VOLUME LXXVI, ISSUE 2, FEBRUARY 2023

ISSN 0043-5147
E-ISSN 2719-342X

Wiadomości Lekarskie
Medical Advances

Official journal of Polish Medical Association has been published since 1928

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

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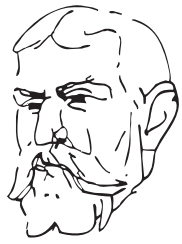
ALUNA Publishing House



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

Wiadomości Lekarskie
Medical Advances

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

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ORIGINAL ARTICLE

IMPLEMENTATION OF THE HOSPITAL DISTRICT FOR THE MEDICAL INSTITUTIONS: MEDICAL AND SOCIAL JUSTIFICATION OF THE FINANCIAL AND ECONOMIC STATE OF KALUSH CENTRAL DISTRICT HOSPITAL BEFORE AND AFTER IMPLEMENTATION

DOI: 10.36740/WLek202302101

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ABSTRACT

The aim: To determine the financial and economic condition before and after the implementation of the hospital district in the Kalush Central District Hospital and to show the medical and social justification of the changes in the institution's finances.

Materials and methods: The object of this study was the activity of the Kalush Central District Hospital, which is a multidisciplinary medical and preventive health care facility, in which medical assistance is provided to patients in surgical, neurosurgical, traumatological, cardiological, gastroenterological, endocrinological, urological departments, in the department of miniinvasive surgery. In order to see how the implementation of hospital districts affected the financial condition of medical institutions, the financial statements of the institution for 2017-2018 were used to study the financial condition of the organization. During this period medical assistance was provided to more than 92,000 patients.

Results: The reform of the health care system in 2017 took place in accordance with the developed concept of the development of medicine, which is based on the creation of hospital districts. On average, the hospital district covers about 60 kilometers of territory. Such a distance allows us to deploy a powerful network of various hospitals that are able to provide almost the entire range of medical services, starting from diagnostics and ending with urgent treatment. The hospital district is headed by an institution that coordinates the work of all institutions and recommends building such organizational and financial structures that allow the medical institution to develop and create a quality medical product. Kalush Central District Hospital coped with the reforms of medicine, the implementation of hospital districts became a significant event that changed not only the organization of the provision of medical services, but also changes in the financial and economic condition of medical institutions. In general, the financial condition of the enterprise shows that the hospital is autonomic, it is financed from its own sources of financing.

Conclussions: The financial condition of the enterprise shows that the Kalush Central District Hospital is autonomous, that is, to a greater extent, it is financed from its own sources of financing. However, liquidity indicators are negative, which require more effective management of cash flows so that the organization can timely repay salary arrears and pay mandatory payments for the use of material resources and energy. At the same time, a large number of patients are coming to the hospital because the income level has increased, which is definitely a positive factor. However, when planning activities for the following periods, it is necessary to take into account the need to update material and technical support, as well as to find sources of increasing staff wages.

KEY WORDS: medical reform, financial and economic state, district hospital, medical services, solvency, financial stability.

Wiad Lek. 2023;76(2):243-250

INTRODUCTION

Any country, when building its own social policy, focuses on the formation of the most advanced model of medical care for the population. It is safe to say that quality medical services are a sign of a developed society. This pattern is also reversed, in particular, the more developed the country, the more resources are allocated to the development of medicine. In Ukraine, 2.7% is allocated to medicine, although developed countries allocate at least 5% [1]. These funds were lacking during the pandemic, and they are even more lacking during the period when Ukraine faced war and daily demands for continuous medical care for people affected by the war.

Ukraine faced reforms in the medical sector in 2017. In general, this reform characterizes changes in the financing of medicine, in particular, the funds are allocated not to meet the needs of the medical institution (including staff wages, utility bills), but to finance the costs necessary to provide medical services to patients who apply to the institution. Among the people, such changes in financing were called «money follows the patient» [2, 3, 4].

In order for such a reform to be implemented, it was necessary to change the structure of medical services, which required the implementation of hospital districts. In essence, hospital districts are a network of medical institutions that serve a certain territorial community, providing them with a full range of medical services [5, 6]. In theory, the essence of the reform assumes that any member of the community has the opportunity to receive full medical care in their region. That is, with this reform, each medical organization must work independently to improve its own material and technical base, which will allow to receive a full range of diagnostic and treatment services, as well as increase and improve the number of employees.

It should be noted that not every community effectively uses the potential of this reform. There are more successful medical institutions, and there are those that are severely lacking in funds to meet their own needs [7, 8]. That is why quite often different communities are faced with the need to close some hospitals or departments, which receives public condemnation. In order to determine the ability of a medical institution to work in new conditions, it is necessary to competently manage finances, and for this, a constant financial analysis should be carried out and directions for improving the financial condition should be studied. As for the medical and social substantiation of the financial and economic condition of the medical institution, it actually requires additional study, and its results will depend on the financial condition of the research object.

THE AIM

To determine the financial and economic condition before and after the implementation of the hospital district in the Kalush Central District Hospital and to show the medical and social justification of the changes in the institution's finances.

MATERIALS AND METHODS

The Kalush Central District Hospital was established in 2005 and is a multidisciplinary medical and preventive health care facility, in which medical assistance is provided to patients in surgical, neurosurgical, traumatological, cardiological, gastroenterological, endocrinological, urological departments, in the department of miniinvasive surgery. The Kalush Central District Hospital also has a diagnostic department, a clinical and diagnostic laboratory, a polyclinic and a maternity hospital. The hospital has 930 inpatient beds, which is 69.4 beds per 10,000 populations, including 288 day inpatient beds at outpatient polyclinic facilities. Currently, 507 doctors and 1,013 medical personnel are providing medical care.

The object of this study is the activity of the Kalush Central District Hospital of the District Councils of Ivano-Frankivsk Oblast (KCDH). In order to see how the implementation of hospital districts affected the financial condition of medical institutions, a number of expert publications were analyzed, which determine the specifics of the change in the organizational structure and funding rules, in particular the work: Blank I.O. [9], Basilinska O.Ya. [10], Belyalov T.E. [11], Chernenok K.P. [12]. The financial statements of the institution for 2017-2018 were used to study the financial condition of the organization. During this period (2017-2018), medical assistance was provided to more than 92,000 patients.

RESULTS

The issue of medical and social substantiation of the financial and economic condition of medical institutions is sufficiently covered in ukrainian and foreign scientific literature [3, 7, 12, 13]. However, in order to understand the profitability of the hospital, it is necessary to carry out a financial and economic analysis of the activity of the medical institution and to understand the specifics of the implementation of reforms in the field of medicine.

The KCDH has been established in the city of Kalush, which includes medical facilities in the city of Kalush, the city of Bolehova of the Kalush district, the towns of Rozhnyativ and Dolyna districts. Accordingly, KCDH belongs to the Kalush Hospital District, which was subject to reform [13].

The main tasks of conducting medical reform in the Kalush Hospital District are: provision of medical services to the people on a free basis, including palliative care; provision of basic types of outpatient services, which is carried out on the referral of a family doctor; provision of basic types of inpatient medical care in medical institutions of the territorial community to which the patient belongs; reimbursement of the cost of purchased medicines, which are purchased by patients in accordance with the instructions of the family doctor; formation of prerequisites for the organization of digital medical services involving the introduction of individual cards, electronic prescriptions, etc.

The medico-social justification of the medical reform in KCDH is carried out on the basis of an analysis of the organization's financial condition. The change in the structure of KCDH property during the implementation of reforms for the period from 2017 to 2018 is presented in the table I.

Table I. Analysis of the property structure of the Kalush Central District Hospital in 2017-2018

Indicators	In the end of the year			Absolute deviation	
	2016	2017	2018	2018-2017	2018-2016
NCA/A	0,65	0,56	0,69	0,13	0,04
CA/A	0,35	0,44	0,31	-0,13	-0,04
OC/L	0,87	0,98	0,60	-0,38	-0,27
CL/L	0,13	0,02	0,40	0,38	0,27

Note: NCA – non-current assets; CA – current assets; A – assets; OC – own capital; CL – Current liabilities; L – liabilities.

Table II. Analysis of own working capital of Kalush Central District Hospital in 2017-2018

Indicators	In the end of the year			Absolute deviation	
	2016	2017	2018	2018-2017	2018-2016
Own working capital	1229	2399	-383	-2782	-1612
Own working capital - Reserves	-648,20	-33,89	-1629,33	-1595,44	-981,13
Own working capital + Long-term debt - Reserves	-648	-34	-1629	-1595	-981
Own working capital + Short-term debt + Long-term debt - Reserves	69	79	102	23	33

Table III. Analysis of liquidity indicators of the Kalush Central District Hospital in 2017-2018

Indicators	At the beginning of the year			Absolute deviation	
	2016	2017	2018	2018-2017	2018-2016
Absolute deviation coverage	2,71	22,25	0,78	-21,47	-1,93
Quick liquidity ratio	0,10	0,70	0,06	-0,64	-0,04
Absolute liquidity ratio	0,08	0,68	0,06	-0,63	-0,02

Table IV. Analysis of indicators of financial sustainability of Kalush Central District Hospital in 2017-2018

Indicators	At the beginning of the year			Absolute deviation	
	2016	2017	2018	2018-2017	2018-2016
Financial risk	0,15	0,02	0,66	0,64	0,51
Maneuverability of own capital	0,26	0,43	-0,15	-0,58	-0,40

According to the results of the research, it can be concluded that the main part of the property of the KCDH consists of non-current assets of the organization. As of 2018, the NCA/A ratio was 0.69. During the last year, there was an increase in the indicator, which indicates that the institution increased its material and technical support, which is definitely a positive factor for the city hospital. However, it should also be noted that the ratio of non-current assets to assets was not distinguished by stability. If the indicator decreased in 2017, then in 2018 the indicator increased.

As for current assets of KCDH, their specific weight in assets was 31%. Accordingly, the indicator also had unstable dynamics, in particular, it increased in 2017 and fell by 0.13 in 2018. To a greater extent, the capital of the hospital was formed at the expense of its own sources of financing. At the same time, the trend of an unstable indicator of autonomy, which requires a more balanced policy of managing financial resources, was observed during the researched period. Thus, as of 2018, the ratio of own capital to liabilities was 0.60 relative to unit. This is a critical value that indicates that the organization is financially stable, but at the same time unstable. Accordingly, the ratio of current debt to liabilities as of 2018 was 0.40.

It should be noted that KCRL did not have its own working capital in 2018. Its size in 2018 was UAH 383,000. The indicator significantly decreased compared to 2017. It should be noted that the analysis of the set of 4 indicators in Table II testifies to the fact that the financial condition is not characterized by stability, and in 3 years there has been a noticeable deterioration of it.

Solvency ratios are of great importance in order for the hospital to be able to settle its own obligations in a timely manner, as well as to purchase the necessary material and technical support. It should be noted that during the studied period, liquidity indicators are not characterized by stability, and their size changes significantly: the absolute deviation coverage ratio in 2018-2017 is -21.47, the quick liquidity ratio and the absolute liquidity ratio are -0.64 and 0.63 in accordance (Table III).

This suggests that fundamental structural changes have taken place in the organization, which required additional funds, as well as reformatting the use of financial resources to address urgent needs. The coefficients of quick liquidity and absolute liquidity testify that KCDH does not have enough funds to solve its own current needs. That is why it is necessary to pay attention to the planning of solvency in the future.

As for the indicators of financial risk, it is necessary to note the following (Table IV): if in 2017 the coefficient of financial risk was 0.02, then in 2018 the indicator was 0.66 relative to units, that is, there was an increase in the indicator by 0.64 relative units. This is an extremely negative value to which more attention should be paid. The tendency of the maneuverability of own capital can also be considered negative. In 2018, own capital ceased to be flexible, and therefore could not be used to solve urgent problems and needs of the organization.

It is natural for any state institution to have a shortage of funds and to end up with a financial result with a deficit. Unlike many hospitals, KCDH has no losses, but this does not mean that its financial condition is stable. In order to confirm the effectiveness of the organization's activity, or to refute it, it is necessary to study the indicators of business activity.

In 2018, the organization received UAH 37,666,000 in revenue, the bulk of which was budget allocations in the amount of UAH 30,188,000. The structure of financial results is shown in Table V.

The budget surplus in 2018 amounted to UAH 1,130,000, which is UAH 350,000 more than in 2017. These funds were mainly directed to the material and technical development of the institution.

According to the results of the study, it was established that the indicators of business activity in general had a positive trend, which is explained by the increase in the amount of income and the search for additional and alternative sources of financing the needs of the medical institution (Table VI).

Thus, the turnover ratio increased, and accordingly, the asset turnover period decreased by almost 4 days. As for current assets, their turnover period also decreased by 2 days, which deserves a positive assessment. The turnover ratio of fixed assets of KCDH also decreased by 2 days.

However, the trend of increasing turnover of accounts payable is negative. In general, in 2018, payables were repaid 5 days longer than in 2017. This is not a significant threat to the organization. However, it should also be taken into account when planning indicators in the next period.

As for the profitability of the hospital, it should be noted that in general, the activity of the KCDH is profitable (Table VII). The coefficient of return on assets is insignificant, but positive and is 0.2. There is a positive trend of change in all indicators of profitability during the studied period, which deserves a positive assessment.

At the same time, in order to understand the medical and social justification of changes in the financial status in accordance with the formation of hospital districts, the main medical and social indicators that determine changes in personnel management and changes in the management of material and technical support were studied (Table VIII).

It should be noted that labor costs have decreased, which is associated with a significant reduction in the costs of maintaining the KCDH. The staff of the organization was also subject to reduction. In particular, in 2018, 31 people were cut so that the organization could not go beyond the established budget.

At the same time, a positive factor is the increase in the average monthly salary of KCDH employees. If in 2017 the salary was about 4,700 hryvnias, then in 2018 the salary increased to 4,840 hryvnias.

As for material support, the organization regularly invested in upgrading equipment. However, this did not have a positive effect on the rate of depreciation of fixed assets, as the rate of depreciation of fixed assets increased by 0.02. This means that in order for the organization to use up-to-date medical equipment, updates must be carried out more often.

It should be noted that KCDH has generally coped with the reforms of medicine, and the administration is searching for ways to improve the financial situation. In particular, the administration is engaged in increasing the volume of paid medical services and uses vacant premises for lease to commercial institutions. Despite this, by necessity, the organization reduces the consumption of budget funds and creates prerequisites for financing needs at its own expense. It requires an additional solution to the issue of strengthening the material and technical base and developing the staff of employees who will be able to provide a wider range of medical services as a result.

Table V. Analysis of the financial results of the Kalush Central District Hospital in 2017-2018

Indicators	Per year, UAH thousands			Absolute deviation	
	2016	2017	2018	2017-2018	2016-2018
Income	44543	38988	37666	-1322	-6877
Budget allocations	35673	31190	30188	-1002	-5485
Income from services	344	322	456	134	112
Other income	8526	7476	7022	-454	-1504
Costs	43652,14	38208,24	36536,02	-1672,22	-7116,12
Costs for implementation of budget programs	43284	37798	36003	-1795	-7281
Production costs	56	66	78	12	22
Other costs	312	344	455	111	143
Budget surplus	891	780	1130	350	239

Table VI. Analysis of indicators of business activity of the Kalush Central District Hospital in 2017-2018

Indicators	Turnover per year, times		Turnover period, days		Absolute deviation
	2017	2018	2017	2018	
Asset turnover ratio	7,0	7,5	51,8	48,1	-3,7
Accounts receivable turnover ratio	5569,7	12555,3	0,1	0,0	0,0
Turnover ratio of current assets	17,5	19,5	20,6	18,4	-2,1
Turnover ratio of fixed assets	11,8	12,4	30,6	28,9	-1,7
Accounts payable turnover ratio	93,9	40,8	3,8	8,8	5,0

Table VII. Analysis of profitability indicators of the Kalush Central District Hospital in 2017-2018

Indicators	Per year		Absolute deviation
	2017	2018	
Rate of return on assets	0,1	0,2	0,1
Coefficient of profitability of the activity	0,02	0,03	0,0
Return on equity ratio	0,15	0,28	0,1

Table VIII. Medical and social indicators of the Kalush Central District Hospital in 2017-2018

Indicators	Per year		Absolute deviation
	2017	2018	
Labor costs, UAH thousands	22678,944	21601,812	-1077,1
Number of personnel, persons	403	372	-31,0
Average monthly salary, UAH thousands	4,69	4,84	0,1
Residual value of fixed assets, UAH thousands	3115	2937	-177,3
Initial cost of fixed assets, UAH thousands	8899	8901	2,0
Depreciation, UAH thousands	5784	5964	179,3
Depreciation of fixed assets, relative unit	0,65	0,67	0,0

DISCUSSION

The reform of the health care system in 2017 took place in accordance with the developed concept of the development of medicine, which is based on the creation of hospital districts. The resolution of the Cabinet of Ministers «On some issues of the creation of hospital districts» defines that they are a functional association of health care institutions located in a certain region and providing the population with primary and secondary medical care [14].

On average, the hospital district covers about 60 kilometers of territory. Such a distance allows us to

deploy a powerful network of various hospitals that are able to provide almost the entire range of medical services, starting from diagnostics and ending with urgent treatment. The hospital district is headed by an institution that coordinates the work of all institutions and recommends building such organizational and financial structures that allow the medical institution to develop and create a quality medical product [15, 16]. At the same time, the main institution does not make decisions on the creation of territorial districts, the availability of appropriate hospitals, staffing and technical support in them.

Only the community of the respective territory decides on the formation of such hospital districts and the activities of certain medical institutions in it. Also, the community, led by the city government, decides how much money it is ready to allocate for the operation of this or that medical institution. That is why, if there are problems with the use of funds in the community, they must be solved jointly, taking into account areas of savings or additional sources of funding.

After the decision of the city council to finance the medical institution, its leaders consider whether they have the opportunity to function under such conditions or not. At the same time, each medical institution has the authority to independently build its own financial system and look for additional sources of funding. Today's medical institutions are financed not only from the local budget, but also from alternative resources, which can be: funds for the provision of paid additional paid medical services; funds received from renting temporarily vacant premises that are not used for their intended purpose; funds from companies and insurance organizations that carry out a comprehensive examination of employees; charitable and sponsorship contributions; financing through grants and other international programs [17].

Moreover, it is the administration that creates the prerequisites for the provision of such commercial services and ensures the possibility of obtaining additional financing. Formation of the commercial part of the medical institution's income is a priority and competence exclusively of the institution's administration.

As for the hospital district, it is essentially not a separate legal institution, but only a form of interregional cooperation [18]. It is actually a regulatory tool that allows creating a network of institutions that are able to provide medical services in full. At the special council, a number of necessary medical facilities, their specialization and the range of medical services are determined in order to provide medical care as fully as possible within the limits of a certain hospital district. The rule for building a network is simple: on the one hand, the patient must receive help in a timely manner, and on the other hand, within the limits of his own territorial district, he must receive the maximum possible medical care, not to turn to hospitals of the regional or regional level.

It should be noted that the reform of the medical system was reflected in the financial condition of various medical institutions in different ways. Yes, some medical institutions were forced to close, because the reform provides for the allocation of funds for specific patients, and there were not many such patients in the hospital. Other institutions received an additional influx of new patients, which contributes to an increase in income and creates prerequisites for the development of a certain medical specialization, building up the material and technical base, expanding the range of services. Some medical institutions rationally use allocated funds and are able to independently expand the range of medical services, create new medical services that are competitive on the market [19, 20]. This means that providing high-quality medical care, which is no worse than the medical service of a commercial institution, the state institution receives an additional influx of patients, which will be followed by an additional amount of funding.

However, despite the numerous advantages, it is worth noting that the general public negatively perceived such administrative reforms, as they were influenced by certain political forces that did not allow the community to understand the specifics of the medical reform. The essence of such a reform is that the community itself takes care of the well-being of the community, and its administration, which skilfully cooperates with the community, is responsible for the well-being of the hospital. However, in fact, in the final result, the population of a certain territorial community saw only the closure of some institutions, in particular narrow-profile medical institutions, and therefore to this day perceives all innovations negatively.

It should be noted that the KCDH coped with the reforms of medicine. In general, the financial condition of the enterprise shows that the institution is autonomous, that is, to a greater extent, it is financed from its own sources of financing.

CONCLUSIONS

The implementation of hospital districts became a significant event that changed not only the organization of the provision of medical services, but also changes in the financial and economic condition of medical institutions. Despite the fact that the Kalush Central District Hospital makes a profit, its financial condition is not characterized by stability. In general, the financial

condition of the enterprise shows that the institution is autonomous, that is, to a greater extent, it is financed from its own sources of financing. However, liquidity indicators are negative, which require more effective management of cash flows so that the organization can timely repay salary arrears and pay mandatory payments for the use of material resources and energy. At the same time, we can see that a large number of patients are coming to the hospital because the income level has increased, which is definitely a positive factor. However, when planning activities for the following periods, it is necessary to take into account the need to update material and technical support, as well as to find sources of increasing staff wages.

