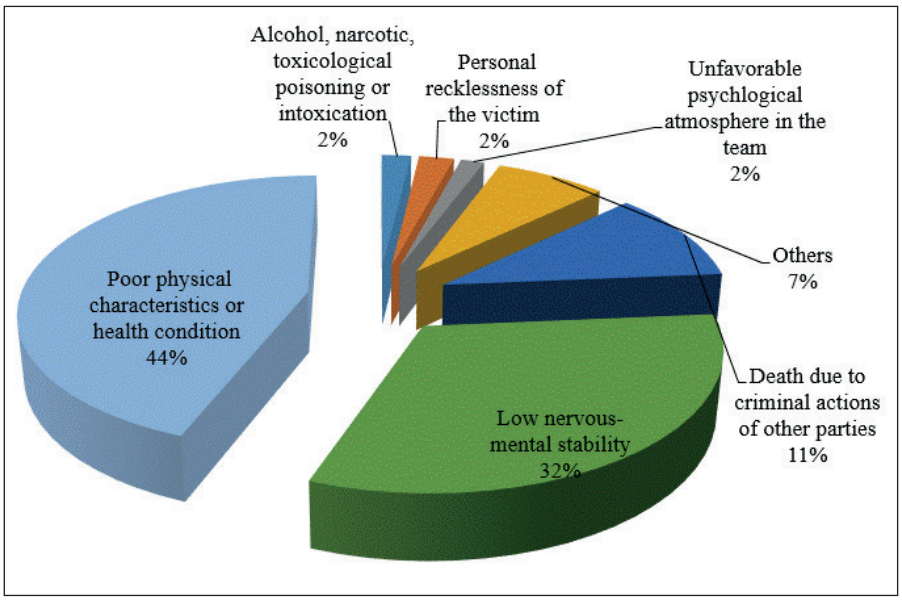


Fig. 5. Relation of occupational injury rate values by their causes in Ukrainian healthcare from 2006 till 2018.

cluding the lethal one, due to criminal actions of the third parties. In 2011 in Ukrainian healthcare establishments 10 medical workers were injured, which included 6 lethal cases. Generally, occupational injury rate due to criminal actions of the third parties takes up the third place among the psycho-physiologically-caused occupational injuries (Fig. 5). This problem has gained extreme importance under the COVID-19 pandemics [20]. Physical protection is another relevant issue, regarding high risk sources situated in hospitals (ionizing radiation sources, highly-toxic substances, strong narcotic medications, etc.).

During the pandemics, due to increase in medical wastes disposal, primarily disposable individual protection facilities, the healthcare establishments environmental problems have also exacerbated [21].

So, due to the conducted authors' research and literature review, we have established that safety issues in the healthcare establishments refer to at least five basic aspects:

1. Patients' safety;
2. Medical staff labour safety and hygiene;
3. Protection of the healthcare establishments risk sources, material assets, staff, patients under everyday conditions;
4. Healthcare establishments resilience to accidents in emergencies;
5. Environmental safety of healthcare establishments.

The authors suppose that the most significant factors of safe hospital environment, joining all five healthcare establishments' safety aspects and providing for the favorable conditions implementation, are developing and preserving high level of the organization culture and its derivative, expressed as the safety culture in healthcare establishments.

Considering all the above-mentioned, safe hospital environment could be defined as the environment of highly organized culture, providing for the safety of the staff, patients, visitors and the surrounding environment under usual circumstances and emergencies, at the acceptable risk level.

The authors' schematic vision of the safe hospital environment is shown in picture 6.

The development of such safe hospital environment is possible only under the common safety program implementation, the program including all components of the hospital environment safety. Such integrated approach would provide for the appropriate safety culture development.

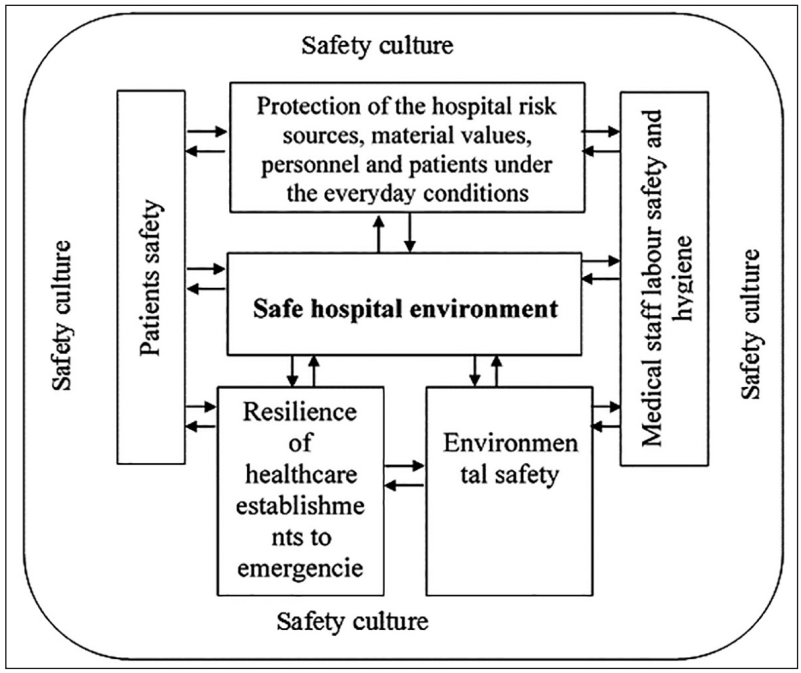


Fig. 6. Scheme of safe hospital environment – components and relation.

Though, transforming the notion "safe hospital environment" into an active instrument of healthcare institutions increased safety requires for more reasoned studies.

CONCLUSIONS

1. The COVID-19 pandemic has acted as a peculiar stress-test for identifying the problems of the hospitals safety and their interdependence.
2. A direct dependence between the occupational risks caused by COVID-19, medical staff values and patients safety values has been registered. The study has found low adherence of the healthcare establishments personnel to the patients' safety issues.
3. Patients' safety, medical staff labour safety and hygiene, medical staff resilience to emergencies and accidents, protection of the healthcare establishments risk sources, material values and patients under everyday conditions, as well as the environmental safety represent the hospital environment safety components.
4. The authors state that the safe hospital environment is the environment of highly organized culture, providing for the safety of the staff, patients, visitors and surrounding environment under the everyday conditions and in emergencies on the acceptable risk level.
5. The most significant factors of the safe hospital environment, joining all aspects of the healthcare establishments, and providing for favorable conditions of their implementation, are: shaping and preserving highly organizated culture level as well as its derivative –safety culture in healthcare establishment.
6. The notion «safe hospital environment» should be present not only in the theoretical guidelines, but realized on practice, through the hospital safety measures, which requires for more substantial study, thus, serving active instrument of safe hospital environment.
7. The values which were studied by the authors may serve as indicators (stress- markers) used for assessment of the personnel and patients safety in the healthcare establishment.

REFERENCES

1. Employer-reported workplace injuries and illnesses – 2018. Bureau of Labor Statistics, U.S. Department of Labor, Survey of Occupational Injuries and Illnesses, in cooperation with participating state agencies. 2019. URL : https://www.bls.gov/news.release/pdf/osh.pdf (Mode of Access : 08.07.2021).
2. Frans Van Poppel, Govert Bijwaard, Mart Van Lieburg, Fred Van Lieburg, et al. The Life Expectancy of Medical Professionals in the Netherlands, Sixteenth to Twentieth Centuries. Population. 2016;71(4):619-40. URL: https://www.cairn-int.info/article-E_POPU_1604_0659--the-life-expectancy-of-medical.htm (Mode of Access : 08.07.2021).
3. Pandey Suresh. Doctor heal thyself. Addressing the shorter life expectancy of doctors in India. Indian Journal of Ophthalmology. 2019;67(7):1248-50.
4. Adam B. Brayne, Ralph P. Brayne, Alexander J. Fowler. Medical specialties and life expectancy: An analysis of doctors' obituaries 1997–2019. Lifestyle Medicine. 2021;2.e23. doi:10.1002/lim2.23
5. Patient Safety Fact File. Geneva: WHO. 2019. URL: https://www.who.int/features/factfiles/patient_safety/patient-safety-fact-file.pdf?ua=1 (Mode of Access : 08.07.2021).
6. Yavorovskyj O.P., Shkurba A.V., Skaletskyi Yu.M. et al. Hihiiena ta okhorona pratsi medychnoho personalu v umovakh podolannia pandemii COVID-19 (pershe povidomlennia) [Hygiene and labor protection of medical personnel in the context of overcoming the COVID-19 pandemic (first report)]. Dovkillya ta zdorov'ya. 2020;3(96):10-18. URL: http://ir.librarynmu.com/handle/123456789/2117 (Mode of Access : 08.07.2021). (in Ukrainian).
7. Summary Table of SARS Cases by Country. WHO. 1 November 2002 – 7 August 2003. URL: https://www.who.int/csr/sars/country/2003_08_15/en/ (Mode of Access : 08.07.2021).
8. Health Worker Ebola Infections in Guinea, Liberia and Sierra Leone – a Preliminary Report. Geneva. WHO. 2015 URL: https://www.who.int/hrh/documents/21may2015_web_final.pdf (Mode of Access : 08.07.2021).

9. Basic Safety Principles for Nuclear Power Plants 75-INSAG-3 Rev. 1 A Report by the International Nuclear Safety Advisory Group. Vienna. 2015. URL: https://www-pub.iaea.org/MTCD/Publications/PDF/P082_scr.pdf (Mode of Access: 08.07.2021).
10. Gromov G.V., Dovbyich A.M., Inyushev V.V. et al. Rezultatyi ekspertnoy otsenki stress-testov deystvuyuschih energoblokov AES Ukrainyi s uchetom urokov avarii na AES «Fukusima-1» v Yaponii. [The results of an expert assessment of stress tests of operating power units of Ukrainian NPPs, taking into account the lessons of the accident at the Fukushima-1 NPP in Japan]. Yaderna ta radiatsiina bezpeka. 2012;1 (53):3-9. (In Russian).
11. Osipenko L., Zhiltsov L., Mormul N. Atomnaia podvodnaia epopeia. Podvyhy, neudachy, katastrofu [Atomic underwater epic. Feats, failures, catastrophes]. «Borhes». Moskva. 1994. (In Russian).
12. A call to action: national governments and the global community must act now. Pan-European Commission on Health and Sustainable Development. 2021. URL: https://www.euro.who.int/en/health-topics/health-policy/european-programme-of-work/pan-european-commission-on-health-and-sustainable-development/rethinking-policy-priorities-in-the-light-of-pandemics-a-call-to-action (Mode of Access: 08.07.2021).
13. Achieving the SDGs in the COVID-19 Pandemic: The Role of Governments and Supreme Audit Institutions. Department of International and Regional Cooperation.2020. URL: https://ach.gov.ru/upload/pdf/Covid-19-SDG.PDF (Mode of Access: 08.07.2021).
14. Hospital Survey on Patient Safety Culture: 2018 User Database Report. Agency for Healthcare Research and Quality U.S. AHRQ Publication №. 18-0025-EF. URL : https://www.ahrq.gov/sites/default/files/wysiwyg/sops/quality-patient-safety/patientsafetyculture/2018hospitalsopsreport.pdf . (Mode of Access: 08.07.2021).
15. Trifonova E.Yu., Kamynina N.N. kulytura bezopasnoy bolnichnoy sredy [Culture of a safe hospital environment]. Mezhdunarodnyiy studencheskiy nauchnyiy vestnik. 2015;5(1):128-134. URL: https://eduherald.ru/ru/article/view?id=12860 (Mode of Access: 08.07.2021). (In Russian).
16. Horbulin V.P. Skaletskyi Yu.M., Kondratov S.I. et al. Kultura bezpeky na yadernykh obiektakh Ukrainy. Naukovo-metodolohichnyi posibnyk [Safety culture at nuclear facilities in Ukraine. Scientific and methodological manual]. DP «NVTs» «Evroatlantykinform». Kiev. 2007. (In Ukrainian).
17. Nordin Anna, Wilde-Larsson Bodil, Nordström Gun, Theander Kersti. Swedish Hospital Survey on Patient Safety Culture – Psychometric properties and health care staff's perception. Open Journal of Nursing, 2013;3.41-50. URL : http://www.scirp.org/journal/ojn/. (Mode of Access: 08.07.2021).
18. Hospital Survey on Patient Safety Culture. AHRQ Publication № 04-0041. 2004. URL:https://proqualis.net/sites/proqualis.net/files/User%20guide%20HSOPSC.pdf (Mode of Access: 08.07.2021).
19. Global action on patient safety. Report by the Director-General. Executive board 148th session. 2021. URL: https://apps.who.int/gb/ebwha/pdf_files/EB148/B148_6-en.pdf (Mode of Access: 08.07.2021).
20. Attacks on health care in the context of COVID-19. WHO. 2020. URL: https://www.who.int/news-room/feature-stories/detail/attacks-on-health-care-in-the-context-of-covid-19 (Mode of Access: 08.07.2021).
21. Water, sanitation, hygiene, and waste management for the COVID-19 virus. Interim guidance. WHO. UNICEF. 2020. URL: https://apps.who.int/iris/bitstream/handle/10665/331846/WHO-2019-nCoV-IPC_WASH-2020.3-eng.pdf?sequence=1&isAllowed=y (Mode of Access: 08.07.2021).

The study was held within the Research Work «Scientific justification of the optimum risk management system used for provision of safe hospital environment», performed by the O.O.Bogomolets National medical university Department of Hygiene and Ecology №2 (state registration number: 0120U101432).

ORCID and contributionship:
Oleksandr P. Yavorovskyi: 0000-0002-4573-8039 [A,B,F]
Oleksandr M. Naumenko: 0000-0002-9001-7580 [A,E]
Yurii M. Skaletsky: 0000-0002-2263-953X [B,C,F]
Roman P. Brukhno: 0000-0002-6666-397X [B,D,F]
Mykhailo M. Rigan: 0000-0003-1244-9801 [B,E]
Sergii T. Omelchuk: 0000-0003-3678-4241 [B,F]
Taisiya V. Loboda: 0000-0001-9926-9449 [A,B]
Oleksandr V. Ivan'ko: 0000-0002-7015-7340 [A,B]
Tetyana O. Zinchenko: 0000-0002-6666-397X [B,F]

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

CORRESPONDING AUTHOR
Roman P. Brukhno
Bogomolets National Medical University, Kyiv, Ukraine
e-mail: bruhnoroman@ukr.net

Received: 22.05.2021
Accepted: 20.09.2021

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

HEALTHY LIFESTYLE PRINCIPLES FORMATION OF CHILDREN AGED 6-7

DOI: 10.36740/WLek202110121

Tetiana I. Pantiuk[1], Mykola P. Pantiuk[1], Olena V. Kvas[2], Nadiya O. Fedchyshyn[3], Olha V. Romanchuk[4], Mariana I. Klym[1], Lesia V. Perkhun[1]

[1]DROHOBYCH IVAN FRANKO STATE PEDAGOGICAL UNIVERSITY, DROHOBYCH, UKRAINE
[2]LVIV IVAN FRANKO NATIONAL UNIVERSITY, LVIV, UKRAINE
[3]TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE
[4]LVIV IVAN BOBERSKIY STATE UNIVERSITY OF PHYSICAL CULTURE, LVIV, UKRAINE

ABSTRACT

The aim of the article is to determine pedagogical conditions of healthy lifestyle principles formation of children aged 6–7.

Materials and methods: Three groups of methods have been used in the research – theoretical – content analysis of scientific literature and modeling; empirical – surveys, observations, questionnaires and pedagogical experiment; statistical – mathematical processing, quantitative and qualitative analysis.

Results: The definition of notion «health» has been analyzed. Attention is focused on the interdisciplinary aspect of the problem. We have conducted pedagogical experiment wcich involved 145 primary school studets (aged 6–7) of Lviv specialized school № 8 (Ukraine) in order to find ways to form the healthy lifestyle principles in general secondary educational institutions. We have identified the levels of formation of healthy lifestyle of primary school children (aged 6 – 7) as high, medium and low.

Conclusions: The organizational and pedagogical conditions for the formation of the healthy lifestyle principles of children aged 6–7during curriculum and extracurriculum activities are: axiological approach application to the formation of healthy lifestyle principles; strengthening positive attitude of primary school children (aged 6–7) toward the healthy lifestyle principles formation; organizational, methodological and pedagogical support of the healthy lifestyle principles of primary school students. It has been found that the implementation of the proposed tools contributes to better understanding of healthy lifestyle importance and develop means to be fit and healthy.

KEY WORDS: health, child's health, children, aged 6–7, health components, pedagogical tools

Wiad Lek. 2021;74(10 p.I):2477-2481

INTRODUCTION

The issue of public health is certainly a priority for every civilized society. These questions are regarded as problems of the national level and efforts of many scientists are directed to their solution. Medicine, valeology, physiology, psychology, philosophy, pedagogy are the fields engaged into the process of healthy lifestyle habits formation. In the modern world human health is determined by a number of global factors and challenges that require balanced, coordinated scientific approaches [1; 2].

Recently lifestyle as an important aspect of health care is paid more attention by researchers [3]. According to WHO, 60% of related factors to individual health and quality of life are correlated to lifestyle [4]. Unfortunally millions of people conduct an unhealthy lifestyle. Hence, they have higher risks of illness, disability and even death. Problems like metabolic diseases, joint and skeletal problems, cardio-vascular diseases, hypertension, overweight, violence and so on, can be caused by an unhealthy lifestyle. The relationship of lifestyle and health should be highly considered [5].

According to Chung J., Chung L., Chen B. [6] the physical fitness levels of school children depends on two main factors contributing to health: genes and lifystyle. Lifestyle could possibly be a key factor and predictor of physical fitness, providing strong evidence to support the interaction between lifestyle and genes in their impact on our health.

The modern human being disregard the laws of nature, natural development of a personality, violation of harmony in the «people-nature» interaction, the unpredictable scientific development often have put civilization on the survival brink. Global warming, air pollution, man-caused disasters, wide spread of new diseases (including COVID-19) and other human issues are prompting the world community for recognizing human life and health as an undisputed priority. Negative trends in the human civilization's life have become threatened and catastrophic nowadays. It is espessialy important to stay physically fit and active during self-isolation caused by the COVID-19 pandemic [7; 8; 9].

The world community has a long positive experience in preserving and promoting human health worldwide. In particular, the activities of such international organization as the World Health Organization (WHO) is proof of it [10].

The notion health is defined in the Charter as «a state of complete physical, spiritual, social well-being and not merely the absence of disease or infirmity» [11]. In the WHO Charter, health is defined as a fundamental human right regardless of race, religion, political views, social and economic status for the first time in international practice [10].

The contemporary science pays special attention to the child's health. The challenges and risks of modern society indicate the urgency of this problem all over world. In many countries, including France, Canada, the United States of America, Austria, Germany, Poland [12] and others, the concept of early formation of a healthy lifestyle is beginning to be implemented in the family and is consistently developped in all levels of education. This concept aims to provide children with knowledge that would help to gain independence, teach them to take care of their physical and mental health [13].

The analysis of scientific theoretical sources on formation of children healthy lifestyle [14] proves the urgency and the necessity to solve above mentioned problem.

THE AIM

The aim of the article is to determine pedagogical conditions of healthy lifestyle principles formation of children aged 6-7.

MATERIALS AND METHODS

Three groups of methods have been used in the research – theoretical – content analysis of scientific literature and modeling; empirical – surveys, observations, questionnaires and pedagogical experiment; statistical – mathematical processing, quantitative and qualitative analysis.

RESULTS

The formation of a healthy lifestyle is usually carried out accorging to a multidisciplinary approach. It includes physiological aspects (basic knowledge on the human body, grounds of hygiene, nutrition etc.), basic knowledge on various activities, self-esteem in everyday life or in the context of specific approaches, such as campaigns to prevent smoking, drug addiction, transmission of infections [15]. The foreign and Ukrainian educators consider valeological and ecological education as one of the important tasks of schooling. The essence is seen in the preservation and strengthening health, care for others and nature [11].

Health is an integral phenomenon that combines the following components: physical, mental, social and spiritual. They are interconnected and determine the state of human health. In addition, the prerequisites for health care are important, ie the factors that determine its current state: «The main prerequisites for health include eight factors: peace, safety, social justice, education, nutrition, income, ecosystem, sustainable resources» [16].

The understanding of human health importance should be formed at the early age as an effective tool. It is regarded as specially and consciously organized human activities aimed at preventing disease, strengthening all body systems, improving well-being, maintaining a reasonable balance between work and rest, extensive usage of nature factors. Children need special attention not only in terms of strengthening and maintaining their health, but the formation of an appropriate philosophy of life, allowing to lead an environmentally friendly lifestyle; be aware of the human capabilities limits and the limits of interference in the natural environment; taking into account valeological and ecological values; formation of the ability to understand health as a holistic and complex phenomenon, covering the physical, mental, spiritual and social components. According to this issue, the formation of children healthy lifestyle habits at the early age requires close attention of researchers in pedagogy, psychology, medicine and valeology. The development of a holistic concept of child's health and the active search for effective methods and health technologies has become urgent.

To optimize the formation of healthy lifestyle principles of primary school children in Ukrainian general secondary education establishments the pedagogical expiriment has been conducted. 145 primary school students (aged 6 – 7 years old) took part at the experiment. They are first and second form students of Lviv specialized school № 8 (Lviv city, Ukraine). The analysis of the structural components of the «healthy lifestyle principles» phenomenon made it possible to determine diagnostic criteria and relevant indicators presented in the table I.

We have identified the levels of formation of healthy lifestyle of primary school children (aged 6 – 7) as high, medium and low. They have been accompanied by the appropriate characteristics based on the criteria and indicators.

The group with high level includes children with an positive attitude to a healthy lifestyle, good health and a high level of motivation to maintain personal health. They consider their health as a priority; follow the rules of personal hygiene; daily routine. They have regular motor activity, attend physical education classes.

The group with average level includes children with a rather low level of motivation for a healthy lifestyle. This category of children don't have a holistic understanding of the essence of health and a healthy lifestyle, don't have a continuous interest in learning about the health value. The health is not a priority, such children mostly follow hygienic rules, physical education lessons are sometimes missed.

The children who aren't motivated to conduct healthy lifestyle and have abnormalities in their health are classified to low level. Children have fragmentary information about maintaining health and do not understand the content of healthy lifestyle concept and its components. They have only basic skills of organizing a healthy lifestyle and use it sporadically. Children often miss physical education classes for no good reason.

Formation results of healthy lifestyle of primary school children (aged 6 – 7) are presented in the table II.

Primary school children (aged 6 – 7) questioning «How do you take care of your health?» has been aimed at iden-

Table I. Criteria and indicators of primary school children (aged 6 – 7) healthy lifestyle principles formation

Criteria	Indicators
Cognitive creteria	– proper understanding of healthy lifestyle; – unterstanding health impact factors; – positive attitude to physical education.
Motivation and values creteria	– understanding importance of health and maintaining healthylifestyle; – understanding the role of health in the hierarchy of general and personal values; – interest in researching healthy lifestyle peculiarities.
Actional creteria	– activation of skills and abilities of healthy lifestyle; – independence during health-related activies; – ability to maintain and promote health in a variety of situations.

Table II. Formation results of healthy lifestyle of primary school children (aged 6–7)

Levels	Criteria					
	Cognitive		Motivational		Actional	
	KG,%	EG, %	KG,%	EG, %	KG,%	EG, %
High	18,7	52,5	17,4	50,2	24,3	61,6
Average	35	39	34,6	36,8	43	34
Low	46,3	8,5	48	13	32,7	4,4

Table III. Total results of distribution of primary school children (aged 6 – 7) of control and experimental groups based on all criteria of healthy lifestyle principles formation

Classes	High		Medium		Low	
	initial,%	final,%	initial,%	final,%	initial,%	final,%
Experimental	21,7	54,6	39,8	37,3	38,5	8,1
Control	20,5	29,2	39,3	40,5	40,2	30,3

tifying behavioral attitudes towards their own health. We have conducted the targeted monitoring of children's energies and independence in various activities aimed at maintaining their health. It has been established that children wash their hands when necessary or when possible, the classroom is aired only on the teacher's instructions. The obtained data show that children do not follow completly the requirements of healthy lifestyle, they just follow the adult's instructions.

The research process of the healthy lifestyle principles, the peculiarities of their formation in classroom and during extracurricular activities, analysis of the observational stage results and the experiment made it possible to determine organizational and pedagogical conditions to form healthy lifestyle principles for primary school students (aged 6 – 7):

1. Axiological approach to the formation of the healthy lifestyle principles based on the values priority. The axiological approach is implemented through the assimilation of folk customs and traditions that contribute to the formation of valeological knowledge.
2. Strengthening the active position of primary school children (aged 6 – 7) to form the healthy lifestyle principles. An active personal attitude is important – awareness of the importance of knowledge about health, components of internal motivation, engaging into health-related activities, taking into account individual interests and abilities, responsibility formation.
3. Organizational, methodological and pedagogical support of the healthy lifestyle principles formation of primary school children (aged 6 – 7). Effective formation of the healthy lifestyle principles needs proper pedagogical support. Mutual understanding, trust and respect among teachers and parents are of great importance. In our opinion, the high-quality performance of primary school teachers functions includes theoretical, methodological and practical training of teachers to form healthy lifestyle principles.

It has also been used the conversation on the topic «Why do you need to be healthy without medicine?». Children have discribed medicine-free means for maintaining and enhancing their health. The analysis of the content of proverbs, sayings, fairy tales gave them information on preserving and strengthening their own health. During the art lessons teachers have used art therapy (for example «Green Forest», «Moments of color»), collage making and painting.

In order to test the effectiveness of such forms of educational activities to form healthy lifestyle principles, a control phase of the experiment has been conducted. In the process of data processing and obtained results analysis we have revealed the expected dynamics in the experimental group of primary school children (aged 6 – 7) in terms of quantitative and qualitative indicators. Generalized results of distribution of primary school children of control and

experimental groups based on the criteria to form healthy lifestyle principles are presented in the table III.

The results of the control experiment (table III) has showed a positive trend in the levels of formation of the foundations of a healthy lifestyle 6 – 7 year olds children due to the implementation of educational work on the formation of the healthy lifestyle principles.

DISCUSSION

The standard educational program for general secondary educational institutions is developed in accordance with the Law of Ukraine «On Education», the State Standard of Primary Education, the Concept of the National Program «Health 2020: Ukrainian Dimension». The conceptual strategy for the formation of a healthy lifestyle is formulated in the Law of Ukraine «Fundamentals of the legislation of Ukraine on health care». The section VII of the document «Maternal and child health» is directly related to children's health. It states that in the process of learning the Ukrainian language, literary reading and mathematics one should use material that enhances the content of these subjects with knowledge about human health, forms a number of competencies accordingly [17].

Acquaintance of primary school children with features of healthy lifestyle formation provides the further development of child's personality. It gives an understanding of significance of physical exercises, ball games, promoting healthy lifestyle, strengthening body and spirit, self-expression, social interaction in the process of physical education and health-related activities [3].

We believe that extracurricular group activities has a significant potential for the formation of a healthy lifestyle principles. We have given the preference to the methods and techniques wich have maximized children interest in healthy living. Further educational activities has been aimed at stimulating motivation for healthy lifestyle – promoting the development of value orientations of children to maintain and strengthen their health.

In order to solve the tasks, posture correction exercises, breathing exercises, health-ralated exercises have been conducted during classes with primary school children. Motor games have been the main means to form healthy lifestyle principles. It contributes to the improvement of physical indixes of the body of children.

CONCLUSIONS

Thus nowadays the world community pays special attention to the child's health. Scientists emphasize the awareness of the importance of good health for human wellbeing. The environmentally friendly support should be formed at the early age, an effective tool should be a healthy lifestyle, ie specially and consciously organized human activities to prevent diseases, strengthen body and spirit, use effectivly various natural factors.

Theoretical analysis of scientific sources on the research problem shows the importance to search the ways to optimize the formation of a healthy lifestyle foundations of primary school children in general secondary educational institutions by means of classroom and extracurricular educational activities.

Assessment of the formation of healthy lifestyle foundations of primary school children has been determined by using the following criteria: cognitive, motivational, actional ones. Based on these criteria and indicators, we have determined three levels of the formation of healthy lifestyle principles: high, medium and low. The organizational and pedagogical conditions for the formation of the healthy lifestyle principles of children aged 6 – 7during curriculum and extracurriculum activities are: axiological approach application to the formation of healthy lifestyle principles; strengthening positive attitude of primary school children (aged 6 – 7) toward the healthy lifestyle principles formation; organizational, methodological and pedagogical support of the healthy lifestyle principles of primary school students.

As the result of the research it has been found that the implementation of the proposed tools contributes to better understanding of healthy lifestyle importance and develop means to be fit and healthy. Such approach is an effective basis to improve children health, to create a holistic valeological standpoint, to realize the importance to be healthy and wellthy.

REFERENCES

1. Melnychuk, I. et al. Philosophical and Cultural Aspects of Medical Profession: Philosophical and Conceptual Peculiarities. Cultura. International Journal of Philosophy of Culture and Axiology. 2019; 16 (1): 165-174.
2. Fedchyshyn N., Vykhrushch A., Bilavych H. et al. Development of Medical Students' Foreign Language Lexical Competence in the Virtual Learning Environment. Analele Universitatii din Craiova. Seria Stiinte Filologice, Lingvistica. 2020; 1-2: 71-79.
3. Denisenko, N. Health technologies in the educational process. Preschool education. 2004; 12. 60.
4. Ziglio, E., Currie, C., Rasmussen, V. The WHO cross-national study of health behavior in school aged children from 35 countries: findings from 2001–2002. J School Health, 2004; 74 (6): 204-206.
5. Farhud D. Impact of Lifestyle on Health. Iranian journal of public health, 2015; 44(11), 1442-1444.
6. Chung J., Chung L., Chen B. The impact of lifestyle on the physical fitness of primary school children. Journal of Clinical Nursury. 2009; 18(7): 1002-1009. doi: 10.1111/j.1365-2702.2008.02420.x. PMID: 19284435.
7. Chtourou, H., Trabelsi, K., H'mida, C. et al. Staying Physically Active During the Quarantine and Self-Isolation Period for Controlling and Mitigating the COVID-19 Pandemic: A Systematic Overview of the Literature. Front Psychol. 2020; 11: 1708. doi: 10.3389/fpsyg.2020.01708.
8. Ammar, A., Brach M., Trabelsi, K. et al. Effects of COVID-19 home confinement on eating behaviour and physical activity: results of the ECLB-COVID19 international online survey. Nutrients. 2020; 12: 1583. doi: 10.3390/nu12061583
9. Hongyan, Guan, Anthony, D. Okely et al. Promoting healthy movement behaviours among children during the COVID-19 pandemic. The Lancet. Child & Adolescent Health. 2020; 4\6: 416-418.
10. World Health Organization. Guidelines on physical activity, sedentary behaviour and sleep for children under 5 years of age. World Health Organization. 2019; https://apps.who.int/iris/handle/10665/311664. License: CC BY-NC-SA 3.0 IGO.

11. United Nations Educational, Scientific and Cultural Organization. Making Education a Priority in the Post-2015 Development Agenda. 2013. Paris: UNESCO.
12. Tremblay, M.S., Gray, C.E., Akinroye, K. et al. Physical activity of children: a global matrix of grades comparing 15 countries. Journal of Physical Act Health. 2014; 11: 113-125.
13. Develay, M. Comment refonder l'école primaire? Regards croisés – Pédagogies en développement: De Boeck Supérieur, 2013; 290.
14. Yaremenko, O., Balakireva, O., Vakulenko, O. Formation of a healthy lifestyle: problems and prospects. Kyiv. Ukrainian Institute for Social Research, 2000; 207.
15. Simons-Morton, B.G., Parcel, G.S., Baranowski, T., et al. Promoting physical activity and a healthful diet among children: results of a school-based intervention study. Am J Public Health. 1991; 81(8): 986. doi: 10.2105/AJPH.81.8.986.
16. Bizzoni-Prévieux, C., Otis, J., Mérini, C. et al. L'éducation à la santé à l'école primaire : approche comparative des pratiques au Québec et en France. Revue des sciences de l'éducation, 2010; 36 (3), 695–715. doi: 10.7202/1006252ar
17. Typical educational program for general secondary educational institutions. Electronic document. Access mode: https://mon.gov.ua/ua/osvita/zagalna-serednya-osvita/navchalni-programi/tipovi-osvitni-programi-dlya-2-11-klasiv. 2011.

The work was carried out according to the research work of Ternopil National Medical University for 2019-2021 on the theme of "Professional training of future specialists in the medical field on the basis of interdisciplinary integration" (state registration number 0116U004031).

ORCID and contibutionship:
Tetiana I. Pantyuk: 0000-0003-0672-9663 [A,D,F]
Mykola P. Pantyuk: 0000-0001-7336-6714 [B,D]
Olena V. Kvas: 0000-0003-1379-3129 [C,E]
Nadiya O. Fedchyshyn: 0000-0002-0909-4424 [E,D]
Olha V. Romanchuk: 0000-0001-8215-9741 [E,F]
Mariana I. Klym: 0000-0001-6992-6563 [B,D]
Lesia V. Perkhun: 0000-0001-9531-1175 [B,F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Nadiya O. Fedchyshyn
Ternopil National Medical University
1 Maidan Volia st., 46002 Ternopil, Ukraine
tel: +30977008085
e-mail: fedushunno@tdmu.edu.ua

Received: 17.05.2021
Accepted: 19.09.2021

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

INTESTINAL MICROFLORA ON THE BACKGROUND OF BACTERIAL VAGINOSIS ON VULVOVAGINAL CANDIDIASIS IN OVERWEIGHT AND OBESE WOMEN

DOI: 10.36740/WLek202110122

Karine A. Hasparian[1], Valentina K. Kondratiuk[1,2], Olha V. Trokhymovych[1], Kateryna O. Kondratiuk[1], Iryna M. Nikitina[3], Inna G. Ponomarova[1]

[1]STATE INSTITUTION «INSTITUTE OF PEDIATRICS, OBSTETRICS AND GYNECOLOGY NAMED AFTER ACADEMICIAN OM LUKYANOV ACADEMY OF MEDICAL SCIENCES OF UKRAINE», KYIV, UKRAINE

[2]NATIONAL UNIVERSITY OF HEALTH OF UKRAINE NAMED AFTER PL SHUPIKA, KYIV, UKRAINE

[3]SUMY STATE UNIVERSITY, SUMY, UKRAINE

ABSTRACT

The aim of our work was to study the intestinal microbiome in obese women and further develop differentiated patterns of exposure to the intestinal microbiota to improve metabolism and reduce excess weight.

Materials and methods: Surveyed 120 overweight and obese women. For comparison, 60 women without overweight and obesity were examined. Group I – women with vulvovaginal candidiasis and overweight and obesity (60 women); group II – women with vulvovaginal candidiasis without overweight and obesity (30 women); group III – women with bacterial vaginosis and overweight and obesity (60 women); IV – women with bacterial vaginosis without overweight and obesity (30 women). The study of the intestinal microbiocenosis included the determination of the species and quantitative composition of the microflora. Quantitative indicators of intestinal microflora were studied by seeding 1 ml from each dilution on differential diagnostic media: Endo, Ploskireva, ICA (bismuth – agar sulfide) to detect pathogenic enterobacteria; ZhSA (yellow – salt agar) for determination of staphylococci. To study the hemolytic activity of bacteria was used agar with 5% erythrocyte content of sheep. The presence of *bifidobacteria* in the test material was studied on Blaurok medium, and *lactobacilli* – on MRS medium. Statistical analysis of the obtained research results was performed using standard computer packages "Data Analysis" Microsoft Excel for Windows 2002.

Results: Based on the results of studies, we can summarize the nature of changes in the intestinal microbiome on the background of vulvovaginal candidiasis and bacterial vaginosis in women with overweight and obesity. In all women with excess body weight and obesity, intestinal microflora disorders have been registered, which are characterized by the replacing the *lactobacilli* and *bifidum bacteria* to opportunistic pathogens. A significant increase in *Firmicutes phylum microorganisms* and a significant decrease in Bacteroidetes phylum were found. The obtained data prove that changes in the species composition of the intestinal microbiota play an important role in the pathogenesis of obesity.