REFERENCES

1. Andronik V. Biudzhet na medytsynu v 2021 rotsi: chomu hroshei znovu ne vystachyt [Budget for medicine in 2021: why there will not be enough money again]. Sohodni. 2021. (in Ukrainian)
2. Hospitalni okruhy utvoreni. Shcho dali? [Hospital districts are formed. What's next?]. Vashe zdorovia. 2017. https://www.vz.kiev.ua/gospitalni-okrugy-utvoreni-shho-dali/
3. Begun JW, Zimmerman B, Dooley KM. Health Care Organisations as Complex Adaptive Systems. In: Mick SM, Wyttenbach M, editors. Advances in Health Care Organisation Theory. San Francisco: JosseyBass; 2003. p. 253–88.
4. Brennan NM, Flynn MA. Differentiating clinical governance, clinical management and clinical practice. Clin Gov: An Int J. 2013;18(2):114–31.
5. Gilson L. Introduction to Health Policy and Systems Research. In: Gilson L, editor. Health Policy and Systems Research: A Methodology Reader. Geneva: Alliance for Health Policy and Systems Research, World Health Organization; 2012.
6. Mason and Barnes. Constructing Theories of Change. Methods Sources Eval. SAGE publications; 2007;13(12):151–70.
7. Making innovation work: how to manage it, measure it, and profit from it. New Jersey: Wharton School Publishing; 2005.
8. Mlotshwa L, et al. Exploring the perceptions and experiences of community health workers using role identity theory. Glob Health Action. 2015;8:28045.
9. Campbell SM, et al. Implementing clinical governance in English primary care groups/trusts: reconciling quality improvement and quality assurance. Qual Saf Health Care. 2003;11:9–4.
10. Bazilinska O. Ya. Finansovyi analiz: teoriia ta praktyka: navch. posib. [Financial analysis: theory and practice: teaching. manual]. Kyiv: Tsentr uchbovoi literatury. 2009; 328 p. (in Ukrainian)
11. Belialov TE, Oliinyk AV. Finansova stiikist pidpryiemstva ta shliakhy yii zmitsnennia. [Financial stability of the enterprise and ways to strengthen it]. Mizhnarodnyi naukovyi zhurnal «Internauka». 2016; 12:22-26. (in Ukrainian)
12. Chernenok KP, Semenenko HM, Lukonin OV. Otsinka finansovoho stanu medychnoho zakladu v umovakh reformy finansuvannia systemy okhorony zdorovia [Assessment of the financial condition of the medical institution in the context of the health care financing reform]. Efektyvna ekonomika. 2020; 12:23-29. (in Ukrainian)
13. Pro zatverdzhennia pereliku ta skladu hospitalnykh okruhiv Ivano-Frankivskoi oblasti [On approval of the list and composition of hospital districts of the Ivano-Frankivsk region]. Rozporiadzhennia Kabinetu Ministriv Ukrainy; Perelik vid 12.07.2017 № 473-p. (in Ukrainian). URL: https://zakon.rada.gov.ua/laws/show/473-2017-p#Text
14. Deiaki pytannia stvorennia hospitalnykh okruhiv. [Some issues of creating hospital districts] Postanova Kabinetu Ministriv Ukrainy; Poriadok, Polozhennia vid 27.11.2019 N1074. https://zakon.rada.gov.ua/laws/show/1074-2019-п#Text
15. Coovadia H, et al., The health and health system of South Africa: historical roots of current public health challenges. Lancet. 2003; 374(9692, 5–11):817–34.
16. Som CV. Clinical governance: a fresh look at its definition. Clin Gov: An Int J. 2004;9(2):87–90.
17. Cascon-Pereira R, Chillas S, Hallier J. Role-meanings as a critical factor in understanding doctor managers' identity work and different role identities. Soc Sci Med. 2016;170:18–25.
18. Model Ivano-Frankivskoho hospitalnoho okruhu proanalizuvaly pid chas robochoi poizdky v oblast zamministra okhorony zdorovia Ukrainy [The model of the Ivano-Frankivsk hospital district was analyzed during a working trip to the region of the Deputy Minister of Health of Ukraine]. Ivano-Frankivska ODA. 2022. https://www.if.gov.ua/news/model-ivano-frankivskoho-hospitalnoho-okruhu-proanalizuvaly-pid-chas-robochoi-poizdky-v-oblast-zamministra-okhorony-zdorovia-ukrainy
19. Tait AR. Clinical governance in primary care: a literature review. Issues Clin Nurs. 2004;13:723–30.
20. Connell L, Durban A. clinical governance handbook for District Clinical Specialist Teams. Durban: Health Systems Trust; 2014.

The work was carried out according to the research work of Shupyk National Healthcare University of Ukraine for 2020-2024 on the theme of "Justification of innovative models of management and optimization of organizational forms of activity of bodies, enterprises and health care institutions" (state registration number 01200U101680).

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Conflict of interest:
The Author declare no conflict of interest

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Received: 08.06.2022
Accepted: 22.01.2023

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

ORIGINAL ARTICLE

DIGITAL IMAGES CLASSIFICATION IN AUTOMATIC LAPAROSCOPIC DIAGNOSTICS

DOI: 10.36740/WLek202302102

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ABSTRACT

The aim: To evaluate the automatic computer diagnostic (ACD) systems, which were developed, based on two classifiers–HAAR features cascade and AdaBoost for the laparoscopic diagnostics of appendicitis and ovarian cysts in women with chronic pelvic pain.

Materials and methods: The training of HAAR features cascade, and AdaBoost classifiers were performed with images/ frames of laparoscopic diagnostics. Both gamma-corrected RGB and RGB converted into HSV frames were used for training. Descriptors were extracted from images with the method of Local Binary Pattern (LBP), which includes both data on color characteristics («modified color LBP»-MCLBP) and textural features.

Results: Classification of test video images revealed that the highest recall for appendicitis diagnostics was achieved after training of AdaBoost with MCLBP descriptors extracted from RGB images – 0.708, and in the case of ovarian cysts diagnostics – for MCLBP gained from RGB images – 0.886 ($P<0.05$). Developed AdaBoost-based ACD system achieved a 73.6% correct classification rate (accuracy) for appendicitis and 85.4% for ovarian cysts. The accuracy of the HAAR features classifier was highest in the case of ovarian cysts identification and achieved 0,653 (RGB) – 0,708 (HSV) values ($P<0.05$).

Conclusions: The HAAR feature-based cascade classifier turned out to be less effective when compared with the AdaBoost classifier trained with MCLBP descriptors. Ovarian cysts were better diagnosed when compared with appendicitis with the developed ACD

KEY WORDS: laparoscopic surgery, images analysis, HAAR features cascade, AdaBoost classifier

Wiad Lek. 2023;76(2):251-256

INTRODUCTION

Automatic computer diagnostic (ACD) / classification of video – images is actual for minimally invasive abdominal surgery and endoscopy [1 - 4]. ACD systems developed for tracking laparoscopic instrumentation [5], and identification of zones of pathology proved their effectiveness [3, 6, 7].

As far as HAAR features exclude analyzing each pixel of an image, the total time for analysis is shortened [5]. It makes the recognition of images congruent with the velocity of video-frames flowing and justifies the exploration of the classifier based on the HAAR-features cascade for video-data analysis. Meanwhile, the main disadvantage of that classifier exploration is confined to a prolonged period of training, which might be measured in months when tens of thousands of images are used. It is hard to avoid that inconvenience as far as increasing the number of images is proportional to the effectiveness of diagnostics [5].

To strengthen the effectiveness of the HAAR - feature-based classifier, we decided to use both color and texture features for training [6, 8]. Thus texture features such as the heightened mediana of greyscale and entropy and contrast might be treated as informative differential indices for normal tissue state identification [3, 9]. Besides, as an alternative to HAAR feature-based classifier, we have explored the AdaBoost classifier trained with a minimal number of descriptors gained from the Local Binary Pattern (LBP) method application [6]. The classical method of LBP manipulates with the greyscale of color and ignores other colors' information. Instead, the modified LBP method, which includes data on color characteristics (modified color LBP- MCLBP) [10], was used in the present investigation to gain color and texture descriptors [11 - 13].

THE AIM

To compare the effectiveness of an ACD based on HAAR-based features cascade classifier with an AdaBoost-based ACD, which both were trained to distinguish between the normal and pathological state of the appendix and ovary in women with chronic pelvic pain syndrome. Besides, the comparison of diagnostic results between RGB and HSV color scales was performed.

THE OBJECT OF THE INVESTIGATIONS

Data on true and false diagnosed appendicitis and ovarian cysts gained in the course of laparoscopic ACD were taken into consideration.

MATERIALS AND METHODS

All observations were performed in accordance with Helsinki Declaration, international laws, and policies. Odesa National Medical University Bioethics Committee (UBC) approval (No17) dated 22/03/2018 was obtained before the start of the study.

The following steps were performed in the course of collecting data and their analysis:

- Calibration of a digital camera, which included white color balance and conversion of color scale into digital code;
- The object was located in the frontal position, which was under inspection. The deviation from the right angle was 15±5 degrees, and the distance to the visualized zone was from 3 to 5 cm [11]. Those images which got in a such a fashion were used for both ACD training and testing;
- Those zones of interest size were 60 x 60 pixels [6, 8]; in the course of the laparoscopic intervention, the speed of video frames was modified via using the low-frequency filter, and the size of the image was artificially modified from 30 x 30 up to 60 x 60 pixels, which was necessary for optimizing classificatory performance.
- Gamma-correction of the gained image performed with the recalculation of gamma-coefficient. The usage of gamma-correction in the preprocessing of primarily gathering information permits to identify relations between quantitative pixel characteristics and their actual brightness [7, 11, 12].
- Conversion of RGB scale into HSV one. Haar features' orientation justifies such a conversion on the estimation of the intensity of pixels.
- Training to HAAR features classifier, using both RGB and HSV images;
- Training AdaBoost classifier with MCLB templates [6, 8]; key features used were confined to mean, entropy, contrast, homogeneity, and excesses.
- Results of classification stored in the database, and additional analysis performed later on.

All laparoscopic videos got a 5 mm aperture diameter Carl Storz Tricam Camera (Carl Storz, Germany) during the 2015-2021 years. That camera had the analogous input (PAL 475 horizontal lines), and the incoming signal was digitalized with the pixel density of 720 x 576 and capture was made with video capture card "AVerMedia HD capture Studio 203" (Avermedia, France) and presented at ACD interface (Fig 1).

FEATURES EXTRACTION AND CLASSIFIER TRAINING

MCLBP calculates LBP for R and G channels of normalized RGB color space [10]. It served to get a more stable RGB – MCLBP under different conditions of illumination intensity.

The texture characteristics calculation using HSV - MCLBP was performed via recalculations on the Hue channel, which was invariant concerning illumination and saturation variability. For LBP calculation, the radius of 1,5 and 12 pixels was applied [10]. The pertinent pattern created for each scale vector, as a result, and the characteristic vector for templates of MCLBP, which included mean, entropy, contrast, homogeneity, and excesses, was determined [10, 11].

For the training of classifiers, 45 laparoscopic video images of patients with appendicitis and 43 with ovarian cysts were used as "positive" ones (Figure 2). Also, 40 videos gained from the normal appendix and 35 from the ovarian surface for the classifier training were used as a control – "negative" images. Each video contained 2500 – 3000 frames, among which manually, those for teaching and testing collections were verified, cropped out, and stored.

Table I. Comparative effectiveness of HAAR features-based and Ada-Boost classifiers trained with RGB and HSV images

Classifier	Frames type used for training	True positive	True negative	False positive	False negative	Precision	Recall	F1 Score	Accuracy
Appendicitis									
Haar-features cascade	RGB	107	113	83	136	0.563	0.440	0.494	0.501
	HSV	116	125	71	127	0.601	0.477	0.527	0.549
AdaBoost	MCLBP (RGB)	172	151	45	71	0.793*#	0.708*#	0.748*#	0.736*#
	MCLBP (HSV)	145	137	59	98	0.711*	0.597*	0.649*	0.642*
Ovarian cysts									
Haar-features cascade	RGB	83	105	45	49	0.654	0.629	0.641	0.653
	HSV	94	110	40	38	0.701	0.712	0.706	0.708
AdaBoost	MCLBP (RGB)	117	129	21	15	0.848*#	0.886*#	0.867*#	0.854*#
	MCLBP (HSV)	106	116	34	26	0.757	0.803*	0.779*	0.771*

N o t e: Fisher's exact test used for statistical comparison *-P<0.05 when compared with the corresponded group diagnosed with Haar–features cascade; #-P<0.05 when results between RGB and HSV data compared in corresponded groups.

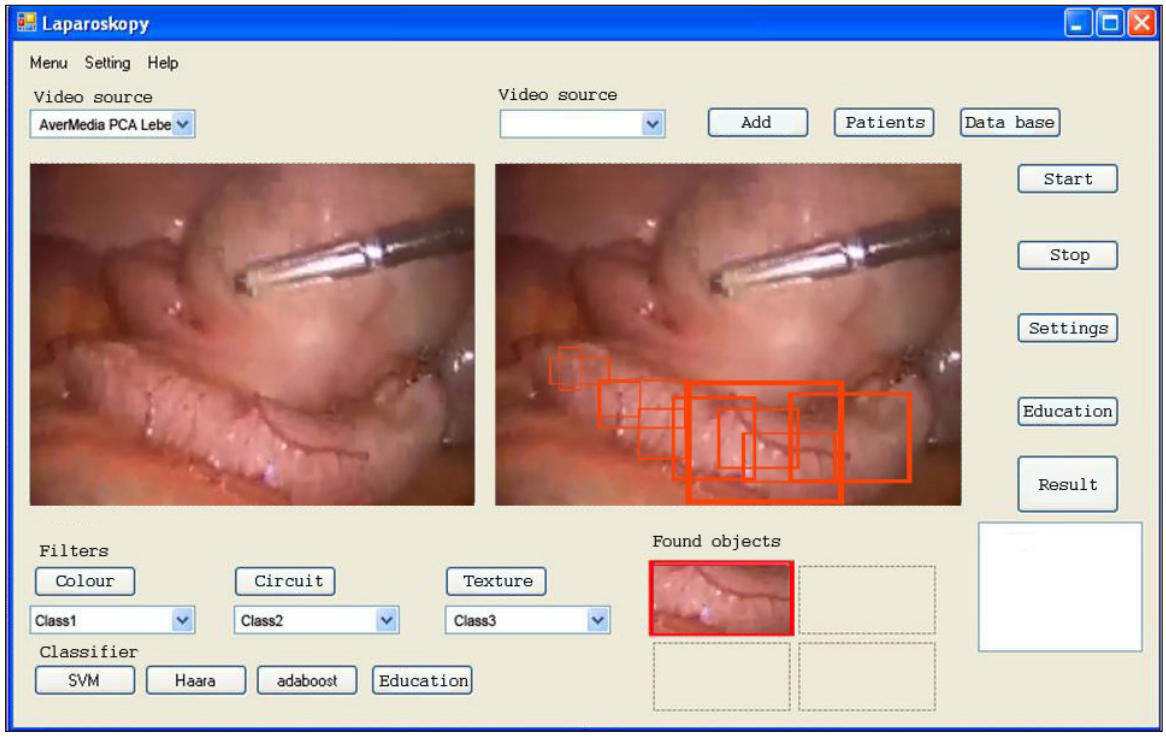

Fig. 1. The interface of the software illustrates zones of appendix inflammation (frames)

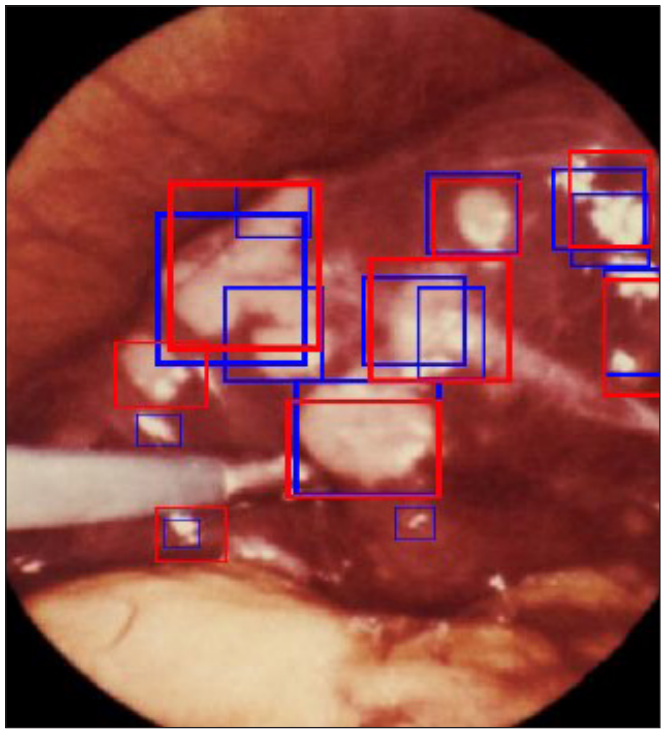

Fig. 2. The general scheme on the ACD work. With the squares, «zones of interest» are automatically defined and tracked

For classifiers training, the next parameters were explored [6]:
- False-positive rate f = 0,3;
- Windows with the size of the frame as 60x60 pixels;
- Number of positive images - n=1000 for each pathology;
- Number of negative images - n=500;

After cessation of training, the tests were performed to estimate the effectiveness of recognition.

Test session images were different from those which have been used for the training of the classifier. Test control sessions were performed with 243 frames containing appendicitis and 132 frames with ovarian cysts. Three 346 frames with the absence of mentioned pathology were used as a control group – normal appendix (196) and ovarium (150).

STATISTICAL PROCEDURES

To assess the performance of our classifiers, we use the measures precision, recall, and F-score [1, 5]. Precision measures the fraction of the detected-positive instances, which are true-positive (TP):

Precision = TP/ (TP + FP),

where FP – false positive instances.

A recall (TP/ P - number of positive instances) is the fraction of all true-positive instances, which are also detected positively.

F-score (also F-measure or F1-score) is the harmonic mean of precision and recall:

F = 2 ∗ Precision ∗ Recall/ Precision + Recall.

Accuracy is the proportion of correctly classified items out of all the items classified:

Accuracy = (TP + TN)/ (TP + TN + FP + FN),

where TN – the number of true-negative, FN – the number of false-negative instances.

Statistical comparisons of gained data were performed using Fisher's exact test. The value of $P<0.05$ was taken as significant.

RESULTS

Presented data showed that appendicitis diagnostics in women with chronic pelvic pain were less effective than diagnostics of ovarian cysts (Table I). Such difference in effectiveness was observed in both training with RGB and HSV types of images. Meanwhile, the highest value of recall was observed for appendicitis diagnostics after training with RGB images for MCLBP – 0.886 and exceeded such one for training with HSV images for MCLBP, which occupied second place - 0.803 ($P<0.05$). The lowest recall was registered for appendicitis diagnostics with HAAR features trained with RGB images (0.440). It should be stressed that in the course of diagnostics of appendicitis as well as diagnostics of cysts MCLBP- based methods revealed better diagnostics results when compared with the based RGB and HSV images training of the classifiers.

It is worth noting that training MCLBP with RGB images raised the number of true positive diagnoses pertained to that gained with HAAR-features cascade classifier in case of appendicitis diagnostics by 1.61 times ($P<0.05$) and in case of ovarian cysts diagnostics – by 1.41 times ($P<0.05$). The corresponded reduction of false-negative diagnoses was 1.92 ($P<0.05$) and 3.27 times ($P<0.05$). A less pronounced increase in the number of true positive diagnoses with MCLBP – HSV training was 1.25 times for appendicitis and 1.13 times for ovarian cysts. The reduction of false-negative diagnoses was 1.18 and 1.08 times correspondently.

DISCUSSION

Hence, gained data favor the relatively high effectiveness of laparoscopic diagnostics of the diseases that cause chronic pelvic pain in women using developed ACD systems. It should stress that such a result corresponds with early gained data in the course of liver pathology diagnostics [6]. Altogether our data prove that only the HAAR-like feature-based classifier is insufficient for reliable object classification [5].

One of the reasons for such a difference is that HAAR feature-based cascade classifier needs more time for the stream of image recalculation, even though better diagnostic results followed training with HSV images. The massive number of variants of pathological manifestations (shape, color, texture) and the role of different orientations are also crucial for correct HAAR feature-based cascade classifier application [14, 15].

Also, our data showed that diagnostics of appendicitis results were weaker than cysts diagnostics independently of image type, which was used for training. Such result points to less potential of HAAR features based classifier for correct video laparoscopic diagnostics when compared with AdaBoost results of diagnostics.

Hence, developing a diagnostic system based on training with modified templates of both RGB and HSV images and minimal MCLBP-derived descriptors substantially improved the results of classification performed with the AdaBoost classifier. Our data showed that MCLBP descriptors from RGB images drastically reduced false-negative diagnoses - by 1.92 for appendicitis and 3.27 times for ovarian cysts compared with the corresponding data gained with Haar feature-based classifier exploration. The number of true positive diagnoses rose by 1.61 times and by 1.41 correspondently. Similar tendencies but less pronounced were registered for training with HSV images.

It should be noted that results on RGB image usage were better than such ones based on HSV image exploration. This fact is in favor of a better description of tissue properties in the RGB color scale [7, 16]. Although the net advantage of HAAR features based classification – shortened number of data for the machine learning as well as preventing overfitting of trained classifier [15, 17], the exploration of MCLBP-based training of AdaBoost proved to be more effective for laparoscopic ACD of diseases which caused chronic pelvic pain in women. It is also possible that a cascade classifier trained with MCLBP descriptors could demonstrate better diagnostics performance, and AdaBoost trained with HAAR descriptors.

Altogether, our data point to a positive perspective with the usage of MCLBP descriptors for AdaBoost classifier training to resolve automatic diagnostics problems in laparoscopic surgery.

CONCLUSIONS

1. The ACD of laparoscopic images based on the AdaBoost classifier permitted effectively classifying appendicitis and ovarian cysts in women who suffered from chronic pelvic pain with the highest recall gained with MCLBP from HSV images used for training – up to 0.803, and for MCLBP RGB – up to 0.886 correspondently.
2. MCLBP used descriptors for training the AdaBoost classifier proved to increase the precision, recall, F1 score, and the accuracy of automatic diagnostics of appendicitis and ovarian cysts.

REFERENCES

1. Albisser Z. Computer-aided screening of capsule endoscopy videos. Master's Thesis, University of Oslo. 2015.https://www.duo.uio.no/handle/10852/47642. [date access 05.08.2022]
2. Lahane A., Yesha Y., Grasso M. et al. Detection of unsafe action from laparoscopic cholecystectomy video. In: Proc. 2nd ACM SIGHIT International Health Informatics Symposium, New York: Association for Computing Machinery Press. 2012. doi: 10.1145/2110363.2110400.
3. Zhang Y., Wang Z., Zhang J. et al. Deep learning model for classifying endometrial lesions. J Transl Med. 2021; 19: 10. doi:10.1186/s12967-020-02660-x.
4. Shu Y., Bilodeau G.A., Cheriet F. Segmentation of laparoscopic images: Integrating graph-based segmentation and multistage region merging. The 2nd Canadian Conference on Computer and Robot Vision (CRV'05). 2005; 429-436. doi: 10.1109/ CRV. 2005.74.
5. Fatiev D. Object tracking for improved telementoring and telestration. Master's Thesis in Telemedicine and E-health, The Arctic University of Norway. 2015. https://hdl.handle.net/10037/7764. [date access 05.08.2022]
6. Lyashenko A.V., Bayazitov N.R., Godlevsky L.S. et al. Informational -technical system for the automatized laparoscopic diagnostics. Radio Electronics, Computer Science, Control. 2016; 4: 90-96.
7. Zhou M., Bao G., Geng Y. et al. Polyp detection and radius measurement in small intestine using video capsule endoscopy. In: Biomedical Engineering and Informatics (BMEI), 2014 7th International Conference on IEEE. 2014. doi: 10.1109/BMEI.2014.7002777.
8. Bayazitov D.N., Kresyun N.V., Buzinovsky A.B. et al. The effectiveness of automatic laparoscopic diagnostics of liver pathology using different methods of digital images classification. Pathologiya. 2017; 14: 182–187. doi: 10.14739/2310-1237. 2017.2.109219.
9. Madad Zadeh S., Francois T., Calvet L. et al. SurgAI: deep learning for computerized laparoscopic image understanding in gynaecology. Surg Endosc. 2020; 34: 5377–5383. doi:10.1007/s00464-019-07330-8.
10. Ledoux A., Losson O., Macaire L. Color local binary patterns: compact descriptors for texture classification. Journal of Electronic Imaging. 2016; 25(6): 061404. doi: 10.1117/1.JEI.25.6.061404.
11. Paramarthalingam A., Mirnalinee T. Extraction of compact boundary normalisation based geometric descriptors for affine invariant shape retrieval. IET Image Processing. 2020; 15: 1093-1104. doi: 10.1049/ipr2.12088.
12. Godlevsky L., Shakun K., Martsenyuk V. et al. Dynamic Changes of the Colour Intensity of Collected Urine as a Basis for a Distant Uroflowmetry," 2019 10th IEEE International Conference on Intelligent Data Acquisition and Advanced Computing Systems: Technology and Applications (IDAACS). 2019, 312 p. doi: 10.1109/IDAACS.2019.8924436.
13. Kresyun N.V., Godlevskii L.S. Superoxide Dismutase and Catalase Activities in the Retina during Experimental Diabetes and Electric Stimulation of the Paleocerebellar Cortex. Bull Exp Biol Med. 2014; 158: 206–208. doi:10.1007/s10517-014-2723-6.
14. Bay H., Tuytelaars T., Van Gool L. SURF: Speeded Up Robust Features. In: Leonardis A, Bischof H, Pinz A, eds. Computer Vision – ECCV 2006. ECCV 2006. Lecture Notes in Computer Science, Berlin, Heidelberg: Springer. 2006. doi:10.1007/11744023_32.
15. Viola P., Jones M. Rapid object detection using a boosted cascade of simple features. In: Proc 2001 IEEE Computer Society Conference on Computer Vision and Pattern Recognition. CVPR 2001. 2001. doi: 10.1109/CVPR.2001.990517.
16. Hashimoto D.A., Rosman G., Rus D. et al. Artificial Intelligence in Surgery: Promises and Perils. Ann Surg. 2018; 268: 70-76. doi: 10.1097/SLA.0000000000002693.
17. Petscharnig S., Schöffmann K. Learning laparoscopic video shot classification for gynecological surgery. Multimed Tools Appl. 2018; 77: 8061–8079. doi: 10.1007/s11042-017-4699-5.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 05.10.2021
Accepted: 14.11.2022

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

DIAPHRAGM FUNCTION IN CHILDREN WITH ACUTE HYPOXEMIC RESPIRATORY FAILURE: THE PROSPECTIVE OBSERVATIONAL COHORT STUDY

DOI: 10.36740/WLek202302103

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ABSTRACT

The aim: To find out whether diaphragm dysfunction might lead to unsuccessful weaning from MV.

Materials and methods: We provided prospective observational cohort study and included 105 patients and divided them in the study and the control groups. To consider diaphragm function, we check amplitude of its movement and diaphragm thickening fraction (Dtf).

The primary outcome was the incidence of successful weaning from MV. The secondary outcomes were changes in diaphragm function parameters.

Results: In the current study, there were found that the incidence of successful weaning from MV was 100% for the day 1 in the control group, while in study group the incidence was significantly lower. Successfully weaned from MV in the study group among children 1 month – 1 year old on day 14 were 20 out 28 patients (71%), in children 1 – 3 years old – 9 out 11 patients (82%), in children 3 – 5 years old – 15 out 15 (100%). However, on day 1 – no one from the study group was weaned (0%), on day 7 – 5 out 28 patients 1 month – 1 year old (18%), 6 out 11 patients (55%) 1 – 3 years old, and 8 out 15 patients (53%) 3 – 5 years old ($p<0.05$).

Conclusions: Diaphragm dysfunction might alter weaning from MV.

KEY WORDS: children, diaphragm, respiratory failure, respiratory physiology

Wiad Lek. 2023;76(2):257-263

INTRODUCTION

Patients who are admitted to an ICU frequently exhibit muscle weakness, and the respiratory muscles are often affected. [1]. On the other hand, mechanical ventilation (MV) is associated with diaphragm injury and its mechanism might be complex and is due to myotrauma [2]. Both these clinical problems frequently occur in mechanically ventilated patients and predisposes them to prolonged ventilator dependence and poor clinical outcomes [3] and the presence of either insufficient or excessive diaphragmatic contractile effort plays a central role in this process [4]. These facts were confirmed for adults, therefore assessing diaphragmatic function with ultrasound is a routine investigation when patient is ready for decreasing MV parameters and weaning from MV.

Significantly, it has been observed that similar pathophysiology changes are present in pediatric patients, who need to be mechanically ventilated due to acute or chronic respiratory failure [5, 6]. In the last decades we observed the improvement in the treatment of pediatric acute respiratory failure [5], and the mortality rates in children under 5 years old have steadily declined in the European Region. However, children are still dying from acute respiratory failure caused by pneumonia [7] despite the presence of modern guidelines on oxygen therapy for children [8] and mechanical ventilation of critically ill children [9]. Nevertheless, there is no a littke knowledge about diaphragm dysfunction in children, admitted to ICU and no evidence that diaphragm dysfunction leads to complicated weaning from MV.

The study hypothesis was that diaphragm dysfunction could not lead to unsuccessful weaning from respiratory support in children with acute respiratory failure.

THE AIM

The aim of the study was to find out whether diaphragm dysfunction in patients with acute respiratory failure might lead to unsuccessful weaning from MV.

MATERIALS AND METHODS

We provided prospective observational cohort study at the Department of Anesthesiology and Intensive Care and included 105 patients 1 month – 5 years old. Study group (n=54) included patients with acute respiratory

failure due to pneumonia, who need to be provided with MV over 72 hours. Control group (n=51) included patients, who underwent short term MV (less than 2 hours) during elective otolaryngology or urology surgery, had no risk factors of diaphragm dysfunction (severe malnutrition, congenital neuro-muscular diseases, onset of ICU admission during last 6 months), and were weaned from MV after surgery were held. Exclusion criteria for the study were: the refusal of the patient's legal representatives to participate in the study at any of its stages, the patient's agonizing state upon admission, and the onset of MV less than 48 h after prior weaning.

The study was conducted in accordance with the requirements of good clinical practice, the Council of Europe Convention on Human Rights and Biomedicine, the Helsinki Declaration of the World Medical Association. The study was approved by the Bioethics Commission, protocol №1, January 30, 2018. All patients` relatives or their legal representatives signed informed consent to participate in the study.

For demographical data we divided all patients into age subgroups (Table I). It was established risk factors of diaphragm dysfunction, selected MV parameters according body weight and height, individualized treatment.

Both groups received lung-protective mechanical ventilation with pressure control ventilation mode (PCV), study group - with "Hamilton C1" or "Hamilton C3", control group – with "Leon" anesthetic machine. Parameters of MV in both groups depended on oxygenation and CO_2 elimination. In addition, in the study group we took into account lung compliance and airways resistance data. The parameters of MV across research in the study group depended on oxygenation, CO_2 elimination, acid-base analysis, course of the disease and ranged: PIP from 18 to 26 cm H_2O; PEEP from 7 to 14 cm H_2O; Rate from 32 to 20 per min; Tin from 0.5 to 1.4 sec; Pplat from 16 to 24 cm H_2O; TV= 5-7 ml/kg; FiO_2 from 0.65 to 0.4; while in the control group MV parameters were more predictable and ranged: PIP = 16-18 cm H_2O; PEEP = 3-4 cm H_2O; Rate = 22 – 28 per min; Tin = 0.5 – 1.1 sec; TV = 6-7 ml/kg; FiO_2 = 0.6.

For the structural and functional assessment of the diaphragm we used ultrasonography of diaphragm during respiration: in the study group – 10:00 – 11:00 AM after suctioning and then 30 minutes rest with the as minimal as possible sedation level and as best as possible spontaneous breathing efforts level but not less than 60% of spontaneous minute volume ventilation; in the control group – 9:00 – 12:00 AM after restoration of spontaneous breathing after surgery with light sedation and achieving over 60% of spontaneous minute volume ventilation. The ultrasound examinations were carried out by two experienced investigators using a commercially available Vivid S60N, GE Healthcare, Milwaukee, WI, USA with curvilinear transducer 3-5 MHz, 60-mm curved array. Patients were in supine position and transducer was placed in L3 (lung zone 3, 4^{th}-10^{th} intercostal spaces, between the anterior and posterior axillary lines), marker pointing cephalad. We search for the interface between the diaphragm and lung at about 5th to 8th intercostal space with identification of lungs, diaphragm, liver, and spleen. Both side examination with B-mode for identification of structures and M-mode for diaphragm function evaluation were used. We measured amplitude of diaphragm movement and inspiratory time with calculation of diaphragm thickening fraction (Dtf), which reflects the magnitude of diaphragmatic effort, for both right and left hemidiaphragms.

Dtf was calculated as percentage from the formula:

(thickness at end-inspiration – thickness at end-expiration)/thickness at end expiration × 100.

The primary outcome was the incidence of successful weaning from MV. The secondary outcomes were changes in diaphragm function parameters, which made the confirmation of the diaphragm dysfunction diagnosis. It was the amplitude of diaphragm movement (and decreasing less than 8 mm was a marker of under-assistance during MV, increasing over 15 mm was a marker of over-assistance during MV), and Dtf (decreasing below 15% was a marker of diaphragm weakness and its increasing over 35% - a marker of high respiratory function and a potentially damaging diaphragm factor).

Stages of the study: 1^{st} day, 7^{th} day, 14^{th} day. We compared study and control group only on the 1^{st} day. Next stages we used to compare changes over time only for the study group.

Results described in this article is the part of the clinical study "Diaphragm ultrasound and trends in electrolyte disorders and transthyretin level as a method to predict ventilation outcome in children: the prospective observational cohort study"; ISRCTN84734652.

Statistical analysis. The D' Agostino test was used to assess the normality of continuous variables. Descriptive statistics are presented as mean ± SD, median [IQR] or numbers (%), as appropriate. Continuous variables were compared with Student's *t*-tests or Mann–Whitney *U*-tests. All statistical analyses were performed using SPSS (SPSS 23.0; IBM Inc., Armonk, New York, USA) software,and *P* value less than 0.05 was considered statistically significant.

RESULTS

All participants completed the study protocol. No adverse events were observed during the study. Dia-

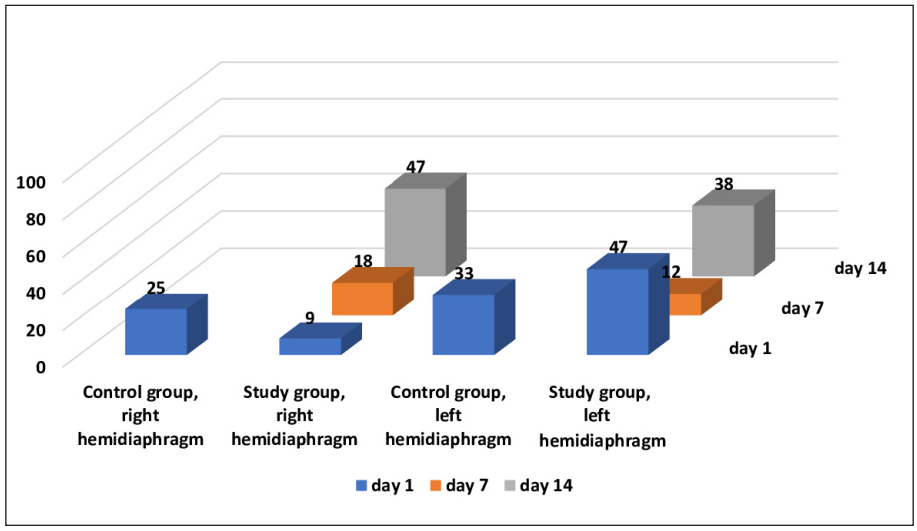

Fig. 1. Children 1 month – 1 year

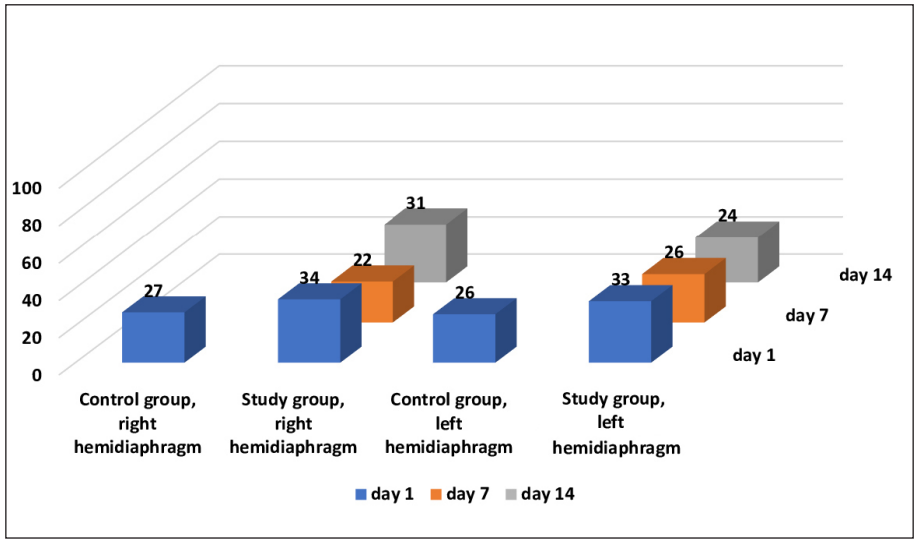

Fig. 2. Children 1-3 years old

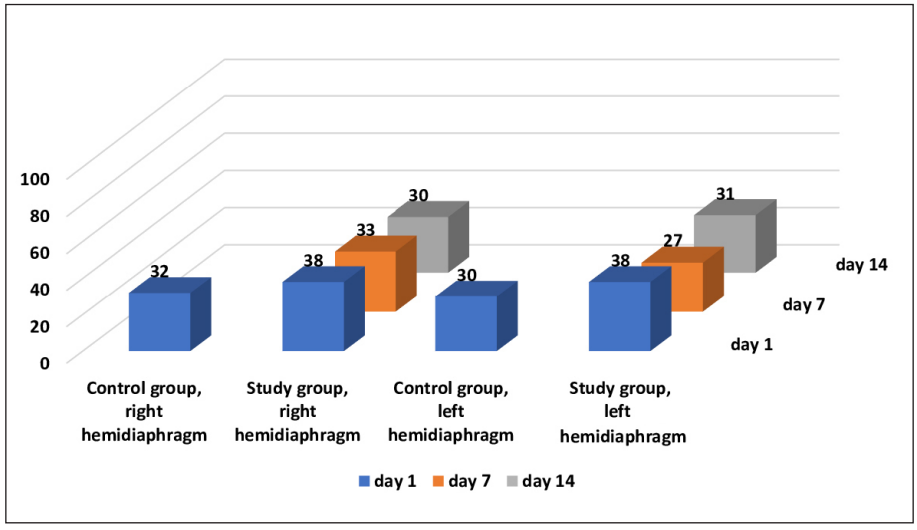

Fig. 3. Children 3-5 years old

phragm ultrasound data showed significant reduction of ventilator-patient asynchrony and, in addition, reduction the need in deep sedation after beginning weaning from MV in both groups but not significant difference between groups.

DIAPHRAGM THICKENING FRACTION

Results are presented as median with IQR. Dtf for right hemidiaphragm was significantly lower in the study group in children 1 month – 1 year old on day 1 compared with the control group ($p<0.05$), and significantly

Table I. Personal data

	Group (age), n					
	Study (1 month – 1 year old), n=28	**Study (1 – 3 years old), n=11**	**Study (3 – 5 years old), n=15**	**Control (1 month – 1 year old), n=15**	**Control (1 – 3 years old), n=16**	**Control (3 – 5 years old), n=20**
Sex (male/female)	18/10	7/4	9/6	10/5	8/8	12/8
Age (months)	1.8 [1.2; 2.9]	14.5 [12.5; 19.0]	39.5 [37.0; 44.5]	2.1 [1.5; 2.8]	15.1 [13.5; 21.0]	41.5 [38.0; 46.5]
Height (cm)	55 [53; 56]	71 [68; 73]	95 [92; 99]	55 [52; 58]	72 [69; 74]	99 [93; 102]
Weight (kg)	4.1 [3.6; 4.3]	9.9 [8.9; 11.5]	14.2 [13.2; 16.5]	4.2 [3.4; 4.4]	11.1 [9.5; 11.8]	15.4 [14.6; 16.9]
BMI, kg/m^2	13.8 [13.4; 14.1]	19.8 [19.2; 20.2]	15.8 [14.9; 16.2]	13.9 [13.6; 14.2]	21.9 [18.7; 22.4]	16.1 [15.4; 18.9]

* Data presented as median with IQR or numbers when applicable.

Table II. Changes in diaphragm movement amplitude for the study and the control groups

Data	**Age**	**Group, study stage**				**P (for control and study groups on day 1)**
		Control, day 1	**Study, day 1**	**Study, day 7**	**Study, day 14**	
Amplitude of diaphragm movement, arithmetic means for right and left sides, mm	1 month – 1 year old	8 [6; 10]	3 [2; 5]	5 [3; 8]	18 [13; 20]	0,04
	1 – 3 years old	10 [9; 11]	6 [5; 9]	8 [6; 11]	11 [9; 15]	0,12
	3 – 5 years old	14 [8; 16]	7 [6; 9]	10 [7; 12]	16 [12; 19]	0,03

Table III. The successful weaning from MV incidence in the control and the study groups

Data	**Subgroup**	**Group, study stage**				**P (for control and study groups on day 1)**
		Control, day 1	**Study, day 1**	**Study, day 7**	**Study, day 14**	
Count of successfully weaned from MV patients/ total patients` count	1 month – 1 year old	15/15	0/28	5/28	20/28	0,04
	1 – 3 years old	16/16	0/11	6/11	9/11	0,02
	3 – 5 years old	20/20	0/15	8/15	15/15	0,03

higher across the research on day 14 in comparison with day 7 in the study group ($p<0.05$). On the other hand, for left hemidiaphragm Dtf was significantly higher on day 1 in the study group compared with the control group ($p<0.05$) (Fig.1).

In children 1 year – 3 years old in the study group Dtf was significantly higher for both right and left hemidiaphragms on day 1 compared with the control group ($p<0.05$) (Fig.2). On day 7 and day 14 data were in normal reference ranges with no significant differences between this study stages for the study group.

Dtf for right and left hemidiaphragms in children 3 years- 5 years old in the study group has no significant differences with the control group on day 1 and no significant differences across the research in study group (Fig.3).

The analysis of the incidence the absence of spontaneous movements of diaphragm in study group showed, that 3 out 28 patients1 month – 1 year old, 1 out 11 patients 1 – 3 years old, and no one out 15 patients 3-5 years old have this deterioration on day 1. No such kind changes were observed on next study stages.

AMPLITUDE OF DIAPHRAGM MOVEMENT

Results are presented as median with IQR. We have made the analysis of data on right and left sides, they were very close for every patient, therefore we present arithmetic means for both sides diaphragm movement amplitude.

No statistically significant differences were found between groups in children 1-3 years old across the research (Table II).

We compared the amplitude of diaphragm movement across the research in children 1 month – 1 year old in the study group and found out that data were significantly decreased on day 1 and day 7 ($p<0.05$), and significantly increased on day 14 in comparison with day 7 ($p<0.05$) (Table II). In addition, this parameter had the tendency for increasing during the research for all patients in the study group. In particular, in children 1 month – 1 year old it increased from 3 [2; 5] mm on day 1 to 5 [3; 8] mm on day 7, and to 18 [13; 20] mm on day 14, while in children 1 – 3 years old - from 6 [5; 9] mm on day 1 to 8 [6; 11] on day 7 and to 11 [9; 15] mm on day 14. In children 3-5 years old these data were 7

[6; 9] mm on day 1, 10 [7; 12] mm on day 7, and 16 [12; 19] mm on day 14.

According to the obtained data, it could be stated that diaphragm dysfunction was detected in children 1 month – 1 year old in the study group on day 1 and day 7 with confirmation of insufficient diaphragm load and on day 14 with confirmation of diaphragm overload. Also, we found subclinical diaphragm overload in children 1 – 3 years old on day 1. There were no found data about diaphragm dysfunction in children 3-5 years old in the study group. There were no found data about diaphragm dysfunction in the control group.