Conclusions: The results indicate the feasibility of monitoring the intestinal microbiota in women with vulvovaginal candidiasis and bacterial vaginosis, especially in the presence of excess body weight and obesity to determine the degree of violations of its components, and timely correction of the detected changes.

KEY WORDS: vulvovaginal candidiasis, bacterial vaginosis, body weight and obesity, intestinal microbiota

Wiad Lek. 2021;74(10 p.I):2482-2488

INTRODUCTION

Today, the problem of obesity on our planet affects more than 250 million people, more than 500 million people are overweight. Changes in dietary habits and increased availability of high-calorie food have made overweight and obesity one of the most serious health problems today [1, 2]. In recent years, changes in the human intestinal microbiome has been shown to play an important role in the development of obesity [3, 4]. There are 4 main phylum bacteria *(Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria)* in the large intestine of healthy people, which are divided into classes, genera and species of microorganisms. The vast majority of microorganisms that inhabit the large intestine are anaerobes. More than 90 % of the microflora of the distal colon in healthy people are microorganisms of the phylum *Bacteroidetes and Firmicutes. Firmicute*s include more than 200 species of gram-positive bacteria, in particular, various species of *Clostridium, Staphylococcus, Streptococcus, Enterococcus* [5]. Phylum *Bacteroidetes* is represented by gram-negative bacteria such as *Bacteroides, Prevotella*.

Bacteroids play an important role in fat metabolism – provide hepato-intestinal circulation of bile acids and cholesterol catabolism. Normally, deoxycholic and chenodeoxycholic acids with participating the secretory IgA, inhibit the growth of pathogenic and opportunistic pathogens, even in natural for the body concentrations [6].

Proteobacteria include such gram-negative opportunistic *enterobacteria* as *E. coli, Klebsiella spp., Citrobacter spp., Proteus spp*.

Actinobacteria phylum consists of gram-positive bacteria, the most important of which are *Bifidobacterium. Bifidobacterium* production of lysozyme, bacteriocins, alcohols and high antagonistic activity against pathogenic bacteria prevent their penetration into the upper gastrointestinal tract [7].

Up to 10% of the total number of intestinal microorganisms are aerobic bacteria – *Escherichia coli, streptococci, staphylococci, enterococci, various types* of opportunistic *enterobacteria*, yeast-like fungi of the genus *Candida*.

In recent years, the development of candidiasis in overweight and obese people is increasingly associated with excessive carbohydrate intake. In addition, chronic inflammation that accompanies obesity affects a person's immune reactivity, increasing the likelihood of developing a fungal infection [8].

Among the fungi of the genus *Candida* which are of major importance as causative agents of candidiasis, are *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei*. Literature data show that against the background of obesity, *Candida albicans and Candida glabrata* are more often isolated from the intestine [9].

The reliability of the intestine ecological barrier is associated with *lactobacilli.* Reducing the number of *lactobacilli* in the intestine leads to the reproduction of pathogenic and opportunistic microorganisms [10].

Many scientists have studied changes in the intestinal microbiota in overweight and obesity. Studies have shown an association between obesity and the composition of the gut microbiome [11].

Thus, a number of studies have found a numerical increase in bacteria of the *Firmicutes* type compared with representatives of the *Bacteroidetes* type and more energy storage from food in overweight people. Some researchers believe that an increase in the microbiome of obese bacteria that ferment polysaccharides with forming the large amounts of short-chain fatty acids (CLA) [12] plays a role in providing an increased energy reserve in the colon and developing the obesity.

Firmicutes, such as those found in large numbers in obese patients, are thought to express genes encoding enzymes that break down undigested polysaccharides. This increases the concentration of KLZHK, providing *colonocytes* with an available source of energy. As a result, the energy value of food increases, which is eventually realized by weight gain [13].

It was found that as the degree of obesity increases, the degree of microecology of the large intestinal cavity increases, which can be explained by the close relationship between the processes of absorption and metabolism in the background of intestinal microecology [14 – 16].

THE AIM

The aim of our work was to study the intestinal microbiome in obese women and further develop differentiated patterns of exposure to the intestinal microbiota to improve metabolism and reduce excess weight.

MATERIALS AND METHODS

In order to solve the tasks set in our work, we surveyed 120 overweight and obese women. For comparison, 60 women without overweight and obesity were examined. All women were divided into groups:

Group I – women with vulvovaginal candidiasis and overweight and obesity (60 women); group II – women with vulvovaginal candidiasis without overweight and obesity (30 women); group III – women with bacterial vaginosis and overweight and obesity (60 women); IV – women with bacterial vaginosis without overweight and obesity (30 women). The data obtained during the examination of 30 healthy women were used as a control.

The study of the intestinal microbiocenosis included the determination of the species and quantitative composition of the microflora. The degree of intestinal biocenosis violations was assessed according to the guidelines by Bondarenko V.M., Matsulevich T.V. "Microbiological diagnosis of intestinal dysbacteriosis" Moscow, 2007.

For quantitative analysis of the intestinal microflora from 1 g of feces delivered without preservatives, a working dilution (1:10) was prepared, from which a number of serial dilutions (103 – 1011) were made. Quantitative indicators of intestinal microflora were studied by seeding 1 ml from each dilution on differential diagnostic media: *Endo, Ploskireva, ICA* (bismuth – agar sulfide) to detect pathogenic *enterobacteria*; ZhSA (yellow – salt agar) for determination of staphylococci; Endo and Simons citrate for the determination of *Escherichia coli* and opportunistic *enterobacteria*; EDDS medium – for the detection of *enterococci*. To study the hemolytic activity of bacteria was used agar with 5% erythrocyte content of sheep. The presence of *bifidobacteria* in the test material was studied on Blaurok medium, and lactobacilli – on MRS medium. The number of *bifidobacteria* and *lactobacilli* was determined by diluting the material.

Saburo medium was used to identify yeast-like fungi. MICROLA-TEST kits "Candida test 21" (Erba Lachema s.r.o. (Czech Republic)) were used for further identification of isolated yeast-like fungi.

The taxonomic position of microorganisms was determined according to the "Determinant of Bergi bacteria". Identification of microorganisms was performed by their cultural and morphological characteristics.

Determination of anaerobic microorganisms was carried out according to the guidelines "Laboratory diagnosis of purulent – inflammatory diseases caused by asporogenic anaerobic microorganisms", Kharkiv, 1985.

Anaerobic intestinal microflora was studied with strict adherence to the technique of anaerobic culture. Solid and liquid nutrient media (thioglycol medium, blood agar with glucose, liver broth, Kitt-Tarozzi medium) were used for the study. The Anaerocult system (Merck, Germany) and the Anaerogas gas packages were used to create anaerobic conditions. MICROLA-TEST kits "Anaero test 23" (Erba Lachema s.r.o. (Czech Republic)) were used for further identification of isolated anaerobic microorganisms.

To assess the degree of intestinal dysbacteriosis, quantitative accounting of colonies grown in dense nutrient media was performed.

The content of UPM in the test material was expressed by the number of colony-forming units in 1 g (CFU / g) of biological material.

Statistical analysis of the obtained research results was performed using standard computer packages "Data Analysis" Microsoft Excel for Windows 2002. The values of the arithmetic mean were calculated – the value (M), the average error of the average value (m), the level of probability of discrepancies (p). The reliability of the obtained data was evaluated by the generally accepted method using the Student's criterion. Reliability was considered established if its probability was at least 95% (0.05).

RESULTS

Intestinal dysbiosis was found in all examined patients with vulvovaginal candidiasis and obesity (group I). II degree of dysbiosis was registered in 41.7% of patients, III degree in 58.3% of women.

When examining women of group I it was found that E. coli was detected in the majority of subjects (95 %), but its quantitative level did not reach the indicators in the group of healthy women and was lg 5.8 CFU / g (Fig. 1). *E. coli* with hemolytic properties was sown with a high frequency – 28.3 %, at a concentration of lg 7.2 CFU / g, which significantly exceeded the diagnostic level. Also, among *Proteobacteria* such opportunistic microorganisms as *Serratia spp*. – 31.7 %, *Klebsiella spp*. – 26.7 %, *Enterobacter spp.* – 25 %, *Citrobacter spp*. – 18.3%.

The quantitative indicators of registrating the citrate-stimulating bacteria exceeded the norm and were within lg 6.2 CFU/g-lg 7.5 CFU/g.

When examining patients of group I was revealed an increase in qualitative and quantitative indicators of staphylococci seeding, streptococci belonging to *Firmicutes*. The seeding frequency of *S. faecalis* reached 70 %, *S. aureus* – 48.3 %, *S. epidermidis* with hemolytic properties – 46.7 %.

A high level of intestinal contamination with gram-positive cocci with pathogenic properties was registered: lg 5.4 CFU/g – lg 5.6 CFU/g. The amount of *S. faecalis* (lg 7.3 CFU/g) also exceeded the diagnostic level.

Fungi of the genus *Candida* were found in the intestine in 100 % of women with vulvovaginal candidiasis at a concentration of lg 6.5 CFU/g. *Candida albicans*-71.7 %, *Candida glabrata* -20.0% were most often sown from the intestines of patients of group I. Other species of fungi were isolated with insignificant frequency (Fig. 2).

Therefore, it can be assumed that the source of highly virulent strains of fungi that infect the mucous membranes of the genitals is the intestine, and without inhibiting the growth of fungi in it, any therapy aimed at treating genital candidiasis is usually unsuccessful and subsequently leads to recurrence of the disease.

The analysis of seeding indicators of indigenous intestinal microflora made it possible to state a significant decrease in its number – *Lactobacillus spp*. was sown in 73.3 % of women at a concentration of lg 5.2 CFU/g, and *Bifidobacterium spp*. in 68.3 % of subjects at a concentration of lg 6.2 CFU/g.

Among the representatives of the anaerobic microflora a significant proportion were bacteria of the phylum *Firmicutes: Peptostreptococus spp*. was detected in 53.3% of patients, *Blautia spp*. in 75%, *Clostridium spp*. in 51.7%, *Eubacterium spp*. in 63.3%. The quantitative level of seeding these microorganisms in women with vulvovaginal candidiasis increased by almost two orders of magnitude compared to normal (Fig. 1).

Among other members of the obligate anaerobic microflora, there was a significant decrease in the qualitative and quantitative level of seeding from the intestine of *Bacteroides spp*. (38.3 %, lg 6.7 CFU/g) and *Prevotella spp.* (13.3 %, lg 6.0 CFU/g).

In 73.3 % of the examined patients of group I, associative forms of bacterial intestinal contamination were revealed. Mostly three – four component associations of staphylococci with opportunistic enterobacteria and fungi of the genus *Candida* were registered.

For comparison, the second group of women with vulvovaginal candidiasis without overweight and obesity was examined. The frequency of registration of grade III dysbacteriosis in patients with vulvovaginal candidiasis without overweight and obesity was 36.7 %, grade II-63.3 %. Intestinal microecology disorders in patients of group II were manifested in an increase in the frequency of seeding the lactose-negative *E. coli* – 16.7 %, *E. coli* (heme +) – 20 %, and certain species of *enterobacteria* (*Klebsiella spp*. – 23.3 %). Gram-positive cocci contaminated the intestines of women with vulvovaginal candidiasis without excess body weight and obesity with a much lower frequency than in women of group I. *S. aureus* was sown in 23.3% of women, *S. epidermidis* with hemolytic properties in 13.3 %. Quantitative seeding rates of opportunistic pathogens were also lower, compared with women of group I, but exceeded the rates of intestinal contamination in the group of healthy women.

Increased intestinal contamination with gram-negative rods and gram-positive cocci was accompanied by a deficiency of *Lactobacillus spp.* in 90% of subjects and *Bifidobacterium spp*. in 83.3% of group II women. Absence of *Lactobacillus spp*. found in 10% of patients with *Bifidobacterium spp*. in 16.7%. Fungi of *Candida* in the II group of examined patients were sown with a lower frequency (93.3%) than in patients of the first group. Quantitative indicators of *Candida* fungi (lg 5.3 CFU / g) also significantly exceeded the diagnostic level. *C. albicans* was found in 56.7% of cases in the species composition of *Candida* fungi. Among *Candida* non-albicans fungi, *C. glabrata* was most often registered in 23.3%, *C. parapsilosis* in 6.7% (Fig. 2).

As in the previous group of patients, the intestinal microflora was in 3-4 component associations. Associations of opportunistic pathogens were found in 66.7% of women in group II.

In women of this group, changes in the anaerobic component of the intestinal microbiocenosis differed from those obtained in patients of group I. In contrast to the results obtained in group I patients, no significant differences were found from the rate of seeding of *Bacteroidetes* phylum bacteria. Frequency and quantitative indicators of

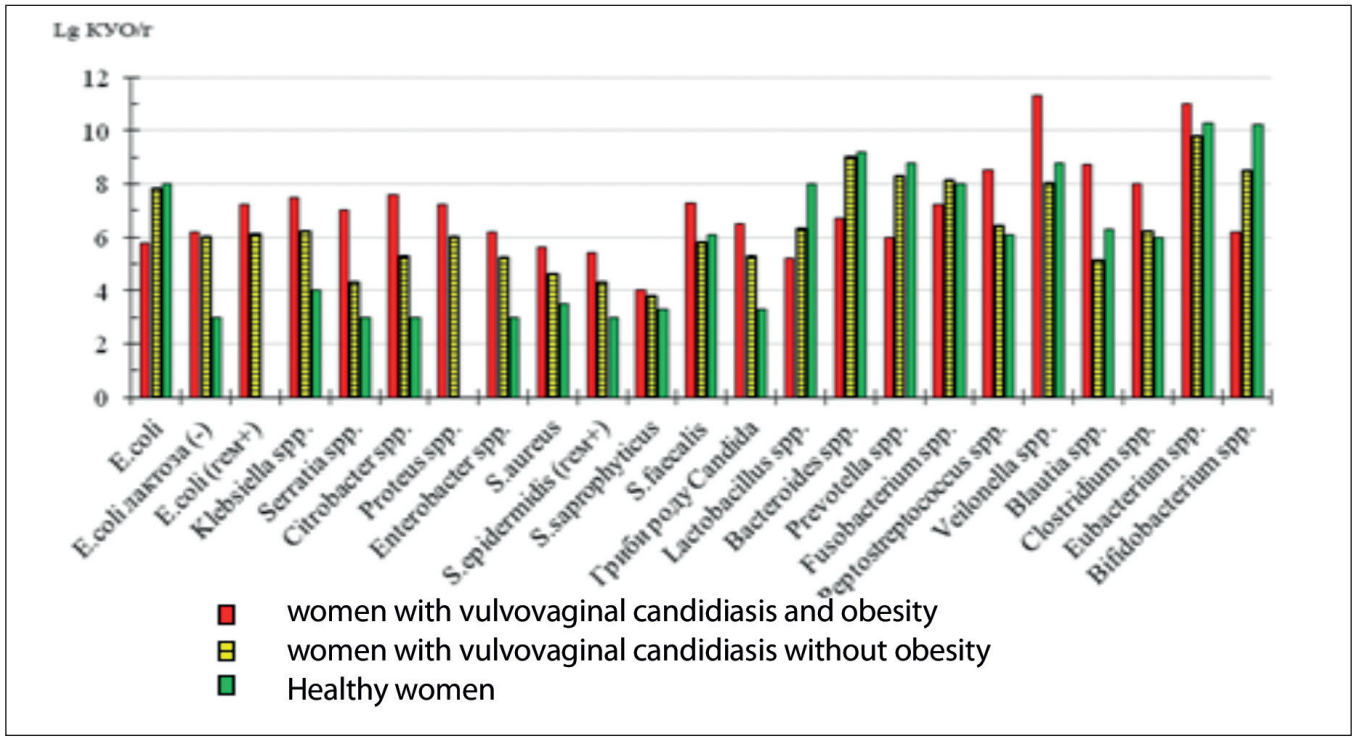


Fig.1. The composition of facultative and obligate anaerobic intestinal bacteria in women with vulvovaginal candidiasis and obesity

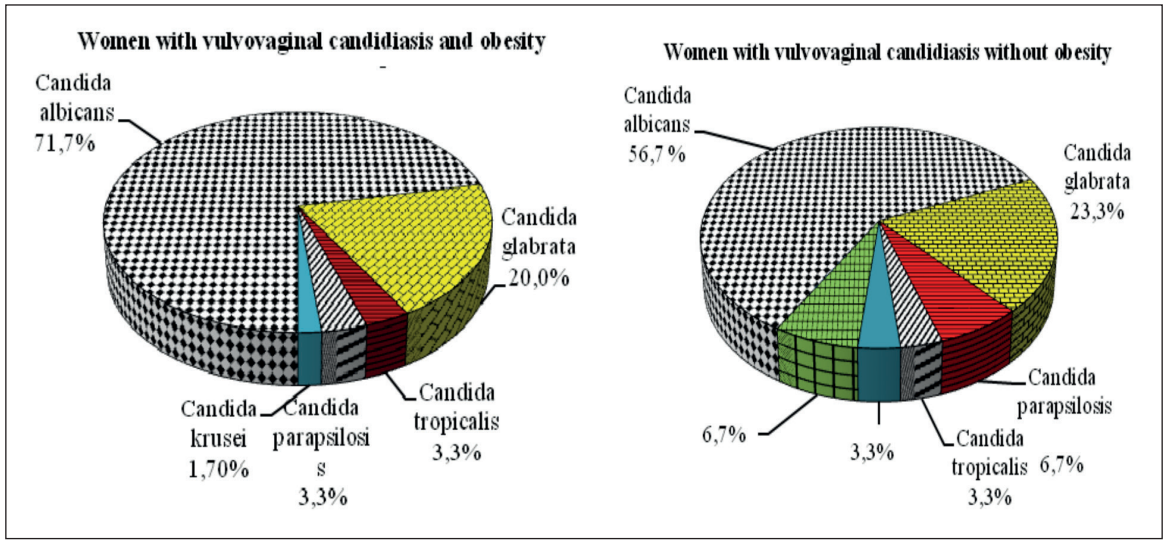


Fig. 2. Species composition of genus *Candida* fungi isolated from the intestine in women with vulvovaginal candidiasis with obesity and without obesity.

seeding *Bacteroides spp*. were in 73.3% (lg 9.0 CFU / g) and *Prevotella spp*. in 30.0% (lg 8.3 CFU / g).

Representatives of anaerobic gram-positive microflora belonging to the *Firmicutes* type, contaminated the intestines of group II patients also with a lower frequency than in patients of group I.

From our studies it can be concluded that in patients of group II the imbalance between bacteroids and representatives of the microflora type *Firmicutes* was less significant than in women of group I.

Thus, the obtained data indicate that the most significant dysbiotic intestinal disorders were registered in the group of patients with vulvovaginal candidiasis in combination with overweight and obesity. Detection of pathological changes in the intestinal microbiocenosis in these patients indicates the need to develop preventive and curative measures aimed at eliminating from the microbial associations of potentially pathogenic microflora and fungi of *Candida*, as well as normalizing the relationship between *Firmicutes* and *Bacteroidetes* towards the latter.

The task also included the study of intestinal microbiome in women with bacterial vaginosis and obesity. The majority of women in group III registered the formation of intestinal dysbiosis, mainly II-III degree (II degree-31.7%, III degree – 68.3%). The amount of *E. coli* in patients of group III did not reach the level of healthy women. In the

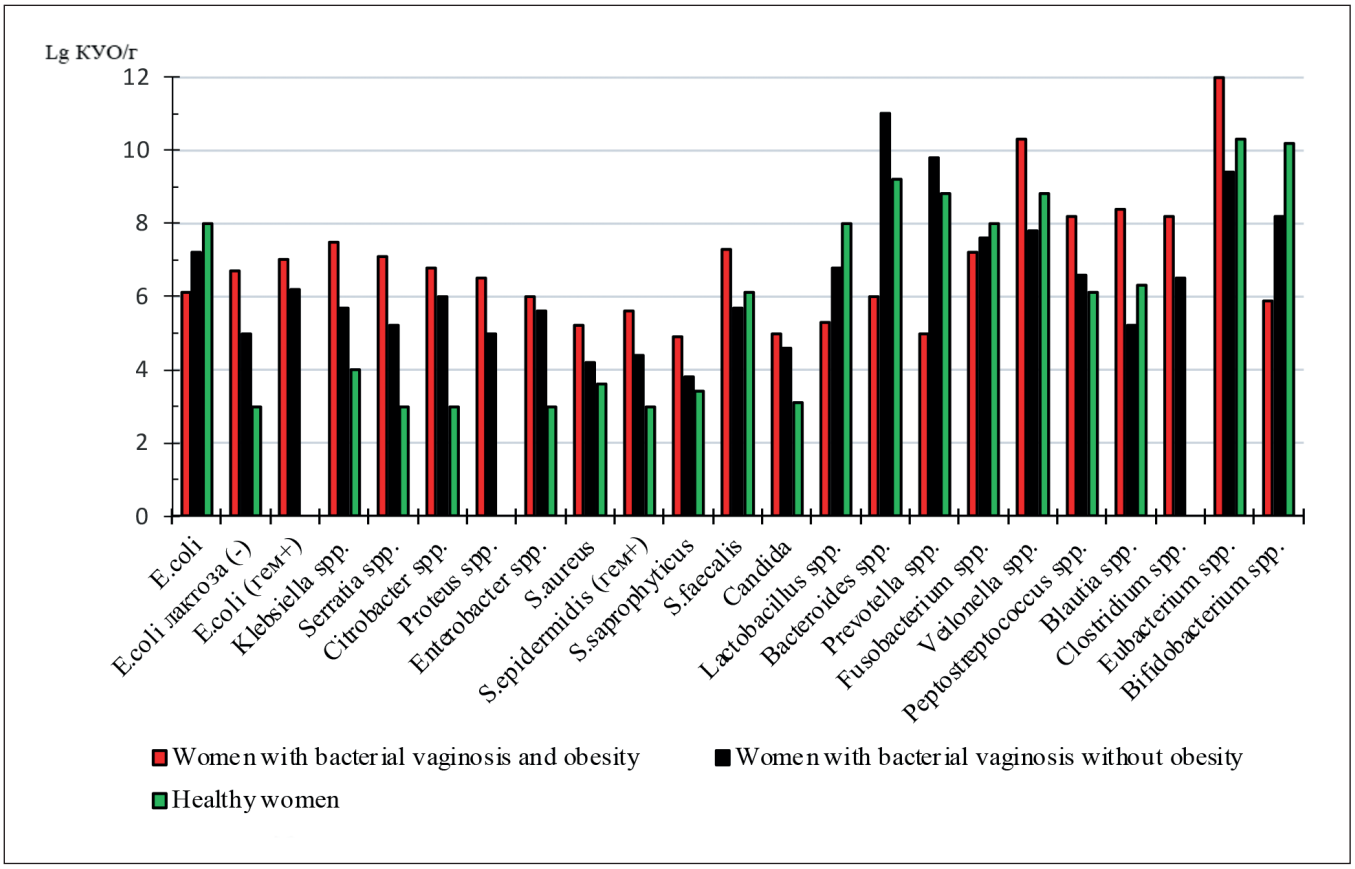


Fig. 3. The composition of facultative and obligate anaerobic intestinal bacteria in women with bacterial vaginosis and obesity

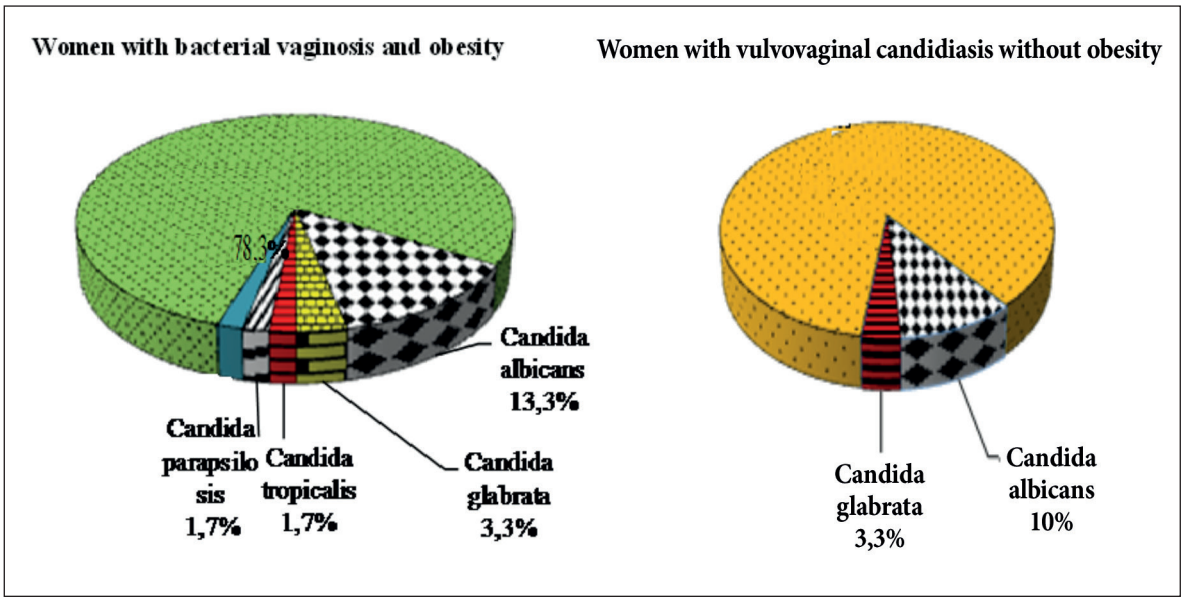


Fig. 4. Species composition of fungi of the genus Candida isolated from the intestines of women, with bacterial vaginosis and obesity and without obesity

spectrum of *Escherichia coli* there is an increase in the frequency of detection and concentration of *E. coli* with hemolytic properties – 25% (lg 6.7 CFU / g) (Fig. 3).

Qualitative indicators of intestinal contamination of women with bacterial vaginosis and obesity (group III) indicate a high frequency of seeding the opportunistic *enterobacteria: Enterobacter spp.*-21.7%, *Proteus spp.* in 16.7%, *Citrobacter spp.* in 28.3%, *Klebsiella spp.* in 23.3%, *Serratia spp.* in 48.3%, in concentrations exceeding the diagnostic level> lg 6.0 CFU / g and data obtained from the examination of healthy women.

Examination of women with bacterial vaginosis and obesity revealed an increase in the qualitative and quantitative indicators of staphylococci seeding, streptococci belonging to *Firmicutes.*

The seeding frequency of *S. aureus* reached 36.7%, *S. epidermidis* (heme +) – 38.3%, *S. saprophyticus* – 41.7%, *S. faecalis* – 76, %. Quantitative indicators of seeding the coccal microflora also reached a significant level: lg 5.2 CFU / g – lg 7.3 CFU / g.

The frequency of detecting the genus *Candida* fungi in female patients of this group was not very high and was 21.7%. *Candida* albicans was found with the highest frequency – 13.3%. Other species of fungi were isolated with insignificant frequency (Fig. 4).

Quantitative indicators of intestinal contamination in women of group III by the genus *Candida* fungi were – lg 5.0 CFU / g and exceeded the diagnostic level (> lg 4.0 CFU / g). Also, in patients of group III there is a significant decrease in the frequency of excretion and the quantitative level of seeding the protective intestinal microflora: *Lactobacillus spp*. –63% (lg 5.3 CFU / g), *Bifidobacterium spp.* –73.3% (lg 5.9 CFU/g).

Studying the intestinal biocenosis in women with bacterial vaginosis and obesity, it was found that the lactobacilli and bifidum bacteria, which are normally dominant in the intestinal microflora, are replaced by opportunistic aerobic microorganisms and obligate anaerobes. Thus, among gram-positive obligate anaerobes, *Peptostreptococus spp*. -56.7%, *Clostridium spp.* -56.7%, *Eubacterium spp*.- 68.3%, *Blautia spp*. – 61.7 %. Quantitative indicators of these microorganisms significantly exceeded the diagnostic level and seeding rates of microorganisms in the group of healthy women and was in the range of lg 8.2 CFU / g – lg 12.0 CFU/g.

Dominant in the intestines of a healthy person Bacteroides spp. were sown only in 41.7 % of surveyed women of group III at a concentration of lg 6.0 CFU/g, and Prevotella spp. in 15% at a concentration of lg 5.0 CFU/g.

In 68.3 % of women with bacterial vaginosis and obesity, opportunistic intestinal microflora was in multicomponent associations. The associations included hemolytic species of gram-negative *enterobacteria, Candida fung*i and *Firmicutes* phylum microorganisms in various combinations.

The results obtained in our study indicate an increase in the relative number of *Firmicutes* against the background of a decrease in the number of *Bacteroidetes* in patients with bacterial vaginosis in combination with obesity. Changes in the *Firmicutes / Bacteroidetes* ratio suggest that there is a relationship between the microflora and the formation of excess body weight and obesity.

Examination of women with bacterial vaginosis without obesity (group IV) also revealed differences in the composition of the intestinal microflora.

In the examined group IV dysbacteriosis of the II degree was found in 56.7 % of cases, dysbacteriosis of the III degree is in 43.3 % of cases.

In patients with bacterial vaginosis without obesity, a lower frequency of intestinal contamination with staphylococci with pathogenic properties (*S. aureus* – 20 %, *S. epidermidis* (heme +) – 16.7 %) was registered.

Among *enterobacteria, E. coli* (heme +) was sown with a lower frequency – 16.7 %, *Klebsiella spp*. – 20 %, *Citrobacter spp*. – 16.7 %, compared with data obtained from patients of group III.

The study of quantitative indicators of intestinal biocenosis in patients with bacterial vaginosis without obesity showed that in this group there are lower concentrations of opportunistic *enterobacteria* (lg 5,2 KUO/g -lg 5,7 KUO/g) and *staphylococci* with pathogenic properties (lg 4, 2 CU/g – lg 4.4 CFU/g), but these indicators exceeded the data obtained in the control group.

Negative changes in the indicators of intestinal microecology in patients of group IV were mainly manifested by low quantitative indicators of normal microflora (*Lactobacillus spp*.- lg 6.8 CFU / g and *Bifidobacterium spp*. – lg 8.2 CFU / g). *Lactobacillus* deficiency was registered in 63.3 % of group IV patients, and their absence in 16.7 % of subjects. Decreased seeding levels of *Bifidobacterium spp*. were found in 56.7 % of group IV patients, in 13.3 % of patients *Bifidobacterium spp*. were not sown.

The frequency of seeding the Candida fungi in patients of group IV was 13.3 %. Among them, fungi of *C. albicans* were found in 10 % of cases, *C. glabrata* in 3.3 % (Fig. 4).

Among the obligate anaerobic microflora in women with bacterial vaginosis without obesity with high frequency and in large numbers sown *Bacteroides spp*. in 83 % (lg 11.0 CFU/g), *Prevotella spp*. in 36.7 % (lg 9.8 CFU/g). *Peptostreptococus spp*. was found in 43.3 % (lg 6.6 CFU/g), *Clostridium spp*. in 40 % (lg 6.5 CFU/g).

In 43.3 % of the IV group women patients the sowing of opportunistic pathogenic microflora in 2 – 3 species associations were recorded.

Therefore, it can be concluded that there are differences between the indicators of intestinal biocenosis in groups of women with bacterial vaginosis and obesity, as well as with bacterial vaginosis and without obesity, which consist in a violation of the ratio of *Bacteroidetes / Firmicutes* towards a significant increase in bacteria *Philou Firmicutes*, a significant increase conditionally – pathogenic *Enterobacteriaceae*, significant intestinal contamination with genus of *Candid*a fungi, the formation of multicomponent associations of conditionally pathogenic microorganisms against the background of a significant decrease or absence of normal intestinal microflora.

Based on the results of studies, we can summarize the nature of changes in the intestinal microbiome on the background of vulvovaginal candidiasis and bacterial vaginosis in women with overweight and obesity.

In all women with excess body weight and obesity, intestinal microflora disorders have been registered, which are characterized by the replacing the *lactobacilli* and *bifidum bacteria* to opportunistic pathogens. A significant increase in Firmicutes phylum microorganisms and a significant decrease in *Bacteroidetes* phylum were found. The obtained data prove that changes in the species composition of the intestinal microbiota play an important role in the pathogenesis of obesity [17].

Detection of pathological changes in the microbiocenosis indicates that the intestinal microflora may play an important role in the occurrence of disorders in the biocenosis of the vagina in women, as well as be of great importance in the pathogenesis of obesity. Considering all this, it becomes necessary to develop preventive and therapeutic measures aimed

at eliminating from the microbial associations of potentially pathogenic microflora and restoring the level of bifido – and lactoflora in the intestines of patients. The most severe dysbiotic processes develop in patients with vulvovaginal candidiasis and overweight and obesity (group I), as well as with bacterial vaginosis and overweight and obesity (group III) due to increased concentrations of hemolyzing, lactose-negative Escherichia coli, pathogenic properties, and *Candida* fungi.

CONCLUSIONS

1. In patients with vulvovaginal candidiasis and bacterial vaginosis and overweight and obesity, there is a decrease in the content of *Bacteroidetes* phylum bacteria and a deficiency of protective microflora (*lactobacilli, bifidum bacteria*), gram-positive anaerobic microorganisms *Philou Firmicutes* and fungi of the genus *Candida.*
2. In groups of patients with vulvovaginal candidiasis, the frequency of intestinal contamination with fungi of the genus *Candida*, including *Candida* non-albicans was 100 % in women of group I and 93.3 % of women in comparison group (group II) in high concentrations that significantly exceeded the diagnostic level > lg 4.0 CFU/g. The spectrum of fungi most often included *Candida* albicans. Other species of *Candida* non albicans were less common.
4. The results indicate the feasibility of monitoring the intestinal microbiota in women with vulvovaginal candidiasis and bacterial vaginosis, especially in the presence of excess body weight and obesity to determine the degree of violations of its components, and timely correction of the detected changes.

REFERENCES

1. VOZ. Izbytochnyj ves I ozhirenie. http://www.who.int/gho/ncd/risk_factors/overweight/en/
2. Gregg E.W., Shaw J.E. Global health effects of overweight and obesity. N. Engl. J. Med. 2017; 377 (1): 80–81. doi: 10.1056/NEJMe1706095.
3. Castaner O., Goday A., Park, Y.M., Lee, S.H., Magkos, F., Shiow, S.T.E., Schröder H. The Gut Microbiome Profile in Obesity: A Systematic Review. Int. J. Endocrinol.- 2018, 4095789.
4. Bell, C.G. The Epigenomic Analysis of Human Obesity. Obesity 2017; 25: 1471–1481.
5. Samojlova Ju.G., Olejnik O.A., Sagan E.V., Vorozhcova I.N., Filippova T.A., Denisov N.S., D'jakov D.A. Sostav i metabolicheskaja aktivnost' mikrobioty pishhevaritel'noj sistemy u detej i podrostkov s ozhireniem. Sibirskij zhurnal klinicheskoj i jeksperimental'noj mediciny. 2020;35(3):38–46. doi:10.29001/2073-8552-2020-35-3-38-46.
6. Barczyńska R., Litwin M., Sliżewska K., Szalecki M., Berdowska A., Bandurska K. et al. Bacterial microbiota and fatty acids in the faeces of overweight and obese children. Pol. J. Microbiol. 2018;67(3):339–345. doi: 10.21307/pjm-2018-041.
7. Milani C., Duranti S., Bottacini F., Casey E., Turroni F., Mahony J. et al. The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev. 2017; 81 (4).
8. Friedman D.Z.P., Schwartz I.S. Emerging fungal infections: New patients, new patterns, and new pathogens. J. Fungi 2019; 5: 67.
9. Surain P., Aggarwal N.K. Candida, a human pathogen and major types of candidiasis. Int J Pharm Sci & Res 2019; 11(1): 41-67. doi: 10.13040/IJPSR.0975-8232.11(1).41-67.
10. Matthew H. Foley, Sarah O'Flaherty, Garrison Allen, Alissa J. Rivera et al. Lactobacillus bile salt hydrolase substrate specificity governs bacterial fitness and host colonization. Proceedings of the National Academy of Sciences. 2021; 118 (6): e2017709118 doi: 10.1073/pnas.201770911.
11. Volkova N. I., Ganenko L. A., Golovin S. N. Rol' mikrobioti kishechnika v razvitii ozhirenija i ego metabolicheskogo profilja (chast' II). Medicinskij vestnik -Severnogo Kavkaza. 2019;14(2). 391396. doi: http://dx.doi.org/10.14300/mnnc.2019.14098
12. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006;444(7122):1022-3. doi:10.1038/4441022a.
13. Blüher M. Metabolically Healthy Obesity. Endocr Rev. 2020; 41(3):405-420. doi:10.1210/endrev/bnaa004.
14. Muscogiuri, G. et al. Gut microbiota: a new path to treat obesity. Int. J. Obes. Suppl. 2019; 9: 10–19. doi: 10.1038/s41367-019-0011-7 .
15. Wang, S.-Z.; Yu, Y.; Adeli, K. Role of Gut Microbiota in Neuroendocrine Regulation of Carbohydrate and Lipid Metabolism via the Microbiota-Gut-Brain-Liver Axis. Microorganisms 2020; 8: 527.
16. Crovesy, L.; Masterson, D.; Rosado, E.L. Profile of the gut microbiota of adults with obesity: A systematic review. Eur. J. Clin. Nutr. 2020; 74: 1251–1262.
17. Magne F, Gotteland M, Gauthier L, Zazueta A et al. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? Nutrients. 2020 May 19; 12(5):1474. doi: 10.3390/nu12051474.