In the current study, there were found that the incidence of successful weaning from MV was 100% for the day 1 in the control group, while in study group the incidence was significantly lower (Table III). Successfully weaned from MV in the study group among children 1 month – 1 year old on day 14 were 20 out 28 patients (71%), in children 1 – 3 years old – 9 out 11 patients (82%), in children 3 – 5 years old – 15 out 15 (100%). However, on day 1 – no one from the study group was weaned (0%), on day 7 – 5 out 28 patients 1 month – 1 year old (18%), 6 out 11 patients (55%) 1 – 3 years old, and 8 out 15 patients (53%) 3 – 5 years old ($p<0.05$).

DISCUSSION

In this prospective observational cohort study, we hypothesized that diaphragm dysfunction could not lead to unsuccessful weaning from respiratory support in children with acute respiratory failure. The results showed, to the contrary, that the presence of diaphragm dysfunction was significantly higher in patients with acute hypoxemic respiratory failure compared with healthy individuals of the same age.

The diaphragm is the primary muscle of inspiration and therefore crucially determines the patient's ability to sustain ventilation in the face of respiratory loads (acute or chronic). By prolonging ventilator dependence, dysfunction predisposes to further diaphragm atrophy and injury, to nosocomial complications (ICU-acquired weakness, nosocomial sepsis, so on), and to a higher risk of long-term morbidity and mortality [3]. It is well known that acute respiratory failure might lead to self-inflicted lungs injury [11] and diaphragm myotrauma [12] therefore the role of spontaneous breathing among patients with acute hypoxemic respiratory failure is debated. On the other hand, there is no possibility to achieve readiness for weaning from MV without continuous training with increasing spontaneous breathing efforts and decreasing mechanical respiratory support. And the balance among these two processes is crucial in surviving patients and as soon as possible weaning from MV. Consequently, diaphragm ultrasound helps to check diaphragm function is highly important modern tool in ICU. Our study adds the important information that the presence of diaphragm dysfunction worsens clinical outcome due to decreasing the incidence of successfull weaning from MV. These results might be expected beforehand, since data from previous studies in adult patients were published, where was established that diaphragm weakness can impact survival and increases comorbidities in ventilated patients [13]. Mechanical ventilation is linked to diaphragm dysfunction through several mechanisms of injury, referred to as myotrauma. By monitoring diaphragm activity and titrating ventilator settings, the critical care clinician can have a direct impact on diaphragm injury [3].

Based on the results of this study, it seems that good diaphragm contraction quality with enough level of its movement amplitude facilitates smooth and quick liberation from respiratory support. So, the amplitude of diaphragm movement from 8 [6, 10] mm in infants in the control group, 10 [12, 13] mm in 1-3 years old children of the same control group, and 14 [11, 18] mm in children 3 – 5 years old in the control group on day 1 give 100% of successful weaning. Of course, these were patients with good lung compliance unlike the study group, where patients need to do high respiratory muscles` work to maintain gas exchange in case of low lung compliance. The reason for lower incidence of weaning from MV till day 14 in children 1 month – 1 year old in the study group might be in physiological features of respiratory system for this age. It is difficult to achieve a good level of ventilator-patient interaction due to psychological issues which lead to patient-ventilator asynchrony with excessive muscles work, what have the confirmation in high level of amplitude of diaphragm movement on 14 day in infants in the study group with the high level of Dtf on the same day 14, when the median for right side was 47% and for the left side - 38%. The theoretical confirmation of harmfulness the underassistance myotrauma are study about the effects of both chronic and acute load-induced diaphragm injury which have been demonstrated by muscle biopsies in healthy subjects and patients with chronic obstructive pulmonary disease (COPD) [11]. Contraction against an excessive load (isotonic/concentric loading) leads to acute diaphragm injury, inflammation, and weakness [13, 14]. Critically ill patients are at especially high risk for load-induced injury as systemic inflammation renders the muscle fiber membrane (sarcolemma) more susceptible to injury [14]. In an experimental

sepsis model, applying mechanical ventilation to relieve inspiratory loading significantly attenuates muscle fiber injury and diaphragm weakness [15]. In addition, patients with a thickening fraction value of 15–30% on average during the first 3 days of ventilation (similar to that of healthy subjects at rest) had stable diaphragm thickness and the shortest duration of ventilation [16].

In conclusion, the optimal effort level to prevent diaphragm dysfunction is uncertain and may vary according to the patient's clinical condition. Several lines of evidence suggest that maintaining a relatively low effort similar to that of healthy study participants breathing at rest might be the most effective approach.

CONCLUSIONS

Diaphragm dysfunction seems to alter the weaning from MV, enabling enough level of respiratory muscles work to maintain spontaneous breathing. Using diaphragm protective MV strategy during weaning process will be helpful to avoid diaphragm myotrauma.

REFERENCES

1. Dres M. et al. Coexistence and impact of limb muscle and diaphragm weakness at time of liberation from mechanical ventilation in medical intensive care unit patients. American journal of respiratory and critical care medicine. 2017;195(1): 57-66.
2. Goligher E., Brochard L., Reid W. et al. Diaphragmatic myotrauma: a mediator of prolonged ventilation and poor patient outcomes in acute respiratory failure. The Lancet Respiratory Medicine. 2019;7(1):90-98.
3. Schepens T., Dres M., Heunks L., Goligher E. Diaphragm-protective mechanical ventilation. Current Opinion in Critical Care. 2019;25(1):77-85.
4. Brochard L. Ventilation-induced lung injury exists in spontaneously breathing patients with acute respiratory failure: Yes. Intensive care medicine. 2017; 43(2): 250-252.
5. Xue Y., Yang C., Ao Y. et al. A prospective observational study on critically ill children with diaphragmatic dysfunction: clinical outcomes and risk factors. BMC Pediatrics. 2020;20(1).
6. Filyk O. Diaphragm Dysfunction in Children with Acute Respiratory Failure. Emergency Medicine. 2017;8(79):73-77.
7. Conti G., Piastra M. Mechanical ventilation for children. Current Opinion in Critical Care. 2016;22(1):60-66.
8. Pneumonia accounts for 12% of all deaths in children under 5 in European Region. Euro.who.int. 2021. https://www.euro.who.int/en/health-topics/environment-and-health/air-quality/news/news/2014/11/pneumonia-accounts-for-12-of-all-deaths-in-children-under-5-in-european-region. [date access 14.07.2022]
9. Organization W. Oxygen therapy for children: a manual for health workers. Apps.who.int. 2021. https://apps.who.int/iris/handle/10665/204584 [date access 14.07.2022]
10. Kneyber M., de Luca D., Calderini E. et al. Recommendations for mechanical ventilation of critically ill children from the Paediatric Mechanical Ventilation Consensus Conference (PEMVECC). Intensive Care Medicine. 2017;43(12):1764-1780.
11. Grieco D., Menga L., Eleuteri D., Antonelli M. Patient self-inflicted lung injury: implications for acute hypoxemic respiratory failure and ARDS patients on non-invasive support. Minerva Anestesiologica. 2019;85(9).
12. Schepens T., Dianti J. Diaphragm protection. Current Opinion in Critical Care. 2020;26(1):35-40.
13. Orozco-Levi M., Lloreta J., Minguella J. et al. Injury of the Human Diaphragm Associated with Exertion and Chronic Obstructive Pulmonary Disease. American Journal of Respiratory and Critical Care Medicine. 2001;164(9):1734-1739.
14. Jiang T., Reid W., Belcastro A., Road J. Load Dependence of Secondary Diaphragm Inflammation and Injury after Acute Inspiratory Loading. American Journal of Respiratory and Critical Care Medicine. 1998;157(1):230-236.
15. Yoshida T., Roldan R., Beraldo M. et al. Spontaneous Effort During Mechanical Ventilation. Critical Care Medicine. 2016;44(8):e678-e688.
16. Goligher E., Dres M., Fan E. et al. Mechanical Ventilation–induced Diaphragm Atrophy Strongly Impacts Clinical Outcomes. American Journal of Respiratory and Critical Care Medicine. 2018;197(2):204-213.

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 27.12.2021
Accepted: 14.11.2022

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

FORMATION OF STUDENTS' HEALTH CULTURE IN THE PROCESS OF PHYSICAL EDUCATION AND HEALTH RECREATION ACTIVITIES

DOI: 10.36740/WLek202302104

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ABSTRACT

The aim: To check the effectiveness of the methodology for the formation of students' health culture in the process of their physical education and health recreation activities

Materials and methods: The following methods were used to achieve the aim: analysis, synthesis and generalization of literary sources, pedagogical observation, questionnaires, testing, pedagogical experiment, methods of mathematical statistics. 368 students took part in the ascertaining experiment, 93 students were involved in the formative experiment (52 – experimental group, 41 – control group).

Results: The existing level of health culture formedness in students was revealed to be insufficient, which stipulated the development and substantiation of the methodology for the formation of students' health culture in the process of their physical education and health recreation activities.

Conclusions: The implementation of the methodology for students' health culture formation into the educational process contributed to an increase in the number of students with a high level of health culture and the level of motivation for a healthy lifestyle. The level of physical fitness of the experimental group students significantly improved during the experiment. All this confirms the effectiveness of the developed methodology.

KEY WORDS: students, physical education, health, health culture, health recreation activities

Wiad Lek. 2023;76(2):264-273

INTRODUCTION

The problem of formation of students' health culture in higher educational institutions (HEIs) in the process of their physical education and health recreation activities finds its imprint in the psychological and pedagogical literature and occupies a leading place in the general complex of scientific research on health [1, 2]. This is due to the fact that a person's working capacity, well-being, intensity and productivity of work depends on the state of his / her health. In addition, the satisfaction of a person's need for creative self-realization depends crucially on his / her health, which is one of the most important prerequisites for effective professional activities. The lack of priority for health culture formation as a leading factor in its preservation and promoting occupies one of the main places among the reasons for the decline in students' health [3, 4]. In this regard, the improvement of the higher education system through its modification with modern systems of health strengthening and formation as an important factor in the creative development of the student's personality acquires special importance.

The analysis of a number of scientific sources [5, 6] indicates that during the course of study, most students of HEIs do not experience significant positive changes in the individual level of health in general and health culture formation in particular. This fact can be explained by the insufficient organization of work on health culture formation and consideration of the individual characteristics of students during such training sessions. Attempts to use physical education as a means of health improvement without realizing

the need for self-education create only prerequisites for the modernization of the process of forming a person's physical development. Otherwise, it is impossible to create a holistic system of health culture formation in an individual. Therefore, currently the problem of forming, preserving and protecting the health of youth in Ukraine is considered from the standpoint of its relevance as a factor of national security and a strategic goal of the state's development, becoming one of the most important directions of national policy in the conditions of the Covid-19 epidemic and the large-scale war.

THE AIM

The aim is to check the effectiveness of the methodology for the formation of students' health culture in the process of their physical education and health recreation activities.

MATERIALS AND METHODS

The research was conducted at Zhytomyr Ivan Franko State University in 2020-2022. 368 (98 men, 270 women) students took part in the ascertaining experiment (2020). The formative experiment lasted one academic year (beginning of the experiment – September 2021, end – May 2022) and involved 93 students (52 – experimental group (EG), 41 – control group (CG)).

The following research methods were used to achieve the aim: analysis, synthesis and generalization of literary sources, pedagogical observation, questionnaires, testing, pedagogical experiment and methods of mathematical statistics. The analysis, synthesis and generalization of scientific literature were carried out to characterize the problem of forming health culture among students of HEIs in the process of their physical education and health recreation activities. We analyzed 15 sources on the topic of the article; most of them are 2019-2021 editions from the scientometric databases PubMed, Scopus, Web of Science Core Collection and others. The observations, questionnaires and testing were conducted to identify the level of students' health culture formedness in the process of physical education and health recreation activities.

The questionnaire was used to obtain subjective information related to the formation of students' health culture, which cannot be obtained by other research methods. The ascertaining stage provided for the application of the author's methodology, which contains 16 questions and concerns the determination of the level (high, average or low) of students' self-assessment of their own health culture by various factors (the level of physical development, physical fitness, adherence to healthy lifestyle, etc.) The students who joined the Faculty of History, the Faculty of Physics and Mathematics as well as the Faculty of Natural Sciences in 2020 (n = 368) took part in the survey.

The formative stage involved the usage of two questionnaires: the first one was used to study the dynamics of the level of students' health culture during the experiment; the second one was aimed at determining the students' motivational factors for the formation of health culture. The first questionnaire contained 20 questions, each of which was evaluated in 3, 4, 5 points, respectively, corresponding to low, average and high levels of health culture formedness according to motivational, cognitive and activity components. The assessment of students' answers in the first questionnaire was carried out by the point-based method, as high, average and low. High score corresponded to 82-100 points, average – 74-81, low – 60-73. A high score characterizes the student's perfect level of knowledge, skills and abilities of a healthy lifestyle, the ability to analyze and interpret the peculiarities of fitness and health recreation activities and factors affecting the quality of health, the ability to set tasks and find means of their implementation in achieving a positive result in health culture formation. An average score characterizes the situational possession of the algorithm for building an effective system of health culture formation, occasional manifestations of the ability to apply the acquired knowledge, skills and abilities during fitness and health recreation activities, partial ability to implement the tasks of health culture formation. A low score characterizes the lack of mastery of the algorithm for building an effective system for health culture formation, the lack of ability to apply the acquired knowledge, skills and abilities during fitness and health recreation activities, not formed ability to implement the tasks of health culture formation. The first year students of the Faculty of Physics and Mathematics who entered the HEI in 2021 took part in the survey during the formative experiment (EG: men = 23, women = 29; CG: men = 21, women = 20). The EG and the CG were formed without any selection criterion, according to the formation of the faculty's study groups i. e. the first two groups were included in the EG, the third and fourth – in the CG. The second questionnaire was used to diagnose the motivational orientation for health culture formation in the EG students (n = 52, men – 23, women – 29), which included a list of motivational factors (8 factors) for each of which students had to give one answer from two options "yes" or "no". The questionnaire was anonymous, which increased the reliability of obtaining true results.

The purpose of the pedagogical experiment was to justify the methodology for health culture formation in

the process of physical education and health recreation activities as well as to check its effectiveness.

The health culture is an integrative personal formation, which includes socially and professionally significant life values and value orientations of the individual, based on individual knowledge about the essence of health, ways and methods of its formation, preservation and strengthening. Health culture of students of HEIs performs value-oriented, regulatory, prognostic and transformative functions. One of the most important directions of health culture formation in students is their involvement in fitness and health recreation activities, which is designed to form a careful attitude to their own health. Therefore, systematic physical exercises that form a healthy lifestyle, both during the academic training process in physical education and during extracurricular fitness and health recreation activities, are relevant now. Taking into account the findings of many scientists and based on the results of our own research, we substantiated the author's methodology for health culture formation in the process of physical education and health recreation activities. The essence of the author's methodology is the formation of students' knowledge, skills, practical abilities in the formation, preservation and promotion of health in the process of educational and future professional activities, as well as in the formation of their strong motivation for a healthy lifestyle and regular fitness and health recreation activities. The content of the author's methodology was aimed at deepening students' knowledge of health culture, the main factors of a healthy lifestyle, studying various means of fitness and health recreation activities, the ability to assess the level of their own health, body structure and physique formation, being able to build a program of motor activities, optimal diet and sleep, rehabilitation and leisure activities, mastering the skills of control and self-control over their own health, etc. The author's methodology was introduced into the academic training process of physical education of students of pedagogical specialties in Zhytomyr Ivan Franko State University. The implementation of the methodology was carried out both during physical education training sessions (one session per week) and during extracurricular physical education training sessions (two sessions per week). The training sessions were conducted by a physical education instructor. All training sessions contained both a practical part (performance of physical exercises) and a theoretical part (mini-lectures, conversations, discussions, keeping a health diary). The duration of the implementation phase of the author's methodology is one academic year. The experiment involved 100 % of the first year students of the Faculty of Physics and Mathematics who joined it in 2021 (n = 98) and were assigned to the main educational department.

The effectiveness of the methodology for health culture formation in the process of physical education and health recreation activities was evaluated according to motivational, cognitive and activity components at three levels (high, average, low). A high level of health culture indicates that students have developed an active, demanding attitude to health; they show high activity, initiative; act on inner conviction; are engaged in physical education and health recreation activities based on deep knowledge about their health. An average level is characterized by students' insufficiently active attitude to health; they do not systematically engage in physical education and health recreation activities. Measures aimed at strengthening the body are carried out periodically, without taking into account individual characteristics. A low level describes students who have a passive attitude to their health. They do not strive for personal health improvement, believing that they are unable to change anything themselves. They are not used to self-observation and self-analysis, they do not feel the need for physical education and health recreation activities and the implementation of tempering measures. In addition, the effectiveness of the author's methodology was evaluated according to the level of students' physical fitness.

The methods of mathematical statistics were applied to correctly process the data and identify the difference between the studied indicators. The results of the questionnaires were evaluated in percentages. The results of students' physical fitness were reported as Mean±SD. The authenticity of the difference between the indicators was determined with the help of the Student's t-test. The statistical significance was set at $p<0.05$. All statistical analyses were performed with the SPSS software, version 21.

This research has been complied with all the relevant national regulations and institutional policies, and has followed the tenets of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. All participants agreed to participate in the research.

RESULTS

A student's way of life is nothing but a way of integrating their needs and corresponding forms of activities. The structure of the way of life is revealed in the relations of subordination and coordination between different types of activities. This is manifested in the distribution of the time budget between types of life activities, giving preference to certain types of work and rest. A

Table I. Students' subjective assessment of the factors of their own health culture (n = 98 men, 270 women; number of people / %)

Factors of health culture	Gender	Students' assessment of the factors of their own health culture		
		High	Average	Low
Physical working capacity	men	9 / 9.2	37 / 37.6	52 / 53.1
	women	17 / 6.3	129 / 47.8	124 / 45.9
Functional status of the body	men	8 / 8.2	29 / 30.0	61 / 62.2
	women	15 / 5.6	119 / 44.1	136 / 50.3
Physical development	men	7 / 7.2	37 / 37.7	54 / 55.1
	women	16 / 5.9	123 / 45.6	131 / 48.5
Physical fitness	men	9 / 9.2	33 / 33.7	56 / 57.2
	women	14 / 5.2	118 / 43.7	138 / 51.1
Body posture and organization of the body	men	8 / 8.2	34 / 34.6	56 / 57.2
	women	22 / 8.2	131 / 48.5	117 / 43.3
Health status	men	11 / 11.2	37 / 37.7	50 / 51.1
	women	27 / 10.0	125 / 46.3	118 / 43.7
Resistance to stress	men	7 / 7.2	26 / 26.5	65 / 66.3
	women	16 / 5.9	98 / 36.3	156 / 57.8
Motor activity	men	12 / 12.2	38 / 38.8	48 / 49.0
	women	29 / 10.7	132 / 48.9	109 / 40.4
Sleep pattern and its quality	men	16 / 16.3	52 / 53.1	30 / 30.6
	women	26 / 9.6	138 / 51.1	106 / 39.3
Dietary pattern and its quality	men	11 / 11.2	42 / 42.9	45 / 45.9
	women	21 / 7.8	143 / 52.9	106 / 39.3
Hygiene and everyday life	men	13 / 13.2	38 / 38.8	47 / 48.0
	women	19 / 7.0	125 / 46.3	126 / 46.7
Smoking	men	– / –	14 / 14.3	84 / 85.7
	women	2/ 0.7	19 / 7.0	249 / 92.3
Alcohol abuse	men	– / –	17 / 17.3	81 / 82.7
	women	– / –	3 / 1.1	267 / 98.9
Healthy lifestyle	men	11 / 11.2	38 / 38.8	49 / 50.0
	women	31 / 11.5	134 / 49.6	105 / 38.9
Rest, leisure	men	4 / 4.1	27 / 27.6	67 / 68.4
	women	13 / 4.8	117 / 43.3	140 / 51.9
Motivation to take care of one's own health	men	5 / 5.1	29 / 29.6	64/ 65.3
	women	17 / 6.3	139 / 51.5	114 / 42.2

student can shape his / her way of acting and thinking having a certain worldview. Life sustaining activities of students are characterized by partial irregularities and chaotic organization: non-compliance with the dietary regime, systematic lack of sleep, insufficient motor activity, little time spent in the fresh air, abuse of smoking, alcoholic beverages, etc. The students' survey conducted at the beginning of the ascertainment experiment showed that the majority of students, both men and women, have a low assessment of their own health culture (Table I).

Subjective assessment of health culture factors by students indicates low important vital indicators, namely: physical working capacity, functional state of the body, physical development, physical fitness, health status (men – 51.1-62.2 %, women – 43.7-51.1 %). More than 50 % of male students and 38.9 % of female ones do not adhere to a healthy lifestyle, more than 80 % of both men and women abuse smoking and alcohol. More than 40 % do not have adequate sleep and rational nutrition; more than 60 % rated their level of stress resistance as low. This indicates that more than 50% of students are aware that

Table II. The dynamics of health culture formation among students during the formative experiment (EG: men = 23, women = 29; CG: men = 21, women = 20; %)

Level of health culture	Experimental group				Control group			
	Before the experiment		After the experiment		Before the experiment		After the experiment	
	men	women	men	women	men	women	men	women
High	8.7	6.9	21.7	20.7	14.3	15.0	14.3	10.0
Average	39.1	27.6	56.6	48.3	38.1	40.0	52.4	45.0
Low	52.2	65.5	21.7	31.0	47.6	55.0	33.3	45.0

Table III. Motivational factors that shape the health culture of the EG students in the process of their physical education and health recreation activities (n = 52, men – 23, women – 29; number of people / %)

Motivational factors	Gender	Before the experiment	After the experiment	Difference
The desire to have a good body posture	men	8 / 34.8	19 / 82.6	11 / 47.8
	women	9 / 31.0	21 / 72.4	12 / 41.4
The desire to be healthy	men	5 / 21.7	14 / 60.9	9 / 39.2
	women	7 / 24.1	17 / 58.6	10 / 34.5
The example of colleagues and public opinion	men	2 / 8.7	9 / 39.1	7 / 30.4
	women	4 / 13.8	13 / 44.8	9 / 31.0
Responsibility and discipline	men	7 / 30.4	13 / 56.5	6 / 26.1
	women	8 / 27.6	15 / 51.7	7 / 24.1
The example of a physical education teacher	men	2 / 8.7	8 / 34.8	6 / 26.1
	women	3/ 10.3	8 / 27.6	5 / 17.3
Health problems	men	3 / 13.0	9 / 39.1	6 / 26.1
	women	5 / 17.2	9 / 31.0	4 / 13.8
Attractiveness of sports facilities and equipment	men	4 / 17.4	7 / 30.4	3 / 13.0
	women	7 / 24.1	12 / 41.4	5 / 17.3
Parents	men	3 / 13.0	4 / 17.4	1 / 4.4
	women	6 / 20.7	8 / 27.6	2 / 6.9

their physical condition and health is insufficient for the full implementation of their educational and life-sustaining activities. At the same time, the vast majority of students (65.3 % of men and 42.2 % of women) did not show interest in the proposed ways to improve their health. All this is a consequence of the imperfection of the development of the motivational sphere, value orientation and social attitudes of the individual towards a healthy lifestyle.

The tasks of physical education and health recreation activities in HEIs are aimed at reducing the negative consequences of the educational load, ensuring a sufficient amount of motor activity of students and training them in various types of motor activity, forming a system of knowledge on the methodology of independent physical exercises, forming a motivational and value attitude to the formation of a healthy lifestyle. This determined the substantiation and development of our author's methodology for students' health culture formation in the process of their physical education and health recreation activities.

When developing the author's methodology, we proceed from the fact that solving the problem of personality development is possible only under the condition of a holistic approach to physical education, which determines: a) the unity of spiritual and physical education; b) the unity of goals and orientation of educational training sessions as well as physical education and health recreation activities. In this regard, it is necessary to emphasize the educational and instructional orientation of physical education, that is, the maximum psychological and pedagogical orientation of all the methods used, on the education and formation of moral, organizational-management, physical culture and sports as well as motor activity of students.

To solve this aim, we used the practice of involving students in the joint management of the educational process. In such a case, we assumed that students have the power to choose the type of physical education and sports training sessions for the development and improvement of both physical and psychological

Table IV. Dynamics of physical fitness of male students during the formative experiment (Mean ± SD; EG = 23, CG = 21)

Tests	Groups	Before the experiment	After the experiment	Rate of growth	The validity of the difference	
					t	p
100 m run (s)	EG	15.46 ± 0.71	14.17 ± 0.83	0.89	2.16	<0.05
	CG	15.33 ± 0.81	15.24 ± 0.76	0.09	1.32	>0.05
Pull-ups on the horizontal bar (times)	EG	6.82 ± 0.83	9.72 ± 1.04	2.90	2.87	<0.01
	CG	6.07 ± 0.92	7.23 ± 0.91	1.16	1.71	>0.05
Push-ups (times)	EG	30.64 ± 2.17	39.17 ± 2.32	8.53	2.90	<0.01
	CG	30.91 ± 2.43	32.74 ± 3.51	1.83	1.39	>0.05
Standing long jump (cm)	EG	217.18 ± 1.96	228.14 ± 1.69	10.06	2.52	<0.05
	CG	219.04 ± 1.72	222.68 ± 1.71	3.64	1.51	>0.05
Lifting torso to the sitting position over 1 minute (times)	EG	30.26 ± 1.69	38.94 ± 1.76	8.68	2.83	<0.01
	CG	31.12 ± 1.83	32.51 ± 1.75	1.39	1.48	>0.05
4 x 9 m shuttle run (s)	EG	10.78 ± 0.95	9.43 ± 0.87	1.35	2.59	<0.05
	CG	10.61 ± 0.89	10.17 ± 0.92	0.44	1.57	>0.05
Torso leaning forward (cm)	EG	9.73 ± 0.91	14.86 ± 0.89	5.13	2.45	<0.05
	CG	9.98 ± 0.88	13.54 ± 0.91	3.56	2.25	<0.05

Legend: Mean: arithmetical average; SD: standard deviation; t: t-test value, p: the significance of the difference between the indicators before and after the experiment

Table V. Dynamics of physical fitness of female students during the formative experiment (Mean ± SD; EG = 29, CG = 20)

Tests	Groups	Before the experiment	After the experiment	Rate of growth	The validity of the difference	
					t	p
100 m run (s)	EG	18.38 ± 1.17	17.13 ± 1.14	1.25	2.28	<0.05
	CG	18.06 ± 1.13	17.51 ± 1.08	0.55	1.72	>0.05
Push-ups (times)	EG	8.41 ± 1.74	14.57 ± 1.34	6.16	3.84	<0.001
	CG	9.13 ± 1.57	10.25 ± 1.52	1.07	1.49	>0.05
Standing long jump (cm)	EG	163.27 ± 7.92	176.68 ±7.61	13.41	2.63	<0.05
	CG	164.58 ± 7.74	167.13 ± 7.72	2.55	1.49	>0.05
Lifting torso to the sitting position over 1 minute (times)	EG	26.25 ± 2.61	37.26 ± 2.73	11.01	3.14	<0.01
	CG	25.67 ± 2.83	29.85 ± 2.78	4.18	1.85	>0.05
4 x 9 m shuttle run	EG	11.98 ± 0.97	10.81 ±0.89	1.17	2.38	<0.05
	CG	11.77 ± 0.84	11.06 ± 0.98	0.71	1.67	>0.05
Torso leaning forward (cm)	EG	12.93 ± 0.92	17.21 ± 0.87	4.28	2.31	<0.05
	CG	13.16 ± 0.86	15.54 ± 0.95	2.38	1.97	>0.05

Legend: Mean: arithmetical average; SD: standard deviation; t: t-test value, p: the significance of the difference between the indicators before and after the experiment

qualities that correspond to their inclinations, interests and requirements of future professional activities. The leading idea of health culture formation by students in the process of their physical education and health recreation activities was to promote the formation of pedagogical components during training sessions, which is an integral, multi-level subsystem united by commonness of goals, tasks and unity of functions. Therefore, the realization of the aim of forming a high level of students' health culture requires the definition of a meaningful resource with the number of pedagogical components in the following sequence: motivational, cognitive and activity.

The motivational component involved the formation of an attitude among students to ensure a high level of health culture in the process of their professional and creative development as well as future professional activities.

The cognitive component provided for the formation of valeological competence, which is manifested in the mastery of a system of knowledge regarding the formation, preservation and strengthening of personal health,

the formation of an optimistic worldview and world perception of students towards physical education and health activities as well as keeping a healthy lifestyle.

The activity component provided for systematic regular physical education and health recreation activities; the presence of positive dynamics of functional readiness in future specialists, bringing the body composition closer to model indicators, ensuring the optimal physical status of the individual, which determines the achievement of a certain level of physical qualities and involvement in a healthy lifestyle.

The developed methodology for health culture formation meets the requirements of the state policy in the field of physical education of young people; contributes to the modernization of traditional and the development of new forms and methods of health culture formation; provides for: preserving and multiplying sports traditions of the university; continuous study of students' interests as well as their motivational and value attitudes towards motor activity; formation of civic self-awareness and behavior of students, their readiness for dignified service to society and the state; creation of optimal material and technical conditions for the development and self-realization of students; forming future specialists' needs and skills to conduct preventive measures for the purpose of preventing antisocial behavior, humanizing and democratizing the style of communication and interaction of teachers and students with the aim of forming their health culture.

The implementation of the author's methodology fundamentally changed the attitude of the students of the experimental group to health culture and physical exercises, contributed to the formation of their motivation and interest in physical education and health recreation activities. The criteria for the formation of students' health culture, in accordance with the defined components, were selected by us on the basis of the concept of health culture, which assumes that health culture is an important constituent component of general human culture, determined by the material and spiritual environment of society's vital activities, and is expressed in the system of values, knowledge, needs, abilities and skills for the formation, preservation and strengthening of health. Assessment of health culture of the experimental group students during the formative experiment according to motivational, cognitive and activity components showed the presence of a high level of formedness in health culture indicators (Table II).

The analysis of the dynamics of students' health culture formation during the formative experiment showed a significant difference between the EG and the CG. In particular, the number of male students with a high level of health culture increased by 13.0 % in the EG, while there were no changes in the CG. The increase was 17.5 % in the EG and 14.3 % in the CG for men in terms of an average level. At the same time, there was a decrease by 30.5 % of male students with a low level of health culture in the EG, and only by 14.3 % in the CG. The increase of female students with a high level was 13.8 % in the EG, and only 5.0 % in the CG; with an average level of health culture in female students it was 20.7 % in the EG, and 5.0 % in the CG. The number of female students with a low level of health culture decreased by 34.5 % in the EG, and by 10.0 % in the CG at the end of the experiment. The obtained data allow us to state that motivation for health culture by means of physical education and health recreation activities among students can be successfully formed during systematic physical exercises.

The studies aimed at researching the phenomena of "students' health" and "students' attitude to their own health" show that they are characterized by a desire for independence, a search for social identification and the formation of a system of life values in accordance with their preferences. This age period is a very difficult and important stage of human development. A significant influence on the formation of a student's personality is exerted by the social environment: the environment in the HEI, fellow students, teachers, colleagues, etc. As the main factors influencing the care of one's own health, students noted: "the desire to have a good body posture" – 82.6 % of men and 72.4 % of women; "the desire to be healthy" – 60.9 % of men and 58.6 % of women; "responsibility and discipline" – 56.5 % of men and 51.7 % of women and others (Table III). Such motivational factors as "the example of a physical education instructor" are effective in 34.8 % of male students and 27.6 % of female ones, and "parents" encourage the formation of health culture in 17.4 % of male students and 27.6 % of female ones.

The obtained data showed that it is possible to significantly improve the manifestations of health culture factors among students in educational as well as physical education and health recreation activities by means of increasing the level of their motivation. In general, the conducted pedagogical experiment confirms the importance of forming students' motivation for fitness and health recreation activities, healthy lifestyle, which, in general, has a positive impact on the process of students' health culture formation.

Purposeful work on health culture formation contributes not only to the increase of life values, knowledge, needs, abilities and skills for the formation and strengthening of students' health, but also significantly affects the improvement of indicators of physical fitness. During the formative experiment, the EG male

students had significantly better results ($p < 0.05$-0.01) on all physical fitness tests, while the CG ones only had reliable results in performing the "torso tilt forward" test (Table IV).

The best results were shown by male students of the EG during the performance of strength exercises, namely: the indicator increased by 2.9 times in pull-ups, by 8.53 times in push-ups, by 8.68 times in lifting torso to the sitting position over 1 minute ($p < 0.01$). The results of the EG students increased by 10.06 and 5.13 cm, respectively in standing long jump, which characterizes speed and strength qualities, and torso leaning forward, which characterizes flexibility.

The EG female students also showed significant improvement in performance on the physical fitness tests at the end of the experiment. The female students of the CG, who were engaged according to the traditional system of physical education, did not manage to reliably improve the level of their physical fitness in any test (Table V).

The highest indicators of the EG female students were demonstrated in push-ups, the increase was 6.16 times ($p < 0.001$); lifting torso to the sitting position over 1 minute – 11.01 times ($p < 0.01$); standing long jump – 13.41 cm ($p < 0.05$) and torso leaning forward – 4.28 cm ($p < 0.05$). All significant changes in the indicators of physical fitness of students (both male and female in the EG) are due to systematic participation in fitness and health recreation activities (regular physical exercises both during physical education training sessions and during extracurricular independent activities) and compliance with healthy lifestyles. All this testifies to the effectiveness of the developed and implemented methodology for the formation of students' health culture.

DISCUSSION

The problem of health culture formation is one of the central problems of modern times, which in the context of the general crisis of culture appears more multifaceted than in any separate scientific aspect [7, 8]. Modern science has more than three hundred definitions of the concept of "health". The definition adopted by the World Health Organization is widely known, according to which health is a state of complete physical, spiritual and social well-being, and not just the absence of diseases and physical defects [9]. H.L. Apanasenko [10] notes that health is expressed in a state that is closely related to such mechanisms of self-organization as resistance to the actions of pathogenic factors and the body's ability to overcome pathological processes. At the same time, health is characterized by plastic, energetic and information provision of self-organization processes. S.L. Marja and A. Suvi [11] give a fundamentally new look at the definition of this term in his research. He considers health through the prism of three streams of information – sensory, verbal, structural and defines it as a state of external and internal balance of a person with himself / herself and the environment in spiritual, mental and physical aspects, as well as the ability to perform biological and social functions at a high level. That is why every student is ambiguous not only externally, but also internally: behavior, perception, thoughts, actions, character, reactions, etc. to various factors. In addition, the very phenomenon of his / her health is very complex in general human and individual being. Therefore, there is no doubt that health depends on the interaction of many factors of physical and mental, social, individual and natural influence.

The conclusions of many scientists [12, 13] indicate that health is the most important for a person, and the value attitude towards it is formed and develops throughout life and is conditioned by the level of health culture formation. In many studies, the priority direction of students' health culture formation is defined as their involvement in modern physical education and health recreation activities. Among the elements of personal physical culture included in the concept of health culture, according to the scientists [1, 2, 14, 15] are:

1. Knowledge in the field of physical culture: the effect of physical exercises on the main body systems; conducting and organizing self-monitoring of physical development and functional state of the body; hygiene of independent physical exercises; hygiene of work and rest, organization of sleep, dietary pattern, personal hygiene.

2. Ability and skills of performing physical exercises: organization and conduct of independent training sessions in physical exercises and sports; morning hygienic gymnastics, physical education minutes during the academic day, independent training sessions; organization and conduct of physical culture and health recreation events and leisure activities; moving and sports games in the fresh air, folk games and fun.

3. Motivational and value orientations in the field of physical education and a healthy lifestyle: the need and awareness of the need for physical perfection and self-education; treating one's health as the value of life.

The introduction of the author's methodology into the educational process contributed to an increase in the number of the EG students at the end of the experiment with a high level of health culture and a level of motivation for a healthy lifestyle. At the same time, the level of physical fitness of the EG students, both men and women, significantly improved during the experiment. All this confirms the effectiveness of

the developed methodology for students' health culture formation in HEIs in the process of their physical education and health recreation activities.

CONCLUSIONS

1. A low level of health culture formedness was revealed in students of pedagogical specialties according to the results of their questionnaire at the ascertaining stage of the experiment. The level of self-esteem of students (both men and women) has low indicators for such factors as physical working capacity (53.1 %, 45.9 % respectively), functional state of the body (62.2 % and 53.3 %), physical development (55.1 % and 48.5 %), physical fitness (57.2 % and 51.1 %), health status (51.1 % and 43.7 %). More than 50 % of male students and 38.9 % of female students do not adhere to a healthy lifestyle, more than 80 % of both men and women abuse smoking and alcohol. At the same time, the vast majority of students (65.3 % of men and 42.2 % of women) did not show interest in the proposed ways to improve their health.
2. The implementation of the author's methodology contributed to improving the level of health culture of the EG students. Thus, in the EG the number of male students with a high level of health culture increased by 13.0 % and by 13.8 % among female students; with a low level – decreased by 30.5 % in male students and by 34.5 % in female ones. There were changes in the CG, but not as pronounced as in the EG. The level of motivation of the EG students improved during the experiment according to the following motivational factors: the desire to have a good posture by 47.8 % in men and 41.4 % in women, the desire to be healthy by 39.2 % in men and 34.5 % in women, the example of colleagues and public opinion by 30.4 % in men and 31.0 % in women and others. The level of physical fitness of the EG students (both men and women), in contrast to the CG, significantly ($p < 0.05$-0.01) improved according to all studied indicators. The greatest influence of the author's methodology was found on the indicators of male students in pull-ups (by 2.9 times), in push-ups (by 8.53 times), in torso lifting (by 8.68 times), in standing long jump (by 10.06 cm). For female students – in push-ups (by 6.16 times), in torso lifting (by 11.01 times).
3. The implementation of the methodology for students' health culture formation into the educational process contributed to an increase in the number of students with a high level of health culture and the level of motivation for a healthy lifestyle. The level of physical fitness of the experimental group students significantly improved during the experiment. All this confirms the effectiveness of the developed methodology.

Prospects for further research are aimed at improving the methodology for the formation of a healthy lifestyle culture of students of HEIs.

REFERENCES

1. Kharissova N., Kharissova L., Smirnov I. et al. Physical culture as the basis of students' healthy lifestyle. Georgian Med News. 2015; (241): 68-72.
2. Hansen A., McGarry D., Johnson A., Roche M.A. The impact of an undergraduate students' culture on their learning about mental health: A scoping review. Nurs Health Sci. 2021; 23(2): 352-361. doi: 10.1111/nhs.12835.
3. 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.
4. Prontenko K., Griban G., Dovgan N. et al. Students' health and its interrelation with physical fitness level. Sport Mont. 2019; 17(3): 41-46. doi 10.26773/smj.191018.
5. Mozolev O., Bloshchynsky I., Alieksieiev O. et al. Influence of modern fitness technologies on the state of health and development of motor abilities of 17-19-year-old female students. J Phys Educ Sport. 2019; 19(3): 917-924. doi:10.7752/jpes.2019.s3132.
6. Griban G., Kobernyk O., Terentieva N. et al. Formation of health and fitness competencies of students in the process of physical education. Sport Mont. 2020; 18(3): 73-78. doi: 10.26773/smj.201008.
7. Cogburn C.D. Culture, race, and health: Implications for racial inequities and population health. Milbank Q. 2019; 97(3): 736-761. doi: 10.1111/1468-0009.12411.
8. The Lancet Planetary Health. Promoting planetary health through culture. Lancet Planet Health. 2018; 2(12): e509. doi: 10.1016/S2542-5196(18)30266-3.
9. World Health Organization. Global action plan on physical activity 2018-2030: more active people for a healthier world. 2018. https://www.who.int/publications/i/item/9789241514187 [date access 02.08.2022]
10. Apanasenko H.L. Individual health: Theory and practice. Valeology. 2006; 1: 5-12.
11. Marja S.L., Suvi A. Cultural competence learning of the health care students using simulation pedagogy: An integrative review. Nurse Educ Pract. 2021; 52: 103044. doi: 10.1016/j.nepr.2021.103044.

12. 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.
13. Mozolev O., Bloshchynskyi I., Prontenko K. et al. Influence of fitness techniques integration on the development of physical qualities and morpho-functional state of adult females. Human Movement. 2021; 22(1): 57-65.
14. Griban G., Tymoshenko O., Arefiev V. et al. The role of physical education in improving the health status of students of special medical groups. Wiad. Lek. 2020; 73 (3): 534-540. doi: 10.36740/WLek202003125.
15. Machul M., Bieniak M., Chałdaś-Majdańska J. et al. Lifestyle practices, satisfaction with life and the level of perceived stress of Polish and foreign medical students studying in Poland. Int J Environ Res Public Health. 2020; 17(12): 4445. doi: 10.3390/ijerph17124445.

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

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Received: 25.08.2022
Accepted: 20.12.2022

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 PECULIARITIES OF BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN THE HEART OF THE CASTRATED RATS IN THE DEVELOPMENT OF ADRENALIN DAMAGE OF HEART

DOI: 10.36740/WLek202302105

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ABSTRACT

The aim of the study was to evaluate the state of oxidation processes and morphological changes in the heart of castrated rats during the development of epinephrine heart damage (EHD).

Materials and methods. The study was performed on 120 white male Wistar rats. The animals were divided into four series: 1 – control, 2 – castration. For EHD, rats were injected once intraperitoneally with a 0.18% solution of adrenaline hydrotartrate at the rate of 0.5 mg/kg of weight. Castration was performed under anesthesia. The concentration of diene and triene conjugates (DC, TC), Schiff's bases (SB), TBA-active products (TBA-ap), oxidatively modified proteins (OMP), activity of superoxide dismutase (SOD) and catalase (CAT) were determined in the heart. A morphological study of preparations stained with Azan-trichrome was carried out. All studies were performed in control, 1, 3, 7, 14 and 28 days after adrenaline injection.

Results: In the I series DC and TC increased after 1 day of EHD, fell to control values after 3 days, and then had wave-like character (highest – after 14 days). SB decreased (minimal after 7 days), TBA-ap increase (maximal after 14 days). OMP_{370} increased after 1 and 3 days, after 7 days they did not differ from the control, after 14 days they were higher than in control, and after 28 days they decreased to the control values. OMP_{430} and OMP_{530}were greater than the control indicators in all terms, except the last; the maximum was noted after 14 days. The activity of antioxidant enzymes was lower than the control indicators at all times of the study.

Castration caused an increase of lipid peroxidation. After 7 days, DC and TC, were lower and SB – higher, than in the I series. Castration caused a decrease in OMP. In EHD all values of OMP, compared to the castrated control rats, were higher at all studed times Castration leads to increase of SOD, and decrase of CAT. All indicators of SOD and CAT exceeded the indicators of animals of the I series at all times of the study.

Biochemical changes are consistent with morphological changes. After injection of epinephrine, severe vascular disorders, adventitia edema, perivasal edema, endothelial cell damage, dilatation of hemicapillaries, full blood vessels, stasis, hemorrhages in the surrounding tissues, and sclerosing of the walls of arteries and venules were observed. Cardiomyocytes were swollen, shortening, necrosis was observed, myocytolysis was noted. Edema of the stroma was noted. In the stroma, around the vessels, located cells of connective tissue elements were observed. Indicate more damage to the myocardium in the process of development of EHD in animals of the I series.

Conclusions: Castration of rats causes an increase of lipid peroxidation products and CAT activity in the heart, but a decrease in the content of OMP. Adrenaline injection causes activation of lipid peroxidation and an increase in the content of OMP. During the development of EHD, the activity of antioxidants is significantly higher in II group. Biochemical changes are consistent with morphological, and indicate more damage to the myocardium in the development of EHD in animals of the I series.