The work is carried out within the framework of the research work "Optimization of diagnosis and prevention of diseases of the reproductive system and development of pathogenically grounded methods for their correction" (state registration number 011U001801).

ORCID and contributionship:
Karine A. Hasparian: 0000-0003-2894-1411 [A, B, D, C]
Valentina K. Kondratiuk: 0000-0001-6220-2116 [A, E, F]
Olha V. Trokhymovych: 0000-0001-7423-528 [B, C]
Kateryna O. Kondratiuk: 0000-0001-5915-1821 [A, E, F]
Iryna M. Nikitina: 0000- 0001-6595-2502 [D, F]
Inna G. Ponomarova: 0000- 0003-0416-0004 [E, F]

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

CORRESPONDING AUTHOR

Iryna M. Nikitina
Sumy State University
Rymskoho-Korsakova 12, 40030 Sumy, Ukraine
tel: +380662947360
e-mail: nikitina1med@gmail.com

Received: 20.05.2021
Accepted: 15.09.2021

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

FEATURES OF HEMODYNAMICS IN HEAD MAGISTRAL AND CEREBRAL ARTERIES IN THE PATIENTS WITH MIGRAINE

DOI: 10.36740/WLek202110123

Valeriy I. Kalashnikov[1], Alexander N. Stoyanov[2], Alexander R. Pulyk[3], Iryna K. Bakumenko[2], Tamara A. Andreeva[2], Svitlana Oliinyk[2]

[1]KHARKIV MEDICAL ACADEMY OF POSTGRADUATE EDUCATION, KHARKIV, UKRAINE
[2]ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE
[3]UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

The aim of the study was to Doppler sonography study of the structural and functional state of head magistral arteries (HMA) and cerebral arteries in the patients with various forms of migraine.

Materials and methods: We conducted the clinical Doppler examination of 124 young patients (18-45 years old), including 55 men and 69 women in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2017-2019. The criteria for involvement of patients in the study were: migraine without aura (group 1 – 63 patients), migraine with aura (group 2 – 61 patients) The control group consisted of 45 patients of the corresponding gender and age. The condition of HMA and cerebral arteries was studied using the ultrasound device.

Results: The presence of extravasal compressions of vertebral arteries (VA) is typical for the patients with migraine, as well as for some cases of the hypoplasia of the VA in the group of the patients with migraine with aura. In the patients with migraine with aura, there was a decrease in the velocity values in the extracranial VA segments. The velocity values in the external carotid arteries (ECA) were slightly reduced in both groups. The most significant were the changes in the hemodynamics in the middle cerebral arteries (MCA), which were manifested by the pattern of the excessive perfusion in the patients of the migraine without aura and the pattern of the hampered perfusion in the MCA in the patients of the migraine with aura.

Conclusions: 1. In the patients with migraine with aura, a decrease in the velocity values in the extracranial segments of the VA was observed, in some cases combined with the hypoplasia of the vertebral artery, the hampered perfusion in middle cerebral arteries.

2. The excessive perfusion in middle cerebral arteries is the most critical hemodynamic pattern in the patients with migraine without aura.

3. The extravasal compression of vertebral arteries, combined with the hyperperfusion in posterior cerebral arteries, is a typical hemodynamic pattern both in the group of the patients with migraine with aura, and in the group of the patients with migraine without aura.

KEY WORDS: migraine, doppler sonography, cerebral hemodynamics, head magistral arteries, cerebral arteries

Wiad Lek. 2021;74(10 p.I):2489-2493

INTRODUCTION

Migraine is the primary neurogenic cerebral dysfunction with the presence of genetically determined stem failure, severe cortical hyperactivity with periodically occurring hypothalamus dysfunctions [1,2]. Migraine is manifested by periodically recurring attacks of an intense pulsating headache, which is commonly located on one side of the head (they tend to fronto-temporal localization), and in most cases it is accompanied by nausea, vomiting, photo and phonophobia, drowsiness, lethargy after the attack. The duration of an attack can last from several hours to 2-3 days, the frequency can vary from 1 time per year to 1 time per week. Two major forms of migraine are migraine without aura, which is characterized by headache attacks with specific accompanying symptoms; migraine with aura, which is characterized by local neurological symptoms, usually preceding or accompanying the headache [3]. A few hours or even days before an attack, precursors of the headache may occur, as well as symptoms after the attack (hyper- or hypoactivity, depression, craving for certain foods, repeated yawning, etc.) [4,5].

A migraine attack begins with the so-called Leo cortical spreading depression. The phenomenon of cortical depression is provoked by various stimuli and occurs in the occipital cortex. Calcium channel dysfunctions and mitochondrial dysfunctions, nitric oxide and glutamate impaired metabolism contribute to the brain overexcitation. The focus of neuronal depolarization progressively spreads over the surface of the cortex from the occipital to the frontal lobe [6].

Depolarization is accompanied by an increase in extracellular potassium, and a decrease in sodium, calcium and chlorine. Cortical depression causes the activation of sensory neurons of the trigeminal complex, increases their sensitivity to nociceptive irritation of the vessels of the dura mater [7].

Activation of brain stem centers leads to depolarization of ascending and descending nociceptive and antinociceptive pathways, dilatation of perimeningeal vessels and neurogenic inflammation. Pain-sensitive nerve fibers can be activated by the leakage of sensitizing vasoactive substances from trigeminal nerve endings or by efferent activity in parasympathetic nerves. Based on these data, we can assume that the release of vasoactive substances around the vessels of the brain can cause sensitization of afferent trigeminal cells, vasodilation and pain during migraines [8]. At the time of the migraine attack, the dorsal region of the midbrain periaqueductal space is activated. These brain areas contain serotonergic, noradrenaline, endorphinergic and GABAergic systems. The trigger factor for migraine is the activation of this area. Before a migraine headache attack, platelet aggregation increases, serotonin is released from them, which leads to vasoconstriction of large-caliber vessels, vasodilation of capillaries (prodromal period) [2]. Changes in cerebral blood flow during the migraine attack are similar to the stages of spreading depression. Pathogenetic factors of the attack are changes in the metabolism and function of the cortex. The rate of oligemia is approximately equal to the rate of pervasive depression during the migraine attack [9].

Various external factors can provoke the development of a migraine attack: mental, nervous or physical overstrain, stress, weather factor, sleep disturbance, intake of certain foods or alcohol, use of hormonal contraceptives.

Migraine pain consists of three components: 1. Vasodilation, mainly intracranial vessels, including vessels of the dura mater and large cerebral arteries. 2. Rapidly developing neurogenic (aseptic) inflammation in the perivascular region. 3. Activation of the central trigeminal system, namely the spinal tract of the trigeminal nerve (nucleus tractus caudalis) and its central connections [1,2,10].

The pathophysiological theory of migraine can be divided into 3 types: 1. Vascular: Wolf's vascular theory, shunt theory, platelet theory. 2. Neurological and neurovascular: the theory of spread depression, trigeminal-vascular theory. 3. Serotonergic theory. [1,2,10,11].

The method of transcranial dopplerography (TCD) of the major vessels of the head has been successfully used to diagnose lesions of extracranial and intracranial sections of the major arteries. Recently, a large number of works have been published on the study of cerebral hemodynamic disorders in patients with migraine with and without aura, during the attack-free period and during the attack [12-15]. Their results are very contradictory. The studies during the attack-free period did not show significant differences in average blood flow velocity in extra- and intracranial arteries in patients with migraine relative to the group of healthy subjects [16].

There is enough available data supporting the vascular hypothesis of the migraine pathogenesis [17-19]. In this model, the vasoconstriction in the intracranial vascular system is attributed to the aura phase of migraine attacks, with the subsequent vasodilation being considered the cause of the headache. The presence of the arteriovenous shunts in this region in migraine has been reported and their role in the development of the headache has been studied [15]. It is possible that vasoconstrictive changes in the posterior regions of the brain are responsible for some of the symptoms of the migraine aura, seizure or postdromic phase [15]. During the migraine attack the linear blood flow velocity (BFV) in middle cerebral arteries (MCA) on the side of the headache was significantly lower than on the side without the headache, without changes in the volumetric blood flow, indicating the intracranial dilatation of the artery on the side of the headache, estimated at approximately 20%. This decrease in the velocity in MCA, indicating the arterial dilatation during the headache, contradicted other studies that left open the question of the pathophysiological role of the vascular dilatation during migraine attacks [20,21].

In connection with the above data, the usage of ultrasound methods of vascular diagnostics gains great importance for the study of this pathology. Available publications do not contain data on the results of the combined study of cerebral and extracerebral hemodynamics in patients with various types of migraine paroxysms.

THE AIM

The aim was to study the structural and functional state of head magistral arteries and cerebral arteries in the patients with various forms of migraine.

MATERIALS AND METHODS

We conducted the clinical Doppler examination of 124 young patients (18-45 years old), including 55 men and 69 women in the conditions of the clinical base of the Kharkiv Medical Academy of Postgraduate Education in 2017-2019. The criteria for involvement of patients in the study were: migraine without aura (group 1 – 63 patients), migraine with aura (group 2 – 61 patients) in accordance with the criteria for the international classification of headache disorders (ICHD-3, 2018) [11]. The exclusion criteria were the presence of occlusions and hemodynamically significant stenoses of brain magistral arteries (BMA). All patients underwent clinical and neurological examinations. The condition of HMA and cerebral arteries was studied using Ultima PA ultrasound device (RADMIR, Ukraine) and Angiodin transcranial Doppler apparatus (BIOSS, Russia). While locating HMA, the study was performed on patency, structure, course, indicators of peak systolic velocity (PSV) and resistance index (RI) of common (CCA), internal (ICA), of external (ECA) carotid arteries, extracranial segments of vertebral (VA) arteries, the size of the intima-media complex (IMC) in CCA, indicators of and linear blood flow velocity (BFV) in middle cerebral (MCA), anterior cerebral (ACA), posterior cerebral (PCA), ICA siphons, intracranial segments of VA, basilar artery (BA). The control group (CG) consisted of 45 patients of the corresponding gender and age.

Statistical analysis and material processing were performed using the Statistic 6.0 software package. Differences recognized to be statistically significant at P <0.05. The study complies with the requirements of the Helsinki Declaration and is approved by the ethics commission of the Kharkiv Medical Academy of Graduate Education.

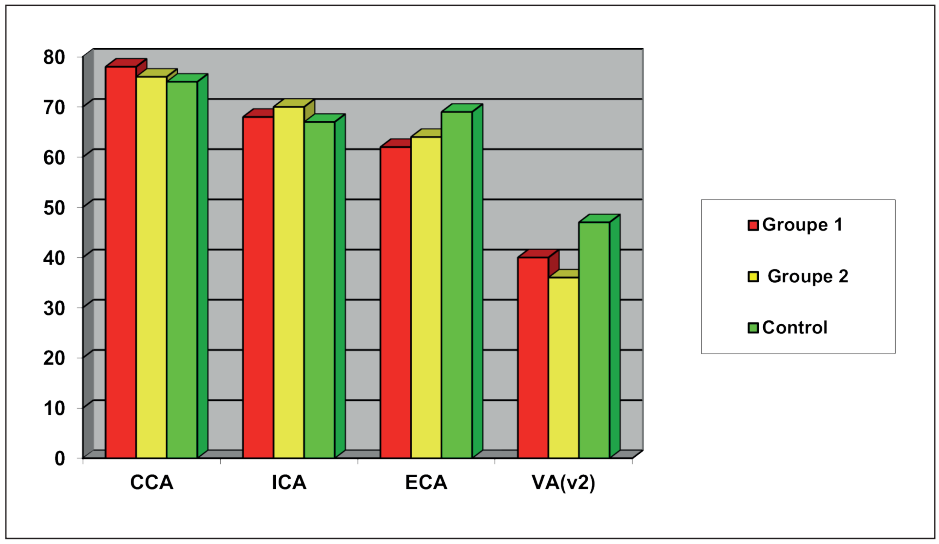


Fig. 1. The indicators of PSV in head magistral arteries in the patients with migraine

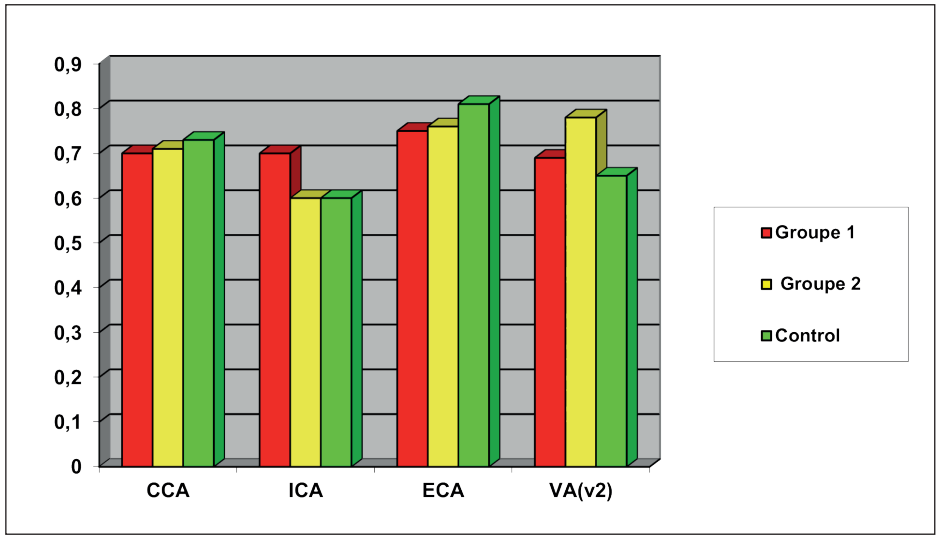


Fig. 2. The indicators of RI in head magistral arteries in the patients with migraine

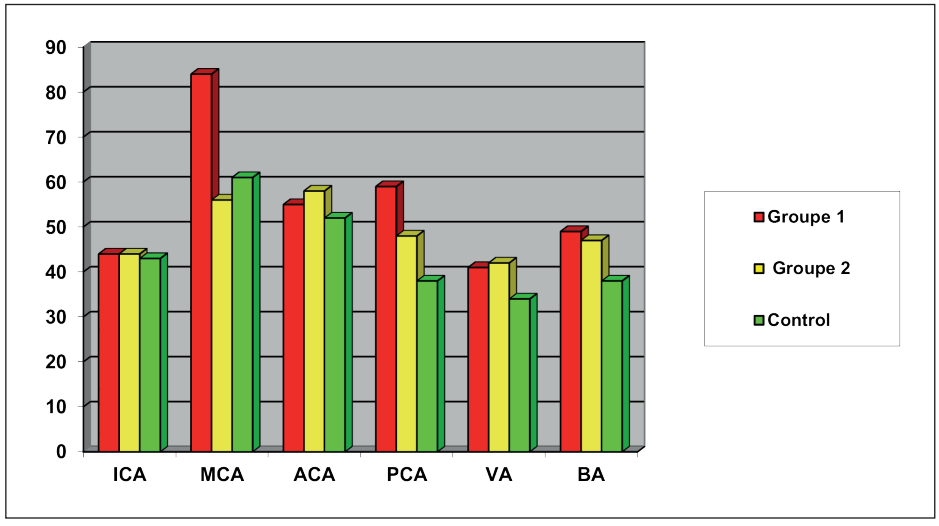


Fig. 3. The indicators of BFV in intracranial arteries in the patients with migraine

RESULTS

The most common patterns of the structural state of HMA were the thickening of the intima-media complex (group 1 – 14.3%; group 2 – 11.5%), the extravasal compression of vertebral arteries (group 1 – 23.8%; group 2 – 34.4%), the hypoplasia of vertebral arteries (group 1 – 6.3%; group 2 – 16.3%). In the patients of both groups, the values of PSV and RI values in the CCA and ICA did not differ from the standard

values. The RI values in the VA in the 1st group also did not differ from the norm. In the patients in the 2nd group, there was a decrease in the velocity values in VA (36.4 ± 9.5 cm/s; CG – 47.8 ± 10.4 cm/s), while the RI values in this group increased (0.78 ± 0.05, CG – 0.65 ± 0.06; p <0.05), also in this group, the majority of the patients had asymmetries in the flow velocity (25-30%) in VA. The PSV values in the patients of the 1st group were also increased. These changes in the velocity values are possibly associated with a higher prevalence of the tortuosity of the extravasal VA compression in the patients with migraine compared with the control group. The velocity and RI values in the external carotid artery ECA were slightly reduced in both groups. Figs. 1,2 show the PSV and RI indicators in HMA in the patients with migraine.

In the patients of the 1st group, the blood flow values in the ICA siphons did not differ or differed insignificantly from the CG data (44.6 ± 7.9 cm/s; CG – 43.4 ± 8.2 cm/s). Similar results were observed during the anterior cerebral artery (ACA) study (55,7 ± 5,4 cm/s; CG – 52.3 ± 6.7 cm/s). We observed significant differences from the CG in terms of BFV in MCA (84,7 ± 11,2 cm/s; CG – 62.6 ± 10,1 cm/s, p< 0,05). Also, significant differences from the CG were found in the velocity values in the PCA (59,6 ± 6,8 cm/s; CG - 36.5 ± 5,7 cm/s, p< 0,05). The flow velocity in the VA and the BA exceeded similar indicators in the CG, while these differences were not significant and were less pronounced than the changes in the MCA and PCA. (VA – 41,2 ± 7,2 cm/s; CG - 34.7 ± 9,1 cm/s, BA – 49,6 ± 6,1 cm/s; CG - 38.9 ± 8,4 cm/s). In the 2nd group in the ICA siphons there were no significant differences in the velocity of the blood flow vs the CG (44,8 ± 8,7 cm/s; CG - 43.4 ± 8,2 cm/s), the ACA values exceeded the reference values slightly (56,3 ± 7,7 cm/s; CG – 52.3 ± 6,7 cm/s). The velocity values in MCA were slightly reduced, in comparison with the CG (56,3 ± 7,7 cm/s; CG - 62.6 ± 10,1 cm/s, p< 0,05), the BFV in PCA was significantly higher than the CG values(48,3 ± 4,7 cm/s; CG - 36.5 ± 5,7 cm/s, p< 0,05). The flow velocity values in the VA and BA exceeded those in the CG (VA – 42,5 ± 6,6 cm/s; CG - 34.7 ± 9,1 cm/s, BA – 47,1 ± 7,5 cm/s; CG - 38.9 ± 8,4 cm/s). Fig. 3 show the indicators of BFV in intracranial arteries in the patients with migraine

DISCUSSION

The presence of extravasal compressions of vertebral arteries is typical for the patients with migraine, as well as for some cases of the hypoplasia of the vertebral artery in the group of the patients with migraine with aura. In the patients with migraine with aura, there was a decrease in the velocity values in the extracranial VA segments. The velocity and RI values in the ECA were slightly reduced in both groups, which is probably due to their expansion at the time of the attack and in the post-attack period. The most significant were the changes in the hemodynamics in the MCA, which were manifested by the pattern of the excessive perfusion in the patients of the 1st group and the pattern of the hampered perfusion in the MCA in the patients of the 2nd group. These conclusions are consistent with the results obtained in a number of studies (12,13,15, 19). We have shown for the first time the presence of the excessive perfusion in the PCA in both groups.

CONCLUSIONS

1. In the patients with migraine with aura, a decrease in the velocity values in the extracranial segments of the VA was observed, in some cases combined with the hypoplasia of the vertebral artery, the hampered perfusion in middle cerebral arteries.
2. The excessive perfusion in middle cerebral arteries is the most critical hemodynamic pattern in the patients with migraine without aura.
3. The extravasal compression of vertebral arteries, combined with the hyperperfusion in posterior cerebral arteries, is a typical hemodynamic pattern both in the group of the patients with migraine with aura, and in the group of the patients with migraine without aura.
4. The indicators of the structural and functional state of head magistral arteries and cerebral arteries in the patients with migraine with aura and without aura can be used to clarify the diagnoses and individualize treatment in these patients.

REFERENCES

1. Burstein R., Noseda R., Borsook D. Migraine: Multiple processes, complex pathophysiology. J Neurosci, 2015; 35: 6619–6629.
2. Goadsby P.J., Holland P.R., Martins-Oliveira M., Hoffmann J., et al. Pathophysiology of migraine: a disorder of sensory processing. Physiol Rev. 2017;97:553–622.
3. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia. 2018; 38(1): 1 – 211
4. Bose P., Goadsby P.J. The migraine postdrome. Curr Opin Neurol. 2016; 29: 299–301.F
5. Giffin N.J., Lipton R.B., Silberstein S.D. The migraine postdrome: an electronic diary study. Neurology. 2016; 87: 309–313
6. Lauritzen M. Cortical Spreading Depression in Migraine. Cephalalgia. 2001 21:7, 757-760
7. Stankewitz A., Aderjan D., Eippert F. Trigeminal nociceptive transmission in migraineurs predicts migraine attacks. J Neurosci. 2011; 31: 1937–1943.
8. Ashina M. Vascular changes have a primary role in migraine. Cephalalgia. 2012 32 : 5, 428-430
9. Asghar M.S., Hansen A.E., Amin F.M. Evidence for a vascular factor in migraine. Ann Neurol. 2011; 69: 635–645.
10. Charles A. The migraine Aura. Continuum (Minneap Minn). 2018;24:1009–22.
11. Hoffmann J., Baca S.M., Akerman S. Neurovascular mechanisms of migraine and cluster headache. J Cereb Blood Flow Metab. 2019;39(4):573–94.
12. Shayestagul N.A., Christensen C.E., Amin F.M. Measurement of blood flow velocity in the middle cerebral artery during spontaneous migraine attacks: a systematic review. Headache. 2017; 57: 852–861.
13. Cheng M.H., Wen S.L., Zhou H.J. Evaluation of headache and regional cerebral flood flow in patients with migraine. Clin Nucl Med. 2013; 38: 874–877.
14. Abdullaiev R.Ya., Kalashnikov V.I., Globa M.V., Posokhov N.F. Dopplerometric Parameters of Cerebral Blood Flow with Migraine. Trends Tech Sci Res. 2018; 1(5): 555574.

15. Hansen J.M., Schankin C.J. Cerebral hemodynamics in the different phases of migraine and cluster headache. J Cereb Blood Flow Metab. 2019;39(4):595–609.
16. Rajan R., Khurana D., Lal V. Interictal cerebral and systemic endothelial dysfunction in patients with migraine: a case-control study. J Neurol Neurosurg Psychiatr. 2015; 86: 1253–1257.
17. Hansen J.M., Schytz H.W., Larsen V.A. Hemiplegic migraine aura begins with cerebral hypoperfusion: imaging in the acute phase. Headache. 2011; 51: 1289–1296
18. Hougaard A., Amin F.M., Christensen C.E. Increased brainstem perfusion, but no blood-brain barrier disruption, during attacks of migraine with aura. Brain. 2017; 140: 1633–1642
19. Kassab M.Y., Majid A., BakhtarO., Farooq M. U., Patel K., Bednarczyk E.M. Transcranial Doppler measurements in migraine and nitroglycerin headache. J Headache Pain. 2007, 8:289-293. doi: 10.1007/s10194-007-0413-8
20. Ashina M., Tfelt-Hansen P., Dalgaard P. Lack of correlation between vasodilatation and pharmacologically induced immediate headache in healthy subjects. Cephalalgia. 2011; 31: 683–690
21. Charles A. Vasodilation out of the picture as a cause of migraine headache. Lancet Neurol. 2013; 12: 419–420

Primary and cervicogenic headache: hemodynamic, structural and functional changes according radiological methods.
State registration number: 0114 U 006460
Index UDK 616.857: [616.831-073.432.19:616.711.1-073.7]
Kharkov Medical Academy of Postgraduate Education, Ukraine

ORCID and contributionship:
Valeriy Kalashnikov: 0000-0002-7012-1698 [A, B, D]
Alexander Stoyanov: 0000-0002-3375-0452 [A,F]
Alexander Pulyk: 0000-0002-8717-047X [A,E]
Iryna Bakumenko: 0000-0002-9278-8936 [F]
Tamara Andreeva: 0000-0002-4357-320X [B,D]
Svitlana Oliinyk: 0000-0002-1197-1116 [B,F]

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

CORRESPONDING AUTHOR
Valeriy I. Kalashnikov
Department of Ultrasound Diagnostics of Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine
58, Amosova str. Kharkiv, Ukraine
tel: +380677057009
e-mail: dr.valkalash@gmail.com

Received: 23.05.2021
Accepted: 15.09.2021

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

NEUROPSYCHOLOGICAL PRINCIPLES OF COGNITIVE AND COMMUNICATIVE ACTIVITIES DIAGNOSIS IN ADULTS WITH EXTRAPYRAMIDAL SYSTEM DISORDERS

DOI: 10.36740/WLek202110124

Nataliya G. Pakhomova[1], Ivan M. Okhrimenko[2], Svitlana V. Kul'bida[3], Tamara A. Yanovska[1], Halyna M. Stanetska[4]
[1]POLTAVA V. G. KOROLENKO NATIONAL PEDAGOGICAL UNIVERSITY, POLTAVA, UKRAINE
[2]NATIONAL ACADEMY OF INTERNAL AFFAIRS, KYIV, UKRAINE
[3]NATIONAL ACADEMY OF EDUCATIONAL SCIENCES OF UKRAINE, KYIV, UKRAINE
[4]LVIV REGIONAL CLINICAL HOSPITAL, LVIV, UKRAINE

ABSTRACT

The aim is to determine the neuropsychological peculiarities of cognitive and communicative activities in adults with the extrapyramidal system disorders.

Materials and methods: The research was conducted during 2018-2021, during which a retrospective analysis of medical treatment records of the patients with extrapyramidal disorders of various etiologies was performed. The research involved 137 adult patients with extrapyramidal disorders: 93 persons with Parkinson's disease, 36 people with manganese encephalopathy, 5 persons with progressive supranuclear palsy and 3 people with Wilson-Konovalov disease.

Results: A significant difference between the indicators of preservation of cognitive and communicative activities and the communicative and semantic component in the group of patients with Parkinson's disease without speech disorders and Parkinson's disease and between the groups of patients with Parkinson's disease without speech disorders and progressive supranuclear palsy indicates the need for experimental correctional and rehabilitation work to restore cognitive and communicative activities of the patients with extrapyramidal disorders.

Conclusions: The most preserved communicative and speech function was found in the patients who had initial and mild stages of the disease, in particular in the patients with Parkinson's disease without speech disorders. It should be emphasized that the diagnosis of cognitive and communicative activities and the communicative and semantic component in adults with extrapyramidal disorders is a necessary prerequisite for the organization of the process of comprehensive rehabilitation treatment.

KEY WORDS: neuropsychology, neuropsycholinguistics, extrapyramidal disorders, cognitive processes, speech disorders

Wiad Lek. 2021;74(10 p.I):2494-2502

INTRODUCTION

Deep assessment and understanding of the current state of implementation of comprehensive medical and psychological, as well as logopedic treatment in the system of medical, psychological and pedagogical rehabilitation of patients with disorders of the central nervous system allows us to consider it as a promising direction, which involves the search for innovative methods and techniques of restoring their cognitive and communicative activities [1-3].

An important condition for ensuring effective correctional and rehabilitation work is the use of an integrative approach, namely: the interpenetration of knowledge from different fields (medicine, psychology, neuropsychology, psycholinguistics, etc.) to achieve the corrective goal [4-9].

Parkinson's disease (PD) is one of the common diseases of the extrapyramidal system. This disease is considered as a neurological condition that occurs due to neurodegenerative changes in the brain. This condition can be hereditary or acquired due to age-related changes or adverse environmental influences, but the causes of PD are still unknown. The analysis of existing research dedicated to the study of this pathology showed that it develops in 40-60 years old people, sometimes earlier, resulting in the disappearance of pigmented dopaminergic neurons of the substantia nigra, which are involved in the transmission of impulses from the brain to the muscles and promote smoothness of movements [10].

The patients with PD have disorders of cognitive activity and verbal communication along with motor disorders; they can also have depression and vivid dreams lasting up to 5 years or more. Motor disorders develop later in the form of bradykinesia, muscle rigidity, rest tremor and postural instability. Symptoms appear on one side of the body first proceeding with the other part within 2-3 years. The main symptoms that indicate the progression of PD include: severe bradykinesia, rigidity, ambulation disorder, parkinsonian posture, disorders of speech, swallowing, intellectual disabilities, depression, dyskinesia, falls and imbalance, changes in handwriting (micrography), stiffness when walking, the face becomes mask-like, etc. [10-12]. Based on a review of the results of a study of patients with PD, it was found that 89% of such individuals have speech or voice disorders, including

disorders of laryngeal, respiratory and articulatory functions and only 3-4 % of them undergo logopedic therapy. People suffering from PD and their families consider restriction in communication to be one of the most difficult consequences of PD due to the following factors: too quiet voice (hypophony), low tonal range (monotonous voice), hoarse voice, blurred articulation, and decreased facial expressions (hypomimia) [12, 13].

The main clinical manifestations of PD and methods of its treatment have been studied and described in the works many scientists [14, 15]. The scientists emphasize that speech disorders are the main symptoms and signs of the extrapyramidal system disorders. Therefore, the issue of diagnosis of cognitive and communicative activities disorders in case of extrapyramidal pathology requires consideration of neuropsychological and neuropsycholinguistic approaches.

Aspects of the functioning, problematics and comprehensive rehabilitation of people with extrapyramidal disorders have always been in the field of view of scientists of different centuries and different fields of knowledge. Thus, numerous investigations of such doctors as neuropathologists, therapists, rehabilitation specialists, etc. are devoted to the restoration of motor functions and the issues of medical treatment and physical rehabilitation of various patients with disorders of the central nervous system [16-18]. All studies of the organization of the correctional and rehabilitation process indicate the need to create special conditions for the restoration of cognitive and communicative function in people with extrapyramidal pathologies. However, the method of psychological and logopedic therapy for adults with extrapyramidal disorders, taking into account the symptoms and signs, severity and complication of the disease was not the subject of special scientific research.

The systematic analysis of the scientific papers shows that, despite the significant developments in the field of rehabilitation, the problem of a comprehensive approach to psychological and pedagogical as well as logopedic therapy for people with extrapyramidal disorders in the process of correctional and rehabilitation work is insufficiently disclosed.

Extrapyramidal motor disorders are the basis of the clinical manifestation of various neurodegenerative diseases with a predominant lesion of the subcortical basal ganglia. Numerous results of studies conducted by various scientists have shown that adults are more likely to have disorders of higher mental functions (memory, attention, imagination, thinking, speaking) and behaviour [15, 19, 20]. The appearance of such disorders indicates the first signs of the disease associated with disorders to the extrapyramidal system. Given that speech activity is an important human achievement that provides one of the main aspects of communicative function, the process of its recovery and normalization should be considered as a process of personal correction according to its individual capabilities, ensuring the success of social rehabilitation and return to normal life.

Deterioration of memory, attention, physical well-being, speech, etc. has been found to be characteristic of a number of diseases, including Alzheimer's disease, Parkinson's disease, and symptomatic forms of parkinsonism, including progressive supranuclear palsy (PSP) and Wilson-Konovalov disease (WKD). All these diseases are based on degeneracy caused by cerebral affection, which is characterized by disorders of mnestic and other cognitive spheres, including speech, spatial orientation, abstract thinking, which leads to complications in everyday life and professional activities [3].

PSP is a neurodegenerative disease caused by damage to the subcortical and stem structures, which is manifested by postural instability, resulting in numerous falls, supranuclear ophthalmoparesis, pseudobulbar syndrome (dysarthria, dysphonia, dysphagia) and dementia of the subcortical-frontal type. Progressive supranuclear palsy is often referred to as atypical parkinsonism, which develops in 1 case per 10 cases of Parkinson's disease, and the annual incidence ranges from 0.3 to 1.1 cases per 100 000 population. PSP is also known as Steele-Richardson-Olszewski syndrome and most often develops in adults after the age of 45 [21, 22]. PSP was first described in 1963-1964 by neurologists, who conducted a detailed clinical and pathomorphological analysis of seven cases of a previously unknown neurodegenerative disease [22]. The cases of PSP have been described in the scientific literature before, but have been misinterpreted as manifestations of postencephalitic parkinsonism, where PSP is characterised by both clinical and pathomorphological similarities. The primary signs of the disease are postural instability with frequent falls (60 % of cases), dysarthria (33 %), slowness of movement (13 %) and visual disturbances (13 %). Much less often, the primary signs of PSP are dysphagia and stiffness when walking. This disease is manifested by paresis of the vertical gaze, akineticorigid syndrome, the development of postural instability with frequent falling back, pseudobulbar syndrome (dysarthria, dysphonia, dysphagia) and dementia of the frontal type [23].

Hypomimia is developed in patients with PSP, which results in masked facies. Due to the spastic tension of facial muscles, facial features are not smoothed, as is often the case with Parkinson's disease, but exacerbated. In this case, the face acquires a characteristic expression, which can be described as "surprise". Motor and speech perseverations, palilalia, echopraxia, echolalia are manifested. Examination of persons with extrapyramidal disorders revealed that such patients have speech disorders, manifestations of pseudobulbar syndrome (dysarthria, dysphonia, dysphagia), in particular, there is a decrease in speech activity, difficulty initiating and understanding speech. Cognitive and emotional disorders were moderate [24]. The authors agree that the main signs of the disease are ambulation disorder, balance disorder, falling back, rocking in the process of walking, body stiffness, slowness of movement, impaired eye motility, along with speech disorders, swallowing and cognitive impairment.

As for WKD, it is an inherited progressive disease with an autosomal recessive type of inheritance, the pathogen-

esis of contraction and progression of which foresees a violation of copper metabolism, which affects the central nervous system, is accumulated in brain tissue and internal organs. It is manifested as neurological and / or psychiatric symptoms and lesions of the kidneys, liver and cornea (Kayser-Fleischer ring) [25]. The symptoms of the disease most often appear when a person is from 5 to 30 years old. This disease requires mandatory medical treatment, without the use of which a fatal case occurs in 5-7 years.

Studies [26] have shown that the causes of the disease are a mutation in ATP7B, P-type ATF protein gene that transports copper cations. One atypical copy of this gene is present in 1 in 100 carriers of the disease. A child can develop Wilson's disease only if he inherits the disease gene from both parents. Usually the symptoms begin to appear at the age of 6 to 20, but cases have been described in much older people. Wilson's disease is found in 1-4 out of 100 000 people. The problems that have arisen in patients with Wilson's disease have hardly been studied in the direction of providing psychological and pedagogical assistance.