KEY WORDS: heart, male rats, adrenalin, castration, biochemical changes, morphological changes

Wiad Lek. 2023;76(2):274-284

INTRODUCTION

The problem of male hypogonadism is constantly growing. Thus, according to the World Health Organization, in the period from 2015 to 2050, the population of the world aged 60 and older will increase more than 2 times and will amount to approximately 2 billion people [1]. Social factors contribute to the growth of male hypogonadism [2]. There is a relationship between corona virus infection and the development of testicular failure [3, 4]. In men with somatic pathologies, in comparison with the general population, androgen deficiency is detected in up to 80% of cases [5, 6, 7].

Currently, the mechanisms of testosterone's action on the cardiovascular system are being studied [8]. It is appropriate to consider the complex impact of male hypogonadism on the development of other various diseases. The pathology of the cardiovascular system continues to occupy the top of three diseases in the world, therefore scientists of many countries are engaged in the study of issues related to cardiovascular

pathology [9, 10]. One of the models reflecting the development of cardiovascular pathology is the catecholamine model [11, 12, 13, 14]. The development of oxidative stress is the most universal mechanism of cell damage. Therefore, the study of this problem is relevant.

THE AIM

To evaluate the state of oxidation processes and morphological changes in the heart of castrated rats during the development of epinephrine heart damage (EHD).

MATERIALS AND METHODS

The work was doing at the Central Research Laboratory of I. Horbachevsky Ternopil National Medical University. Morphological investigation of heart doing in Histology and Embriology of I. Horbachevsky Ternopil National Medical University.

All experiments were performed in the morning in a specially designated room at a temperature of 18-22 °C, relative humidity of 40-60% and illumination of 250 lux. Animals were kept and experiments on them in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [15].

The study was performed on 120 white Wistar line male rats, which were kept in one room on a standard diet and a vivarium regime. All animals were divided into two series: 1 – control, 2 – castration. To modulate EHD, rats were injected once intraperitoneally with a 0.18% solution of adrenaline hydrotartrate at the rate of 0.5 mg/kg of weight (Pharmaceutical company "Darnytsia", Ukraine) [16]. Such dose of adrenaline causes 1 hour after injection responsible regulatory changes in the morphology and functioning of the cardiovascular system, without causing lethality among animals.

Experimental modeling of the decrease in the level of sex hormones in rats was carried out using surgically castration under sodium thiopental anesthesia (40 $mg \cdot kg^{-1}$ of the animal's body weight) according to the method of Ya. D. Kirshenblatt through a median incision of the anterior abdominal wall [17, 18]. The age of the operated animals was 3 months. At the time of the initiation of EHD reproduction, all animals were 4 months old, after injection of adrenaline hydrotartrate in volumes corresponding to body weight, after 1, 3, 7, 14 and 28 days, animals were euthanized under sodium thiopental anesthesia. Design of experiment show in table I.

Euthanasia of rats was carried out by total bleeding from the heart after previous thiopental-sodium anesthesia (60 $mg \cdot kg^{-1}$ of the animal's body weight intraperitoneally).

In the heart of animals, the concentration of diene and triene conjugates (DC, TC), TBA-active products (TBA-ap), Schiff's bases (SB), oxidatively-modified proteins (OMP), superoxide dismutase and catalase activity (SOD, CAT) were determined.

The concentration of DC, TC and SB was determined according to the method [19], which is based on the fact that hydroperoxides extracted with a heptane-isopropyl mixture have a certain absorption maximum for DC at λ = 232 nm, for TC – at λ= 278 nm, for SB – at λ = 400 nm. The content of diene and triene conjugates, Schiff bases was expressed in units/g. TBA-ap was determined at a wavelength of 535 nm according to the method [19], expressed in micromoles per kilogram (μmol/kg).

The method of determining the oxidative modification of proteins is based on the interaction of oxidized amino acid residues with 2,4-dinitrophenylhydrazine (2,4-DNPH) with the formation of 2,4-dinitrophenylhydrazones [20]. Ketone-dinitro-phenylhydrazones of a neutral character are registered at 370 nm (OMP_{370}), ketone-dinitro-phenylhydrazones of a basic character – at 430 nm (OMP_{430}), aldehyde-dinitrophenylhydrazones of a neutral character – at 530 nm (OMP_{530}), expressed in nmol/g of protein.

Superoxide dismutase activity in heart homogenate was determined according to the method [19], expressed in conventional units per 1 g. Catalase activity in heart homogenate and blood serum was determined according to the method [19], expressed in mcat/kg.

Transverse sections of the heart, made at the level of both ventricles, were also taken for morphological examination. The preparations were taken immediately after taken blood from the heart of the animal, fixed in a 10% solution of neutral formalin. No earlier than two weeks later, the preparations were washed in the tap water and held in alcohol, poured into paraffin blocks. Sections were stained with azan-trichrome and examined under a light microscope [21].

The significance of the obtained differences between the results (minimum level of significance $p<0.05$) was assessed using the Kruskal–Wallis and Newman–Keuls tests (BioStat program, AnalystSoft Inc.). All results prresented in $M \pm \sigma$.

RESULTS

The products of lipid peroxidation have the following changes (Table II). When analyzing indicators in I series of rats after 1 day after adrenaline injection, compared to control, the following was noted an increase in DC by

Table I. Design of experiment – division of experimental animals

Group	Characteristics of the experimental model group	Number of animals
I – Control white rats	Control (Intact)	10
	1 day after adrenaline injection	10
	3 days after adrenaline injection	10
	7 days after adrenaline injection	10
	14 days after adrenaline injection	10
	28 days after adrenaline injection	10
II – Castrated white rats	Control (Castrated)	10
	1 day after adrenaline injection	10
	3 days after adrenaline injection	10
	7 days after adrenaline injection	10
	14 days after adrenaline injection	10
	28 days after adrenaline injection	10
Total		120

Table II. Changes in the content of products of lipid peroxidation in the heart of rats during the development of epinephrine heart damage (M ± σ, n=10)

Group	Index			
	DC, units/g	TC, units/g	SB, units/g	TBA-ap, μmol/kg
Series I – Control				
Control (Intact)	1.58 ± 0.15	1.40 ± 0.12	1.28 ± 0.12	1.82 ± 0.10
1 day EHD	2.04 ± 0.20*	2.01 ± 0.14*	1.00 ± 0,05*	3.17 ± 0.25*
3 days EHD	1.42 ± 0.17**	1.11 ± 0.21**	0.69 ± 0.12*,**	3.58 ± 0.25*
7 days EHD	2.42 ± 0.22*,**	2.40 ± 0.18*,**	0.37 ± 0.02*,**	2.67 ± 0.22*,**
14 days EHD	3.46 ± 0.27*,**	3.47 ± 0.15*,**	0.49 ± 0.02*,**	7.42 ± 0.49*,**
28 days EHD	2.74 ± 0.21*,**	2.73 ± 0.21*,**	0.58 ± 0.03*,**	4.64 ± 0.24*,**
Series II – Castration				
Control (Castration)	2.47 ± 0.22$^{\#}$	2.64 ± 0.23$^{\#}$	1.65 ± 0.11$^{\#}$	2.74 ± 0.14$^{\#}$
1 day EHD	3.52 ± 0.27$^{*,\#}$	3.59 ± 0.27$^{*,\#}$	0.99 ± 0.03*	3.22 ± 0.32*
3 days EHD	1.46 ± 0.26*,**	1.57 ± 0.15$^{*,**,\#}$	0.62 ± 0.04*,**	3.73 ± 0.25*
7 days EHD	1.28 ± 0.12$^{*,**,\#}$	1.36 ± 0.13$^{*,\#}$	0.90 ± 0.03$^{*,**,\#}$	4.44 ± 0.25$^{*,**,\#}$
14 days EHD	2.21 ± 0.13$^{**,\#}$	2.26 ± 0.11$^{*,**,\#}$	1.01 ± 0.03$^{*,**,\#}$	4.85 ± 0.22$^{*,\#}$
28 days EHD	1.81 ± 0.12$^{*,**,\#}$	1.81 ± 0.12$^{*,**,\#}$	0.87 ± 0.02$^{*,**,\#}$	4.34 ± 0.31*

Notes: * – differences with the control within the series;
** – differences with the results of the previous term of the study within the series;
# – differences with the corresponding term of series I.

29.1% (p<0.001), an increase in TC by 43.6% (p<0.001), a decrease in SB by 21.9% (p<0.001) , an increase in TBA-ap by 74.2% (p<0.001). After 3 days of EHD, compared to control, DC and TC indicators have not change, SB decreased by 46.1% (p<0.001), TBA-ap increased by 96.7% (p<0.001). The DC, TC, SB were decrease at that time, compare to results after 1 day. After the 7 days EHD, compared to control, the indicators of DC increased by 53.2% (p<0.001), TC by 71.4% (p<0.001), TBA-ap by 46.7% (p<0.001), the most significant decrease in SB indicators was by 71.1% (p<0.001). The DC and TC were increase, but SB and TBA-ap were decrease at that time, compare to results after 3 days EHD. After 14 days, compared to control, indicators increased most significantly: DC – by 2.2 times (p<0.001), TC – by 2.5 times (p<0.001), TBA-ap – by 4.1 times (p<0.001), SB indicators decreased by 61.7% (p<0.001). All indexes were increase, compare to results after 7 days EHD. After 28 days of EHD, compared to control, the indicators of DC, TC and TBA-ap increased by 73.4% (p<0.001), by 95.0% (p<0.001) and by 2.5 times (p<0.001), respectively; SB was decreased by 54.7% (p<0.001). The DC, TC and TBA-ap were decreased, nd SB was increased at that time, compare to results after 14 days EHD.

Table III. Changes in the content of oxidatively-modified proteins in the heart of rats during the development of epinephrine damage to the heart, nmol/g of protein, (M ± σ, n=10)

Group	Index		
	OMP_{370}, nmol/g of protein	OMP_{430}, nmol/g of protein	OMP_{530}, nmol/g of protein
Series I – Control			
Control (Intact)	0.14 ± 0.01	0.15 ± 0.01	0,23 ± 0,01
1 day EHD	0.22 ± 0.01*	0.23 ± 0.02*	0.26 ± 0.01*
3 days EHD	0.24 ± 0.02*	0.27 ± 0.02*,**	0.31 ± 0.02*,**
7 days EHD	0.15 ± 0.02**	0.23 ± 0.05*	0.35 ± 0.02*,**
14 days EHD	0.18 ± 0.01*,**	0.33 ± 0.02*,**	0.40 ± 0.01*,**
28 days EHD	0.15 ± 0.02**	0.16 ± 0.02**	0.27 ± 0.01*,**
Series II – Castration			
Control (Castration)	0.09 ± 0.01#	0.08 ± 0.01#	0.13 ± 0·01#
1 day EHD	0.17 ± 0.03*,#	0.18 ± 0.01*,#	0.26 ± 0.02*
3 days EHD	0.19 ± 0.02*,#	0.24 ± 0.02*,**	0.33 ± 0.01*,**
7 days EHD	0.16 ± 0.02*	0.18 ± 0.02*,**,#	0.28 ± 0.02*,**,#
14 days EHD	0.16 ± 0.01*,#	0.18 ± 0.01*,#	0.27 ± 0.02*,#
28 days EHD	0.17 ± 0.,03*	0.18 ± 0.02*	0.24 ± 0.02*,#

Notes: * – differences with the control within the series;
** – differences with the results of the previous term of the study within the series;
# – differences with the corresponding term of series I.

Table IV. Changes of the antioxidants activity in the heart of rats during the development of epinephrine heart damage (M ± σ, n=10)

Group	Index	
	Superoxide dismutase activity, unit/g	Catalase activity, mcat/kg
Series I – Control		
Control (Intact)	3.05 ± 0.29	1.48 ± 0.13
1 day EHD	1.89 ± 0.12*	1.01 ± 0.07*
3 days EHD	2.27 ± 0.17*,**	1.07 ± 0,08*
7 days EHD	1.56 ± 0.12*,**	1.06 ± 0.02*
14 days EHD	1.72 ± 0.13*	1.06 ± 0.01*
28 days EHD	2.03 ± 0.10*,**	1.02 ± 0.01*,**
Series II – Castration		
Control (Castration)	2.75 ± 0.23	3.12 ± 0.10#
1 day EHD	2.25 ± 0.11*,#	2.39 ± 0.14*,#
3 days EHD	3.56 ± 0.35*,**,#	1.67 ± 0.10*,**,#
7 days EHD	4.79 ± 0.24*,**,#	1.71 ± 0.07*,#
14 days EHD	5.37 ± 0.22*,**,#	1.68 ± 0.02*,#
28 days EHD	4.58 ± 0.19*,**,#	1.51 ± 0.02*,**,#

Notes: * – differences with the control within the series;
** – differences with the results of the previous term of the study within the series;
# – differences with the corresponding term of series I.

Analyzing the indicators in II series of rats after 1 day EHD, compared to castration control, was noted an increase in the indicators of DC, TC and TBA-ap by 42.5% (p<0.001), by 36.0% (p<0.001) and by 17.5% (p<0.05); reduction of SB indicators by 40.0% (p<0.001). After 3 days of EHD, compared to castration control, DC indicators decreased by 40.9% (p<0.001), TC – by 40.5% (p<0.001), SB – by 62.4% (p<0.001), but TBA-ap indicators increased by 36.1% (p<0.001). The DC, TC, SB were decrease at that time, compare to results after 1 day. After 7 days EHD, compared to castration control, there was the most significant decrease in DC

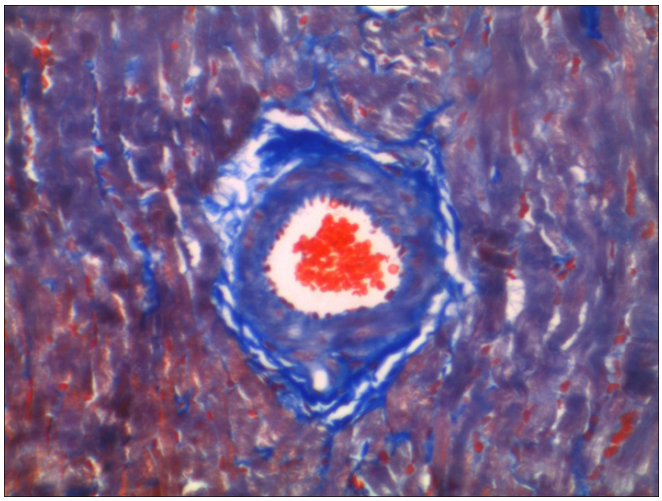

Fig. 1. Structural organization of the heart of the animals of group I, 1 day after adrenalin injection. Stained with Azan-trichrome x 200.

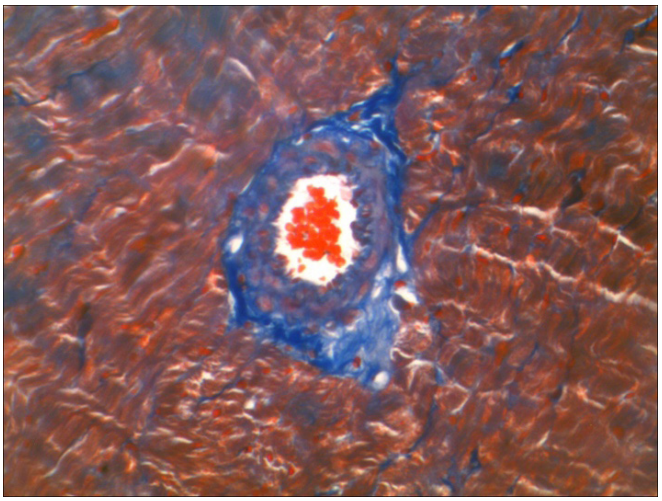

Fig. 2. Structural organization of the heart of the animals of group II, 1 day after adrenalin injection. Stained with Azan-trichrome x 200.

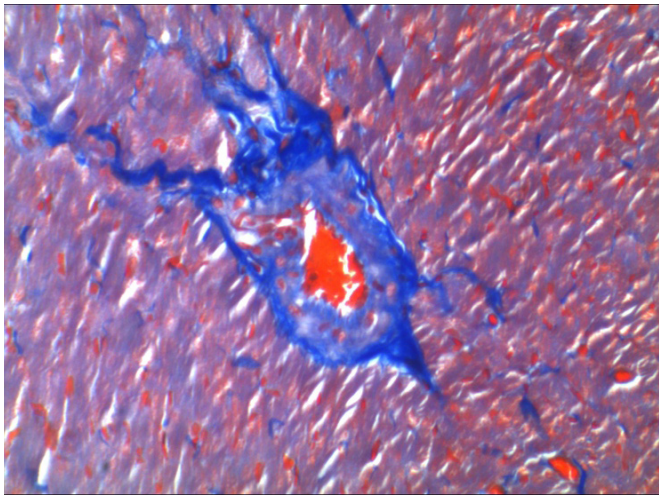

Fig. 3. Structural organization of the heart of the animals of group I, 3 days after adrenalin injection. Stained with Azan-trichrome x 200.

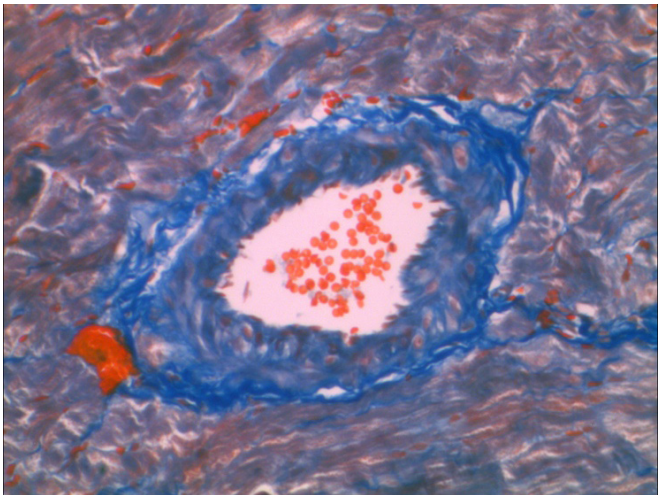

Fig. 4. Structural organization of the heart of the animals of group II, 3 days after adrenalin injection. Stained with Azan-trichrome x 400.

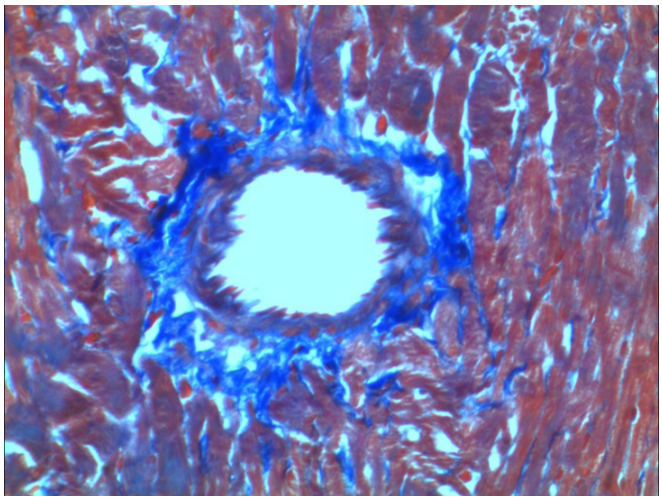

Fig. 5. Structural organization of the heart of the animals of group I, 7 days after adrenalin injection. Stained with Azan-trichrome x 200.

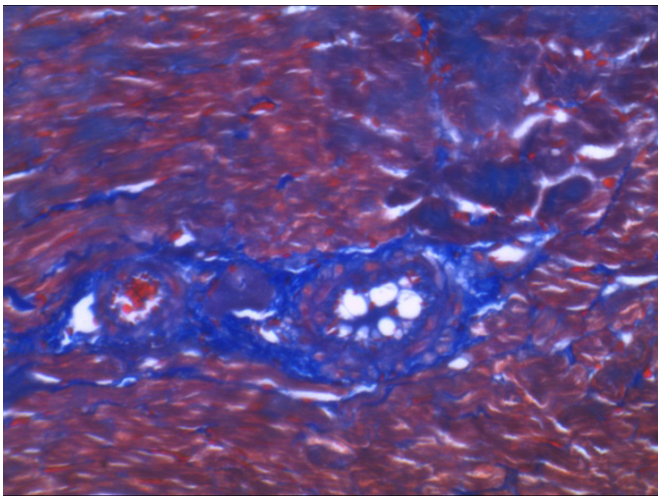

Fig. 6. Structural organization of the heart of the animals of group II, 7 days after adrenalin injection. Stained with Azan-trichrome x 200.

indicators by 48.2% ($p<0.001$), TC – by 48.5% ($p<0.001$), a less significant decrease in SB – by 45.5% ($p<0.001$), an increase of TBA-ap by 62.0% ($p<0.001$). The DC and TC were decrease, but SB and TBA-ap were increase at that time, compare to results after 3 days EHD. After 14 days of EHD, compared to castration control, the DC

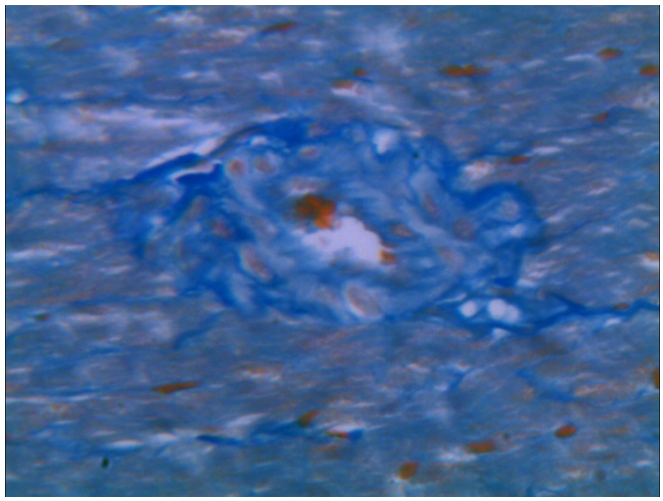

Fig. 7. Structural organization of the heart of the animals of group I, 14 days after adrenalin injection. Stained with Azan-trichrome x 200.

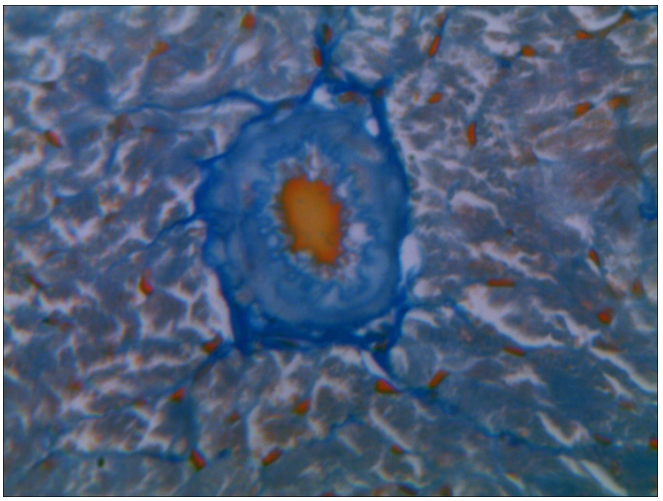

Fig. 8. Structural organization of the heart of the animals of group II, 14 days after adrenalin injection. Stained with Azan-trichrome x 200.

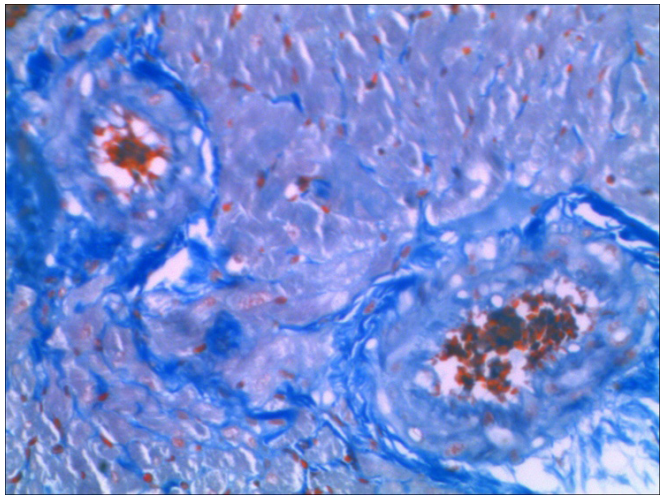

Fig. 9. Structural organization of the heart of the animals of group I, 28 days after adrenalin injection. Stained with Azan-trichrome x 200.

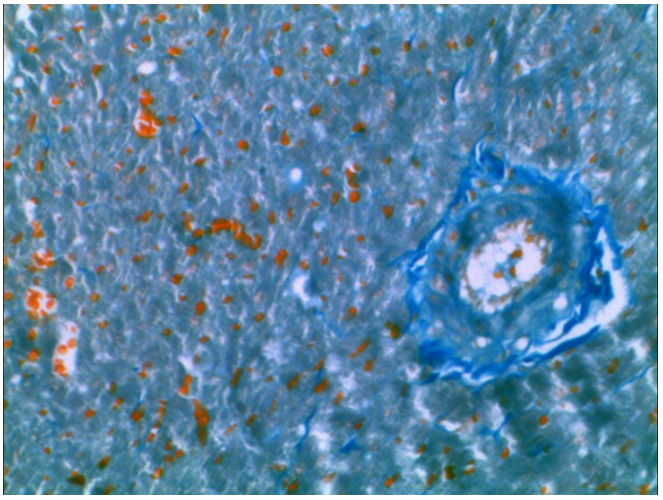

Fig. 10 . Structural organization of the heart of the animals of group II, 28 days after adrenalin injection. Stained with Azan-trichrome x 200.

was not change from control level, TC and SB indicators were decrease by 14.4% ($p<0.01$) and 38.8% ($p<0.001$), respectively, while the TBA-ap indicator increased by 77% ($p<0.001$). The DC, TC and SB were increased at that time, compare to results after 7 days EHD. After 28 days EHD, compared to castration control, the indicators of DC, TC and SB was less by 26.7% ($p<0.01$), 31.4% ($p<0.001$) and 47.3% ($p<0.001$), respectively, and TBA-ap indicators increased by 58.4% ($p<0.001$). Compared to 14 days after EHD, amount of DC, TC and SB was less.

When we compared results of I and II series, we saw, that castration lead to increase levels of DC, TC, SB and TBA-ap by 56.3% ($p<0.001$), by 88.6% ($p<0.001$), by 28.9% ($p<0.002$), by 50.5% ($p<0.001$), respectively. After 1 day EHD levels of DC and TC were higher in II group of rats by 72.5% ($p<0.001$) and 78.6% ($p<0.001$) respectively. After 3 days EHD level of TC was higher in II group by 41.4% ($p<0.001$). After 7 days EHD levels of DC and TC were less in II group of rats by 47.1% ($p<0.001$) and 43.3% ($p<0.001$), respectively, and levels of SB and TBA-ap were higher in II group by 2.4 times ($p<0.001$) and 66.3% ($p<0.001$), respectively. After 14 days EHD levels of DC, TC and TBA-ap were less in II group of rats by 36.1% ($p<0.001$), 34.9% ($p<0.001$) and 34.6% ($p<0.001$), respectively, and level of SB and was higher in II group by 2.1 times ($p<0.001$). After 28 days EHD levels of DC and TC were less in II group of rats by 33.9% ($p<0.001$) and 33.7% ($p<0.001$), respectively, and levels of SB was higher in II group by 1.5 times ($p<0.001$).

The changes of oxidatively-modified proteins (OMP) we show in table III.

When analyzing indicators in I series of rats after 1 day after adrenaline injection, compared to control, the following was noted an increase in all cases: in OMP_{370} by 57.1% ($p<0.001$), in OMP_{430} – by 53.3% ($p<0.001$) and in OMP_{530} – by 13% ($p<0.05$). After 3 days of EHD, compared to control, the indicators of OMP_{370} increased by 71.4% ($p<0,001$), OMP_{430} by 80% ($p<0.001$), OMP_{530}

by 34.8% (p<0.001). The level of OMP_{430} and OMP_{530} were higher, compared to previous term of investigation. After 7 days of EHD, compared to control, the indicators of OMP_{370} not changed, OMP_{430} increased by 53.3% (p<0.001), OMP_{530} – by 52.2% (p<0.001). The OMP_{370} was decrease, OMP_{430} – no changed, OMP_{530} was increase at that time, compare to results after 3 days of EHD. After 14 days of EHD, compared to control, the indicators of OMP_{370} increased by 28.4% (p<0.001), OMP_{430} increased most significantly by 2.2 times (p<0.001), and OMP_{530} be 73.9% (p<0.001). Compared to 7 days after EHD, amount of all indexes were higher. After 28 days of EHD, compared to control, the indicators of OMP_{370} and OMP_{430} were similas to control data, OMP_{530} increased by 17,4% (p<0.002). All indexes were less, compared to time after 14 days of epinephrine injection.

When analyzing indicators in II series of rats after 1 day after adrenaline injection, compared to castration control, was noted an increase in the indicators of OMP_{370} by 88.9% (p<0.001), OMP_{430} by 2.2 times (p<0,001), OMP_{530} – by 2 times (p<0.001). After 3 days of EHD, compared to castration control, the indicators increased most significantly: OMP_{370} – by 2.1 times (p<0.001), OMP_{430} – by 3 times (p<0.001), OMP_{530} – by 2.5 times (p<0.001). Compared to 1 day after EHD, amount of OMP_{430} and OMP_{530} were higher. After 7 days of EHD, compared to castration control, the indicators of OMP_{370} increased by 77.8% (p<0.001), OMP_{430} by by 2.2 times (p<0.001), OMP_{530} by 2.1 times (p<0.001). Compared to 3 days after EHD, amount of OMP_{430} and OMP_{530} were less. After 14 days of EHD all indexes were similar to previous time of invstigation. After 28 days of EHD, compared to castration control, the indicators of OMP_{370} increased by 88,9% (p<0.001), OMP_{430} by 2.2 times (p<0.001), OMP_{530} – by 84.6% (p<0.001) and only OMP_{530} decrased by 12.5% (p<0.01), compared to 14 days after EHD.

When we compare results of both series, we saw, that castration lead to decrease in all indicators: OMP_{370} by 35.7% (p<0.001), OMP_{430} – by 46.7% (p<0.001), OMP_{530} – by 43.5% (p<0.001). After 1 day of EHD in II series, compared to I series, the indicators of OMP_{370} was less by 22.7% (p<0.001), OMP_{430} – by 21.7% (p<0.001), OMP_{530} was similar. After 3 days of EHD in II series, compared to I series, only the indicators of OMP_{370} was less by 20.8% (p<0.001). After 7 days of EHD in II series, compared to I series, the indicators of OMP_{370} was similar, OMP_{430} decreased by 21.7% (p<0,001) and OMP_{530} – by 20% (p<0.001). When analyzing indicators after 14 days of EHD in II series, compared to I series, the following was noted an decrease in all cases: in OMP_{370} by 11,1% (p<0,05), in OMP_{430} – by 45.5% (p<0.001) and in OMP_{530} – by 32.5% (p<0.001). After 28 days of EHD in II series, compared to I series, only the indicators of OMP_{530} was less by 11.1% (p<0.05).

The changes of superoxide dismutase and catalase activities we demonstrated in table IV.

Analyzing the indicators of superoxide dismutase activity after adrenaline injection in I series, compared to control, demonstrated it decrease in all term of investigation. Indexes was less after 1 day by 38% (p<0.001), after 3 days – by 25.6% (p<0.001), after 7 days – by 48.9% (p<0.001), after 14 days – by 43.6% (p<0.001), after 28 days – by 33.4% (p<0.001). It increase, compares to previous term, after 3 anf 28 days. Analyzing the indicators of catalase activity after adrenaline injection in I series, compared to control, after 1 day was decreased by 31.8% (p<0.001), after 3 days – by 27.7% (p<0.001), after 7 and 14 days – by 28.4% (p<0.001), after 28 days – by 31.1% (p<0.001).

Superoxide dismutase activity after adrenaline injection in II series, compared to castration control, after 1 day was lower by 18.2% (p<0.001), after 3 days was higher by 29.5% (p<0.001), after 7 days – by 74.2% (p<0.001), after 14 days – by 95.3% (p<0.001), after 28 days – by 66.5% (p<0.001). Catalase activity after adrenaline injection in II series, compared to castration control, after 1 day was lower by 23.4% (p<0.001), after 3 days – by 46.5% (p<0.001), after 7 days – by 45.2% (p<0.001), after 14^{th} day – by 46.2% (p<0.001), after 28^{th} day – by 51.6% (p<0.001).

Analyzing the indicators of superoxide dismutase activity in both series of rats without adrenaline injection, the following in II series with castration rats, compared to I series, was not changed, but catalase activity was higher by 2.1 times (p<0.001) after castration. At all terms of invstigation antioxidant activity was higher in II series of rats. Indicators of superoxide dismutase activity after adrenaline injection in II series, compared to I series, after 1 day increased by 19% (p<0.001), after 3 days – by 56.8% (p<0.001), after 7 days – by 3.1 times (p<0.001), after 14 days – by 3.1 times (p<0.001), after 28 days – by 2.7 times (p<0.001). Indicators of catalase activity after adrenaline injection in II series, compared to I series, after 1 day increased by 2.4 times (p<0.001), after 3 days – by 56.1% (p<0.001), after 7 days – by 61.3% (p<0.001), after 14 days – by 58.5% (p<0.001), after 28 days – by 48.0% (p<0.001).

The studied biochemical changes in the heart are accompanied by alterative changes in the structural components of the myocardium in the experimental groups.

When studying histological specimens stained with Azan-trichrome, the following was noted after the injection of adrenaline. Pronounced vascular disorders, edema of the adventitia, perivasal edema, damage to the endothelial cells, dilatation of the capillaries lumen, full blood vessels, stasis, hemorrhages, sclerosing of the

walls of arteries and venules were observed. Destructive changes in cardiac muscle cells were also noted. Blood vessels wall edema, their shortening, necrosis was partially observed, and myocytolysis was noted in some fields of vision. Edema of the stroma was noted.

When studying the histological specimens of the hearts of animals of the I and II series, the following was noted. 1 day after the injection of adrenaline (Fig. 1), in the rats of the I series, there was more intensive growth of connective tissue, edema of the stroma, edema of the arteries wall, structural desorganization of the endothelium, and in the II series (Fig. 2), there was visiblea more prominent overstretching and edema of cardiac muscle cells.

3 days after the injction of adrenaline in rats of the I series (Fig. 3), a more pronounced growth of connective tissue, edema of the stroma, cardiac muscle cells, and arteries wall were identifed microscopically. Intravascular hemolysis of erythrocytes was noted. In the II series of rats (Fig. 4), a structural desorganization of the endothelium was observed in the blood vessels, which was noted in the previous period of the study to be removed in the animals of the I series. Changes in the tinctorial properties of cardiac muscle cells were noted.

7 days after the injection of adrenaline, microscopically in the myocardium of rats of the I series (Fig. 5), there was more prominent edema of stroma and cardiomyocytes, and in some places their necrotic changes were observed. Fibrosis of wall of the arteries, damage of the endothelial cells also was noted. The lumen of blood vesells were empty, without any formed elements of blood. In the II series of rats (Fig. 6), edema and contracture changes of cardiac muscle cells, prominent fibrosis were microscopically observed.

14 days after the injection of adrenaline microscopically in the myocardium of rats of the I series (Fig. 7) there was more prominent growth of vascular adventitia and perivascular space, edema of the cardiac muscle cells. Intravascular hemolysis of erythrocytes was noted. In the II series of rats (Fig. 8), edema of the stroma and wall of arteries, intravascular hemolysis of erythrocytes was microscopically observed.

28 days after the injection of adrenaline microscopically in the rats' heart of the I series (Fig. 9), there was a more proprominent growth of connective tissue, edema of the stroma and cardiac muscle cells, wall of the arteries. Blood clots were noted in the blood vessels. In the II series of rats (Fig. 10), endothelial structural desorganization, wall and stromal edema were observed in the vessels. Violation of the tinctorial properties of the myocardium was noted.

So, morphological studies of the hearts of experimental animals established more prominent alterative changes in the I series of animals. They appeared faster than in the II series of rats.

DISCUSSION

In the I series of animals, DC and TC indicators increased already after 1 day of EHD, fell to control values only after 3 days, and then the changes had a wave-like character. The highest indicators of DC and TC were registered after 14 days. Such results may indicate an active process of development of adrenaline damage in the heart. It is obvious that after the initial acute phase of the alteration, there was a slight decrease in the activity of the inflammatory process, cardiosclerosis was formed, an active process of fibrosis, which progressed. The connection between increase level of epinephrine, protein oxidation, antioxidant status and inflammation was described by another scientist [22]. As for changes in SB, this indicator decreased, with a minimum after 7 days, and then the values increased, but did not recover to control indicators. It is obvious that the decrease in the formation of SB indicated the incomplete neutralization of lipid peroxidation products at all times of the study. This was indicated by the increase in TBA-ap, which exceeded the control indicators at all times of the study. As with the DC and TC values, the maximum TBA-ap was noted after 14 days.

In the I series of rats, all values of OMP_{370} increased after 1 and 3 days, after 7 days they did not differ from the control, after 14 days they were higher than control level, and after 28 days they decreased and did not differ from the control values. OMP_{430} were greater than the control indicators in all terms, except the last; the maximum was noted after 14 days. The indicators of OMP_{530} in all terms of the study were higher than the control, gradually increased from the first term of the study and decreased in the last; the maximum was noted also after 14 days. The obtained data indicate that, like the processes of lipid peroxidation, the maximum destruction of proteins also occurs after 14 days from adrenaline injection.

The activity of antioxidant enzymes was lower than the control indicators at all times of the study, which could indicate their use at all stages of the development of EHD.

It is known, that female has reduced cardiovasclar risk and a better prognosis [23]. That is why castration leads to better prognosis in males, that is showed in our exeriment.

In the II series of rats, castration caused an increase in all indicators of lipid peroxidation. During the development of EHD, after 1 and 3 days, similar changes were noted as in rats of the I group. After 7 days, DC

and TC, in contrast to the I series of rats, were less than the control values, and smaller than in the animals of the I series; SB and TBA-ap were increased. It is possible that during this period the higher activity of antioxidants caused less accumulation of products of lipid peroxidation, but the process of fibrosis increased, as evidenced by the growth of DC and TC after 14 days of adrenaline injection. DC at this time were at the level of control castrated rats. The increase in SB indicates the detoxification of lipid peroxidation products to a greater extent than in the I group of rats. After 28 days, a decrease in DC and TC was noted, relative to the control of the II series and the indicators of the animals of the I series at that study period; SB was higher, compared to the values of the I series of rats. Such results indicate a more favorable course of EHD in the II series of rats as a result of less damage to the bilipid layer of cardiomyocyte membranes.

In the II series of rats, castration caused a decrease in OMP indicators. After injection of epinephrine, all values of OMP, compared to the values of castrated control rats, were higher at all times of the study. The values of OMP in II series of rats, compared to I, did not exceed the indicators of the corresponding periods of the study. Such results indicate a more favorable condition of EHD in the II series of rats as a result of less damage to cardiomyocyte membrane proteins.

In the II series of rats after castration, an increase in superoxide dismutase noot changed, but catalase activity was noted. Only catalase activity was observed to decrease with the development of EHD. Superoxide dismutase activity decreased only 1 day after the adrenaline injection, and then, at all subsequent times, it exceeded the control value. Compared with the I series of rats, in the II series, all indicators of SOD and CAT exceeded the indicators of animals of the I series at all times of the study.

Biochemical changes are consistent with morphological changes, and indicate more damage to the myocardium in the process of development of EHD in animals of the I series. Vascular and endotelial changed show the development of hypoxia, deseminated intravascular coagulative syndrome, and cardioscleosis. The similar effect, with functional changes of hart properties, saw other scientist [24]. Also, such changes of lipid peroxidation, OMP, antioxidant and moprphological picture in endothelium, the aterosclerosis and atrial fibrillation development can occure in the case of chaanges of steroid horrmones [25], that can decrease the duration of life.

The obtained results of the biochemical study can be explained obviously by the fact that castration causes a decrease in testosterone, which leads to a relative increase in the level of estrogens. Estrogens have a cardioprotective effect, which is why they prevent damage to cardiomyocytes under the action of a cardiotoxic dose of adrenaline. The mechanism is the anti-inflammatory action of estrogens [26]. On the other hand, a decrease in the level of testosterone leads to an increase in antioxidant activity, a high level of which remains even under the action of a cardiotoxic dose of adrenaline.

CONCLUSIONS

Castration of rats causes an increase of lipid peroxidation products and catalase activity in the heart, but a decrease in the content of oxidatively modified proteins. Adrenaline injection causes activation of lipid peroxidation and an increase in the content of oxidatively modified proteins. A more significant increase in the content of products of lipid peroxidation and a higher content of oxidatively modified proteins is observed in animals of the I series of rats. During the development of epinephrine heart damage, the activity of antioxidants is significantly higher in castrated animals. Biochemical changes are consistent with morphological changes, and indicate more damage to the myocardium in the process of development of epinephrine heart damage in animals of the I series.

REFERENCES

1. World Health Organization. World health statistics 2016: monitoring health for the SDGs sustainable development goals. World Health Organization, 2016.
2. Lopuszanska-Dawid M., Kołodziej H., et al. Age, Education, and Stress Affect Ageing Males' Symptoms More than Lifestyle Does: The Wroclaw Male Study. Int J Environ Res Public Health. 2022; 19(9):5044.
3. Yang M., Chen S., Huang B., et al. Pathological Findings in the Testes of COVID-19 Patients: Clinical Implications. European Urology Focus.2020; 6(5): 1124-1129.
4. Kharbach Y., Khallouk A. Male genital damage in COVID-19 patients: Are available data relevant? Asian Journal of Urology. 2021;8(3): 324-326.
5. Orshal J.M., Khalil R.A. Gender, sex hormones, and vascular tone. Am J Physiol Regul Integr Comp Physiol. 2004; 286(2): R233-249.
6. Tivesten A., Mellström D., Jutberger H., et al. Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. The MrOS Study in Sweden. J Am Coll Cardiol. 2007;50(11):1070-1076.