The acquired disorders that lead to the presence of movement disturbances (extrapyramidal disorders in general) include the excessive use of harmful chemicals. It has been established that the use of surrogate drugs obtained by treating medical preparations with potassium permanganate can lead to severe organic brain damage caused by the toxic effects of manganese compounds. Excess of manganese in the body leads to loss of appetite, hallucinations, memory impairment, atony, fatigue, depression, encephalopathy, muscle atrophy, the risk of parkinsonism.

Manganese belongs to a neurotropic poison that can cause the most severe forms of occupational neurotoxicosis. A feature of the pathogenesis of manganese intoxication is its tropism to the extrapyramidal (striopalidar) system. Manganese disrupts the metabolism of biogenic amines, a number of enzymes, inhibits adrenoreactivity and activates the cholinoreactivity of the nervous system, increases the content of acetylcholine in the synapses of the subcortical nodes and the hypothalamus. The main thing concerning the toxic effect of manganese is a violation of the synthesis and deposition of dopamine, resulting in impaired muscle tone, accuracy, agility and smoothness of voluntary movements [27].

The accumulation of manganese in the brain structures (pale bullet and substantia nigra) disrupts functioning of the cortico-subcortical pathways and pallid-stem ligaments, which leads to the development of a complex combination of motor and neuropsychological disorders. Parkinsonism is the leading syndrome of chronic manganese intoxication. A rapidly increasing pseudobulbar syndrome with the development of severe hypokinetic-dystonic dysarthria is often the first manifestation of manganese encephalopathy. Cognitive disorders such as bradyphrenia, decreased attention, tendency to perseveration are often observed.

There are three degrees of severity of surrogate drug poisoning as in the case of chronic manganese poisoning. Mild degree is characterised by the patient's slight postural instability, focal dystonia, mild manifestations of pseudobulbar syndrome in the form of mild dysarthria and dystonia. Moderate degree is characterised by moderate postural instability, moderate multifocal dystonia and moderately manifested pseudobulbar syndrome, characterized by decreased voice volume, impaired pronunciation and monotony of speech. Severe degree causes a pronounced disability i. e. intense postural instability with very frequent falls, pronounced multifocal dystonia, which significantly impairs the gait and other motor functions of the patient, a pronounced pseudobulbar syndrome and severe dysarthria, which leads to speech loss. Thus, the analysis of existing studies on the causes and symptoms of disorders of the extrapyramidal system makes it possible to state that numerous works by scientists in various fields have been devoted to the study of disorders of cognitive processes and speech. However, insufficient attention has been paid to the problem of comprehensive research of cognitive and communicative activities in patients with extrapyramidal pathology using neuropsychological, psycholinguistic and neuropsycholinguistic approaches.

Therefore, various disorders of the cognitive and communicative system are manifested depending on the severity and the form of extrapyramidal disorders, which requires a detailed study of symptoms and signs, clinical manifestations in order to work out ways and directions of correctional and rehabilitation work and develop special methods for comprehensive rehabilitation taking into account medical, neuropsychological, psycholinguistic and neuropsycholinguistic approaches.

THE AIM

The aim of this study is to determine the neuropsychological peculiarities of cognitive and communicative activities in adults with the extrapyramidal system disorders.

MATERIALS AND METHODS

Participants. During 2018-2021, we conducted an experimental research of 137 adult patients with extrapyramidal disorders who were examined and treated in the neurology department and the advisory clinic department involving 93 people with Parkinson's disease, 36 patients with manganese encephalopathy (ME), 5 people with progressive supranuclear palsy and 3 people with Wilson-Konovalov disease.

Methods. The research is based on neuropsychological and neuropsycholinguistic approaches, which involve a systematic analysis of the brain and psyche in unity and interdependence [28]. It allows you to correlate the maturity and integrity of brain structures with the formation and preservation of mental functions involved in various human activities. Since cognitive processes and speech are forms of mental activity carried out on the basis of appropriate motives, regulated by appropriate goals and programs and subject to all laws of mental activity, the process of their recovery and normalization is the main

goal of comprehensive rehabilitation and social integration of patients with central nervous system (CNS) disorders.

To achieve the purpose of the research it was necessary to solve the following tasks:

– to identify neuropsychological peculiarities of cognitive and communicative functions in various extrapyramidal pathologies;
– to investigate the state of preservation of cognitive and communicative activities;
– to carry out a complex assessment of the revealed violations of the basic preconditions of communicative and speech activities (neuropsychological bases);
– to conduct a thorough study of cognitive and communicative activities as well as its speech and semantic component, to characterize them in order to organize effective correctional and rehabilitation work.

The first stage – diagnostic-clinical – involved acquaintance with the patients' medical cards, conclusions of neurologists about the results of clinical researches and the established diagnoses about extrapyramidal disorders. In addition, general clinical examinations, otolaryngological examinations, computed tomography and magnetic resonance imaging, and other medical examinations were performed as prescribed by a physician.

The second stage – neuropsychological and pedagogical – involved a neuropsychological and neuropsycholinguistic examination in order to study disorders of cognitive activities as well as expressive and impressive speech and to identify speech and non-speech disorders in adults with extrapyramidal disorders. The quality of cognitive and communicative function was assessed audioperceptively in the process of the conversation with the patient. The purpose of the second stage of the experiment was to assess the performance of tasks according to diagnostic methods and determine the level of preservation of neuropsychological bases of cognitive and communicative activities and basic speech characteristics (fluency, intelligibility, respiration, speech rate, etc.).

The neuropsychological research was performed using the MMSE (Mini-Mental State Examination) scale described in the examination of patients with extrapyramidal disorders and presented in many scientific sources. This scale of assessment of mental status was used to assess cognitive functions due to its comprehensiveness, as it covers its main components. Respondents were offered tasks consisting of 30 points, which were divided into groups according to the studied cognitive components: time orientation, spatial orientation, perception, attention, arithmetic, memory, speech (nominative function, impressive and expressive speech, understanding of complex instructions, reading, writing, etc.). This examination is recommended for the diagnosis of adult patients with disorders of the extrapyramidal system, as it allows to identify the impact of cognitive functions disorders, comprehension of addressed and written speech. Assessment of the level of preservation of cognitive processes during testing is indicated by the authors as a characteristic of patients according to their type i. e. attentive, sleepy, in a stupor, in a coma. The results of the tasks were assessed according to the scoring system: 29-30 points – no cognitive disorders, 24-28 points – moderate cognitive disorders (slight neuro-cognitive impairment), 20-23 points – mild dementia (medium neuro-cognitive impairment), less than 19 points – severe dementia (pronounced neuro-cognitive impairment).

Therefore, the examination consists of two blocks. The first provides voice answers related to orientation, memory, attention and it is rated at a maximum of 21 points. The second block determines the ability to name objects, perform oral and written commands, spontaneously write sentences, copy a picture of a complex geometric figure and it is rated at 9 points. The total maximum score is 30 points.

This test takes very little time to perform and includes answers to 11 questions and is therefore especially practical for one-time and serial examinations. It is possible to draw conclusions about the patient's cognitive ability on the grounds of this test.

Thus, based on neuropsychological and neuropsycholinguistic approaches the indicators of preservation of cognitive and communicative activities are determined as follows: cognitive activity, preservation of cognitive activity, comprehension of addressed speech, carrying out elementary verbal instructions, preservation of writing skills. The communicative and semantic component defined by us acquires special significance for full-fledged realization of communicative and speech activity. The study of the communicative and semantic component of communicative activity involved the implementation of tasks to study the state of preservation of the main components of speech activity (lexical-grammatical, syntactic, etc.). The indicators of preservation of the communicative and semantic component are the ability to maintain conversation, understand and use lexical and grammatical constructions of varying complexity, talk about interesting (monologue speech), communicate on different topics, culture of speech behaviour, communication initiative, use of different forms of speech activity.

Statistical analyses. We used the coefficient of rank correlation according to the bilateral Student's t-test (for independent, unrelated samples) in order to compare the results of the research. The results were considered reliable at $p < 0.05$.

Ethics approval. The research was performed according to the requirements of the Regulations on Academic Honesty of Poltava V. G. Korolenko National Pedagogical University, which were developed on the basis of Ukrainian and world experience of ethical rulemaking. This document was approved by the Academic Council of Poltava V. G. Korolenko National Pedagogical University (Protocol No. 2 of 10.09.2020) and implemented by the order of the Rector of the University (Order No. 1098 of 10.09.2020). According to its provisions, the members of the scientific community are guided by the rules of ethical conduct and professional communication; respect the principles, values, norms, rules, and conditions of academic honesty in their activities. The consent to participate in the research was obtained from all participants.

RESULTS

The results of the research of cognitive and communicative activity in 36 patients with manganese encephalopathy (namely the assessment of memory and cognitive processes) on Mini-Mental State Exam scale of mental status of patients revealed that the functions of all patients concerning their orientation in time and space, memory, the ability to concentrate and remember the events that happened to them before, the ability to perform a three-stage task, reading and writing a simple sentence were preserved almost completely.

That is, cognitive functions in patients with manganese encephalopathy were maintained at the level of a healthy person in the range of 27-30 points on the MMSE scale.

Almost all patients had hypomimia combined with a constant smile on their face associated with dystonia, reminiscent of a natural joyful expression ("Manganese Mask"). Patients showed micrography i. e. the size of the letters when writing became reduced. Severe dysarthria was developed in 28 patients (77.8 %). There was a feeling of tightness in the throat. Forced involuntary manganese laughter was observed in most patients. Lip movements were reduced with a characteristically frozen (not mobile, "frozen") upper lip. The movements of the tongue were slow and jerky (intermittent). Six patients (16.7 %) had moderate salivation and only four (11.1 %) had minor difficulty in swallowing.

Hypokinetic dysarthria was observed in 16.7 % of patients, and hypokinetic dysarthria with dystonic component (with varying degrees of muscle tightness and compression) was observed in 72.2 % of the patients. Four patients (11.1 %) had a persistent dystonic voice without a hypokinetic component. Two patients (5.6 %) had mutism with complete loss of speech volume and speech initiation. Palilalia and the spastic component were identified during the pronunciation of individual words ("YES" – "NO"), which required great effort.

The research included 93 patients with Parkinson's disease, with the average age of 65.5 ± 0.75 years old ranging from 31 to 83. There were two relatively young patients (31 and 36 years old) and five patients older than 75 among those involved in the research. The patients were offered to perform tasks also on the MMSE scale in order to determine the level of preservation of their cognitive and communicative activities.

Diagnosis of memory and cognitive functions using the MMSE scale revealed that memory of the patients with Parkinson's disease was reduced to 25-29 points at a rate of 30 points, indicating mild cognitive impairment. We found 28 (30.1 %) patients without pronounced speech disorders and 65 (69.9 %) patients with pronounced speech pathology among the examined patients with Parkinson's disease. We selected these groups as separate for the sake of purity of the experiment. Moderate neurocognitive disorders were diagnosed in 9 people (13.9 %), mild neurocognitive disorders were found in 35 people (63.8 %) and a high level of preservation of cognitive processes was found in 21 people (32.3 %) in the group of patients with Parkinson's disease having pronounced speech disorders. A high level of cognitive and communicative tasks performance was found in 20 people (71.4 %) and a mild neurocognitive disorder was found in 8 people (28.6 %) in the group of patients with Parkinson's disease without pronounced speech disorders (28 people). The results of tasks performance on the cognitive and communicative criterion confirm a certain interdependence of the state of cognitive processes and speech activity. The level of preservation of cognitive processes is higher in the group of people with Parkinson's disease without pronounced speech disorders than in the group of people with PD with speech disorders (SD).

MMSE scale for assessing the mental status of patients was offered to determine the preservation of cognitive and communicative activities in the process of examining of 5 patients (2 men and 3 women) with progressive supranuclear palsy aged 48, 60, 62, 68, 72. It was found that all patients have a mild neurocognitive deficit (25-26 points) according to the results of the tasks performance.

The patients with PSP were diagnosed with impaired memory and concentration and writing quality. A score of 26 points indicates cognitive impairment in the patient.

The diagnosis of cognitive and communicative activities in three patients with Wilson-Konovalov disease: one patient was born in 1992, the second one was born in 1994 and the third patient was born in 1979. These are quite young people who have significant problems in communication at the social level.

The diagnosis of cognitive and communicative activities of two patients (66.7 %) with Wilson's disease (according to the MMSE scale) revealed a high level of preservation of cognitive processes (30 points), and one patient showed reduced level (27 points) (33.3 %), indicating a mild neurocognitive impairment.

The study of the preservation of cognitive and communicative activities revealed that most patients with extrapyramidal pathology have a high and sufficient level of preservation of cognitive functions. Most patients with Parkinson's disease (77.7 %) have mild and moderate neurocognitive impairment. Generalized results of preservation of cognitive and communicative activities of all patients with extrapyramidal disorders are presented in Table I.

The research revealed that the preservation level of cognitive and communicative activities is significantly lower in patients with extrapyramidal pathology, in particular with PSP, PD, WKD and ME than the patients with Parkinson's disease without speech disorders. The category of these patients is very heterogeneous in terms of cognitive and speech activities. The following levels of preservation of cognitive and communicative activities of patients are revealed: high, sufficient, medium and low. The coefficient of rank correlation by Student's t-test between the experimental groups is 1.987, 2.04, 2.045 at a significance level of $\alpha = 0.05$.

The generalized results of tasks performance on the communicative and semantic component in patients with manganese encephalopathy allowed to establish that 3 (8.3 %) patients have a high level, the majority of patients

Table I. The state of preservation of cognitive and communicative activities

Levels of cognitive and communicative activities	Experimental groups									
	Persons	%	Persons	%	Persons	%	Persons	%	Persons	%
	Persons with ME 36 people		Persons with PD 65 people		Persons with PD without SD 28 people		Persons with PSP 5 people		Persons with WKD 3 people	
High (age norm)	23	63.8	21	32.3	20	71.4	-	-	2	66.7
Sufficient (mild neurocognitive impairment)	13	36.2	35	63.8	8	28.6	5	100	1	33.3
Medium (moderate neurocognitive impairment)	-	-	9	13.9	-	-	-	-	-	-
Low (severe neurocognitive impairment)	-	-	-	-	-	-	-	-	-	-

Table II. The state of preservation of communicative and semantic component in patients with extrapyramidal pathology

Levels of communicative and speech activities	Experimental groups									
	Persons	%	Persons	%	Persons	%	Persons	%	Persons	%
	Persons with ME 36 people		Persons with PD 65 people		Persons with PD without SD 28 people		Persons with PSP 5 people		Persons with WKD 3 people	
High	3	8.3	5	7.7	10	35.7	-	-	-	-
Sufficient	29	80.6	12	18.5	15	53.6	2	40	3	100
Medium	4	11.1	18	27.7	3	10.7	3	60	-	-
Low	-	-	30	46.1	-	-	-	-	-	-

29 (80.6 %) have a sufficient level, 4 (11.1 %) patients have a medium level, no patients with a low level.

Certain patients had difficulties in performing tasks that focused on studying the level of understanding and use of lexical and grammatical as well as syntactic constructions of medium and high complexity. The patients did not always recreate the story without the help of an experimenter. There were errors in cause-and-effect relations in the narrative.

Analysis of the results of the tasks performance according to the communicative and semantic criterion of 65 patients with PD revealed that 5 (7.7 %) patients are characterised by a high level, 12 (18.5 %) patients – by a sufficient level, 18 (27.7 %) patients – by a medium level and most patients 30 (46.1 %) – by a low level. Most patients had difficulties in understanding logical and grammatical constructions with direct and inverted meaning, in performing tasks for semantic analysis and synthesis, in reproducing the text and creating a story. The generalized results of the tasks performance according to the communicative and semantic criterion of 28 patients with the initial stage of PD allowed to establish that 10 (35.7 %) patients are characterised by a high level, most patients 15 (53.6 %) – by a sufficient level, 3 (10.7 %) patients – by a medium level and no patients were detected with a low level. No communication and speech disorders were detected in these patients. Speech activity had a somewhat slow nature, but in general a high and sufficient level according to the communicative and semantic criterion.

The results of the tasks performance according to the communicative and semantic criterion of 5 patients with PSP allowed to establish that 2 (40 %) patients are characterised by a sufficient level, 3 (60 %) patients – by a medium level and no patients were detected with high and low levels. Such patients showed no significant disorders of communicative and speech activities according to the communicative and semantic criterion. The analysis of the results of the tasks performance allowed to establish that all 3 patients with WKD were characterised by a sufficient level of preservation of speech according to the communicative and semantic criterion.

The levels of preservation of the communicative and semantic component in the patients with extrapyramidal disorders were identified according to the results of the tasks performance (Table II).

Thus, a certain correspondence has been revealed for all patients with extrapyramidal pathology between the levels of preservation according to the communicative and semantic criterion as well as the severity and degree of the patient's disease, its duration. It was established that extrapyramidal disorders cause cognitive and speech disorders that are common to all such patients. The com-

municative and semantic component of speech activity is most impaired in patients with Parkinson's disease, which confirms the need for specially organized correctional and rehabilitation treatment to restore communicative and speech activities.

The results of the experimental research have proved the expediency of application of comprehensive diagnostics of cognitive and communicative activities. Significant differences between the indicators of preservation of cognitive and communicative activities in the group of patients with Parkinson's disease without speech disorders and with Parkinson's disease and between the groups of patients with PD without speech disorders and with PSP indicate the need to organise experimental correctional and rehabilitation work to restore cognitive and communicative activities of the patients with extrapyramidal disorders.

DISCUSSION

The revealed neuropsychological peculiarities of cognitive and communicative activities of the patients with extrapyramidal disorders, in particular with PD, manganese encephalopathy, PSP and WKD are characterized by the presence of psychophysiological, phonological, prosodic, articulatory and innervation, motorial, psychological, morphological, semantic and other groups of cognitive and speech disorders (dysfunction of the extrapyramidal system characterized by metabolic lesions of the basal ganglia or related structures of the speech-motor analyser, kinesthetic apraxia, lack of motivation for speech activity, etc.).

The results of the research of disorders of higher cortical functions (cognitive disorders) and olfactory functions in 20 patients with PD depending on the form, stage and duration of the disease revealed that such patients are characterized by slow cognitive processes, impaired visual and spatial perception, memory and olfaction at all stages of their disease. According to scientists [29], the presence of voice and speech disorders in patients may be a clinical tool for early detection of PD. At the same time other scientists note in their research that PD impairs cognitive functions such as memory, thinking, attention and speech, which correlate with the severity of the disease [10, 12, 17, 18, 30].

The results of our research allowed discovering the fact that the deterioration of memory, attention, physical well-being, speech, etc. is a characteristic feature of a number of diseases of the extrapyramidal system. The disorders of communicative and speech activities identified by us of prosodic, phonological, morphological and syntactic aspects in the process of speech generation are manifested in impaired communicative function due to damage to extrapyramidal pathways, impaired motor coordination and innervation of the articulatory apparatus characterised by hypokinesia, hypomimia, bradykinesia, rigidity which reduce speech activity. These peculiarities determine the search for optimal integrative medical, psychological and pedagogical approaches to the restoration and normalization of cognitive and speech activities in people with extrapyramidal disorders.

The developed method of diagnosing the state of preservation of cognitive activity and speech in extrapyramidal disorders should be considered as a necessary condition for the development of a unified comprehensive system of correctional treatment for persons with extrapyramidal pathology in the process of medical, psychological and pedagogical rehabilitation.

CONCLUSIONS

The comparative analysis of the research results on cognitive and communicative activities revealed that the experimental groups' patients with ME, with PD without speech disorders, PSP and WKD have mostly sufficient and high levels, the group of patients with PD who made up the vast majority of all patients with extrapyramidal pathology were characterised by mostly sufficient level (63.8 % – 35 persons), high in 32.3 % (21 persons), medium in 13.9 % (9 persons).

The communicative and semantic component of speech activity turned out to be most impaired in the patients with PD, in particular: a low level was found in 46.1 % (30 people), a medium level in 27.7 % (18 people), a sufficient level in 18.5 % (12 people) and a high level in only 7.7 % (5 people). Other patients with disorders of the extrapyramidal system with ME, with PD without speech disorders, PSP and WKD revealed mostly sufficient and high levels of preservation of the communicative and semantic criterion of communicative and speech activities.

Thus, in general, the most preserved communicative and speech function was found in the patients who had initial and mild stages of the disease, in particular in the patients with PD without speech disorders. It should be emphasized that the diagnosis of cognitive and communicative activities and the communicative and semantic component in adults with extrapyramidal disorders is a necessary prerequisite for the organization of the process of comprehensive rehabilitation treatment. The success and pace of recovery and normalization of cognitive and speech activities in patients with extrapyramidal disorders are stipulated by the stepping of content-related aspect of the correctional treatment in medical institutions and the need to create special integrative medical, psychological and pedagogical conditions for rehabilitation. The effectiveness of the restoration of communicative and speech activities in people with extrapyramidal disorders is determined by the comprehensiveness, integrativeness and communicative orientation of special treatment, taking into account neuropsychological, psycholinguistic and neuropsycholinguistic approaches and the use of tango therapy, kinesiotherapy, physiotherapeutic means, etc. They also depend on the motive, psycho-emotional state and immersion of the patient in the communicative and speech situation both during special classes and in everyday life, stimulation of communicative activity in social and domestic situations. The results of the experimental research confirmed the need to create a system of integrative psychological and pedagogical conditions for the restoration of communicative and speech activities of patients with extrapyramidal disorders on the basis

of modern comprehensive integration approaches to the process of correctional and rehabilitation work based on neuropsychological and neuropsycholinguistic concepts.

We see the **prospect of further research** in the development of experimental methods for the development of social and communicative skills in patients with extrapyramidal disorders.

REFERENCES

1. Boriak O., Pakhomova N., Okhrimenko I., Odynchenko L., Usyk D. Psycholinguistic research of speech activity of junior students with intellectual disorders. Applied Linguistics Research Journal. 2021; 5(6): 104-112. doi: 10.14744/alrj.2021.03360.
2. Mannapova K., Okhrimenko I., Tverdokhvalova I., Bychkova S., Makarenko P., Melnychuk V. Peculiarities of providing psychological assistance to abused children. BRAIN. Broad Research in Artificial Intelligence and Neuroscience. 2020; 11(2Sup1): 139-156. doi: 10.18662/brain/11.2Sup1/100.
3. Pakhomova N.G. Neyropsykholinhvistyka [Neuropsycholinguistics]. Poltava: ASMI. 2010. (in Ukrainian).
4. Aleksandrov D.O., Okhrimenko I.M., Serbyn R.A. Psychological factors of post-stress psychotraumatic feelings susceptibility of combanants' children. Science and Education. 2017; 9: 16-25. doi: 10.24195/2414-4665-2017-9-3.
5. Bloshchynskyi I., Griban G., Okhrimenko I. et al. Formation of psychophysical readiness of cadets for future professional activity. The Open Sports Sciences Journal. 2021; 14: 1-8. doi: 10.2174/1875399X02114010001.
6. Griban G., Yavorska T., Tkachenko P. et al. Motor activity as the basis of a healthy lifestyle of student youth. Wiad Lek. 2020; 73(6), 1199-1206. doi: 10.36740/WLek202006123.
7. Okhrimenko I., Boiko-Buzyl Y., Kisil Z., Katolyk H., Miloradova N. Main components of communicative activities of future legists with sense of loneliness. Applied Linguistics Research Journal. 2021; 5(4): 91-103. doi: 10.14744/alrj.2021.87360.
8. Okhrimenko I., Pasko O., Prudka L. et al. The influence of modern sports technologies on health and professional activity of law enforcement officers. Wiad Lek. 2021; 74(6):, 1365-1371. doi: 10.36740/WLek202106115.
9. Prontenko K., Griban G., Aloshyna A. et al. The physical development and functional state as the important components of the students' health. Wiad Lek. 2019; 72(12a): 2348-2353. doi: 10.36740/WLek201912115.
10. Levin O.S., Artem'yev D.V., Bril' Ye.V., Kulua T.K. Bolezn' Parkinsona: sovremennyye podkhody k diagnostike i lecheniyu [Parkinson's disease: modern approaches to diagnosis and treatment]. Prakticheskaya meditsina. 2017; 1 (102): 45-51. (in Russian).
11. Okhrimenko I., Hrebeniuk M., Borovyk M. et al. Sport classes as effective means for psychophysical health improvement of representatives of the security and defense sector. Wiad Lek. 2021; 74(5): 1142-1146. doi: 10.36740/WLek202105118.
12. Plowman-Prine E.K., Okun M.S., Sapieza C.M., Shrsvastan R., Fernandez H.H., Foote K.D. Perceptual characteristics of Parkinsonian speech: A comparison of the pharmacological effects of levodopa across speech and non-speech motor system. Neuro Rehabilitation. 2009; 24: 131-144.
13. Rusz J., Cmejla R., Ruzickova H., Ruzicka E. Quantitative acoustic measurements for characterization of speech and voice disorders in early untreated Parkinson's disease. The Journal of the Acoustical Society of America. 2011; 29(1): 350-367.doi: 10.1121/1.3514381.
14. Hartelius L., Svensson P. Speech and swallowing symptoms associated with Parkinson's disease and multiple sclerosis: A survey. Folia Phoniatrica Logopedia. 1994; 46: 9-17.
15. Trail M., Fox C., Ramig L.O., Sapir S., Howard J. Speech treatment for Parkinson's disease. Neuro Rehabilitation. 2005; 20: 205-221.
16. Brabenec I., Mekyska J., Galaz Z., Rektorova I. Speech disorder in Parkinson's disease: early diagnostics and effect of medication and brain stimulation. Journal of Neural Transmission. 2017; 124: 303-334.
17. Griban G., Kuznietsova O., Tkachenko P. et al. Formation of the students' volitional qualities in the process of physical education. International Journal of Human Movement and Sports Sciences. 2020; 8(6); 505-517. doi: 10.13189/saj.2020.080625.
18. Okhrimenko I., Lyhun N., Pryimak V., Korol Ya., Myroshnychenko M. Negative factors of management activities of the security and defence sector representatives and directions of their overcoming. Wiad Lek. 2021; 74(4): 891-895. doi: 10.36740/WLek202104115.
19. Tripoliti E., Strong L., Hickey F., Foltynie T., Zrinzo L., Candelario J. Treatment of dysarthria following subthalamic nucleus deep brainstimulation for Parkinson's Disease. Movement Disorders. 2011; 26(13): 2434-2436. doi: 10.1002/mds.23887.
20. Griban G., Lyakhova N., Tymoshenko O. et al. Current state of students' health and its improvement in the process of physical education. Wiad Lek. 2020; 73(7): 1438-1447. doi: 10.36740/WLek202007124.
21. Zhamardiy V., Shkola O., Okhrimenko I., et al. Checking of the methodical system efficiency of fitness technologies application in students' physical education. Wiad Lek. 2020; 73 (2), 332-341. doi: 10.36740/WLek202002125.
22. Richardson J., Steele J., Olszewski J.J. Supranuclear ophthalmoplegia, pseudobulbar palsy, nuchal dystonia and dementia. A clinical report on eight cases of heterogeneous system degeneration. Transactions of the American Neurological Association. 1963; 88: 25-29.
23. Litvan I., Agid Y., Jankovic J. Accuracy of clinical criteria for the diagnosis of progressive supranuclear palsy. Neurology. 1996; 46: 922-930.
24. Kluin K.J, Foster N., Berent S., Gilman S. Perceptual analysis of speech disorders in progressive supranuclear palsy. Neurology. 1993; 43: 563-569.
25. Ala A., Walker A.P., Ashkan K., Dooley J.S., Schilsky M.L. Wilson's disease. The Lancet. 2007; 369: 397-408. doi: 10.1016/S0140-6736(07)60196-2.
26. Senatorova A.S., Omel'chenko E.V., Uryvaeva M.K. Khvoroba Vil'sona-Konovalova [Wilson-Konovalov disease]. Zdorov'ya dytyny. 2012; 6(41): 201-205. (in Ukrainian).
27. Ellingsen D.G., Haug E., Ulvik R.J., Thomassen Y. Iron status in manganese alloy production workers. Journal of Applied Toxicology. 2003; 23: 239-247.
28. Pakhomova N.G., Baranets I.V., Pakhomova V.A., Scherban O.A., Boryak O.V. Comprehensive approach to the treatmentof motor alalia in preschool children. Svit Medytsyny ta Biolohiyi. 2021; 1(75): 125-129. doi: 10.26724/2079-8334-2021-1-75-125-129. (in Ukrainian).
29. Ciucci M.R., Grant L.N., Rajamanickam E.S.P. Early identification and treatment of communication and swallowing deficits in Parkinson disease. Seminars in Speech and Language. 2013; 34 (3): 185-202.
30. Pakhomova N.G., Pakhomova V.A. Intehratsiya medyko-psykholohichnykh i pedahohichnykh znan' v protsesi korektsiynoyi roboty [Integration of medical-psychological and pedagogical knowledge in the process of correctional work]. Naukovyy Chasopys Natsional'noho Pedahohichnoho Universytetu imeni Drahomanova. 2015; 30: 285-291. (in Ukrainian).

This scientific article is a fragment of a research project of the Poltava V. G. Korolenko National Pedagogical University for 2021-2024 "Psychology of personality development in the educational space" (state registration number 0119U002283).

ORCID and contibutionship:
Nataliya G. Pakhomova: 0000-0002-8332-8188 [A,F]
Ivan M. Okhrimenko: 0000-0002-8813-5107 [B,E]
Svitlana V. Kul'bida: 0000-0002-0194-3495 [C,A]
Tamara A. Yanovska: 0000-0002-7144-689X [D,B]
Halyna M. Stanetska: 0000-0003-1623-6459 [E,F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Ivan M. Okhrimenko
National Academy of Internal Affairs, Kyiv, Ukraine
tel: +380679903905
e-mail: ivango-07@ukr.net

Received: 03.06.2021
Accepted: 27.09.2021

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

FEATURES OF DENTAL STATUS AND METABOLISM IN CHILDREN WITH EARLY CHILDHOOD CARIES AGAINST THE BACKGROUND OF CONNECTIVE TISSUE DYSPLASIA

DOI: 10.36740/WLek202110125

Iryna I. Sokolova, Olena H. Yaroshenko, Svitlana I. Herman, Tetiana V. Tomilina, Karyna V. Skydan, Maxym I. Skydan
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim: To assess the dental status of infants suffering from connective tissue dysplasia, with the analysis of some aspects of hydrocarbon and amino acid metabolism (blood, urine) and internal organs status.

Materials and methods: 81 infants (aged 14 – 36 months) with multiple dental caries were examined. Among them 39 infants were suffered from connective tissue dysplasia.

Results: High prevalence of caries in infants against the background of connective tissue dysplasia compared to their peers in the control group ($p < 0.05$) is established: the caries intensity index and the caries intensity growth index are high in all age groups. Disorders of amino acid and carbohydrate metabolism were observed in infants of the main group. Thus, simultaneous increase of amino acids in the blood and urine was observed in 34 children of the main group in different age groups, and simultaneous increase of amino acids in the blood and urine and carbohydrates in the urine was observed in 25 children in different age groups. In infants of the main group the ultrasound examination of abdominal organs revealed changes in the liver, gallbladder, spleen, pancreas and kidneys.

Conclusions: When carrying out endogenous prophylaxis of dental caries in infants with connective tissue dysplasia, it is necessary to take into account the internal organs' status and thin-layer chromatography data of amino acids and carbohydrates in the blood and urine and to prescribe peroral drugs together with the doctors geneticists.

KEY WORDS: connective tissue dysplasia, infants, caries, level of hygiene, amino acid metabolism

Wiad Lek. 2021;74(10 p.I):2503-2509

INTRODUCTION

It's well known that children are the most vulnerable part of population [1]. It has been proved by many investigations that dental health is also highly influenced by geochemical condition of the region, somatic health, psychological status of the children and heredity [2, 3].

Hereditary amino acid metabolism occupies a special place in a wide range of genetically determined childhood pathologies [4-6]. This group of pathology is common and it is possibly due to the complexity of the protein metabolism biochemical organization, which also involves amino acids, that the probability of various metabolism units disruption is growing [7, 8].

In patients with congenital "metabolism error" there is a primary enzymatic defect and there is an increase in the level of one or more amino acids (AA) in the blood and / or urine, which leads to multiple disorders in functions of various organs and systems [9, 10]. Excess levels of AA or their metabolites cause toxic effects on the body and cause severe clinical disorders [11].

The connective tissue in the human body occupies a special place and is present in all organs and systems, accounting for more than 50% of the body weight. Most of the tissues in the maxillofacial area have also connective tissue origin. Connective tissue dysplasia (CTD) is a condition that is caused by structural changes in individual components of the connective tissue as a result of disorders in the embryogenesis process. CTD is a systemic process that is the basis for the development of pathology.

First of all, CTD is the basis of many structural and shaping changes in organs and systems that determine dysplastic-dependent disorders of functions [12-15]. Dysplastic-dependent changes in the internal organs are of greatest interest to researchers, as they determine the prognosis for the patients' lives. The presence of a "defective" connective tissue alters and reduces the body's ability to adapt when exposed to adverse environmental factors or changes in the conditions of the body's existence.

Many authors believe that the generalized form of CTD is more common. The main markers are changes in the valvular heart apparatus, hypermobility of the joints, pathology of the musculoskeletal system, skin hyperelasticity, anomalies in the structure of internal organs [6, 7].

Taking into account that different disorders of formation depend on the connective tissue condition in the body as a whole [7, 8], as well as following the principle of structural and functional unity of organs and tissues and given the high prevalence of connective tissue pathology, the study of its effect on the condition of hard of tooth tissue in younger children is relevant and timely.

Table I. Indices of the hard tooth tissues and oral hygiene status in infants against the background of CTD.

Age of infants, months	14-18		19-23		24-29		30-36	
Number of infants in groups, n	Control group	Main group	Control group	Main group	Control group	Main group	Control group	Main group
	10	7	12	8	7	7	13	17
Number of teeth in oral cavity, n	10.00± 0.96	10.00± 1.12	14.3± 1.38	14.00± 1.39	17.14± 0.27	17.14± 0.18	20.00± 0.00	20.00± 0.00
Index CF, points	1.7± 0.42	3.00± 0.92*	2.25± 0.41	3.00± 0.92*	3.27± 0.92	5.14± 1.80*	3.37± 0.85	6.29± 0.50*
CIGI index, points	0.15± 0.03	2.25± 0.59	0.15± 0.02	2.08± 0.59*	0.18± 0.04	2.34± 0.83*	0.16± 0.04	2.27± 1.18*
Carious teeth in oral cavity, %	15.64± 3.38	30.24± 4.83*	14.91± 2.09	22.83± 4.14*	19.2± 4.46	36.30± 9.35*	16.5± 4.26	33.0± 4.83*
Number of teeth with complicated caries (P and Pt), n	0	0	0	2.0± 0.0	0	2.41± 0.64	0	2.51± 0.89
Teeth health index (THI), points	0.04± 0.67	0.51± 0.04*	0.14± 0.02	0.49± 0.10*	0.11± 0.03	0.64± 0.13*	0.12± 0.04	0.59± 0.06*
Index interpretation	good	poor	satisfactory	poor	satisfactory	poor	satisfactory	poor

Notes: * – statistical difference between the main and the control groups.

Table II. Number of infants with CTD who have changes in abdominal organs.

Age of infants with CND	Number of infants	Number of infants with changes in				
		liver	gallbladder	pancreas	spleen	kidneys
14-18 months	7	7	7	2	1	6
19-23 months	8	8	8	3	2	7
24-29 months	7	7	7	6	1	7
30-36 months	17	17	17	11	2	15
Total	39	39	39	22	6	35

THE AIM

The aim of our study was to assess the dental status of infants suffering from connective tissue dysplasia, with the analysis of some aspects of hydrocarbon and amino acid metabolism (blood, urine) and internal organs status.

MATERIALS AND METHODS

The survey included 81 infants (aged from 14 to 36 months). The children were divided into two groups: the main and the control ones. The main group included 39 infants with multiple caries against the background of CTD. At the Department of Genetics and Prenatal Diagnosis of KhNMU (Doctor of Medical Sciences, Prof. O.Ya. Grechanyna), these infants were studied for the blood and urine carbohydrates and amino acids by means of thin layer chromatography (TLC) and ultrasound (U/S) diagnostics of internal organs. The diagnosis of "connective tissue dysplasia" was established by a geneticist for a selected contingent of infants. The control group included 42 infants of the similar age who were somatically healthy but had carious dental lesions.

All children were divided into four age groups, depending on the number of incised teeth. Thus, the first group included 7 infants in the main group and 10 in the control one at the age of 14 – 18 months, the second group – 8 infants in the main group and 12 in the control one at the age of 19 – 23 months, the third group included 7 infants in the main group and 7 – in the control at the age of 24 – 29 months, and finally the fourth group – 17 infants in the main group and 13-in the control at the age of 30 – 36 months.

The study was carried out in compliance with the basic bioethical standards of the World Health Association's Helsinki Declaration on Ethical Principles for Scientific and Medical Research and its materials were reviewed by the local Ethics and Bioethics Committee of Kharkiv National Medical University. Written informed consent was obtained from all participants (parents of infants).

All infants were determined their Temporal Tooth Caries Intensity Index (CF), the Temporal Tooth Caries Intensity Growth Index (CIGI) [16]. Additionally, all infants were assessed for hygienic condition of the oral cavity using the teeth health index (THI) according to the method of E.M. Kuzmina [17].

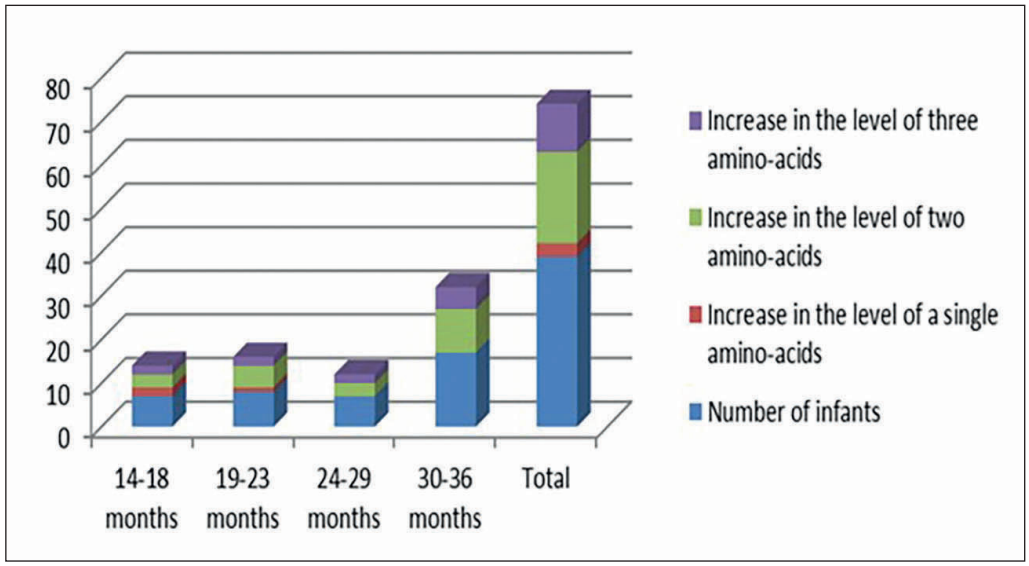


Fig 1. Amount of amino acids in the blood of the main group infants whose level is increased.

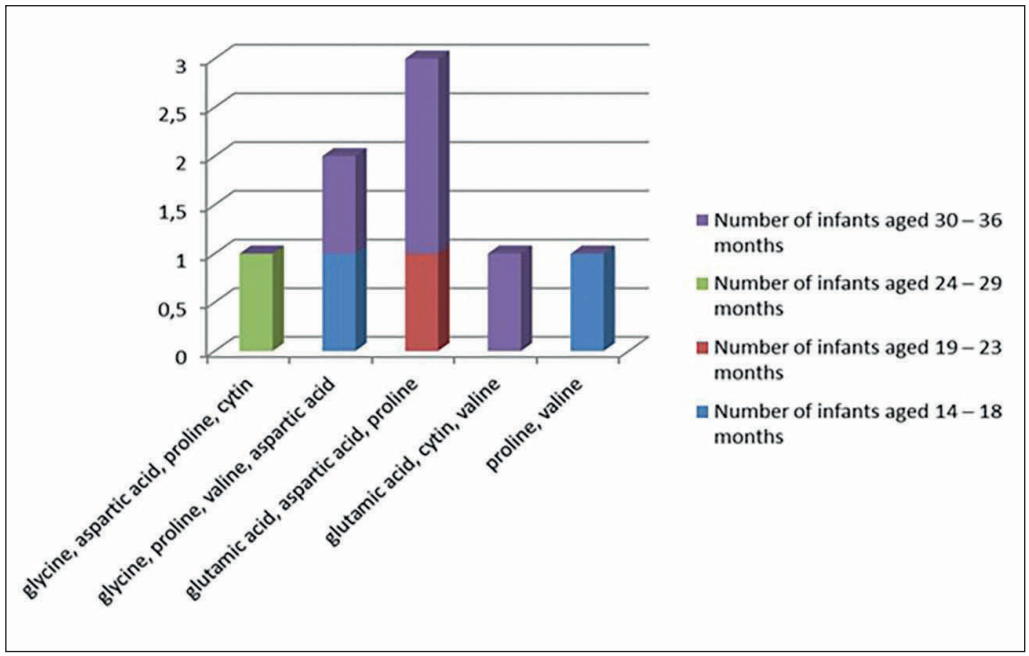


Fig 2. Increased amino acids level in the main group infants' urine.

RESULTS

The results of the studies indicate a high prevalence of caries in infants against the background of CTD. The caries intensity index (CF) is very high at all ages, and the caries intensity growth index (CIGI) is also high. As a result of the oral hygiene study in the selected contingent of infants, a high index of plaque was established, which indicates poor oral hygiene. All data are presented in table I.

As it can be seen from the data in table 1, the caries intensity index (CF) of temporary teeth has a very high level in infants of both the main and the control groups, especially in infants aged from 24 to 36 months. Besides, in all children with CTD, the index values were higher than those in children without the above pathology of amino acid metabolism. Thus, in infants aged 14-18 months, the intensity of caries was by 1.8 times higher than that in the control group ($p = 1.285$); in infants aged 19-23 months – by 1.3 times ($p = 0.744$); in infants aged 24-29 months – by 1.6 times ($p = 0.925$) and in those aged 30-36 months – by 1.9 times ($p = 2.961$) (table I).

The CIGI index for temporary teeth up to 0.4 points is considered low, from 0.5 to 0.8 points – average, from 0.9 to 1.2 points – high and above 1.3 points – very high. According to table 1, the CIGI index in infants of the main group aged 14-18 months is higher than that in infants of the control group by 15 times ($p = 3,554$); in infants aged 19-23 months – by 13.8 times ($p = 3.369$); in infants at the age of 24-29 months – by 13 times ($p = 2.599$) and in those at the age of 30-36 months – by 14.1 times ($p = 1.787$). Therefore, the CIGI index in the surveyed infants of the main group was on average by 1.7 times higher than the very high level of this index ($p < 0.05$) (table I).

The percentage of carious teeth in the main group infants aged 1 to 36 months was 30.59±5.58%, which is by 1.8 times

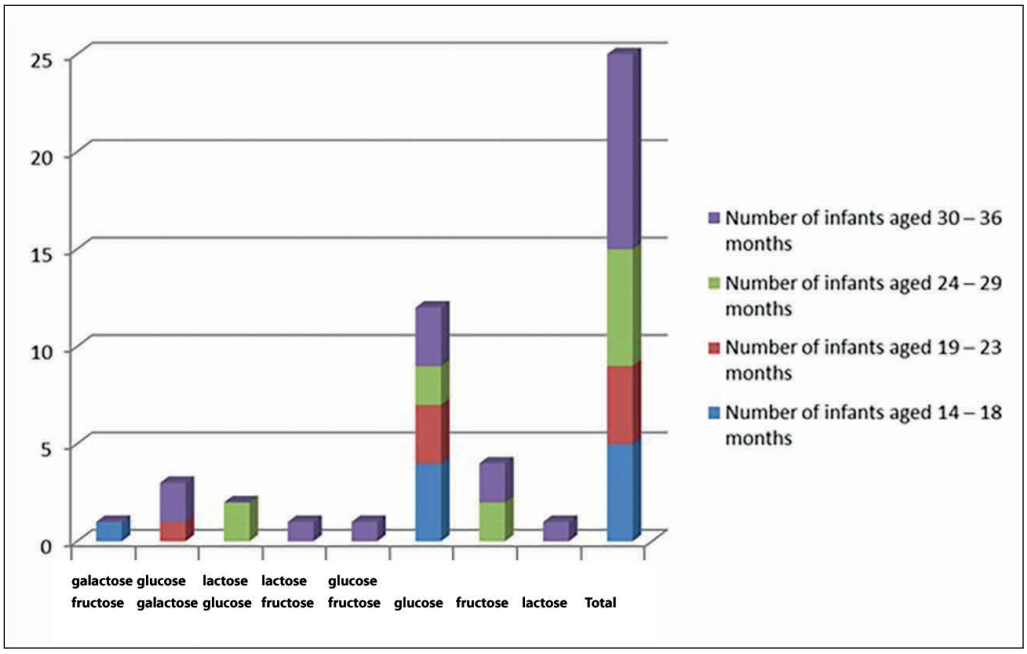


Fig 3. Increased carbohydrates level in the urine of the main group infants.

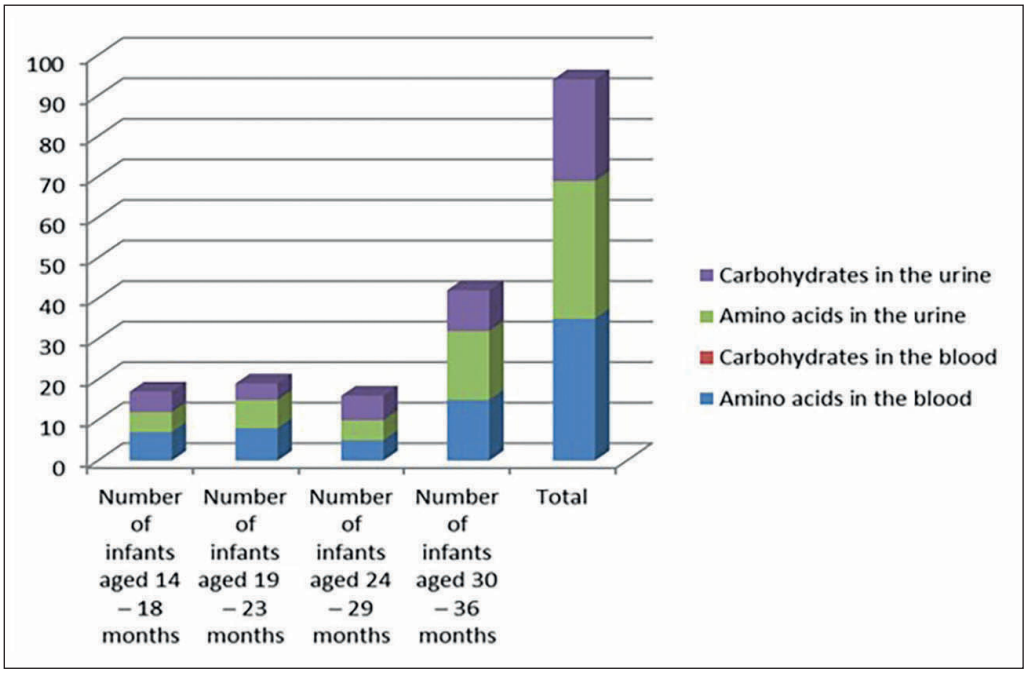


Fig 4. The number of children in the main group with a simultaneous increase in the level of amino acids and carbohydrates in the blood and urine.

higher than that in the control group (p = 2.476). Also in the main group of age categories such as 19-23, 24 -29 and 30-36 months there are already teeth lesions with chronic forms of pulpitis and periodontitis (table I).

With regard to oral hygiene, the main group infants of all ages were found to have its poor level (p <0.05). But it should be noted that in infants of the control group the level of oral hygiene is good only in the first age period, and in the last three age periods it was satisfactory (table I).

Also at the Department of Genetics and Prenatal Diagnosis of KhNMU, children with CTD were performed thin-layer chromatography (TLC) of the blood and urine amino acids and carbohydrates of (data are presented in fig.1, fig.2, fig.3, fig.4 and table II).

Data of the TLC studies on the blood amino acids in the main group infants indicate that 36 infants out of 39 showed an increase in amino acid levels (fig. 1). Thus, the level increase in a single amino acid in the blood (aspartic acid and glycine) was observed in three infants. A simultaneous increase in the level of two amino acids in the blood was observed in 21 infants. Increase in the level of two amino acids was in the folloing combination: glycine and glutamic acid; glycine and aspartic acid; glycine and proline; glycine and alanine; glutamic acid and aspartic acid; glutamic acid and alanine; aspartic acid and proline; aspartic acid and alanine; aspartic acid and valine; proline and alanine; proline and valine.

A simultaneous increase in the level of three amino acids in the blood was found in 11 infants. The increased amino

acids were in the following combination: glycine, proline, alanine; glycine, proline, valine; glutamic acid, aspartic acid, alanine; glycine, aspartic acid, proline.

An increase of the carbohydrates level in the blood was not observed.

With regard to amino acids in the urine (fig. 2), the result of the analysis indicates a simultaneous increase in their quantitative and variational indices. Thus, an increase in the levels of four amino acids such as glycine, aspartic acid, proline and cytin was observed in one infant in the third age period (24-29 months). An increase of four amino acids, but in another combination (glycine, proline, valine, aspartic acid) was observed in two infants in the first (14 18 months) and fourth (30 – 36 months) age periods. An increase in another combination of four amino acids (glutamic acid, aspartic acid, proline, valine) was observed in one child of the second age group (19-23 months) and two children of the fourth age group (30 – 36 months). An increase of three amino acids (glutamic acid, cytin, valine) was observed in one child of the fourth age group (30 – 36 months) and an increase of two amino acids (proline, valine) was observed in one child of the first age group (14-18 months) (fig.2).

The next item to be analyzed was the level of carbohydrates in the urine (fig. 3). We found that in the first age period (14-18 months) the increase in carbohydrate level was observed in five infants, in the second age period (19-23 months) – in four infants, in the third age period (24-29 months) – in six infants and in the fourth age period (30 -36 months) – in ten infants. Carbohydrates, the level of which was increased in the urine, were fructose, galactose, glucose, lactose (fig. 3).

Elevation of carbohydrates level in the urine of the main group patients was observed in 15 infants.

Therefore, changes in the urine were observed in all the 39 children of the main group and were manifested in an increase in the amino acids and carbohydrates level.

It should be noted additionally, that we also found a simultaneous increase in amino acids in the blood and urine observed in 34 children of different age groups, and a simultaneous increase in amino acids in the blood and urine, and carbohydrates in the urine observed in 25 infants of different age groups (fig. 4).

During the ultrasound examination of abdominal organs in infants with CTD changes were observed in the liver, gallbladder, pancreas, spleen and kidneys (table II).

As it is seen from table II, changes in the liver and gallbladder condition were observed in all the 39 infants suffering from CTD. The second place in the incidence of the changes detected during ultrasound study is occupied by the kidneys (35 infants out of 39), the third – by the pancreas (22 infants out of 39) and, finally, the last position belongs to the spleen (6 infants out of 39).

According to the ultrasound study, changes in the liver were manifested in the form of venous plethora, perivascular infiltration, moderate diffuse changes. Condition of the bile ducts was characterized by the presence of diffuse changes, bending of the gallbladder, hypotension; condition of pancreas and spleen – presence of reactive changes; and the state of the kidneys – by the presence of completely dysplastic metabolic changes, perivascular infiltration, pyelectasis of one or two kidneys.

DISCUSSION

Thus, our data on disorders of amino acid and carbohydrate metabolism indicate a significant disorder of metabolism, which is a symptom of some genetically determined connective tissue diseases, including connective tissue dysplasia [6, 10, 12].

In general, the results of our studies coincide with the opinion of other researchers on disorders of amino acid metabolism in hereditary connective tissue diseases. Thus, according to Smolnova TYu, Adamyan LV. (2013) the impairment of amino acid biosynthesis is the leading link in the formation of pathology of intermediate metabolism [13]. When the transport of amino acids is disturbed, various forms of genetically determined diseases can be observed – from asymptomatic to those with severe clinical manifestations. And the basis of increased excretion of amino acids in the urine, as a rule, is an impairment of tissue metabolism of amino acids or their transport at the level of cell membranes in the renal tubules [7].

The importance of amino acids in the metabolism of the human body is difficult to overestimate. Thus, alanine, aspartic acid, glutamic acid, proline, glycine – are substituted amino acids and in the biosynthesis of substituted amino acids, their carbohydrate part is formed from intermediates of glucose oxidation, which can affect carbohydrate metabolism. This is confirmed by the results of our studies and this coincides with the opinion of Hrechanina O.Ya. et al. [9]. Aspartic and glutamic amino acids are important in the body's metabolism, participating in the processes of protein biosynthesis, the formation of other amino acids. Alanine is a part of muscle extractives. «Conditionally substitutable» acids include cystine, glycine, which are also involved in most biochemical processes in the body. In particular, glycine is involved in the synthesis of the most important substances for the body – nucleic acids. Also, these amino acids are part of proteinoids – simple proteins that are part of the supporting tissues, collagen [12].

The amino acids mentioned are glucogenic, i.e. those that give their carbohydrate fragments for the formation of carbohydrates. And as you know, carbohydrates, which are part of mucopolysaccharides, perform structural, protective and regulatory functions. Thus, mucopolysaccharides make up the bulk of extracellular tissue, are part of the skin, cartilage, synovial fluid [7, 9, 11]. The importance of amino acid metabolism in the development of genetically determined connective tissue pathology is also emphasized by such geneticists as Nikolayev KYu, Oteva EA, Nikolayeva AA, BugayevaYeV, Vasilyeva OV, Korenev NM [4, 6, 8].

The importance of carbohydrate metabolism in connective tissue pathology is also based on the fact

that they directly affect the formation of phosphorus compounds. Thus, metabolism of calcium and phosphorus in the body is regulated by calcitonin – a peptide consisting of a chain of amino acid residues [12,13].

Castori M. et al. concluded that an increase in the level of one or more amino acids in the blood and/or urine can lead to multiple dysfunction of various organs and systems [14]. The works of other researchers suggest that as a result of mutations in the regulatory or structural gene, amino acids or their metabolites accumulate, which causes toxic effects on the body [15-17].

Today, the most common is to determine the level of amino acids in the daily sample of urine or blood serum. All of these are markers of collagen breakdown, which are considered to be objective and accurate criteria for connective tissue dysplasia (CTD). Therefore, determination of the level of amino acids in the daily sample of urine or blood serum can be used both for the purpose of diagnosis and for control of further rehabilitation. This is confirmed by the results of our study, as well as the work of other scientists [12, 15].

Therefore, analyzing the known data on the occurrence of disorders of amino acid and carbohydrate metabolism in patients with connective tissue dysplasia, we can conclude that it is appropriate to further study these pathogenetic aspects in order to timely identify risk groups for possible complications.

CONCLUSIONS

1. In the main group infants with poor cf (3) and high levels of caries intensity growth index (CIGI), poor oral hygiene is observed.
2. In young children, high levels of dental caries intensity and its growth, high rates of plaque deposition may be both a consequence of connective tissue dysplasia and one of its first clinical manifestations.
3. In infants with multiple lesions of the teeth against the background of CTD impaired amino acid metabolism was observed, which was detected during the TLC of carbohydrates and amino acids in the blood and urine. During ultrasound examination of the abdominal organs, changes in the liver, gallbladder, spleen, pancreas and kidneys were detected.
4. In infants with CTD and the presence of complicated caries in the oral cavity, a foci of chronic infection may be formed, which may further enhance the impact of the pathological factor in the presence of dysplastic changes in the liver and kidneys.
5. When carrying out endogenous prophylaxis of dental caries in infants with CTD, it is necessary to take into account the internal organs' status and TLC data of amino acids and carbohydrates in the blood and urine and to prescribe peroral drugs together with the doctors geneticists.
6. For infants with CTD it is necessary to develop and carry out individual methods of exogenous prevention of the dental caries development.

REFERENCES

1. Popovych Z., Rozhko M., Ostapyak I. et al. Biochemical indexes of mineral metabolismin patients from the polluted regionaffected by chronic periodontitis. Pharmacia 2020; 67(1): 23–8. doi: 10.3897/pharmacia.67.e36150.
2. Trufanova V., Polishchuk T., Kazakova K. et al. Monitiring of efficiency of dental caries management in children`s temporary teeth throughout Poltava oblast. Wiad Lek, 2018; 71(3): 761-67.
3. Kas'kova L.F., Popyk K.M., Ulasevych L.P. et al. Korelyatsiyni zv"yazky psykhoemotsiynoho stanu ditey iz sotsial'no-hihiyenichnymy chynnykamy vynyknennya kariyesu zubiv. Ukrayins'kyy stomatolohichnyy al'manakh. 2019; 4: 57–62. (in Ukrainian)
4. Nikolayev K.Yu., Oteva E.A., Nikolayeva A.A. et al. Displaziya soyedinitelnoy tkani i poliorgannaya patologiya u detey shkolnogo vozrasta. Pediatriya. 2006; 2: 89-93. (in Russian)
5. Belyakov Yu.A. Nasledstvennyye zabolevaniya i sindromy v stomatologicheskoy praktike. Moskva: Ortodent-Info, 2000, 294 p. (in Russian)
6. Bugayeva Ye.V., Vasilyeva O.V. Displaziya soyedinitelnoy tkani – aktualnaya problema sovremennoy meditsiny (Obzor literatury). Ultrazvukova perinatalna díahnostyka. Materialy mizhnarodnoyi konferentsiyi "Plid yak chastyna rodyny". Kharkív-Odesa. 2015; 27-28: 266-281. (in Russian)
7. Klemenov A.V. Hereditary connective tissue disorders: nomenclature and diagnostic algorithm. The Clinician. 2015;9(1):42-49. doi:10.17650/1818-... (in Russian)
8. Korenev N.M. Patologiya soyedinitelnoy tkani u detey i podrostkov – osnova problemy i perspektivy. Patolohiya spoluchnoyi tkanyny – osnova formuvannya khronichnykh zakhvoryuvan ditey ta pidlitkiv: mat. konf. – Kharkiv: 2014; 3-5. (in Russian)
9. Hrechanina O.Ya., Hrechanina Yu.B., Molodan L.V. et al. Zistavlennya klinichnykh ta biokhimichnykh fenotypiv pry syndromi Elersa-Danlosa. Ultrazvukova perynatalna diahnostyka. Materialy mizhnarodnoyi konferentsiyi "Plid yak chastyna rodyny" Kharkiv-Odesa. 2014; 27-28: 32-44. (in Ukrainian)
10. Malfait F. The 2017 International Classification of the Ehlers-Danlos Syndromes. Am J Med Genet (Part C Seminars in Medical Genetics). 2017;175;1:8-26. doi: 10.1002/ajmg.c.31552
11. Arsentyev V.G., Baranov V.S., Shabalov N.P. Nasledstvennyye zabolevaniya soyedinitelnoy tkani kak konstitutsionalnaya prichina poliorgannykh narusheniy u detey. Sankt Peterburg: SpetsLit. 2014, 188 p. (in Russian)
12. Nesterenko Z.V. Klassifikatsionnyye kontseptsii displazii soyedinitelnoy tkani. Zdorovye rebenka. 2010; 5(26): 131-133. (in Russian)
13. Smolnova T.Yu., Adamyan L.V. Dinamika fenotipicheskikh priznakov sindroma displazii soyedinitelnoy tkani v razlichnyye vozrastnyye periody. Aktualnost problemy v akusherstve i ginekologii. Akusherstvo i ginekologiya. 2013; 4: 74-79. (in Russian)
14. Castori M., Morlino S., Ghibellini G. et al. Connective tissue, Ehlers-Danlos syndrome, and head and cervical pain. Am. J. Med. Genet. C. Semin. Med. Genet. 2015; 169(1): 84-96. doi:10.1002/ajmg.c....
15. Mosca M., Tani C., Vagnani S. et al. The diagnosis and classification of undifferentiated connective tissue diseases. J. Autoimmun. 2014; 48(49): 50-52. doi:10.1016/j.jaut....
16. Udovytska O.V., Leporska L.B., Spiridonova T.M. et al. Dytyacha stomatolohiya. Kyiv: Zdorovya. 2000, 296 p. (in Ukrainian)
17. Khomenko L.A., Kiselnikova L.P., Smolyar Yu.B. et al. Terapevticheskaya stomatologiya detskogo vozrasta. Kiev: "Kniga-plyus". 2013, 859 p. (in Russian)

The work is a fragment of the research project "Development of new methods for diagnosis, treatment and prevention of maxillofacial pathology in children and adults" (0115U000230, 01.2015-12.2017) and "Formation and implementation of modern scientific approaches to the diagnosis, treatment and prevention of dental pathology in children and adults" (0118U000939, 01.2018 -12.2020).

ORCID and contributionship:
Iryna I. Sokolova: 0000-0002-2339-4909 [A,D,F]
Olena H. Yaroshenko: 0000-0001-5700-692X [B,C]
Svitlana I. Herman: 0000-0002-8122-1459 [C]
Tetiana V. Tomilina: 0000-0002-8267-533X [E]
Karyna V. Skydan: 0000-0001-5579-0896 [C]
Maxym I. Skydan: 0000-0002-4141-4432 [F]

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

CORRESPONDING AUTHOR
Iryna I. Sokolova
Kharkiv National Medical University
4 Nauky avenue, 61022 Kharkiv, Ukraine
tel: +380503433988
e-mail: sokdentirina@gmail.com

Received: 20.10.2020
Accepted: 15.08.2021

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

METABOLIC SYNDROME. ETIOLOGY AND PATHOGENESIS

DOI: 10.36740/WLek202110126

Inna Diemieszczyk[1], Paulina Głuszyńska[1], Pawel Andrzej Wojciak[2], Jerzy Robert Ładny[1], Hady Razak Hady[1]
[1]1ST DEPARTMENT OF GENERAL AND ENDOCRINE SURGERY, UNIVERSITY HOSPITAL OF BIALYSTOK, BIALYSTOK, POLAND
[2]GENERAL SURGERY DEPARTMENT, GENERAL HOSPITAL, WYSOKIE MAZOWIECKIE, POLAND

ABSTRACT

The aim of the study was to assess the impact of individual components of the metabolic syndrome on the human body, taking into account their etiology and pathogenesis. This article is analytical analysis of scientific and medical literature basing on aspects of the etiology and pathogenesis of the metabolic syndrome. The key role in the pathogenesis of the metabolic syndrome is played by insulin resistance, which may be a result of lifestyle conditions (low physical activity, overweight or obesity) or genetic background. A certain role in the pathogenesis of the metabolic syndrome is also attributed to disorders of the hypothalamic-pituitary-adrenal axis in the form of increased cortisol control, which may initiate the development of abdominal obesity, insulin resistance, hypertension and dyslipidemia. Aforementioned factors (environmental, hormonal and genetic) lead to excessive fat tissue gathering. The excess of abdominal fat tissue – abdominal obesity – leads to insulin resistance, the concentration of which causes body mass gain. Such mechanism is dangerous for our health and may lead to the occurrence of type 2 diabetes and premature development of atherosclerosis with all its consequences such as atherosclerotic cardiovascular diseases including coronary artery disease.

KEY WORDS: metabolic syndrome, insulin resistance, obesity

Wiad Lek. 2021;74(10 p.I):2510-2515

INTRODUCTION

Metabolic syndrome (MS), also known as syndrome X, is defined by the WHO (World Health Organization) as a pathological condition characterized by abdominal obesity, insulin resistance, hypertension, and hyperlipidemia. The interpretation of the metabolic syndrome has been defined somewhat differently by different organizations over the course of nearly 100 years. The first observations of coexisting metabolic disorders, including abnormal carbohydrate and lipid metabolism, obesity, arterial hypertension and impaired renal function, started in 1923 [1]. The name of the metabolic syndrome and the definition as well as diagnostic criteria were first formulated by the WHO in 1999. Diagnostic criteria were then defined as glucose intolerance, impaired glucose tolerance or diabetes and/or insulin resistance with the coexistence of 2 or more of the following criteria [2]: elevated blood pressure, increased plasma triacylglycerol levels and/or low HDL-C cholesterol levels, central obesity defined by waist-hip ratio (WHR) or body mass index (BMI), microalbuminuria, or albumin/creatinine ratio. In the following years, a group of experts operating within the European Group on Insulinresistance Research (EGIR) decided that one of the foundations of the etiopathogenesis of metabolic syndrome was the phenomenon of insulin resistance, i.e. an insufficient response of target tissues, mainly adipose tissue, to endogenously secreted insulin. It was then established that insulin resistance is a necessary condition for the diagnosis of the metabolic syndrome. Moreover, a relationship was established between the excess of abdominal and intraperitoneal fat tissue as well as the development and intensification of insulin resistance and an increase in cardiovascular risk [3]. However, the diagnostic criteria established by EGIR were associated with the determination of plasma insulin levels, which is too problematic in everyday clinical practice. Another approach in the interpellation of the metabolic syndrome was the publication in 2001 of the Third Report on the "Detection, Assessment and Treatment of Hypercholesterolaemia in Adults" by experts of the National Cholesterol Education Program (NCEP-ATP III), where the definition of MS was simplified and based on the diagnosis of 3 out of 5 risk factors: central obesity (also assessed on the basis of waist circumference measurements), dyslipidemia (elevated levels of TG-triacylglycerols) or reduced levels of HDL (high density lipoprotein), increased blood pressure and impaired fasting glucose (IFG) [4]. Another update of the metabolic syndrome criteria was published in 2003 by the American Association of Clinical Endocrinologists (AACE). According to the new guidelines, MS could be diagnosed with any of the above metabolic disorders, with the primary condition again being insulin resistance (understood as abnormal fasting glycemia or impaired glucose tolerance), and obesity was defined as a BMI value> 25 kg/m^2. Additional risk factors were also given, such as: positive family history of type 2 diabetes (DM 2), polycystic ovary syndrome (PCOS), a sedentary lifestyle or elderly age [5]. In 2005, the International Diabetes Federation (IDF) modified the diagnostic criteria of MS, where central obesity definitione was specified, assessed using waist circumference, however, specific norms of this

Table I. Consensus on the definition of metabolic syndrome according to IDF and AHA/NHBLI (2009)

Consensus on the definition of metabolic syndrome according to IDF and AHA/NHBLI (2009)	
Required diagnosis of at least 3 out of 5 following metabolic disorders:	
Criterion	**Treshold values**
Excessive waist circumference (dependent on population group) central/abdominal obesity	Populations: – Caucasian ≥ 94 cm (M), ≥ 80 cm (K) – American (USA, Canada) ≥ 102 cm (M), ≥ 88 cm (K) – the Middle East and the Mediterranean Basin ≥ 94 cm (M), ≥ 80 cm (K) – Asian ≥ 90 cm (M), ≥ 80 cm (K)
TG concentration	≥ 150 mg/dl or ongoing lipid-lowering treatment
HDL concentration	< 40 mg/dl (M), < 50 mg/dl (K) or ongoing lipid-lowering treatment
Blood pressure	≥ 130/85 mm Hg or ongoing hypotensive treatment
Fasting glycemia	≥ 100 mg/dl or hypoglycemic treatment

parameter were introduced for individual ethnic groups of patients, and BMI> 30 kg/m^2 was also given as a criterion for the diagnosis of obesity.

The other criteria were consistent with those established by NCEP-ATP III, except for the IFG value, which was lowered to <100 mg/dl [6]. Simultaneously to the above, recommendations of the American Society of Cardiology (AHA) and the National Institute of Cardiology, Pulmonology and Hematology (NHLBI) have been published, where the main pathogenetic factor of MS has not been taken into account and it is necessary to identify 3 out of 5 previously used criteria that form MS. In 2009, the diagnostic criteria were updated to the current form, taking into account the definitions established by AHA-NHLBI and IDF. MS is diagnosed when 3 out of the 5 following criteria can be found (Table I).

The metabolic syndrome is a group of metabolic disorders, such as abdominal obesity, arterial hypertension, dyslipidemia and hyperglycemia, which tend to coexist and have a common etiopathogenetic basis.

THE AIM

The aim of the study is to evaluate each of the criteria for MS. Investigation of factors influencing the emergence and development of central obesity, hyperglycemia, insulin resistance, dislipidemia and arterial hypertension.

MATERIALS AND METHODS

The work is based on the analysis of medical literature on the etiology and pathogenesis of MS.

REVIEW AND DISCUSSION

METABOLIC SYNDROME IN OBESITY

The development of obesity is caused by the action of three groups of factors [7]:

– environmental (extrinsic, extramural), including: sedentary lifestyle, lack of physical activity, improper nutrition, disturbances in the circadian rhythm of sleep.
– intrinsic (internal): age, stress, aging processes and related changes in the hormonal balance, immobilization for traumatic or disease reasons, mental disorders related to eating disorders (depression, anxiety disorders, post-traumatic stress disorder, compulsive overeating, night overeating and others) and the type of intestinal microbiome-genetic predisposition that determines the susceptibility to the occurrence of metabolic disorders in patients exposed to the above environmental and endogenous factors.

Considering etiology of the metabolic syndrome, it is important to determine the factors influencing the formation and development of a specific type of obesity – abdominal (visceral, central) obesity, most associated with the occurrence of MS [8]. Endocrine conditions related to the activity of sex hormones make the physiological distribution of adipose tissue in the upper body (i.e. mainly around the abdomen) and the tendency to a central type of obesity is characteristic for adult men, while body fat storage in lower parts and gynoid obesity most often affect premenopausal adult women, but this does not exclude the possibility of coexistence nor the development of central obesity in this type of patients. Typical endocrine disorder leading to abdominal obesity are also all conditions with hypercortisolemia, because the action of cortisol in higher than physiological concentration results in the induction of metabolic disorders characteristic for the metabolic syndrome: central obesity, hypertension, dyslipidemia and hyperglycaemia, as well as due to insulin antagonism: induces and intensifies insulin resistance. Another common endocrine disorder that may be related to obesity is hypothyroidism, which leads to a decrease in metabolic rate, decrease in basal metabolism, decrease in thermogenesis and oxygen consumption, and the deposition of glycosaminoglycans in the extracellular space. All these metabolic disorders are closely related to the increase in body weight due to the increase in fat deposits.

INSULIN RESISTANCE – THE BASIS OF THE METABOLIC SYNDROME

Insulin resistance is the disturbance of glucose homeostasis, namely a decrease in the sensitivity of target tissues to insulin, despite its normal or elevated levels in blood serum. Langerhans' islet β cells compensate the decreased tissue insulin response and decreased glucose uptake from plasma by increased insulin secretion (compensatory hyperinsulinemia), so that blood glucose is initially normal. Currently, the HOMA-IR homeostatic assessment model (homeostatic model for assessing insulin resistance) is most often used to assess the severity and measure insulin resistance.

Physiologically, this index takes the value of 1, while insulin resistance is diagnosed at values ≥2.5.

Among the tissues and organs involved in glucose metabolism and insulin response, 3 can be distinguished: muscles, liver and adipose tissue. The pathomechanism of insulin resistance development in the course of central obesity, where adipose tissue is an important organ of glycemic control, consists in the uptake of glucose molecules from plasma and intracellular metabolic changes in adipose tissue (mainly visceral) in response to insulin binding by specific receptors associated with the GLUT intracellular glucose transporter-4, occurring only in adipocytes of adipose tissue, skeletal muscles and myocardium, which largely determines the insulin-dependent hypoglycemic effect [9].

On the other hand, the endocrine and paracrine activity of adipocytes involves the secretion of numerous cytokines from the adipokine group, the spectrum of which covers, among others, regulation of appetite, nutrient metabolism and insulin response (e.g. leptin, resistin, adiponectin) and pro-inflammatory and pro-thrombotic effects (e.g. IL-6, TNF-α, TF, PAI-1) [10]. In people constantly exposed to the above-mentioned extrinsic and intrinsic factors promoting the development of obesity and genetically predisposed, there is an excessive increase in adipose tissue.

Skeletal muscles are the primary target organ of insulin in the body and accounts for almost 80% of insulin-stimulated tissue glucose uptake [11]. The most important defect leading to the development of insulin resistance in muscles is connected with disorders of insulin post-receptor signaling, which are associated with impaired GLUT-4 translocation to the cell membrane and reduced transport of glucose to the interior of myocytes [12]. Lipotoxicity plays an important role in the pathogenesis of myocyte insulin resistance. Increased blood NEFA (Non-Esterified Fatty Acids) levels and their increased uptake by myocytes result in the accumulation of toxic forms of muscle lipids, including ceramides and diacylglycerols (DAGs), which interfere with insulin signaling [13]. Muscle lipids lead to the development of insulin resistance by activating 2 pro-inflammatory pathways: the kinase-βIκB (IKK-β)/NF-κB pathway, activation of which leads to the stimulation of the expression of many genes whose products are responsible for the development of insulin resistance [14]. Another activated pathway is the JNK1 kinase cascade (Jun kinase 1), which shows phosphorylase activity against the IRS-1 protein (Insulin receptor substrate 1) and the insulin receptor itself, which results in impaired signaling of the intracellular insulin response and increased insulin resistance [15].

The liver is one of the most important organs involved in the metabolism of lipids and glucose, and is recognized as the key tissue for the pharmacotherapy of insulin resistance (IRes) and type 2 diabetes [16]. Hepatic insulin resistance is manifested by an uncontrolled increase in hepatic glycogenolysis and gluconeogenesis, which increases endogenous glucose production [17]. As a result of insulin resistance of adipocytes, there is an increased release of free fatty acids (NEFA) from adipose tissue into the blood, which causes their increased inflow to the liver. Increased inflow of NEFA and hyperinsulinemia accompanying obesity intensify intrahepatic lipogenesis with a simultaneous, relative slowing down of the rate of fatty acid oxidation [18]. The result is an excessive accumulation of lipids in hepatocytes, which are mainly rich in the triacylglycerol (TAG) fraction. The rate of fatty acid oxidation is relatively reduced in relation to the excessive influx of fatty acids, and under these conditions there is an increased oxidation of fatty acids in the peroxisomes, which results in the intensification of peroxidation processes [19-20]. Lipids undergoing increased peroxidation increase the oxidative stress of hepatocytes, which leads to their necrosis, and peroxidized lipids released from damaged hepatocytes induce inflammation and damage to the hepatic veins. This contributes to the development of nonalcoholic fatty liver disease (NAFLD) with complications such as fibrosis, cirrhosis and hepatocellular carcinoma.

HYPERGLYCEMIA AND PRE-DIABETES IN THE METABOLIC SYNDROME

One of the first symptoms of worsening carbohydrate metabolism and prediabetes is abnormal fasting glycemia (IFG), which is one of the diagnostic criteria of the metabolic syndrome and also a predictor of the development of non-insulin-dependent diabetes [21]. Another pre-diabetes condition is impaired glucose tolerance (IGT), defined as the plasma glucose level in the range of 140 – 199 mg/dl after 2 h of OGTT (Oral Glucose Tolerance Test). On the other hand, the current diagnostic criteria for the diagnosis of diabetes include:

- random glycemia ≥11.1 mmol/l (200 mg/dl) and typical symptoms of hyperglycaemia (increased thirst, polyuria, weakness);
- random blood glucose ≥11.1 mmol/l (200 mg/dl) without typical symptoms of hyperglycaemia and once (on another day) fasting blood glucose ≥7.0 mmol / l (126 mg/dl);
- 2 times (measured on other days) fasting blood glucose ≥7.0 mmol/l (126 mg/dl);
- glycemia in 2h OGTT ≥11.1 mmol/l (200 mg/dl) [22].

Patients with metabolic syndrome have been shown to be 5 times more likely to develop type 2 diabetes than subjects who do not meet its criteria [23]. The mechanisms of non-insulin-dependent diabetes mellitus include genetic predisposition, as well as metabolic and cellular factors. At

the cellular level, the glucotoxic effect of prolonged hyperglycemia has been demonstrated, reducing the action of GLP-1 (glucagon-like peptide 1), resulting in ineffective regeneration and apoptosis of β-cells, leading to a decrease in insulin production and the development of DM 2. Metabolic mechanisms that lead to the development of DM 2 are similar to those that result in insulin resistance in sarcomocytes, hepatocytes, and adipocytes. Increased secretion of NEFA into the blood in the course of the development of insulin resistance results in lipotoxicity and the death of β cells. Moreover, the constant excess of energy substrates leads to the development of intracellular metabolic and oxidative stress, which induce apoptosis of pancreatic islet cells. These mechanisms result in a gradual loss of the physiological β-cell reserve of pancreatic islets over time, which results in the appearance and worsening of hyperglycemia, pre-diabetes, and finally full-blown DM 2 [24].

ARTERIAL HYPERTENSION IN THE METABOLIC SYNDROME

Like obesity, dyslipidemia and diabetes, arterial hypertension (HT) is a very important risk factor, promoting the development of cardiovascular diseases and increasing the mortality of patients. Multicenter population studies have identified obesity as one of the most important factors promoting the development of HT, and also showed the prevalence of HT in patients with BMI <25 kg/m^2 at 15%, with a noticeable increase to about 40% with a BMI exceeding 30 kg/m^2, which indicates a significant correlation of blood pressure values with body weight, not only in obese patients, but also in those with normal weight [25]. Moreover, a statistically significant relationship between abdominal obesity and the development of arterial hypertension has been demonstrated, and it has been proved that increased waist circumference is an independent predictor of the development of hypertension [26]. Insulin resistance is the etiopathogenic factor linking arterial hypertension with other components of the metabolic syndrome. The reduced action of insulin leads to a decrease in vascular compliance and an increase in blood pressure (RR) [27]. Hyperinsulinemia causes an increase in the activity of the sympathetic nervous system and renal blood pressure-regulating enzyme renin-angiotensin-aldosterone (RAA), which results in increased sodium retention and excessive vasoconstriction of angiotensin, resulting in an increase in RR [28].

DYSLIPIDEMIA IN THE METABOLIC SYNDROME

In order to diagnose dyslipidemia in the metabolic syndrome, it is required to find disturbances in the concentration of lipid fractions of triglycerides and high-density lipoproteins in the patient's plasma: TG (Triglyceride) ≥ 150 mg/dL and/or HDL (High-density lipoprotein) <40 mg/dL (M), <50 mg/dL (K). The lipid profile accompanying the metabolic syndrome, consisting in a decrease in HDL fraction and an increase in TG and LDL (Low-density lipoprotein) concentration, is called atherogenic dyslipidemia. The pathomechanism of the development of atherogenic dyslipidemia in the metabolic syndrome is closely related to insulin resistance and the excess of free fatty acids in the bloodstream. The excess of free fatty acids results in their accumulation in the liver, re-esterification to triglycerides and increased synthesis of TG-rich VLDL (very low density lipoprotein) particles.

Under physiological conditions, the function of the VLDL lipoprotein is to transport triglycerides into adipose tissue, where the lipoprotein is broken down and transfers TG for storage in adipocytes. At the adipocyte level, lipoprotein lipase (LPL) is an important membrane protein responsible for lipid uptake and transport into the cell and for the breakdown of low-density lipoproteins. Expression of this protein is induced by insulin, and under conditions of insulin resistance it is significantly reduced, which results in the retention of VLDL in the plasma [29]. At the same time, the influx of lipids supplied with food is greater in the case of insulin resistance than in healthy subjects, which results in the phenomenon of postprandial hyperlipidemia.

Another effect that increases triglyceridemia is hepatic de novo lipogenesis, which is not inhibited under insulin resistance conditions and does not directly increase the amount of circulating VLDL, but results in an increase in the amount of TG [30]. Through the cholesterol ester transporting protein (CETP), cholesterol esters are replaced from HDL and LDL with triglycerides derived from VLDL. As a result, the amount of cholesterol esters in VLDL increases as well as the amount of triglycerides in HDL and LDL. Structurally abnormal HDL loses the properties of cholesterol re-transport from tissues to the liver, and also has a high plasma clearance, which is why it is quickly removed from the bloodstream and its concentration decreases. On the other hand, structurally abnormal LDL (sdLDL) has a lower affinity for hepatocyte receptors than normal LDL, and therefore remains in the plasma for longer [31]. Thus, lipid metabolism disorders characteristic of the metabolic syndrome occur.

So, the metabolic syndrome is a pathological condition characterized by the coexistence of central obesity, hyperlipidaemia, hyperglycemia, and hypertension. Complications of the metabolic syndrome are often associated with the coexistence of several of these conditions. Numerous meta-analyzes of studies conducted for many years on large population groups have shown a close statistical correlation of the increase in BMI with an increase in overall premature mortality from all causes, significantly lower mortality in groups of patients with normal body mass index, as well as an increase in mortality by as much as 30% in the case of an increase in BMI as low as 5 kg/m^2 above 25 kg/m^2 [32-33].

Depending on gender, race, severity and complications, obesity shortens the patient's life by 5-20 years. According to WHO data in Europe, 35% of ischemic heart disease, 55% of HT and 80% of type 2 diabetes are associated with overweight and obesity [34]. Comparative statistics, based on meta-analyzes of multicentre clinical trials, showed a 2-fold increase in the total risk of cardiovascular events in the population groups

of subjects suffering from metabolic syndrome compared to healthy subjects, more than 2.5-times higher risk of myocardial infarction and death for cardiac reasons it is the most common cause of death in morbid obesity (BMI>40 kg/m^2) and the average life expectancy of these patients is 6.5–13.7 years shorter than in those with normal BMI [35-37].

Aterogenic dyslipidemia causes the formation and intensification of atherosclerotic lesions both by the formation of dangerous forms of LDL, but also by reducing the physiological defense against atherogenesis as a result of HDL damage, which results in an increased incidence of atherosclerotic diseases. Increased cardiovascular risk in the course of carbohydrate metabolism disorders is noticeable from the stage of pre-diabetes and is strongly associated with insulin resistance [38]. Chronic hyperglycemia, which may accompany the metabolic syndrome complicated by type 2 diabetes, leads not only to the development of cardiovascular disorders known as macroangiopathies (e.g. myocardial infarction, stroke), but also to microangiopathies at the organ level, such as: nephropathy , retinopathy or neuropathy [39]. In patients diagnosed with metabolic syndrome, myocardial infarction, stroke and type 2 diabetes occur more frequently, which significantly increases the cardiovascular risk and contributes to an increase in mortality compared to the general population [40].

MS therapy is a complex and multidisciplinary process. It should include a thorough history of lifestyle, eating habits, family burdens, anthropometric measurements, physical examination, including blood pressure measurement, determination of basic laboratory tests, especially lipid profile and fasting glucose. The main goals of the modern therapeutic approach to patients with metabolic disorders are the reduction of short- and long-term cardiovascular risk and the prevention of acute vascular events, as well as the prevention of type 2 diabetes. It is a difficult, long-term therapy that requires the cooperation of many specialists and a high commitment of the patient.

CONCLUSIONS

1. Metabolic disorders that occur in the body and lead to the formation of the metabolic syndrome – abdominal obesity, hypertension, dyslipidemia and hyperglycemia – tend to coexist and have a common etiopathogenetic basis.
2. Insulin resistance plays one of the key roles in the pathogenesis of the metabolic syndrome leading to the development of type 2 diabetes.
3. Abdominal obesity is the most dangerous type of obesity associated with the occurrence of the metabolic syndrome and predisposes to the development of arterial hypertension.
4. The development of atherogenic dyslipidemia in the metabolic syndrome is closely related to insulin resistance and excess free fatty acids in the bloodstream.
5. The treatment of the metabolic syndrome is multidisciplinary and requires close cooperation between the doctor and the patient.
6. The analysis of etiopathogenic connections points that metabolic syndrome should be treated by an interdisciplinary team of specialists. It also requires close and disciplined cooperation of the patient in the treatment process.

REFERENCES

1. Kylin E. Studies of the hypertension hyperglycemia hyperuricemia syndrome. Zentralbl Innere Med. 1923;44:105-127.
2. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. World Health Organization: Geneva: Switzerland, 1999.
3. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med. 1999 May;16(5):442-443.
4. III, NCEP – ATP. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001;285:2486-2497.
5. Einhorn D, Reaven GM, Cobin RH, et al. American College of Endocrinology position statement on the insulin resistance syndrome. Endocr Pract. 2003;9:237-252.
6. Alberti KG, Zimmet PZ, Shaw J, et al. Metabolic syndrome -a new world-wide definition. A Consensus Statement from the International Diabetes Federation Diabet Med. 2006 May;23(5):469-80.
7. Gale EA. The myth of the metabolic syndrome. Diabetologia 2005;48:1679-1683.
8. Phillips LK, Prins JB. The link between abdominal obesity and the metabolic syndrome. Curr Hypertens Rep. 2008;10(2):156-164.
9. Contreras AM, Berrío GB, Velásquez CM. An approach to the etiology of metabolic syndrome. Colomb Med (Cali). 2013;44(1):57-63.
10. Juhan-Vague I, Alessi MC, Mavri A, et al. Plasminogen activator inhibitor-1, infl ammation, obesity, insulin resistance and vascular risk. J Thromb Haemost. 2003;1:1575-1579.
11. Jazet IM, Schaart G, Gastaldelli A, et al. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signaling in obese insulin-treated type 2 diabetic patients. Diabetologia 2008;51:309-319.
12. Zaid H, Antonescu CN, Randhawa VK et al. Insulin action on glucose transporters through molecular switches, tracks and tethers. Biochem J. 2008; 413:201-215.
13. Timmers S, Schrauwen P, de Vogel J. Muscular diacylglycerol metabolism and insulin resistance. Physiol Behav. 2008;94: 242-251.
14. Shoelson SE, Lee J, Yuan M. Inflammation and the IKKb/IkB/NF-kB axis in obesity- and diet-induced insulin resistance. Int J. Obes. 2003, 27(suppl. 3):49–S52.
15. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. Nature 2002 420:333-336.
16. Zabielski P, Daniluk J, Hady RH, et al. The effect of high-fat diet and inhibition of ceramide production on insulin action in liver. J Cell Physiol. 2019:234(2):1851-1861.
17. Lewis GF, Vranic M, Harley P, et al. Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes 1997;46:1111-1119.
18. Hellemans K, Kerckhofs K, Hannaert JC, et al. Peroxisome proliferator-activated receptor alpha-retinoid X receptor agonists induce beta-cell protection against palmitate toxicity. FEBS J. 2007;274:6094-6105.
19. Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005;115:1343-1351.

20. Machado M, Cortez-Pinto H. Non-alcoholic fatty liver disease and insulin resistance. Eur J Gastroenterol Hepatol. 2005;17:823-826.
21. Muoio DM, Newgard CB. Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9(3):193-205.
22. Sieradzki J, Płaczkiewicz-Jankowska E. Cukrzyca. In: Szczeklik A (ed.). Medycyna Praktyczna – Interna. https://www.mp.pl/interna/chapter/B16.II.13.1. [Access: 10.08.2020]
23. Ramli AS, Daher AM, Nor-Ashikin MN, et al. JIS definition identified more Malaysian adults with metabolic syndrome compared to the NCEP-ATP III and IDF criteria. Biomed Res Int. 2013;2013:760963.
24. Levine F, Itkin-Ansari P. beta-cell Regeneration: neogenesis, replication or both? J Mol Med (Berl). 2008;86(3):247-258.
25. Brown CD, Higgins M, Donato KA, et al. Body mass index and prevalence of hyper-tension and dyslipidemia. Obesity Res. 2000;8:605-619.
26. Poirier P, Lemieux I, Mauriège P, et al. Impact of waist circumference on the relationship between blood pressure and insulin. The Quebec Health Survey. Hypertension. 2005;45:363-367.
27. Chaudhary K, Buddineni JP, Nistala R, et al. Resistant hypertension in the high-risk metabolic patient. Curr Diab Rep. 2011;11(1):41-46.
28. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005; 365:1415 -1428.
29. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 2009;297(2):E271-288.
30. Wang Y, Viscarra J, Kim SJ, et al. Transcriptional regulation of hepatic lipogenesis. Nat Rev Mol Cell Biol. 2015;16(11):678-689.
31. Manjunath C N, Rawal JR, Irani PM et al. Atherogenic dyslipidemia. Indian J Endocr Metab. 2013;17:969-976.
32. Flegal KM, Kit BK, Orpana H, et al. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA. 2013;309:71-82.
33. Prospective Studies Collaboration, Whitlock G, Lewington S, Sherliker, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;373:1083-1096.
34. Diemieszczyk I, Woźniewska P, Gołaszewski P, et al. Does weight loss after laparoscopic sleeve gastrectomy contribute to reduction in blood pressure? Polish Arch Inter Med. 2021 :131(7-8):693-700.
35. Gami AS, Witt BJ, Howard DE, et al. Metabolic syndrome and risk of incident cardiovascular events and death. A systematic review and meta-analysis of longitudinal studies. J Am Coll Cardiol. 2007;49(4):403-414.
36. Mente A, Yusuf S, Islam S et al. Metabolic syndrome and risk of acute myocardial infarction a case-control study of 26,903 subjects from 52 countries. J Am Coll Cardiol. 2010; 55(21):2390-2398.
37. Kitahara CM, Flint AJ, Berrington de Gonzalez A et al. Association between class III obesity (BMI of 40-59 kg/m2) and mortality: a pooled analysis of 20 prospective studies. PLoS Med. 2014;11(7):e1001673.
38. Balkau B, Eschwège E, Papoz L. Risk factors for early death in non-insulin dependent diabetes and men with known glucose tolerance status. BMJ. 1993;307:295-299.
39. Petrie JR, Guzik TJ, Touyz RM. Diabetes, Hypertension, and Cardiovascular Disease: Clinical Insights and Vascular Mechanisms. Can J Cardiol. 2018;34(5):575-584.
40. Wozniewska P, Diemieszczyk I, Hady RH Complications associated with laparoscopic sleeve gastrectomy – a review. Przegl Gastroenterol. 2021:16(1):5-9.

ORCID and contributionship:
Inna Diemieszczyk: 0000-0002-0494-1719 [A-B,D]
Paulina Głuszyńska: 0000-0002-3952-3154 [D-E]
Pawel Andrzej Wojciak: 0000-0002-0241-0885 [B,D]
Jerzy Robert Ładny: 0000-0003-4167-1962 [E]
Hady Razak Hady: 0000-0002-0838-3478 [E-F]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Inna Diemieszczyk
1st Department of General and Endocrine Surgery,
University Hospital of Bialystok, Poland
e-mail: demeschik.inna@gmail.com

Received: 30.06.2021
Accepted: 30.09.2021

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

THE EPIDEMIOLOGICAL PROBLEM OF GLOBAL EPIDEMICS DANGER TO ACUTE RESPIRATORY VIRAL INFECTIONS, ESPECIALLY CORONAVIRUS, FOR THE ORGANIZATION OF MASS SPORTING EVENTS: SOLUTION WAYS

DOI: 10.36740/WLek202110127

Yaroslav V. Pershehuba[1], Oleh I. Tsyganenko[2], Olena V. Maslova[2], Natalia A. Sklyarova[2]
[1]SHUPYK NATIONAL HEALTHCARE UNIVERSITY OF UKRAINE, KYIV, UKRAINE
[2]NATIONAL UNIVERSITY OF UKRAINE ON PHYSICAL EDUCATION AND SPORT, KYIV, UKRAINE

ABSTRACT

The aim: To find solutions for the ecological problems of epidemics danger to acute respiratory infections, especially coronavirus infections, during the organization of mass sporting events by establishing the features of its development and providing epidemiological measures to reduce the negative impact of epidemics for human health and activities, including athletes at both national and international levels.

Materials and methods: The methodological basis of this study is general and special scientific methods: dialectical, analysis and synthesis, synergism, historical and legal, formal and logical, systematic and structural, comparative and legal, formal and legal. Empirical data were scientific works, international regulations, EU legislation, United States and other countries.

Conclusions: The conclusions that the organization of mass sporting events must take into account the epidemiological situation of the disease in acute respiratory infections primarily on coronavirus infection at the national level to ensure contact with the institutions of the public health national system, use data "Hazard determination and risk assessment systems" (HDRAS) to determine the risk degree of infectious diseases, to conduct timely observation activities.

KEY WORDS: mass sports events, coronaviruses, acute respiratory viral infections, epidemics danger

Wiad Lek. 2021;74(10 p.I):2516-2519

INTRODUCTION

It is known that epidemics of acute respiratory viral infections (ARI), which belongs to the group of the most common infectious human diseases and are able to cause epidemics on a global scale, not only a significant problem in terms of negative impact on public health and health of athletes too, as a separate group of it, but also for the organization of mass events, including sports. It is no accident that the problem of preventing the negative impact of ARI for the organization of mass events, including sports, deals with the WHO Regional Office for Europe and its experts (Examples: Poland – "World Youth Day", 2016; Spain – "World Pride Parade", 2017; Turkmenistan – "Asian Indoor and Martial Arts Games", 2017) in collaboration with WHO Headquarters and the WHO Network Centers. However, according to the WHO definition during public events should be understood "measures the number of participants which is large enough to make a risk mechanism for planning and regulation of the society, the state or the people". All these measures have been implemented by the WHO Regional Office for Europe to improve the public health systems of the countries concerned [1-8].

The features of the sports events organization is that it quite often has an international character, which increases the risk of infectious disease and epidemics causing people from around the world arrival, including countries with a difficult epidemiological situation regarding infectious diseases [1-3].

Determined that ARI is a group of clinically and morphologically similar acute inflammatory diseases of the human respiratory system, the causative agents of which are pneumotropic viruses. These rather large groups of viral pathology other than influenza viruses (orthomyxoviruses) include parainfluenza viruses (paramyxoviruses), adenoviruses, rhinoviruses (enteroviruses), respiratory syncytial virus (PSV), and coronaviruses [9].

It is important to note that until recently, regarding ARI, experts from the WHO Regional Office for Europe to mass events, including sports (athletes' health), the greatest attention was paid to influenza viruses and much less to other pneumotropic viruses. In general, ARI coronavirus have been caused regarding athletes and their sports activities, discussed in the scientific literature, textbooks and materials of international organizations, mostly in a generalized form along with other pneumo viruses, without sufficient coverage of the epidemiology of coronaviruses in sports practice [10-11].

However, the outbreak of the COVID-19 coronavirus epidemic in China in 2019-2020, which began to spread to many countries around the world, including Japan, where

the XXXII Summer Olympics were planned in Tokyo in 2020 (and there was a proposal to move the XXXII Summer Olympics to London) made it very important to consider the problem of the epidemiology of coronaviruses and their dangers to both the health of athletes and their sports activities. In this regard, there is even a thesis that "sport has contracted the coronavirus" [12].

It should be borne in mind that, as modern practice shows, epidemics of coronaviruses can take the form of quarantine (convection) viral infections, such as contagious viral fever (Lassa, Marburg, Ebola), yellow fever. This is indicated by fairly high mortality, virulence, the ability to spread widely in such species of coronaviruses as SARS – COV, MERS – COV, COVID – 19 (SARS – COV – 2), and most importantly, that in practice quarantine measures are already imposed in areas where coronavirus infections occur (China, Italy, etc.). It should be noted that the term "quarantine infections" is not identical to the term "particularly dangerous infections" and means only a conditional group of infections in which quarantine may be imposed [13-16].

However, taking into account the peculiarities of the coronaviruses epidemiology, including the sports mass events, has not yet been emphasized so that they are separately provided for in the "International Health Regulations 2005" and the "WHO Health Emergencies Programme". This was not provided for the recommendations of the WHO Regional Office for Europe regarding the mass events holding, including sports [5, 8, 17].

It is important to note that we have not yet developed the ways to reduce the negative impact of coronaviruses and its epidemics on the athlete's health and their performance, making such development relevant and timely.

THE AIM

The article purpose – to find solutions for the ecological problems of epidemics danger to acute respiratory infections, especially coronavirus infections, during the organization of mass sporting events by establishing the features of its development and providing epidemiological measures to reduce the negative impact of epidemics for human health and activities, including athletes at both national and international levels.

MATERIALS AND METHODS

The methodological basis of this study is general and special scientific methods: dialectical, analysis and synthesis, synergism, historical and legal, formal and logical, systematic and structural, comparative and legal, formal and legal, and statistical [18]. Empirical data were scientific works, international regulations, EU legislation, United States and other countries.

REVIEW AND DISCUSSION

The analysis of various sources of information, including documents and scientific developments of WHO experts showed that when applying epidemiological measures in organizing mass events, including sports, it is necessary to pay special attention to acute respiratory infections (ARI), especially coronavirus infection and its epidemiological features [5, 6, 8, 19, 20].

Coronaviruses family (Coronoviride) according to the latest data has two subfamilies and includes 40 types of RNA – containing coronaviruses that affect humans and animals. The term "crown" is associated with their specific mechanism of penetration through the cell membrane by postiche the so-called "fake molecules" of molecules to which the body's cell receptors respond [21, 22].

From an epidemiological point of view, including in relation to athletes, the greatest danger is posed by three types of coronaviruses [23, 24]:

- SARS virus – COV – known as the causative agent of SARS since 2002;
- MERS virus – COV – known as the causative agent of Middle Eastern respiratory syndrome since 2015;
- virus COVID – 19 (SARS – COV – 2) – known as the causative agent of pneumonia of a new type since the end of 2019.

The analysis of scientific, scientific-methodical literature and other sources of information showed that there is a special problem – the problem of possible negative effects of the epidemic, which is caused by coronaviruses not only on athlete's health but also directly on their sports activities due to various epidemiological restrictive measures [12, 25, 26].

In this regard, one of the reasons for the negative impact of the epidemic caused by coronaviruses on sports activities may be restrictions in the form of quarantine followed by special observational measures, which in turn are defined as a set of special epidemiological measures, including restrictive aimed at reducing the negative effects of viruses, in this case coronaviruses on human health [27].

In determining the level of quarantine and observation measures, it should be borne in mind that the effects of coronavirus COVID – 19 on the human body on average are: 80% – mild symptoms that are not significantly life-threatening; 14 – serious problems, pneumonia, difficulty breathing; 5% – critical conditions – respiratory failure, septic shock; 2% – deaths. It is very important to use an electronic platform called the "Hazard determination and risk assessment systems" (HDRAS) to determine the extent of an epidemic to public health. It allows you to analyze information about dangerous factors from various sources of information, including the media (media), as well as social networks and more. The system used by experts of the European Regional Office to collect information on infections and other factors during mass events, including sports and the subsequent provision of advisory services, including the Chief Sanitary Inspectorate in Poland. The effectiveness of WHO's work in preparing and conducting mass events was noted by the WHO Executive Board following the report "Global Mass Events: Their Importance and Opportunities for Health in the World" (Document EB 130/17) [5, 28, 29].

So according to information sources during the epidemic of 2020 in China, which was caused by the coronavirus COVID – 19, a significant number of athletes through the introduction of quarantine observation, was forced to train alone at home, that is not based on personal housing. This negative impact on their training process and as a result all their sports performance [30].

In addition, a coronaviruses epidemic can lead to the postponement and even disruption of sports competitions. For example, the Chinese men's tennis team because of coronavirus COVID – 19 and the use of observational measures was forced to refuse to participate in the World Group playoffs and Davis Cup [31].

If there coronaviruses infection and quarantine and observational measures necessary to follow with the provisions for the mass sporting events organization:

- to provide close contact at the national level for mass sports events organizers with the institutions of the national public health system, and at the international level, if necessary, with WHO experts and the WHO Regional Office for Europe (the latter for European countries);
- in planning for the mass sporting events organization in conditions of coronavirus epidemic it is necessary to take into account the general state of public health regarding the availability of coronavirus infection and quarantine and the application of observation measures for the territories and settlements (cities, etc.) where the mass sports events planned;
- if necessary to ensure timely evacuation of athletes from quarantine zones, as their presence in these areas can not only be dangerous to their health, but also affect the quality of training, competitive process;
- use the "Hazard determination and risk assessment systems" (HDRAS) to assess the danger degree of the epidemiological situation regarding coronavirus infection;
- to use diagnostic test methods of coronavirus infection to assess the epidemiological situation and to determine the athlete's health state;
- do not to allow spectators and fans from quarantine areas to places of mass sports events;
- to maximum limit as much as possible the presence of elderly people and children at sports events as high-risk groups for coronavirus infections;
- do not to allow domestic and farm animals as possible sources of coronavirus infections on the territory and in the premises where sports events are held;
- to perform general hygiene requirements, hands cleaning and disinfection, dressing protective face masks and its timely replacement with new ones and so on.

CONCLUSIONS

The epidemiological problem of epidemics of acute respiratory viral infections (ARI), especially coronavirus in the organization of mass sports events can pose a significant threat to the health of athletes and their sports activities and therefore requires a comprehensive, systemic solution, with special attention to coronavirus infections.

Comprehensive, systematic solution of this epidemiological problem should include such measures as observation and quarantine, zoning of areas according to the degree of danger of SARS, the use of rapid diagnostic methods, vaccination, the maximum possible provision of athletes with the opportunity to engage in sports activities, epidemiological assessment their dangers, fulfillment of the general sanitary – epidemiological requirements.

REFERENCES

1. Meijer A., Webster C., William R. et al. The COVID-19-crisis and the information polity: An overview of responses and discussions in twenty-one countries from six continents. Information Polity. 2020;25 (3): 243-274. doi: 10.3233/IP-200006.
2. Blackburn J. K., Kracalik I. T. and Fair J. M. Applying Science: Opportunities to Inform Disease Management Policy with Cooperative Research within a One Health Framework. Front. Public Health. 2016;3:276. doi: 10.3389/fpubh.2015.00276.
3. Chinazzi M., et al. The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science. 2020; 368 (6489): 395–400. doi: 10.1126/science.aba9757.
4. Brukner P. Clinical Sports Medicine. Third edition. «McGraw-Hill». 2008, 1084 p.
5. Ute E., Regm J. Ukrepleniye sistemy obshchestvennogo zdravookhraneniya: opyt organizatsii massovykh meropriyatiy. [Strengthening public health: making the case for mass gatherings]. 2018. Panorama obshchestvennogo zdravookhraneniya. 2018;4(1):67-71.
6. Bounce Back Better: Sustainable Strategies for a Healthy Recovery from the Pandemic. 2020. https://www.iseepi.org/common/Uploaded%20files/2020%20Website%20Edits/Policy/final%20ats%20ers%20isee%20covid%20air%20pollution%20and%20health_08182020.pdf Accessed October 03, 2020.
7. Malchow H., Petrovskii V., Venturino E. Spatiotemporal Patterns in Ecology and Epidemiology: Theory, Models, and Simulation. USA: CPS – Press, Series: Matematical and Computational Biology. 2019, 469 p.
8. Salamans P. WHO's new emergencies program bridges two worlds. Bull World Health Organ. 2017; 95(1):8–9. doi: 10.2471/BLT.17.030117.
9. van Doorn H.R., Yu H. Viral Respiratory Infections. Hunter's Tropical Medicine and Emerging Infectious Diseases. 2020:284–8. doi: 10.1016/B978-0-323-55512-8.00033-8.
10. Roberts J. A. Viral Illnesses and Sports Performance. Sports Medicine 1986;3: 296–303. doi: 10.2165/00007256-198603040-00006.
11. Martin S. A., Pence B. D., Woods J. A. Exercise and respiratory tract viral infections. Exercise and sport sciences reviews. 2009; 37(4): 157–164. doi: 10.1097/JES.0b013e3181b7b57b.
12. Ying-Ying Wong A. et al. Impact of the COVID-19 pandemic on sports and exercise, Asia-Pacific Journal of Sports Medicine, Arthroscopy, Rehabilitation and Technology. 2020; 22:39-44. doi: 10.1016/j.asmart.2020.07.006.
13. Day T. et al. When Is Quarantine a Useful Control Strategy for Emerging Infectious Diseases?, American Journal of Epidemiology. 2006;163(5):479–485. doi: 10.1093/aje/kwj056.
14. Tognotti E. Lessons from the history of quarantine, from plague to influenza A. Emerging infectious diseases. 2013;19(2): 254–259. doi: 10.3201/eid1902.120312.
15. Bloom D. E., Cadarette D. Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response. Frontiers in immunology. 2019;10: 549. doi: 10.3389/fimmu.2019.00549.

16. Ewald P. W. Guarding against the most dangerous emerging pathogens. Emerging infectious diseases. 1996; 2(4): 245–257. doi: 10.3201/eid0204.960401.
17. International Health Regulations. The official portal of Verkhovna Rada of Ukraine. https://zakon.rada.gov.ua/laws/show/897_007. 2005. Accessed October 03, 2020.
18. Danyl'yan O. H. Orhanizatsiya ta metodolohiya naukovykh doslidzhen [Organization and methodology of scientific research]. Kharkiv: Pravo, 2017; 448 p. (in Ukrainian)
19. Wiersinga W.J., et al. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020;324(8):782–793. doi: 10.1001/jama.2020.12839/
20. Aleta A., Martín-Corral D., Pastore y Piontti A. et al. Modelling the impact of testing, contact tracing and household quarantine on second waves of COVID-19. Nat Hum Behav. 2020; 4,: 964–971. doi: 10.1038/s41562-020-0931-9.
21. Chorba T. The Concept of the Crown and Its Potential Role in the Downfall of Coronavirus. Emerging Infectious Diseases. 2020;26(9):2302-2305. doi: 10.3201/eid2609.ac2609.
22. Liu C. et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. ACS Central Science. 2020; 6 (3): 315-331 doi: 10.1021/acscentsci.0c00272.
23. Peeri N.C. et al. The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned?, International Journal of Epidemiology. 2020; 49(3): 717–726. doi: 10.1093/ije/dyaa033.
24. Zheng J. SARS-CoV-2: an Emerging Coronavirus that Causes a Global Threat. International journal of biological sciences. 2020; 16(10): 1678–1685. doi: 10.7150/ijbs.45053.
25. Cauchemez S. et al. Closure of schools during an influenza pandemic, The Lancet Infectious Diseases. 2009;9(8):473-481. doi: 10.1016/S1473-3099(09)70176-8.
26. Samuel R. D., Tenenbaum G., and Galily Y. The 2020 Coronavirus Pandemic as a Change-Event in Sport Performers' Careers: Conceptual and Applied Practice Considerations. Front. Psychol. 2020; 11:567966. doi: 10.3389/fpsyg.2020.567966.
27. Yen-Hao Chu I., Alam P., Larson H. J. et al. Social consequences of mass quarantine during epidemics: a systematic review with implications for the COVID-19 response, Journal of Travel Medicine. 2020;27(7):192. doi: 10.1093/jtm/taaa192.
28. Yi Y., Lagniton P., Ye S. et al. COVID-19: what has been learned and to be learned about the novel coronavirus disease. International journal of biological sciences. 2020; 16(10): 1753–1766. doi: 10.7150/ijbs.45134.
29. Shang Y., Pan C., Yang X. et al. Management of critically ill patients with COVID-19 in ICU: statement from front-line intensive care experts in Wuhan, China. Ann. Intensive Care. 2020; 10: 73. doi: 10.1186/s13613-020-00689-1.
30. Mendell E. Rooftop Drills and Home Games on Foreign Turf: Coronavirus Alters China's Olympic Prep. The Wall Street Journal. 2020. https://www.wsj.com/articles/rooftop-drills-and-home-games-on-foreign-turf-coronavirus-alters-chinas-olympic-prep-11582730587. Accessed October 03, 2020.
31. Coronavirus: sporting events in China under threat as virus spreads, Deutsche Welle. 2020. https://www.dw.com/en/coronavirus-sporting-events-in-china-under-threat-as-virus-spreads/a-52165953 Accessed October 03, 2020.

ORCID and contributionship:
Yaroslav V. Pershehuba: 0000-0003-4592-3047 [A, E, F]
Oleh I. Tsyganenko: 0000-0002-0485-6979 [A, C, E]
Olena V. Maslova: 0000-0003-4926-7681 [B, C, D]
Natalia A. Sklyarova: 0000-0002-1897-5960 [B, E, F]

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

CORRESPONDING AUTHOR
Yaroslav V. Pershehuba
Shupyk National Healthcare
University Of Ukraine
9 Dorohozhytska St., 04112 Kyiv, Ukraine
tel: +380669916299
e-mail: igme@ukr.net

Received: 04.10.2020
Accepted: 02.06.2021

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

THE CASE OF JEUNE SYNDROME AMONG THE PRECARPATHIAN POPULATION

DOI: 10.36740/WLek202110128

Nadiya M. Fomenko[1], Olha B. Synoverska[1], Lesia I. Marchuk[2], Yaroslav S. Dziubynsky[2]
[1]IVANO-FRANKIVSK NATIONAL MEDICAL UNIVERSITY, IVANO-FRANKIVSK, UKRAINE
[2]IVANO-FRANKIVSK REGIONAL CHILDREN'S HOSPITAL, IVANO-FRANKIVSK, UKRAINE

ABSTRACT

Despite the fact that Jeune syndrome is rather rare, neonatologists and pediatricians need to be aware of this pathology. This will facilitate early diagnostics of the condition and aid in the choice of the most adequate algorithms for its monitoring and treatment. The aim: To describe the case of Jeune syndrome among the Precarpathian population. Infant patient with Jeune syndrome and relevant medical records. Methods used in the study: clinical-genealogical and syndromal analysis, general clinical examination, radiologic method, including computed tomography (CT) scan with 3D image reconstruction, methods of ultrasound diagnostics. The study was conducted in accordance with the Declaration of Helsinki Ethical Principles. The newborn baby was diagnosed with asphyxiating thoracic dystrophy on the basis of personal observation and conducted complex examination. According to the literature, this syndrome is rarely diagnosed in this age group. The diagnosis was based on the clinical and phenotypic manifestations of the syndrome, primarily on the characteristic association of symptoms of specific chest deformity and severe respiratory failure with oxygen dependence in the patient. Skeletal and pulmonary changes on radiographs and computed tomography scans were rather indicative. Brief follow-up data on the patient at the age of nine months are given.

KEY WORDS: asphyxiating thoracic dystrophy, ciliopathy, Precarpathian population, oxygen dependence

Wiad Lek. 2021;74(10 p.I):2520-2524

INTRODUCTION

Jeune syndrome (synonym: asphyxiating thoracic dystrophy/ dysplasia, Eng.: Jeune asphyxiating thoracic dystrophy). ICD10 code – Q77.2; OMIM codes – 208500, 611263, 613091, 613819, 613376, 615630, 615633, 616300, 617088; ORPHA code: 474 – orphan genetically determined disease. Disease incidence is 1:110000 – 1:130000. This syndrome was first described by the French pediatrician M. Jeune, after whom the syndrome is named. He described a familial case of asphyxiating thoracic dystrophy in siblings with a very narrow chest in 1955. [1-3]. The type of inheritance of the syndrome is autosomal recessive. The main complex of symptoms includes osteochondrodystrophy with dominating affection of the chest, limbs, pelvic bones in combination with the damage to internal organs (kidneys, liver, pancreas), eyes. Growth retardation and underweight body are characteristic for the condition. Central nervous system disorders in the form of psychomotor retardation are observed in a significant number of children, but are secondary. Jeune syndrome is characterized by significant clinical polymorphism, but all cases are defined by the development of respiratory failure (RF), which manifests itself since birth, and is of stable, often progressive nature, and determines the prognosis in children under two years of age [1-6]. In cases of significant limb shortening by the type of metaphyseal dysplasia and the presence of postaxial polydactyly, there is a need for differential diagnosis with the syndrome of chondroectodermal dysplasia, also known as Ellis van Creveld syndrome. The latter, despite the fact of some similarity of clinical manifestations, is not characterized by severe respiratory failure in the neonatal period, and at the same time the significant percentage of cases is characterized by congenital heart defects and damage to skin, nails, hair, teeth, which makes it possible to differentiate these diseases at the clinical level. Occasionally, particularly in the presence of renal and hepatic pathologies, a differential diagnosis is made with Sensenbrenner's syndrome, which, unlike the Jeune syndrome, is characterized by an abnormal skull shape and symptoms of skin pathology and its derivatives, limb changes such as brachy- and syndactyly.

Etiologically, Jeune syndrome is associated with mutations in IFT80, IFT140, DYNC2H1, WDR19, TTC21B genes, located in the loci of 3q25.33, 11q22.3, 4p14, 2q24.3 chromosomes, respectively, as well as the mutations in the locus of 15q13 chromosome, that is, the disease is characterized by significant genetic heterogeneity [3-8], while the search for new mutations and their description still continues. [4,5] It is considered, that all these genes are responsible for the functioning of the so-called cilia – organelles in the form of flagella (cilia), which are present in most eukaryotic cells, including human. They perform both motor and sensory functions. Due to the prevalence of cilia in the body, their structural or functional defects cause a wide range of pathologies and syndromes (ciliopathies). It is assumed that the malfunction of cilia due to the defect

of a particular protein leads to disruption of connections between cells in the process of embryo- and fetogenesis, as well as in the postnatal period. Jeune syndrome also belongs to this group of pathologies. However, the exact causes and mechanism of the predominant damage to certain organs and systems in ciliopathies have not been fully studied yet.[4,7,8]

THE AIM

Taking into account the extreme rarity of the syndrome and the limited number of publications on this pathology, we consider it useful to describe the case of Jeune syndrome in the Precarpathian population to raise awareness of general practitioners on this issue.

MATERIALS AND METHODS

The study involved infant patient with Jeune syndrome observed by the authors since birth and relevant medical records. Methods used in the study: clinical-genealogical and syndromal analysis, general clinical examination, radiologic method, including computed tomography (CT) scan of thoracic organs, ultrasound diagnostic methods: neurosonography (NSG), ultrasound examination of abdominal organs, and Doppler ultrasonography of heart (Echocardiography). The study was conducted in accordance with the Declaration of Helsinki Ethical Principles. The research protocol was approved by the Local Ethics Committee (LEC) of all the institutions mentioned in the study. Informed consent from parents was obtained for the study.

CASE STUDY

Proband is a boy born first of a bichorionic, biamniotic twins. Third pregnancy, second birth, premature at 35 weeks of gestation, pathological: rupture to delivery interval of 38 hours, hyperthermia in labor. During pregnancy: gestational pyelonephritis, polyhydramnios, and inpatient treatment in this regard at 32-33 weeks of gestation. The second twin and the elder sibling are healthy; between births, one pregnancy ended in a therapeutic abortion. The proband-baby was born with dysplastic manifestations of fetal growth retardation, body weight – 1930 g, body length – 44 cm, head circumference (HC) – 31.5 cm, chest circumference (CC) – 24 cm, with Apgar score of 7/7. Manifestations of respiratory failure were present since birth (4 points by the Silverman scoring system, tachypnea 80/min., the baby required mask oxygen therapy, decreased breath sounds over the lungs). On the third day the baby was transferred to the regional children's clinical hospital, the department of premature and low birth-weight children in a severe condition due to II degree of respiratory failure (RF), neurological symptoms, with the pronounced oxygen dependence. Phenotypically, the child had a significant lag in the growth of the chest circumference (7.5 cm) as compared to the head circumference: HC-31.5 cm, CC-24 cm, shortening of the lower limbs due to the proximal parts. Calvarial bones are pliable, thinned, open large and soft fontanel, slightly below the skull bones. The chest is narrow, rigid, almost does not participate in the act of breathing, tachypnea up to 100-120 / min., perioral – and acrocyanosis, which increases during crying, nasal flaring, abdominal breathing, oxygen saturation (SaO2) – 79-83 %. During auscultation, the areas of decreased breath sounds alternated with the areas of harsh breathing with the presence of scattered dry and mixed moist rales. Heart sounds are slightly weakened, systolic murmur at the base of the heart. The abdomen is soft, takes an active part in the act of breathing. The liver is located + 2 cm below the edge of the costal arch, the spleen is not palpable. Respiratory system changes were clinically regarded as a manifestation of respiratory distress syndrome complicated by pneumonia. Changes in the skeletal system indicated the presence of osteochondrodysplasia in the child. The child underwent the complete physical examination check-up and consultations of specialized doctors (geneticist, neurologist, cardiologist, ophthalmologist), multidisciplinary team discussions were organized. The following findings were obtained in the process of screening: complete blood count (CBC) test showed no pathologic changes, though, predisposition to thrombocytopenia was observed during the first week of baby's life; biochemical blood assay revealed hyperbilirubinemia due to indirect fraction, which was regarded as conjugational, tendency to hypoproteinemia, decrease in the concentration of active transport form of vitamin D (25OH $Д_3$). Considering the burdened history of infection in the mother (pyelonephritis, fever in labor), the child was examined for the most common TORCH infections by ELISA test, which showed elevated levels of IgG antibodies to cytomegalovirus and herpes virus, however, the PCR for these pathogens was negative. Bacterial culture test of sputum, blood and urine was performed to rule out bacterial contamination and showed no pathogenic micro flora. The level of procalcitonin was 0.141 ng/dl and indicated local infection.

ULTRASOUND EXAMINATION OF THE ABDOMINAL ORGANS (AGE – 5 DAYS)

The *liver* is located typically, protruding by 2cm below the costal margin. Structurally without changes. Bile ducts are indurated. The wall is not thickened, the content is homogeneous. *The pancreas* visualizes all through, the structure is homogeneous. *The spleen* is not enlarged, 37x14 mm, structurally homogeneous. *The kidneys* are located typically, and have smooth and distinct wall borders. Medial complex is not thickened, caval system is somewhat extended, renal pelvis: on the right – 5mm, on the left – 6mm.

Echocardiography was performed several times: at the age of 4, 10, 18 days and at 1.5 months. Conclusion: The path of great vessels is correct. The coronary sinus is slightly dilated, the chambers of the heart are not dilated. The

walls of the myocardium are not thickened. The general contractility of the myocardium is good. The blood flow is pulsating. Functional foramen ovale is 5 mm with overload of the right heart chambers. Open arterial duct of 2.2 mm (closed at 1.5 months). Moderate pulmonary hypertension.

Neurosonography was performed twice, at the age of 5 and 18 days (without negative dynamics): echogenicity of brain tissues is normal. No midline shift revealed. The third and lateral ventricles are not dilated. The vascular plexuses of the right and left ventricles are heterogeneous. Cisterna magna is within normal limits, longitudinal cerebral fissure – 5mm. Hyperechogenicity is observed in periventricular areas, close to the vascular plexuses.

Radiography (series of examinations) was performed during the first week of hospital stay at the age of 10 days and revealed irregular pneumatization of the pulmonary fields with the areas of increased pneumatization in the upper divisions of the left lung and lower lateral right lung areas. Increased lung markings with infiltrative changes in the medial divisions of both lungs were observed. Sinuses and domes are not clearly differentiated. Cardiac borders are dilated in the breadth. The thorax is deformed, narrowed, cylindrical, the ribs are shortened and thickened, high riding clavicles ("handlebar clavicles"). Iliac wings are squarewise; a characteristic "trident" in the areas of acetabulum is noticed. At the age of 19 days, changes in the respiratory system without positive dynamics with increasing infiltrative changes.

Radiological conclusion: X-ray pattern is characteristic of bilateral bronchopneumonia, pulmonary hypoplasia and osteochondrodysplasia are not excluded (Fig.1 **a,** b).

The child's condition remained severe for a long time. Manifestations of pneumonia and respiratory failure progressed, which correlated with radiological changes. Constant dyspnea at rest and oxygen dependence were observed, thus, the patient required continuous mask oxygen therapy. On auscultation, patient's breathing is severely weakened, crepitation and moist rales are heard on both sides. Heart tones are somewhat weakened, intermittent systolic murmur, heard mainly on the basis of the heart. Ultrasound examination of the heart, as mentioned above, showed moderate pulmonary hypertension, open arterial duct, which closed at the age of 1.5 months, functional foramen ovale with overload of the right chambers. During the first month of life the child slowly gained weight, there was also low tolerance to food. Baby's feeding involved small portions of expressed breast milk, supplemented with partially hydrolyzed formula; partial parenteral nutrition (PPN) was necessary during the first two weeks. Patient's neurologic state showed weakening and mosaicism of inborn reflexes, muscular dystonia, decreased motor activity.

Considering clinical and radiological changes, namely the combination of severe respiratory failure, which required constant oxygen therapy, and pathology of the skeletal system with predominant affection of the chest, we performed the diagnostic search of various literature sources. Based on this search and clinical syndrome analysis, the diagnosis of Jeune syndrome (asphyxiating thoracic dystrophy) was established, which was also confirmed by computed tomography (CT) of the chest. The description of CT is given below.

CT OF THE THORACIC ORGANS WITH 3D RECONSTRUCTION (AGE – 25 DAYS)

The chest is narrow, deformed due to the reduction of its frontal size and increase of the sagittal one. The ribs are shortened, expanded, horizontally placed, II-V ribs are fused in the anterior segments (Fig. 2 a, b), the clavicles are wide, deformed and high riding. Marked decrease in pneumatization of both lungs due to infiltrative changes, more pronounced in the basal parts of the increased lung markings, decrease in amount of lung tissue. The contours of the diaphragm are smooth and flattened.

Radiologist's conclusion: CT picture is characteristic of Jeune asphyxiating thoracic dystrophy, bilateral bronchopneumonia and bronchopulmonary dysplasia (BPD).

Thus, on the basis of clinical and phenotypic data, laboratory and instrumental examination methods, consultations with specialists, the child was diagnosed with: asphyxiating thoracic dystrophy (Jeune syndrome) with autosomal recessive type of inheritance. Congenital pneumonia of unspecified etiology, respiratory failure – II degree.

Complications: severe bronchopulmonary dysplasia. Pulmonary hypertension of moderate degree.

Concomitant pathology: functional fetal communications: open arterial duct (2.2 mm), functional foramen ovale (5 mm) with overload of the right heart chambers;

Hypoxic-ischemic CNS injury, acute period, CNS depression syndrome;

Conjugative jaundice of a premature baby with low body weight before gestation.

The patient received comprehensive treatment, which included oxygen therapy, infusion therapy, phototherapy, partial parenteral nutrition, antibiotic therapy using reserve group antibiotics (III and IV generation cephalosporins, carbapenems), intravenous immunoglobulin, dexamethasone, euphyllin, sildenafil, probiotics, antifungal drugs, vitamin therapy, hepatoprotectors, inhalations with pulmicort, berodual. On treatment, the patient's condition stabilized; there was a positive clinical and radiological dynamics of pneumonia (the comparison X-ray at the age of 1 month 11 days showed no inflammatory infiltration signs). But at the same time shortness of breath of the mixed type still persisted: respiratory rate (RR) about 80 per min. at rest, oxygen dependence. Oxygen saturation remained reduced: at rest it ranged at 88-90-93%, while at minimum load it dropped to 77%. The mother refused to consult the child in the medical institutions of III-IV level. The child was discharged home at 1 month and 3 weeks with recommendations to continue mask oxygen therapy in an outpatient unit under the supervision of a district pediatrician and the course of rehabilitation and symptomatic therapy, as well as regular consultations with specialists, namely geneticist, pulmonologist, orthopedist, neurologist, ophthalmologist.

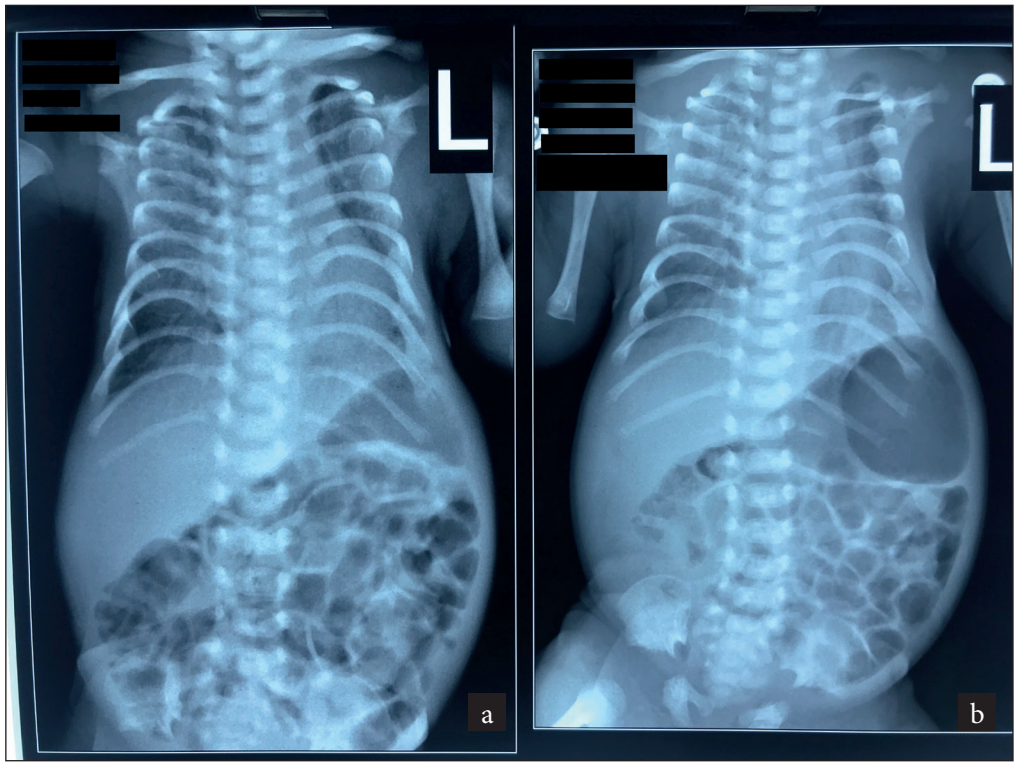


Fig1. Radiographs of the chest, abdomen and pelvis of a patient aged 10 (a) and 19 (b) days.

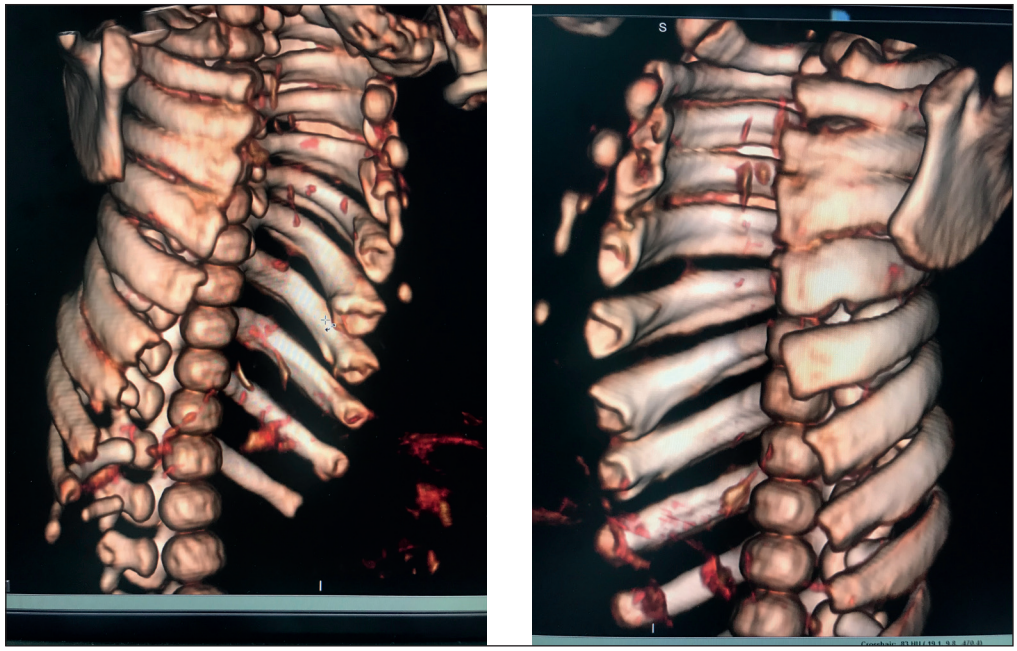


Fig. 2. CT of the patient's chest with 3D-reconstruction performed at the age of 25 days: narrowed chest, wide, short, horizontally placed ribs with the fusion of II-Vth ribs in the anterior divisions.

Follow-up data: At the age of nine months, the patient still has the phenotypic features of Jeune syndrome: narrowed and deformed chest, significant lag of head circumference from the chest circumference – 6.5 cm (HC – 39cm, CC – 32.5cm). Lag in height and weight are still observed: height – 64cm (-3sigma), body weight – 5.9kg (body mass index 14.4), limbs are somewhat shortened. Failure to gain height and weight reduced as compared to the first half a year of the baby's life. The use of anabolic medications (agvantar, trimetabol, retabolil) showed positive effect. Shortness of breath decreased in the dynamics: RR 45-50 breaths/min. at rest, SaO2 levels ranged between 93-95%. The neurological status is characterized by the delay in statokinetic development: the baby sits only with support, weak legs resistance, and muscular hypotonia. Up to nine months, the patient has no recurrent diseases of the bronchopulmonary system, which gives hope for a more favorable prognosis in this case.

DISCUSSION

Analyzing this case and comparing it with the observations of patients with Jeune syndrome made by other authors [1-3,6],

we can reasonably state the presence of the most characteristic disease symptoms in our patient. The combination of specific deformity and rigidity of the chest with severe respiratory disorders in the form of shortness of breath, low oxygen concentration in the blood, oxygen dependence, both during pneumonia and without it, are the leading symptoms in the diagnosis of this syndrome. Such contributing factors as chronic intrauterine hypoxia of the fetus, infectious diseases of the mother during pregnancy, premature birth, reduced weight and growth rates, a significant delay in chest circumference as compared to the head circumference in children with Jeune syndrome, both at birth and later in life, which were stated by other authors, were also observed in our case. In most of the described cases [1-3] the authors note patients' severe state in the neonatal period, complex concomitant pathologies to other organs and systems, which were also registered in our observation. However, the diagnosis of Jeune syndrome is still rarely established in the neonatal period, so we consider it appropriate to remind about it. Ideally, the final verification of the diagnosis should be based on molecular genetic analysis, but today it is limited to widespread clinical use, and in Ukraine it is not carried out at all. According to the literature, the prognosis of the state is serious, often unfavorable with significantly reduced life expectancy of patients. At early manifestations of severe respiratory failure, oxygen dependence, recurrent bronchopulmonary diseases, the lethal outcome occurs at the age of about two years. With more favorable course, children live over two years, but they usually develop severe renal pathology such as interstitial nephritis, nephronophthisis, nephrocalcinosis with the development of renal failure. It is believed that in such cases, death occurs at the age of 2-10 years or older from renal pathology [3,6,8]. In some cases, liver fibrosis is also revealed. Treatment is symptomatic, with the development of surgical methods to manage chest and lung pathologies, including their transplantation. Medical and genetic family counseling on the prognosis is carried out classically, based on the 25% risk for offspring in autosomal recessive pathology. Prenatal diagnosis is more informative with the molecular genetic analysis of the proband, and is based on a purposeful search for the main manifestations of the disease by means of ultrasound examination[6,7]. This is how the medical and genetic consultation of the family was carried out in our case.

CONCLUSIONS

Despite the fact that Jeune syndrome is rather rare, neonatologists and pediatricians need to be aware of this pathology and first of all, pay special attention to the association of characteristic chest deformity and severe respiratory failure. This will facilitate early diagnostics of the condition and aid in the choice of the most adequate algorithms for its monitoring and treatment.

REFERENCES

1. Kenneth L. Jones. Hereditary syndromes according to David Smith, translation from English. M.: Praktika; 2011, 408 p.
2. Kosolapova N.V., Pakhomova Ye.V., Havryliuk V.P., Dobrokhodova D.V., Romashyna M.S. Clinical observation of Jeune syndrome. Vesnik Rentgenologii i radiologii. 2015;(1):40-42.
3. Ovsiannikov D.Yu., Stepanova Ye.V., Beliashova M.V., Dehtiariova Ye.A. Jeune Syndrome: Description of a Series of Observations. Vesnik RAMS. 2016;71(1):61–67.
4. Lehman A.M., Langlois S., Yong S.L. et al. Co-occurrence of Joubert syndrome and Jeune asphyxiating thoracic dystrophy. Am. J.Med. Gen. 2010;152(6):14119.
5. Miller K.A., Ah-Cann C.J., Welfare M.F. et al. Cauli: a mouse strain with an IFT140 mutation that results in a skeletal ciliopathy modelling Jeune syndrome. PLoSGenetics. 2013;9(8):1003746.
6. Vriesde J., Yntema J.L., Dievan C.E. et al. Jeune syndrome: description of 13 cases and a proposal for follow-up protocol. Eur. J. Pediatr. 2010;169(1):77-88.
7. The Portal for Rare Diseases and Orphan Drugs. © everythingpossible/ Fotolia: https://www.orpha.net/consor/cgi-bin/Disease_Disability_Simple.php?lng=EN (Access data: 2020-04-24)
8. Das Portal für seltene Krankheiten und Orphan Drugs. © everythingpossible/Fotolia: https://www.orpha.net/consor/cgi-bin/Disease.php?lng=DE (Access data: 2020-04-24)

ORCID and contributorship:

Nadiya M. Fomenko: 0000-0001-6992-9182 [A,D,E]
Olha B. Synoverska: 0000-0003-1072-3782 [F]
Lesia I. Marchuk: 0000-0001-5149-2357 [B]
Yaroslav S. Dziubynskyi: 0000-0002-5965-1460 [B]

Conflict of interest:

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Nadiya M. Fomenko
Ivano-Frankivsk national medical university
2 Galitska st., 76000 Ivano-Frankivsk, Ukraine
tel: +48579158001
e-mail: fomenko.nm@gmail.com

Received: 13.10.2020
Accepted: 02.08.2021

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

INHERITED EPIDERMOLYSIS BULLOSA IN NEWBORN (CASE STUDY)

DOI: 10.36740/WLek202110129

Veronika M. Dudnyk, Olga I. Izumets, Oksana S. Rubina, Valentin H. Furman
VINNYTSIA NATIONAL PIROGOV MEMORIAL MEDICAL UNIVERSITY, VINNYTSIA, UKRAINE

ABSTRACT

Inherited epidermolysis bullosa (IEB) is a group of genetically and clinically heterogeneous diseases characterized by the formation on the skin and mucous membranes blisters and erosion due to injury. Different forms of IEB can be accompanied by various extracutaneous complications, such as blisters and erosion on the cornea and mucous membranes, stenoses and strictures of the respiratory system, gastrointestinal tract, urinary system, muscle dystrophy, and malignant tumors. Therefore diagnosis and prescribing appropriate treatment and follow-up care is an important task for neonatologists and pediatric dermatologists. Because the manifestations of IEB are numerous, a specialized center is required for optimal care, where multidisciplinary care will be provided (neonatologists, pediatric surgeons, pediatric dermatologists, etc.). The purpose of this case report is to pay attention of specialists to a disease that is rare, to present clinical case of IEB in newborn who was admitted to the intensive care unit of newborns of Vinnitsa Regional Children's Clinical Hospital.

KEY WORDS: inherited epidermolysis bullosa, newborns

Wiad Lek. 2021;74(10 p.I):2525-2529

INTRODUCTION

Inherited epidermolysis bullosa (IEB) is a group of genetically and clinically heterogeneous diseases characterized by the formation of blisters on the skin and mucous membranes and erosions due to injury. Different forms of IEB can be accompanied by various extradermal complications, namely the formation of blisters and erosions on the cornea and mucous membranes, stenosis and strictures of the respiratory system, gastrointestinal tract, urinary system, muscle dystrophy and malignant tumors. The term "epidermolysis bullosa" was introduced in 1886 by Kebner. IEB is divided into three types depending on the level of blistering: simplex, junctional and dystrophic. There is a stratification of the epidermis due to cytolysis of keratinocytes in simplex IEB. Blisters are formed on the border of the epidermis and dermis due to splitting of the light plate of the basement membrane (lamina lucida) in junctional IEB type. There is blisters formation under the dense plate of the basal membrane in dystrophic IEB. Nowadays we know more than 30 subtypes of IEB [1, 2], main of them are present in table I.

Currently, mutations have been identified in more than 10 genes encoding the structural proteins of keratinocytes and the basal membrane of the skin and mucous membranes. A common feature of these proteins is their involvement in the formation of strong bonds between the epithelium and the basement membrane. The nature of the mutations and their localization determine the severity of the clinical manifestations of IEB. Mutation information is a prerequisite for effective medical and genetic counseling, prenatal and pre-implantation DNA diagnosis.

Most variants of simplex IEB are inherited autosomal dominantly. The most common – localized – is known as the Weber-Cockayne subtype. The disease manifests itself in the neonatal period, extradermal manifestations in the form of blisters on the oral mucosa. The most difficult variant is the autosomal dominant generalized BE, the Dowling-Meara subtype.

Junctional IEB is inherited autosomal recessively. Severe generalized form, Herlitz subtype is called lethal IEB due to the high risk of premature death. The cause of death is considered to be sepsis, pneumonia, obstruction of the larynx and trachea.

Dystrophic IEB can be inherited autosomally dominantly and autosomally recessively. This form is characterized by generalized blisters, erosions, scars, contractures of the hands, feet, elbows and knees. Typical are damage to the gastrointestinal tract, urinary system, eyes, chronic anemia, developmental delay, high risk of squamous cell skin cancer.

The frequency of IEB is unknown. Mild variants are registered with a frequency of 1 in 50,000 births, heavy – 1 in 500,000. The incidence does not depend on gender. Examination of a child with suspected IEB should include a study of the pedigree. Importantly, individual cases may be due to spontaneous mutation or incomplete penetration of the autosomal dominant gene.

The main treatment is skin care. All forms of IEB are characterized by skin damage, respectively, wound healing is central to treatment. Due to the formation of blisters, constant inflammatory process, polymicrobial colonization with

infectious complications, poor nutritional status and trophic disorders, skin damage turns into chronic wounds. They cause severe pain, and changing bandages makes it worse.

Delicacy and a minimum of touches are important in care. Preventing overheating and lubricating the skin to reduce friction can limit blistering. Auxiliary is the use of a special water or air mattress. The child should not be taken under the armpits, it is taken in the arms, holding the neck and buttocks. Clothing should be made of soft material, simple cut. It is not advisable to use diapers, it is better to keep the crotch open.

Wound healing occurs in four stages: inflammation, reepithelialization, tissue formation and renewal. The ideal bandage should provide a sufficient level of moisture, not stick, be atraumatic, protect against infection, reduce pain, and have the appropriate size. Soft silicone bandages meet these requirements. Hydrogel dressings are recommended for dry wounds. Absorbent dressings are used for wet wounds. Bacterial colonization and infection inhibit wound healing. Local antiseptics should be used for a short period of time. When healing does not occur due to the above actions, use the biological equivalents of the skin – xenografts.

Severe forms of IEB are accompanied by a deep protein-energy deficiency. [3, 4] Eating disorders are caused by the following reasons: increased catabolism on the background of chronic inflammation – open wounds with loss of blood and serous fluid, increased protein breakdown, heat loss, infection, and complications from the mouth, esophagus and other gastric departments. -intestinal tract limit food intake and disrupt the absorption of nutrients. Therefore, the main objectives of therapeutic nutrition are as follows: to prevent nutrient deficiencies, reduce stress during feeding, maintain normal body fat, normalize intestinal function and immune status.

The daily energy requirement of babies with IEB is from 130 to 180 kcal / kg, and in some cases up to 225 kcal / kg. The need for protein is 2.5-4 g / kg, the liquid is 150-200 ml / kg. It is necessary to support breastfeeding, applying Vaseline on the lips and nipple reduces friction. When breastfeeding is not possible, use expressed breast milk with fortifiers. Severely ill children with IEB need a subsidy for all vitamins, especially vitamin C, which plays an important role in iron absorption and collagen synthesis.

Constant loss of blood from the wound surface leads to chronic anemia, so it requires correction with iron supplements. Zinc is a cofactor of more than 200 enzymes, so due to its antioxidant properties it plays an important role in the processes of growth, wound healing, immune protection, membrane stabilization. [5,6] Recommended liquid dosage forms in the form of zinc sulfate (30 mg in 5 ml). In all children with impaired nutritional status, it is recommended to monitor the level of selenium and carnitine, which is used as a solution at a rate of 50-100 mg / kg / day. In children with IEB, bone metabolism is impaired due to increased concentrations of cytokines on the background of chronic inflammation, as well as impaired calcium absorption due to gastrointestinal complications. Therefore, such children are offered a combination of calcium and vitamin D.

Thus, bullous epidermolysis is a severe disabling disease that adversely affects the quality and life expectancy of patients. Patients die between the ages of 3 and 30, depending on the form of the disease.

THE AIM

The purpose of this case report is to draw the attention of specialists to a disease that is rare, to share their own experience of managing patients with IEB of newborns.

CASE STUDY

Child L., born from the first normal pregnancy, first physiological delivery, Apgar score 7 and 9 points. Mother is 23 years old, father 28 years old, no inherited diseases in both families. Child's body weight is 2930 g, length is 49 cm.

The general condition of a child was severe; the child is routinely anesthetized with IV paracetamol. Unaffected areas of the skin were without impaired microcirculation. Self-breathing, RR – 48 per minute. Hemodynamic was not disturbed, HR – 128 per min, blood pressure – average 60 mm Hg. Swollen, holds through a probe 80 ml of breast milk. Diuresis 760 ml per day, bowel movements 6 times was not disturbed.

The presence of skin aplasia at birth (both lower extremities from the fingers to the lower third of the thighs, lesions of the mucous membranes of the mouth, the presence of blisters on the skin 0.5-1 cm serous-hemorrhagic content on the skin of the forehead.

Wounds on the skin of the cheeks, elbows, palms, knees, legs, feet are closed by xenoskin, single erosions are open, there are cracks on the legs in areas of exfoliation of xenoskin. New damage occurs due to friction.

Additional examination which was performed during hospitalisation:

- CBC (leukocytosis, mild anemia);
- Measuring level of proteins (normal), bilirubin (normal), ALT and AST (normal), thymol test (normal), urea and creatinine(normal);
- CRP – 14 mg/l;
- ASLO (normal),
- *electrolyte* imbalance;
- normal serum level of zinc and low amount of serum $25(OH)D_3$;
- thyroid hormones (T4, TSH, ATPO) were in normal ranges;
- General urine analyses was performed 2 times a week (normal);
- ECG / EchoCG (sinus arrhythmia, patent foramen ovale);
- ultrasound of the thyroid gland and kidneys hadn't show anomalies;
- bacterial investigation of the wounds content and sensitivity to antibacterial drugs.

Diagnosis: Inherited epidermolysis bullosa, dystrophic type.

Treatment we used included daily skin care, management of infected and non-infected wounds, management of the oral mucosa membranes and rehabilitation. It was per-

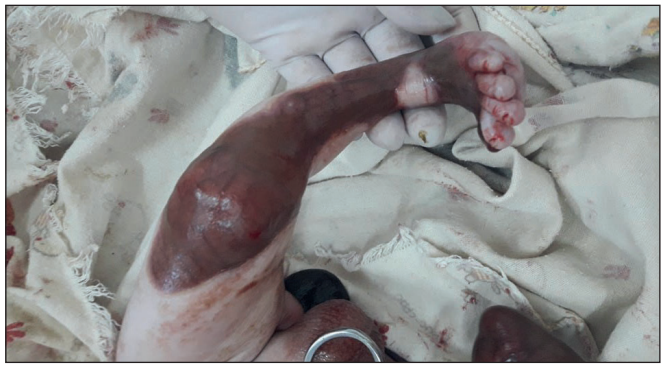


Fig. 1. Skin aplasia of the left leg

Fig. 2. Skin aplasia of the right leg

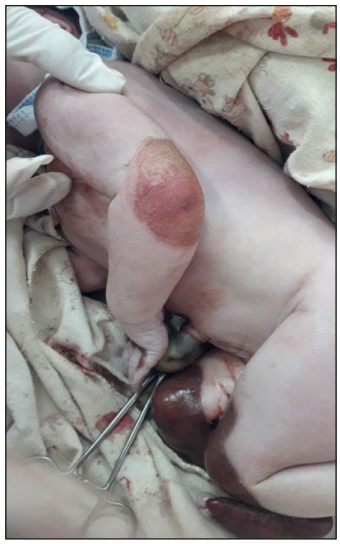


Fig. 3. Skin aplasia of the elbow

Fig. 4. Damage of the skin closed with xenoskin

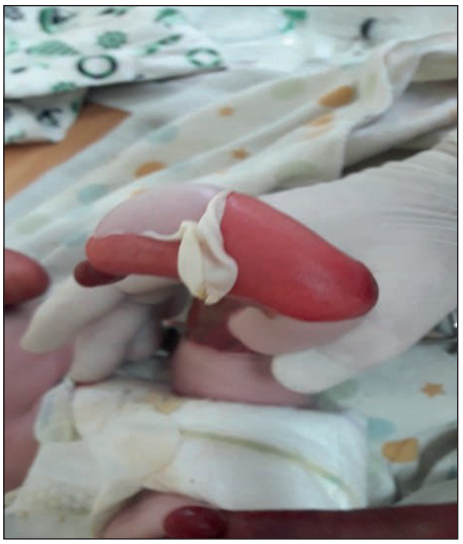


Fig. 5. Damage of the foot skin closed with xenoskin

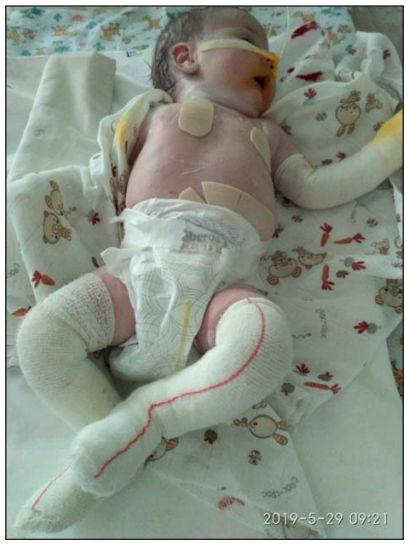


Fig. 6. Damage of the skin closed with xenoskin during treatment

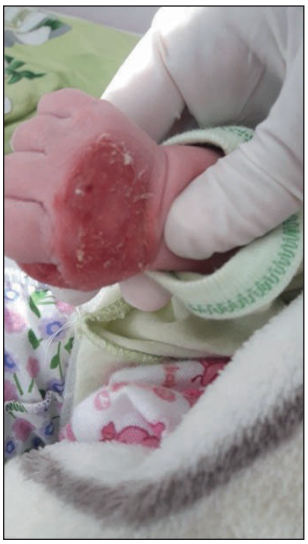


Fig. 7. Child with IBE during treatment

formed according to the "Adapted evidence-based clinical guidelines. Bullous epidermolysis"[7].

Management of skin folds (neck, ears, elbows, thighs and knees) is carried out daily 1-2 times a day with special liquids gently. Management of dry elements and those that are at the stage of epithelialization is carried out with ointment 2-5 times a day. Wounds in the perineum were treated as needed after using the toilet.

Care for blisters and wounds included bathing (0.9% salt solution: hypo (less than 90 g) or 90 g per 10 liters

Table I. Classification of the main types and subtypes of inherited epidermolysis bullosa

Main type	Subtypes	Proteins in whose genes occur mutations
Simplex IEB	Simplex Weber-Cockayne IEB	Keratin 5, 14
	Simplex Koebner IEB	Keratin 5, 14
	Simplex Dowling-Meara IEB	Keratin 5, 14
	Simplex IEB with muscle dystrophy	Plectin
Junctional IEB	Junctional Herlitz IEB	Laminin 332
	Junctional Non-Herlitz IEB	Laminin 332, Collagen 17 type
Dystrophic IEB	Dominant dystrophic IEB	Collagen 7 type
	Recessive dystrophic Hallopeau-Siemens IEB	Collagen 7 type
	Recessive dystrophic non-Hallopeau-Siemens IEB	Collagen 7 type

of water for clean wounds); in case of wound infection, octenidine dihydrochloride/chlorhexidini bigluconas/povidone-iodine solution 1:20 in the bath, combine solution (hexamidine diisothionate 100 mg, chlorhexidine bigluconate solution 20% 0.5 ml, chlorocresol 300 mg) as a detergent (requires rinsing).

Care of the infected wounds included using of creams or ointment with bandages which are listed above:

– argosulfan (silver sulfathiazole 20 mg, cetostearyl alcohol (cetyl alcohol 60%, stearyl alcohol 40%) 84.125 mg, liquid paraffin – 20 mg, white vaseline – 75.9 mg, glycerol – 53.3 mg, sodium lauryl sulfate – 10 mg, methyl parahydroxybenzoate – 0.66 mg, propyl 0.33 mg, potassium dihydrogen phosphate – 1.178 mg, sodium hydrogen phosphate – 13.052 mg, water d/i – up to 1 g.)

or

– Tyrosur gel (1 g of gel contains 1 mg of thyrotricin; cetylpyridinium chloride, propylene glycol, ethanol 96%, carbomer, trometamol, purified water)

or

– Bactroban (mupirocin 2.2 g, macrogol 400 – 58.7 g, macrogol 3350 – 39.1 g)

or

– Octenilin (100 ml of solution contain: 0.050% octenidine dihydrochloride, 9.90% propylene glycol, 2.50% hydroxyethylcellulose, 87.55% distilled water)

or

– Levomikol (chloramphenicol 7.5 mg, dioxomethyltetrahydropyrimidine (methyluracil) 40 mg, macrogol 1500 – 190.5 mg, macrogol 400 – 762 mg).

There were performed steps describing below in case of formation of "fresh" elements:

– evacuation of the vesicular element contents without removing the epidermal film;
– wound care (description below);
– applying a bandage the size of the wound or slightly larger than it (description below).

Puncture of blisters in 2 points or the lower point with a sterile Microlance G18-G19 needle or sterile scissors with liquid evacuation and preservation of a tire with the following processing:

– Creams containing zinc oxide 15.25%, benzyl benzoate 1.01%, benzyl alcohol 0.39%, benzyl cinnamate 0.15% in combination with dexpanthenol and/or miramistin;
+ Antibacterial sponge bandage with silver
+ Absorbent wipes and tampons
+ Ointment bandage (Peruvian balm, white vaseline, cetomacragol 1000, glycerol monostearate 40–50%, hydrogenated fat, medium triglycerides).
+ Fixation with lightweight elastic tubular bandage that stretches in radial and longitudinal directions

Treatment of the oral mucosa membranes included oral antiseptic for 7 days such as:

– Benzydamine hydrochloride 0.255 mg 0.15 g (with ethanol 96% – 10 ml, glycerol – 5 g, methyl parahydroxybenzoate – 0.1 g, menthol flavor (flavoring) – 0.03 g, saccharin – 0.024 g, sodium bicarbonate – 0.011 g, polysorbate 20 – 0.005 g, purified water – qs up to 100 ml)

or

– Solkoseril (deproteinized dialysate from the blood of healthy dairy calves (in terms of dry matter) 4.15 mg, methyl parahydroxybenzoate, propyl parahydroxybenzoate, sodium carmellose, propylene glycol, calcium lactate pentahydrate, water d/i)

or

– Octenidine dihydrochloride, water-based.

During hospitalization specialists of the multidisciplinary team trained parents how to provide daily care of the child's skin and make correct rehabilitation. It included information about how to

rehabilitate the body and wounds carefully, avoiding significant pressure and pulling movements; avoid "erasing" movements when handling/cleaning the body with wipes, to change them for "wetting" movements; making correct bandages.

It was recommended to provide after discharge:

– continuous care of the child's skin
– social assistance as a child with a disability
– supervision by a dermatologist and pediatrician/family doctor;
– consultation of a dentist, surgeon, ophthalmologist, psychologist, nutritionist after 6 months.

CONCLUSIONS

Inherited epidermolysis bullosa requires the patient to fight the disease throughout his life, which is associated not only with the severity of the disease, but also with the development of secondary complications, such as deformity of the musculoskeletal system or eating problems. Despite the fact that patients with IEB need special living conditions, they try to live a normal life with its ups and downs, successes and failures, desires and dreams, overcoming daily difficulties. Dissemination and clarification of information about the disease and the difficulties caused by it, in most cases, help to understand the problems of the patient, evoking respect for him and help to form the attitude to him that he really deserves.

REFERENCE

1. Pope E., Lara-Corrales I., Mellerio J.E. et al. A Consensus Approach to Wound Care in Epidermolysis Bullosa J Am Acad Dermatol. 2012; 67(5):904-17.
2. Fine J.D., Bauer E.A., Gedde-Dahl T. In: Inherited epidermolysis bullosa: definition and historical overview. Epidermolysis Bullosa: Clinical, Epidemiologic, and Laboratory Advances, and the Findings of the National Epidermolysis Bullosa Registry Baltimore: Johns Hopkins University Press; 1999. 305 p.
3. Moss C., Wong A., Davies P. The Birmingham Epidermolysis Bullosa Severity score: development and validation. Br J Dermatol. 2009;160:1057-1065.
4. Anderson S.H., Meenan J., Williams K.N. et al. Efficacy and safety of endoscopec dilatation of esophageal strictures in epidermolysis bullosa. Gastrointest Endosc. 2004;59:28-32.
5. Springer-Verlag W. Occupational Therapy in Epidermolysis Bullosa. A Holistic Concept for Intervention from Infancy to Adult. Hedwig Weib, Mphil. Florian Prinz. 2013, 275p.
6. Alissa E.M., Bahijri S.M., Ferns G.A. The controversy surrounding selenium and cardiovascular disease: a review of the evidence. Med Sci Monit. 2003;9:RA9-RA18.
7. Adapted evidence-based clinical guidelines. Epidermolysis bullosa. Kiev. 2016. 76p.

The work was carried out within the framework of the research work of the Department of Pediatrics No. 2 of the Vinnytsia National Medical University named after N.I. Pirogov "Improvement of diagnostics, treatment and determination of prediction of various somatic and orphan diseases in children."

ORCID and contributionship:
Veronika M. Dudnyk: 0000-0003-2164-8204 [A, F]
Olga I. Izumets : 0000-0002-6421-4863 [B, D]
Oksana S. Rubina: 0000-0003-4410-7910 [C]
Valentin H. Furman: 0000-0001-6544-3273 [E]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olga I. Izumets
Vinnytsia National Pirogov Memorial Medical University
56 Pirogova st., 21018 Vinnytsia, Ukraine
tel: +380675094714
e-mail: izyumets.olga@gmail.com

Received: 15.10.2020
Accepted: 02.08.2021

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

COVID-19 CHALLENGE, IN WHICH A THREE-YEAR-OLD GIRL WON

DOI: 10.36740/WLek202110130

Halyna O. Lytvyn, Mariia V. Stasiv, Volodymyr R. Mishchuk, Iryna Yu. Avramenko
DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE

ABSTRACT

Since March 11, coronavirus infection has become an intercontinental problem – a pandemic has developed.Ukraine (until December 2019) ranks 17th in the world in the number of Covid-19 cases. Although according to statistics, the children are the least vulnerable group for coronavirus infection, unfortunately, severe and serious complications such as pneumonia, Kawasaki disease and Kawasaki-like syndrome, Multisystem inflammatory syndrome in children, toxic shock syndrome, myocarditis occur in children, too. As of the end of November, according to the Ministry of Health in Ukraine, 732,625 cases of coronavirus were laboratory- confirmed, including 13,720 children. According to the Lviv Regional Laboratory Center of the Ministry of Health, in the Lviv region since the beginning of the Covid-19 pandemic, among 46078 of all infected were about 5-6% of children. To analyze clinical, laboratory features of severe coronavirus infection complicated by bilateral pneumonia with acute respiratory distress syndrome (ARDS) in a three-year-old girl who was on V-V ECMO for one week and mechanical ventilation of the lungs for 28 days. The diagnosis was confirmed by detection of SARS-CoV-2 virus RNA by PCR, X-ray and ultrasound examination of the lungs. The disease had a dramatic course but a successful outcome. Life-threatening conditions associated with COVID-19 in children are much less common than in adult patients. However, in some cases, when critical hypoxemia is not eliminated by traditional methods of respiratory support, ECMO can become a life-saving technology and with its timely usage in pediatric patients.

KEY WORDS: SARS-CoV-2, children, extracorporeal membrane oxygenation (ECMO), respiratory failure, ACE2

Wiad Lek. 2021;74(10 p.I):2530-2534

INTRODUCTION

Since the end of 2019, the new coronavirus infection (COVID-19) has spread from China to other countries, and the number of cases is growing rapidly. The virus has become a global threat. On January 30, the WHO declared an international emergency over the coronavirus. Since March 11, coronavirus infection has become an intercontinental problem – a pandemic has developed [1].

Ukraine (until December 2019) ranks 17th in the world in the number of Covid-19 cases. Although according to statistics, the children are the least vulnerable group for coronavirus infection, unfortunately, severe and serious complications such as pneumonia, Kawasaki disease and Kawasaki-like syndrome, Multisystem inflammatory syndrome in children, toxic shock syndrome, myocarditis occur in children, too [2]. As of the end of November, according to the Ministry of Health in Ukraine, 732,625 cases of coronavirus were laboratory confirmed, including 13,720 children. According to the Lviv Regional Laboratory Center of the Ministry of Health, in the Lviv region [3] since the beginning of the Covid-19 pandemic, among 46078 of all infected were about 5-6% of children.

At the beginning of the epidemic, COVID-19 in children did not cause concern to doctors, as there was a mild or asymptomatic course of the disease. Clinically, it is similar to acute respiratory viral infections (ARVI) and was not threatening to the pediatric group [4]. In the future, there were more reports of a wider clinical spectrum of pediatric COVID-19: from asymptomatic to critically severe disease. The most common symptoms in children and adolescents are fever and cough, headache, sore throat, myalgia, shortness of breath, nausea, abdominal pain, vomiting and diarrhea. Other clinical features, such as renal, cutaneous, olfactory, gustatory, neurological, and ocular, are less common in the COVID-19 pediatric population [5]. No significant differences in demographic and clinical indicators were found in patients with and without background allergic diseases, but severe forms of coronavirus infection in children were more common in obese children [6]. Subsequently, severe cases of SARS-CoV-2 have been reported more frequently in various countries in children with pneumonia complicated by distress syndrome [7, 8]. Some children developed shock with biochemical signs of myocardial dysfunction and multisystem inflammatory response syndrome in children with multiple organ failure [8, 9]. The development of such a severe process is associated with hemophagocytic syndrome (HPS), or hemophagocytic lymphohistiocytosis (HLH) – a disease based on uncontrolled activation of the effects of cellular immunity. Activation of cytotoxic T lymphocytes and tissue macrophages (histiocytes) is accompanied by hyperproduction of proinflammatory cytokines and leads to severe organ dysfunction [10]. Correction of severe hypoxic and metabolic conditions requires intravenous (venous-venous) extracorporeal membrane oxygenation (ECMO). The main indications for ECMO are severe, life-threatening disorders of pulmonary gas exchange, which are accompanied by impaired oxygenating (arterial hypoxemia) and / or ventilation (arterial hypercapnia) lung function [11].

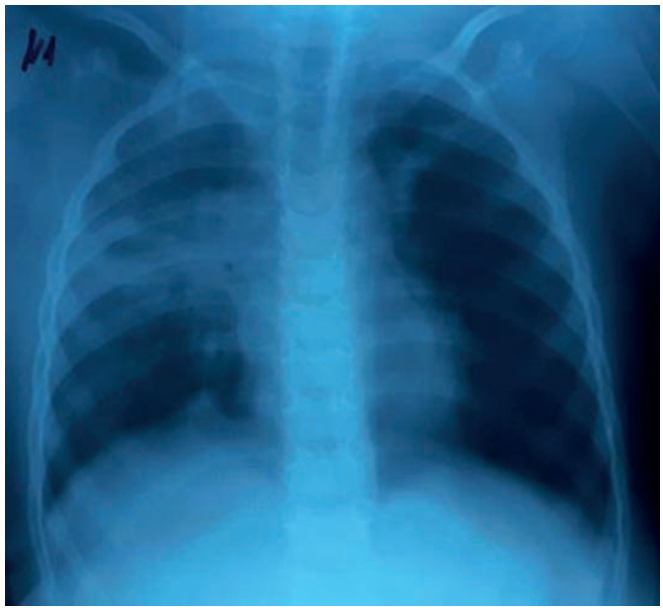


Fig. 1. Right-sided focal pneumonia.

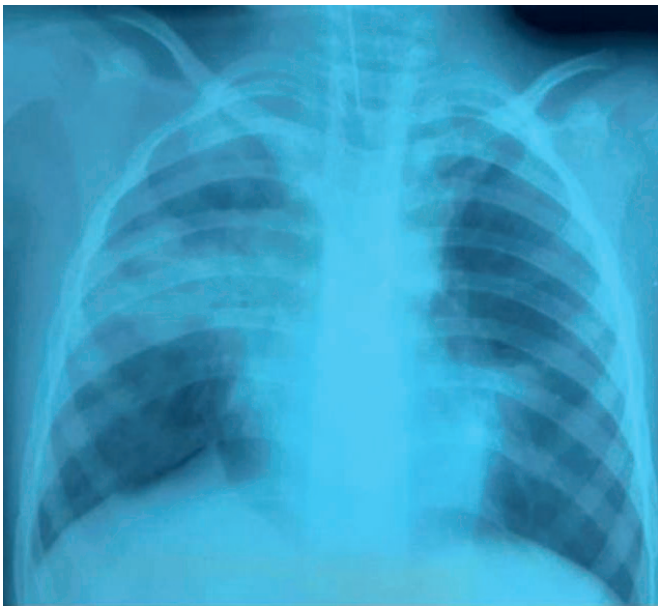


Fig. 2. Increase of the intensity of opacities on the right side and the emergence of new bilateral infiltrates.

To analyze clinical, laboratory features of severe coronavirus infection complicated by bilateral pneumonia with acute respiratory distress syndrome (ARDS) in a three-year-old girl who was on V-V ECMO for one week and mechanical ventilation of the lungs for 28 days.

The diagnosis was confirmed by detection of SARS-CoV-2 virus RNA by PCR, X-ray and ultrasound examination of the lungs.

CASE STUDY

A 3-year-old girl (body weight 15 kg) was admitted to the intensive care unit of the Lviv Regional Children's Clinical Hospital on the 14 of September 2020. History: the child had been ill for two days as fever and shortness of breath developed. She was not in contact with infectious patients. Hereditary, allergic history is not complicated. She was born from the second full-term pregnancy. Bodyweight at birth – 4200 g. She developed according to age.

14.09.2020 Physical examination revealed: condition – severe due to respiratory failure, shortness of breath -72/min, retraction of intercostal spaces, heart rate (HR) – 170/min, SpO2 – 87%, body temperature – 36,8°C. Crackles on both sides were detected during auscultation of the lungs, breathing remained weakened on the right side. Heart tones were clear, sinus tachycardia. Chest X-rays revealed signs of right-sided focal pneumonia.

14.09. 2020 – Treatment was prescribed: oxygen therapy through a face mask (O2 flow 10 l/min), antibiotic therapy: (ceftriaxone + azithromycin), correction of water-electrolyte disorders. Because oxygen therapy was not accompanied by a significant improvement of oxygenation (SpO2 – 90-91%, PaO2 – 68 mmHg, PaCO2 – 55 mmHg, pH – 7), shortness of breath with additional muscles persisted, the child was administered non-invasive lung ventilation: NIV CPAP/PSV: FiO2 -60%, PEEP – 8 cmH2O, PIP – 5 cmH2O). A temporary improvement of oxygenation was obtained: PaO2/FiO2-133, PaO2 –80 mmHg, PaCO2- 57 mmHg.

15.09.2020 there were further progression of respiratory failure and hypoxemia (PaO2/FiO2-117) on the background of NIV CPAP/PSV in the child, as well as deterioration of neurological status on the Glasgow coma scale (decrease in GCS scale from 14 to 12 points). Control Chest X-rays revealed a deterioration of the radiological imaging: increase of the intensity of opacities on the right side and the emergence of new bilateral infiltrates. The child was administered mechanical ventilation: P/SIMV, FiO2- 50%, PIP-12 cmH2O, PEEP – 10 cm H2O, TV 6 ml/kg, RR-25/min, I/E 1:1,5. Given the data of acid-base balance (BE-8), infusion of dobutamine 5 μg/kg/min was prescribed.

CBC (15.09.2020): HGB 9,7g/dl., RBC 3,8 ×1012/l, PLT 320 ×109/l, WBC 12,5×109/l, neutrophils 78%, lymphocytes 19%, monocytes 3%. Procalcitonin test (15.09.2020) – 0,28 ng / ml, IL-2 -0, IL-6 – 23pg/ml.

16.09.20 – a positive PCR test for SARS-COV-2 was obtained.

Based on a positive PCR test for SARS-COV-2, clinical symptoms, radiographic picture and, PaO2/FiO2 – 117, the diagnosis was made: SARS-COV-2 infection, bilateral polysegmental pneumonia, acute respiratory distress syndrome (ARDS) of the moderate degree.

16.09.2020-19.09.2020 The child was on P/SIMV convection respiratory support in the prone position. Required strict parameters of mechanical ventilation: PIP-15 cm H2O, PEEP – 14 cm H2O, TV 6 ml/kg, RR-25 / min, I/E 1:1,2. The patient's condition deteriorated, the patient needed an increase of FiO2 from 60% to 100%. Negative dynamics of PaO2/FiO2 from 117 to 80 were noted. Ultrasound examination (Lung ultrasound – Blue protocol) was used as bedside monitoring of lung condition. Ultrasound examination of

the lungs showed signs of interstitial syndrome (a large number of B-lines, single A-lines) and a symptom of consolidation in the basal areas of the lungs on both sides (Lung consolidation, shred sign). There was a decrease in the ejection fraction of the left ventricle (IF – 45%). Clinically, there was a decrease in urine output to 0,5 ml/kg/h. The child received medication: meropenem, fluconazole, dobutamine – 7,5 μg/kg/min, furosemide 7 mg/kg/d, dexamethasone – 8 mg/d, heparin – 5 IU/kg/h. Laboratory CRP-20 mg / l, Procalcetonin test – 0,123 ng/ml.

20.09.20 The child was subjected to the high-frequency oscillatory ventilation: HFOV: FiO2 – 100% Paw – 25 cm-H2O, ΔP33%, Frequency 7 Hz. However, no significant improvement in oxygenation was achieved (PaO2/FiO2 -72).

23.09.2020 There was a decrease in blood oxygenation (PaO2/FiO2 – 60) . It was decided to apply the method V-V ECMO for the child. The surgeon cannulated the right internal jugular vein (Return cannula: 14 Fr) and the left femoral vein (Access cannula: 16 Fr). V-V ECMO settings: –RPM (Pump speed) 3125, LPO (Blood flow rate) – 0,7-0,8, FiO2 – 100%. Convection mechanical ventilation of the lungs continued: P/SIMV, FiO2 – 30%, PIP-14 cm H2O, PEEP – 10 cm H2O, TV 6 ml/kg, RR-15/min, I/E 1:1,5. The child required prolonged analgesia and sedation (thiopental sodium, fentanyl) and heparinization (target values APTT 45-50 s,)

As a result of V-V ECMO there was an improvement in oxygenation of PaO2 170-180 mmHg., as well as respiratory mechanics – Cst increased from 8 ml / cmH2O to 22 ml / cmH2O. Duration V-V ECMO – 7 days. Subsequently, convection ventilation continued: P/SIMV: PIP 18 cmH2O, PEEP – 10 cmH2O, FiO2 – 40%, RR – 20/min, Cst – 14 ml/cmH2O. Satisfactory oxygenation (PaO2/FiO2 -310) and hemodynamics (IF-60%) were achieved.

After 6 days, the child was weaned from mechanical ventilation . After 10 days he was transferred from the intensive care unit. Complications were observed during treatment:

1. Hematoma of the anterior abdominal wall (as a consequence of heparin therapy). Surgical drainage of the hematoma was performed.
2. Thrombocytopenia (as a consequence of heparin therapy). Platelet transfusion was administered.
3. Steroid diabetes (patient needed insulin infusion).
4. Cognitive disorders.

The child was discharged with minimal neurological deficit. The total duration of hospitalization was 67 days.

DISCUSSION

The coronavirus pandemic (COVID-19) affects all age groups, but is less severe in children [4, 12, 13]. A possible reason for this difference in severity between adults and children may be related to the difference in receptors in the renin-angiotensin system (RAS) and the altered inflammatory response to pathogens [14]. Angiotensin I converting enzyme 2 (ACE2) is a protein encoded by the ACE2 gene located on the human X chromosome. It is a membrane or secreted enzyme protease, the main function of which is the cleavage of the peptide hormone angiotensin I to a more active form of angiotensin II. ACE2 destroys the peptide bradykinin and is its natural regulator in humans. Also, this protein is a receptor for the entry into the cell of several viruses, including coronaviruses SARS-CoV and SARS-CoV-2 [14]. Changes in immune function and key receptors in RAS occur in children, which may explain the milder course of the disease [15]. SARS -CoV-2 uses the ACE2 receptor to penetrate cells, and is mainly distributed through the respiratory tract. These receptors are present in many cell types in the body, including immune cells such as monocytes, neutrophils and lymphocytes. The RAS system is associated with inflammation through angiotensin II, and ACE2 alters RAS activity from a pro-inflammatory to an anti-inflammatory response [16]. But patients with different levels of ACE2 may have an abnormal immune response and pneumonia. In the lungs, SARS-CoV-2 mainly affects pneumocytes and macrophages, activating them, and can lead to multiple organ failure. The pathophysiology of macrophage activation syndrome is the cytolytic activity of lymphocytes [10]. Cytokines storm (that is, IL-1, IL-6, IL-18) leads to the activation of macrophages, creating hemophagocytosis and contributing to organ dysfunction [17]. Clinical signs of HPS are characteristic of hyperinflammatory syndromes, or cytokine storm syndrome. Hyperinflammatory syndromes are the same pathophysiological condition in the final stage, which is the result of the developed initiation of triggers of uncontrolled inflammation. Hyper inflammatory syndromes are classified based on known major triggers or defects [18]. Implementation of the prediction of severe infectious process in children is also possible in the study of levels of pro-inflammatory and anti-inflammatory cytokines, procalcitonin and C-reactive protein, troponin [7, 19, 20]. Mohamed Jeljeli and others [21] studied the ontogenesis of cytokine production in response to phytohemagglutinin from newborns to adults and noted a change from elevated IL-10 in neonates to a balanced level of IL-10 / T helper type 1 (Th1) / Th2 / Th17 in early life. This protects against pathogens, but improves the storm of cytokines. Changes in ACE2 are critical for neutrophil influx and pneumonia. Severe COVID-19 infection is characterized by a massive inflammatory reaction or a cytokines storm, leading to ARDS and multiorgan dysfunction. This result suggests that the etiology may be hemophagocytosis or macrophage activation syndrome [10]. Based on the understanding of this syndrome, and many studies that have determined the indicators of proinflammatory cytokines in severe clinical forms of coronavirus infection, glucocorticosteroids, intravenous immunoglobulin and other immunosuppressive drugs are used. Lesions of the cardiovascular system, lungs (interstitial changes) and kidneys are observed in the most severe cases, when there is infiltration by macrophages of tissues of internal organs [20]. Extracorporal membrane oxygenation should be considered as rescue therapy for patients in cases when traditional respiratory support methods, including conventional ventilation and HFOV, do not provide adequate blood oxygenation. According to Euro-ELSO [22], the frequency of ECMO utilization during the

COVID-19 pandemic was 0.5% – 1% from all hospitalized patients. In Europe, as on May 7, 2020, 1068 adult patients required ECMO support. Although, cases of pneumonia due to SARS-CoV-2 have been reported in infants [23], children [24] and young adults [25], these patients generally had good outcomes and rarely required extracorporeal life support. Thus, according to the EURO ELSO survey, conducted on 28 January 2021 in European countries, only 10 cases of ECMO in children were officially registered, while more than 1,000 cases of ECMO were registered in adult patients [22].

Adult patients with COVID-19 required from 20 to 50 days of ECMO to recover [26]. In the case of our patient, 7 days of ECMO was sufficient to maintain oxygenation and allowed protective ventilation to be carried out until the inflammatory changes in the lungs regressed. A group of Spanish physicians led by Gimeno-Costa. R has also reported a shorter duration of ECMO in children with COVID-19 compared to adults. Their report noted the duration of ECMO 7 days in a female patient aged 16 years, which led to the patient's survival.

Concerning the children's survival, the results of ECMO treatment in the pediatric population are better than in adults and are on average 57% [27]. Mechanical ventilation more than 2 weeks before ECMO, as well as comorbid conditions, are considered by different authors as predictors of mortality in patients requiring ECMO. As for the most common complications that develop in children on ECMO, most authors point to the threatening bleeding as a result of anticoagulant therapy. Our patient developed a large hematoma of the anterior abdominal wall, which led to intra-abdominal hypertension and required surgical drainage. According to H. J. Dalton, et al. bleeding occurred in 70.2% of children on ECMO, including intracranial haemorrhage in 16% of patients, which was independently associated with a higher daily risk of mortality [28].

CONCLUSIONS

In conclusion, life-threatening conditions associated with COVID-19 in children are much less common than in adult patients. However, in some cases, when critical hypoxemia is not eliminated by traditional methods of respiratory support, ECMO can become a life-saving technology and with its timely usage in pediatric patients, successful treatment results are observed in more than half of cases.

REFERENCES

1. Worldometer. Coronavirus Disease Cases. Corona Virus. 2021. https://www.worldometers.info/coronavirus/
2. Dufort E.M., Koumans E.H., Chow E.J. et al. Multisystem inflammatory syndrome in children in New York State. N Engl J Med. 2020;383(4):347-58. doi: 10.1056/NEJMoa2021756.
3. Lviv Regional Laboratory Center of the Ministry of Health. Up to 5% of patients with coronavirus in the region are children. 2021. https://loda.gov.ua/news?id=55572
4. Huang C., Wang Y., Li X. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.
5. Song W., Li J., Zou N. et al. Clinical features of pediatric patients with coronavirus disease (COVID-19). J Clin Virol. 2020;127:104377. doi: 10.1016/j.jcv.2020.104377.
6. Pouletty M., Borocco C., Ouldali N. et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS- CoV-2 mimicking Kawasaki disease (Kawa- COVID-19): a multicentre cohort. Ann Rheum Dis. 2020;79(8):999-1006. doi: 10.1136/annrheumdis-2020-217960.
7. Li Y., Zheng Q., Zou L. et al. Kawasaki disease shock syndrome: clinical characteristics and possible use of IL-6, IL-10 and IFN-γ as biomarkers for early recognition. Pediatr Rheumatol Online J. 2019;17(1):1. doi: 10.1186/s12969-018-0303-4.
8. Verdoni L., Mazza A., Gervasoni A. et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. Lancet. 2020;395(10239):1771-8. doi: 10.1016/S0140-6736(20)31103-X.
9. Belhadjer Z., Méot M., Bajolle F. et al. Acute heart failure in multisystem inflammatory syndrome in children (MIS-C) in the context of global SARS-CoV-2 pandemic. Circulation. 2020;142(5): 429-436. doi: 10.1161/CIRCULATIONAHA.120.048360.
10. Crayne C., Cron R.Q. Pediatric macrophage activation syndrome, recognizing the tip of the Iceberg. Eur J Rheumatol. 2019;7(1):1-8. doi: 10.5152/eurjrheum.2019.19150.
11. Götzinger F., Santiago-García B., Noguera-Julián A. et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. Lancet Child Adolesc Health. 2020;4(9):653-661. doi: 10.1016/S2352-4642(20)30177-2.
12. Badal S., Thapa Bajgain K., Badal S. et al. Prevalence, clinical characteristics, and outcomes of pediatric COVID-19: A systematic review and meta-analysis. J Clin Virol. 2021;135:104715. doi: 10.1016/j.jcv.2020.104715.
13. 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):271-80.e8. doi: 10.1016/j.cell.2020.02.052.
14. Bunyavanich S., Do A., Vicencio A. Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. JAMA. 2020;323(23):2427-9. doi: 10.1001/jama.2020.8707.
15. Iwasaki M., Saito J., Zhao H. et al. Inflammation Triggered by SARS-CoV-2 and ACE2 Augment Drives Multiple Organ Failure of Severe COVID-19: Molecular Mechanisms and Implications. 2021;44(1):13-34. doi: 10.1007/s10753-020-01337-3.
16. Ni W., Yang X., Yang D. et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care. 2020;24(1):422. doi: 10.1186/s13054-020-03120-0.
17. McGonagle D., Sharif K., O'Regan A. et al. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun Rev. 2020;19(6):102537. doi:10.1016/j.autrev.2020.102537.
18. Mehta P., McAuley D.F., Brown M. et al. Across Speciality Collaboration, UK. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020;395(10229):1033-4. doi: 10.1016/S0140-6736(20)30628-0.
19. Riphagen S., Gomez X., Gonzalez-Martinez C. et al. Hyperinflammatory shock in children during COVID-19 pandemic. Lancet. 2020;395(10237):1607-8. doi: 10.1016/S0140-6736(20)31094-1.
20. Rizzo P., Vieceli Dalla Sega F., Fortini F. et al. COVID-19 in the heart and the lungs: could we «Notch» the inflammatory storm? Basic Res Cardiol. 2020;115(3):31. doi: 10.1007/s00395-020-0791-5.

21. Jeljeli M., Guérin-El Khourouj V., Pédron B. et al. Ontogeny of cytokine responses to PHA from birth to adulthood. Pediatr Res. 2019;86(1):63–70. doi: 10.1038/s41390-019-0383-y
22. EURO ELSO. European Survey on ECMO in COVID-19 pts at 7/05/20. https://www.euroelso.net/covid-19/covid-19-survey/.
23. Alonso Díaz C., López Maestro M., Moral Pumarega M.T. et al. First case of neonatal infection due to SARS-CoV-2 in Spain. An Pediatr. 2020;92(4):237–8. doi: 10.1016/j.anpedi.2020.03.002
24. Ong J.S.M., Tosoni A., Kim Y.J. et al. Coronavirus Disease 2019 in critically ill children: a narrative review of the literature. Pediatr Crit Care Med. 2020;21(7):662-666. doi: 10.1097/PCC.0000000000002376.
25. Guan W.J., Ni Z.Y., Hu Y. et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382:1708.
26. Gimeno-Costa R., Barrios M., Heredia T. COVID-19 respiratory failure: ECMO support for children and young adult patients. An Pediatr (Barc). 2020;93:202-205.
27. Zabrocki L.A., Brogan T.V., Statler K.D. et al. Extracorporeal membrane oxygenation for pediatric respiratory failure: survival and predictors of mortality. Crit Care Med. 2011;39(2):364-70. doi: 10.1097/CCM.0b013e3181fb7b35.
28. Dalton H.J., Reeder R., Garcia-Filion P. et al. Factors associated with bleeding and thrombosis in children receiving extracorporeal membrane oxygenation. Am J Respir Crit Care Med. 2017;196(6):762-771. doi: 10.1164/rccm.201609-1945OC.

ORCID and contributionship:
Mariia V. Stasiv: 0000-0002-7110-0557 [A-C,F]
Halyna O. Lytvyn: 0000-0002-6902-1024 [A-D,F]
Volodymyr R. Mishchuk: 0000-0003-0542-0891 [B,D,F]
Iryna Yu. Avramenko: 0000-0002-7812-6602 [C]

Conflict of interest:
The Authors declare no conflict of interest

CORRESPONDING AUTHOR
Mariia V. Stasiv
Danylo Halytsky Lviv
National Medical University
69 Pekarska st, 79010 Lviv, Ukraine
tel: +380673419081
e-mail: mariatanchuk@gmail.com

Received: 23.02.2021
Accepted: 15.08.2021

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