7. Panach-Navarrete J., Martínez-Jabaloyas J.M., DE-SDT study group. The influence of comorbidities on the aging males' symptoms scale in patients with erectile dysfunction. Aging Male. 2017; 20(3):146-152.
8. Diaconu R., Donoiu I., Mirea O., Bălşeanu T.A. Testosterone, cardiomyopathies, and heart failure: a narrative review. Asian J Androl. 2021; 23(4):348-356.
9. Fioranelli M., Bottaccioli A.G., Bottaccioli F., Bianchi M., Rovesti M., Roccia M.G. Stress and Inflammation in Coronary Artery Disease: A Review Psychoneuroendocrineimmunology-Based. Front. Immunol. 2018;9:2031.
10. Kivimäki M., Steptoe A. Effects of stress on the development and progression of cardiovascular disease. Nat Rev Cardiol. 2018;15(4):215-229.
11. Salama A., Mansour D., Hegazy R. The cardio and renoprotective role of ginseng against epinephrine-induced myocardial infarction in rats: Involvement of angiotensin II type 1 receptor/protein kinase C. Toxicol Rep. 2021;8: 908-919.
12. Khdhiri E., Mnafgui K., Ncir M., et al. Cardiopreventive capacity of a novel (E)-N'-(1-(7-methoxy-2-oxo-2H-chromen-3-yl)ethylidene)-4-methylbenzenesulfonohydrazide against isoproterenol-induced myocardial infarction by moderating biochemical, oxidative stress, and histological parameters. J Biochem Mol Toxicol. 2021;35(6):e22747.
13. Ghalwash M., Elmasry A., Omar N.M.A. Possible cardioprotective role of NaHS on ECG and oxidative stress markers in an unpredictable chronic mild stress model in rats. Can J Physiol Pharmacol. 2021;99(3):321-327.
14. Daniela B.D., Aurelian M.G., Sânziana I., Ioana C.C. Experimental research in rats on the reactivity of new corneal blood vessels to adrenaline. Rom J Ophthalmol. 2021;65(1):64-69.
15. Makarenko T.M., Radchenko O.M. Spivvidnoshennia biokhimichnykh pokaznykiv krovi v medychnii praktytsi: kliniko-diahnostychne znachennia [The ratio of blood biochemical parameters in medical practice: clinical and diagnostic value]. Practicing physician. 2017;6(2): 49–53. (In Ukrainian).
16. Denefil O.V. Zminy avtonomnoho balansu sertzevoho rytmu tvaryn pry diyi adrenalinu za riznykh typiv pohody [Changes in the autonomous balance of the heart rhythm of animals under the action of adrenaline under different types of weather]. Zaporizhia Medical Journal. 2008;4:14-15. (In Ukrainian).
17. Aloisi A.M., Ceccarelli I., Fiorenzani P. Gonadectomy affects hormonal and behavioral responses to repetitive nociceptive stimulation in male rats. Ann N Y Acad Sci. 2003;1007(1):232–237.
18. Joshi S.A., Shaikh S., Ranpura S., Khole V.V. Postnatal development and testosterone dependence of a rat epididymal protein identified by neonatal tolerization. Reproduction. 2003;125(4):495–507.
19. Vlizlo V.V., Fedoruk R.S., Ratych I.B. Laboratorni metody doslidjen' u biologii, tvarynnytstvi ta veterynarnii medytsyni: dovidnyk [Laboratory research methods in biology, animal husbandry and veterinary medicine: a handbook]. 2012:764. (In Ukrainian).
20. Meshchyshen I.F. Metod vyznachennia okysniuvalnoi modyfikatsii bilkiv plazmy (syrovatky) krovi [Method of determining the oxidative modification of plasma proteins (serum) blood]. Bukovynian Medical Journal. 1998;2(1):156-158. (in Ukrainian).
21. Horalskyi L.P., Khomych V.T., Kononskyi O.I. Osnovy histolohichnoj tekhniky i morfofunktsionalni metody doslidjnnya u normi ta pry patolohij: navch. posib. [Fundamentals of histological technique and morphofunctional research methods in normal and pathology: textbook.]. Zhytomyr: ZhNAEU, 2019: 286 p. (in Ukrainian).
22. Barbas I., Fatouros I.G., Douroudos I.I., et al. Physiological and performance adaptations of elite Greco-Roman wrestlers during a one-day tournament. Eur J Appl Physiol. 2011;111(7):1421-36.
23. Liccardo Dю, Arosio Bю, Corbi Gю, Cannavo A. Sex/Gender- and Age-Related Differences in β-Adrenergic Receptor Signaling in Cardiovascular Diseases. J Clin Med. 2022; 11(15):4280.
24. Hackenhaar F.S., Fumagalli F., Li Volti G., et al. Relationship between post-cardiac arrest myocardial oxidative stress and myocardial dysfunction in the rat. J Biomed Sci. 2014; 21(1): 70.
25. Theorell T. A long-term perspective on cardiovascular job stress research. J Occup Health. 2019; 61(1): 3-9.
26. Najjar F., Ahmad M., Lagace D., Leenen F.H.H. Sex differences in depression-like behavior and neuroinflammation in rats post-MI: role of estrogens. Am J Physiol Heart Circ Physiol. 2018;315(5):H1159-H1173.

The work is carried out within the framework of the initiative research work of the I. Horbachevsky Ternopil National Medical University (Ternopil, Ukraine) "0116 U003390 Systemic and organ disorders due to the action of extraordinary factors on the body, the mechanisms of their development and pathogenetic correction".

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Conflict of interest:
The Authors declare no conflict of interest

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Received: 02.07.2022
Accepted: 20.01.2023

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

ORIGINAL ARTICLE

THE EFFECT OF PHYSICAL TRAINING ON HEALTH AND PSYCHO-EMOTIONAL STATE OF MANAGERS OF LAW ENFORCEMENT AGENCIES

DOI: 10.36740/WLek202302106

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ABSTRACT

The aim: To investigate the effect of physical training sessions on health and psycho-emotional state of managers of law enforcement agencies.

Materials and methods: The research was conducted in 2019-2021. The research involved 155 managers of law enforcement agencies (men) of different age groups. Research methods: analysis and synthesis of literature, pedagogical observation, testing, methods of mathematical statistics, including correlation analysis (Pearson's correlation coefficient was used).

Results: An insufficient level of general physical fitness of managers of law enforcement agencies of all age groups was revealed. The worst level was found among managers of older age groups. Among physical qualities, the worst level was found in the development of endurance. A reliable relationship between the indicators of health and psycho-emotional state of managers of law enforcement agencies and the level of their general physical fitness was revealed. The highest correlation coefficients of these.

Conclusions: It was established that the use of general physical training with the predominant use of endurance and strength exercises, taking into account the age of managers of law enforcement agencies is the direction of solving the problem of promoting health, improving indicators of psycho-emotional state and professional activities of managers of law enforcement agencies.

KEY WORDS: physical training, health, managers of law enforcement agencies, psycho-emotional state

Wiad Lek. 2023;76(2):285-291

INTRODUCTION

Modern changes taking place in the political, military, economic, social and other spheres of society are accompanied by reformatting the law enforcement system, forasmuch as in conditions of military aggression, as well as threats to internal order and legality of our state professionalism of employees of law enforcement agencies of Ukraine, especially their officers-in-charge (managers) comes to the fore. The professionalism of managers of law enforcement agencies (MLEAs) is manifested in the systematic improvement of their qualification, creativity, ability to productively meet the growing demands of society and system of government, in personal and professional development which includes self-education, self-regulation, self-cultivation and self-development [1, 2]. Today there is a significant expansion and complication of management tasks that require MLEAs to have a high degree of development of specific management skills, good health, a high level of professionally important psychological and physical qualities [3, 4]. The research by the scientists [5-7] showed that the activities of superior personnel of law enforcement agencies at all levels of management are carried out, as a rule, in difficult, and sometimes – extreme conditions. Professional management environment is accompanied by the emergence of stressful states in managers due to specific professional relationships, unforeseen situations, instability of the socio-economic situation in the country, uncertainty about the future, shortcomings in the organization of activities of law enforcement agencies and units. All this results in the deterioration of the physical and mental health of MLEAs, losses, especially in personnel policy, provokes conflict

situations, hinders the performance of professional and functional duties in the subordinate unit [8-10]. Exercise in the process of physical training can be one of the possible ways to promote health, relieve psycho-emotional stress and reduce stress levels in MLEAs [11, 12].

Physical training of law enforcement officers is divided into general and special [13, 14]. The tasks of general physical training (GPT) are: development and improvement of physical qualities; improving physical development, promoting health, increasing working capacity and service activities; ensuring professional longevity and resistance of the body to adverse environmental factors and professional activities, reducing labour costs associated with diseases; education of courage, determination, initiative, persistence, independence, self-confidence, mental stability; involvement of law enforcement officers in regular physical exercises and sports [15, 16]. GPT is the basis for special qualities formation i. e. improvement of special physical training (SPT). The tasks of the SPT are defined as: mastering the skills of performing measures of physical impact, including after significant physical activities, in conditions as close as possible to the real ones, and their improvement; developing the ability to perform special exercises; development of endurance, orientation on the ground; education of ingenuity and perseverance in achieving the goal [17, 18]. The practice convincingly shows that, firstly, it is impossible to improve special qualities without a high level of GPT, and, secondly, insufficient level of law enforcement officers' GPT results in deterioration of physical condition and health, emergence of various diseases and reduced efficiency of service activities. With regard to management activities of MLEAs, a low level of general physical fitness of instructors leads to overweight, stagnation in the body, decreased muscle strength and tone, disruption of basic life support systems, reduced efficiency and the emergence of such diseases. All this has a negative impact on the process of managing subordinate units and the effectiveness of the tasks assigned to the unit. Therefore, improving the level of general physical fitness is the main task of physical training of MLEAs.

Many scientists [19, 20] have confirmed that GPT takes important place in order to promote the health, improve physical and mental working capacity as well as professionally important psychological qualities and psycho-emotional state of law enforcement officers, whose activities are characterized by high nervous tension, stress, low motor activity and bad habits (smoking, alcohol). To verify these conclusions, we conducted the investigation of the level of general physical fitness of MLEAs of different age groups and correlation analysis of the indicators of general physical fitness and health and psycho-emotional state of MLEAs.

THE AIM

The aim is to investigate the effect of physical training sessions on health and psycho-emotional state of managers of law enforcement agencies.

Research hypothesis: using correlation analysis, it is planned to prove the effect of general physical training on health and psycho-emotional state of managers of law enforcement agencies of different age groups.

MATERIALS AND METHODS

The research was conducted in 2019-2021. The research involved 155 managers of law enforcement agencies (men) of different age groups, who held superior positions in various police departments of Ukraine: under 30 years old – officers-in-charge of sectors of territorial police units (n = 39), 31-35 years old – officers-in-charge of divisions of territorial police units (n = 33), 36-40 years old – officers-in-charge of stations of territorial police units (n = 30), 41-45 years old – officers-in-charge of directorates within the main police departments (n = 29), 45 years and older – officers-in-charge of departments and other units of the central office of the National Police (n = 24). This distribution of officers is determined by the Order of the Ministry of Internal Affairs of Ukraine dated January 26, 2016 No. 50 "On approval of the Regulation on the organization of official training of employees of the National Police of Ukraine" (https://zakon.rada.gov.ua/laws/show/z0260-16#Text). This study was organized and conducted at the Department of Legal Psychology of National Academy of Internal Affairs (NAIA), Kyiv, Ukraine.

Research methods: analysis and synthesis of literature, pedagogical observation, testing, methods of mathematical statistics.

With the help of the analysis and synthesis of literature, we decided on the topic of the research, got acquainted with the researches of leading scientists and scientifically substantiated the factual material that we received during the research. The main attention was paid to the following issues: peculiarities of professional activity of MLEAs and requirements for their physical fitness and health; the place of physical training in the system of professional training of law enforcement officers; the impact of physical exercises on reducing the negative factors of professional activity, strengthening health and restoring the working capacity of law enforcement officers. 27 publications from the databases PubMed, Scopus, Web of Science Core Collection, Crossref and others were analyzed. Most of them are 2020-2021.

Pedagogical observation as a method of scientific research was used during the analysis of the professional activity of MLEAs, during the evaluation of the effectiveness of the current system of organization of physical training with MLEAs, during sessions and inspections of MLEAs on physical training, during the study of their attitude to physical

training sessions as a means of promoting their health and improving psycho-emotional state.

In order to carry out quality testing, a research card was created for each manager, in which indicators of physical fitness, health, morphofunctional state, psycho-emotional state, and professional training were recorded. The level of physical fitness of MLEAs was tested based on the results of 100 m run, complex strength exercise, 1000 m run. It was evaluated according to the Order of the Ministry of Internal Affairs of Ukraine dated January 26, 2016 No. 50 "On approval of the Regulation on the organization of official training of employees of the National Police of Ukraine. Testing was carried out by physical training specialists. We obtained health and morphofunctional indicators at the Polyclinics of the Ministry of Internal Affairs of Ukraine, where all law enforcement officers are required to take an annual medical examination. We obtained indicators of psycho-emotional state in the departments of psychological support of the relevant structural subdivisions, where psychological testing of all law enforcement officers of the subdivision is conducted annually. The efficiency of professional training of MLEAs was assessed by the indicators of the average grade of professional training and professional skills regarding the use of measures of physical coercion.

The methods of mathematical statistics were used in order to prove the regularities discovered in the research process. One-dimensional and two-dimensional statistical analyses were used. One-dimensional statistical analysis included the calculation of the following characteristics: arithmetical average, standard deviation, significance of the difference between the studied indicators according to the Student's t-test. The use of two-dimensional statistical analysis made it possible to calculate the correlation coefficient (Pearson's correlation coefficient was used). Pearson correlation coefficient is an indicator of the linear relationship between two variables, which takes values from – 1 to + 1 inclusive. All studied indicators corresponded to a normal distribution.

This study followed the regulations of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. The procedure for organizing this study was previously agreed with the committee on compliance with Academic Integrity and Ethics of the NAIA. The topic of the study was approved by the Academic Council of the NAIA (No. 01 dated 16.09.2019). Informed consent was received from all participants who took part in this study.

RESULTS

Physical training of law enforcement officers is one of the important components of improving their health and increasing the efficiency of their professional activity only if it is systematically carried out. Pedagogical observation of MLEAs attendance at physical training sessions showed that more than 70% of MLEAs miss scheduled physical training sessions. Some MLEAs may be absent from many sessions in a row. It was also revealed that more than 50% of MLEAs miss more than a third of physical training sessions for various reasons.

The results of the study of physical fitness level of MLEAs of different age groups during 2019-2021 are shown in Table. I.

The analysis of the results in 100 m run showed that the level of speed qualities of the MLEAs generally meets the regulatory requirements (it is rated mostly as "good" and "satisfactory"). During the three years of the study, the indicators of MLEAs of all age groups worsened – the difference is 0.2 s for MLEAs under 30 years old ($p>0.05$), for MLEAs of other age groups – 0.4-0.7 s and is reliable ($p<0.05-<0.01$).

In the complex strength exercise, the indicators of MLEAs of most age groups, with the exception of those under 30 years old, significantly ($p<0.05-<0.001$) deteriorate during the research. The difference between the indicators of 2019 and 2021 for MLEAs under 30 years old is 1.7 times ($p>0.05$), in the age group of 31-35 years – 4.2 times ($p<0.01$), 36-40 years – 4.2 times ($p<0.01$), 41-45 years – 5.3 times ($p<0.001$), over 45 years – 6.4 times ($p<0.001$). At the same time, the level of development of strength qualities significantly ($p<0.001$) worsened with age: the worst indicators were found in MLEAs over 45 years old (22.7-29.1 times). However, in all age groups of MLEAs, the level of strength qualities is assessed as "good" and "satisfactory".

Of particular interest is the level of development of general endurance of MLEAs, because based on indicators of endurance, a conclusion is made about the level of human health. According to the results of the analysis of endurance level, it can be concluded that the results of MLEAs of all age groups in 1000 m run has an insufficient level of development. The results of MLEAs of the age groups under 30 and 31-35 years old are rated as "satisfactory", and those of other age groups as "unsatisfactory". In addition, indicators of endurance development are characterized by negative trends both during the research (in 2019-2021) and with increasing the age of MLEAs.

In order to study the effectiveness of general physical training sessions to promote health, improve morphofunctional status, psycho-emotional state, indicators of professional activity in MLEAs, we conducted the correlation analysis between the results of 100 m run, complex strength exercise, 1000 m run and the indicators of body mass index, Robinson index, vital and strength indices, the level of physical health, professionally important psychological qualities, emotional state, mental working capacity and efficiency of professional activities of MLEAs (Table II).

Table I. The level of physical fitness of managers of law enforcement agencies (2019-2021 pp., n = 155, Mean±SD)

Grade, Years of research	Age groups				
	Under 30 years old	31-35 years old	36-40 years old	41-45 years old	45 years and older
	n=39	n=33	n=30	n=29	n=24
100 m run, s					
2019	14.8±0.08	15.1±0.11	15.6±0.13	16.1±0.15	16.5±0.16
2020	14.9±0.09	15.3±0.11	15.8±0.14	16.4±0.15	16.9±0.16
2021	15.0±0.10	15.5±0.12	16.1±0.15	16.7±0.16	17.2±0.17
t; p	1.56; >0.05	2.46; <0.05	2.52; <0.05	2.74; <0.05	2.99; <0.01
Complex strength exercise, times					
2019	46.4±0.64	43.5±0.81	39.1±0.84	34.8±0.95	29.1±1.04
2020	45.3±0.60	41.2±0.83	36.3±0.86	31.1±0.96	25.0±1.05
2021	44.7±0.58	39.3±0.82	34.9±0.87	28.9±0.96	22.7±1.05
t; p	1.97; >0.05	3.64; <0.01	3.74; <0.01	4.37; <0.001	4.37; <0.001
1000 m run, s					
2019	246.04±4.09	254.19±4.01	267.20±4.21	278.00±4.32	289.05±4.46
2020	25110±4.16	261.02±4.06	273.11±4.27	285.14±4.35	296.13±4.51
2021	257.07±4.22	268.08±4.15	281.04±4.30	293.19±4.37	305.21±4.59
t; p	1.88; >0.05	2.41; <0.05	2.30; <0.05	2.47; <0.05	2.84; <0.05

Legend: M: arithmetical average; SD: standard deviation; t: t-test value, p: the significance of the difference between the indicators of each age groups in 2019 and 2021

Table II. Relationship between the level of general physical fitness of managers of law enforcement agencies (n = 155) and indicators of their health, morphofunctional status, psycho-emotional state, indicators of professional activity (2021, c. u.)

Indicators of their health, professionally important psychological qualities and professional activities	Indicators of general physical fitness		
	100 m run	Complex strength exercise	1000 m run
	Correlation coefficients		
Body mass index	0.162	-0.204*	0.461**
Robinson index	-0.145	0.183	-0.397**
Vital index	-0.118	0.524**	-0.243*
Strength index	0.153	-0.172	0.480**
Heart rate recovery time	0.172	-0.151	0.514**
Level of physical health	-0.126	0.182	-0.505**
Distribution and scope of attention	-0.083	-0.113	0.073
Visual short term memory	0.065	0.049	0.136
Peculiarities of thinking	0.087	-0.075	0.091
Emotional stability	0.134	-0.191*	0.239*
Concentration and stability of attention	-0.092	0.141	-0.174
Mental working capacity	-0.167	0.173	-0.212*
Well-being	0.211*	-0.219*	0.340**
Activity	0.249*	-0.245*	0.288**
Mood	0.222*	-0.310**	0.296**
Grade of professional training	-0.160	0.388**	-0.678**
Professional skills regarding the use of measures of physical coercion	-0.509**	0.768**	-0.844**

Legend: * – r critical = 0.196 at p < 0.05; ** – r critical = 0.258 at p < 0.01

The analysis of the correlation coefficients between the studied indicators of MLEAs and their results in 100 m run suggests a reliable relationship between the results of speed exercise with indicators of emotional state (well-being (r = 0.211), activity (r = 0.249), mood (r = 0.222); p<0.05) and the level of formedness of professional skills regarding the use of measures of physical coercion (r = -0.509; p<0.01). The correlation of the results in 100 m run with the rest of the studied indicators (health, morphofunctional status, professionally

important psychological qualities, grade of professional training) is either absent or low.

The study of the correlation coefficients between results of MLEAs in complex strength exercise and indicators of morphofunctional status and health shows that the performance of strength exercise has a significantly high relationship with the strength index ($r=0.524$; $p<0.01$) and a significantly low relationship with the body mass index ($r= -0.204$; $p<0.05$). According to other indicators of morphofunctional status and health, there is a relationship with the results in complex strength exercise, but it is not reliable ($p>0.05$). The structure of the relationship between the results of MLEAs in complex strength exercise with indicators of psychological qualities is complex. Thus, the result of complex strength exercise performance has a significant relationship with the indicators of emotional states (well-being – $r = -0.219$; $p<0.05$; activity – $r = -0.245$; $p<0.05$; mood – $r = -0.310$; $p<0.05$) and emotional stability ($r = -0.191$; $p<0.05$). Significant influence of the exercise on the development of strength qualities has been established on the level of professional training ($r = 0.388$; $p<0.01$) and the level of professional skills in the application of measures of physical coercion ($r = 0.768$; $p<0.01$).

The correlation analysis of the results of 1000 m run showed that there is a high reliable relationship between the result of endurance exercise performance and the level of professional training and the level of professional skills in the application of measures of physical coercion ($r = -0.678$; $p<0.01$; $r = -0.844$; $p<0.01$). The results of 1000 m run are significantly correlated with all indicators of morphofunctional status and physical health of OLEBs, but a significant high relationship was found with the time of recovery of heart rate to baseline ($r = 0.514$; $p<0.01$) and the level of health ($r = -0.505$; $p<0.01$). The correlation relationship between the level of endurance development and the body mass, vital and Robinson indices is also significant, but average ($r = 0.461$; $r = -0.397$; $r = -0.243$; $p<0.01$), and it is low with the strength index ($r = 0.243$; $p<0.05$). The presence of a reliable correlation relationship between endurance exercise was also found with indicators of emotional stability ($r = 0.239$; $p<0.05$), mental working capacity ($r = -0.212$; $p<0.05$), well-being ($r = 0.340$; $p<0.01$), activity ($r = 0.288$; $p<0.01$) and mood ($r = 0.296$; $p<0.01$). The relationship is unreliable with other indicators of professionally important psychological qualities ($p>0.05$).

The analysis of the correlation coefficient between the results of general physical training exercises with the effectiveness of service activities of MLEAs showed that grades in professional training subject areas have a significantly close relationship with the results of physical exercises such as 1000 m run and pull-ups on the crossbar ($r = -0.678$ та $r = 0.388$ respectively).

DISCUSSION

Physical training in law enforcement agencies is aimed at comprehensive harmonious development of law enforcement officers, maintaining good health, education of high moral and volitional qualities, formation of special skills, instilling personnel in the need for physical and moral improvement, formation of readiness for duty [11]. Performance of physical exercises by MLEAs should provide preservation of their health, creative and labour activity, comprehensive development of physical qualities, the vital skills necessary for performance of service tasks [21, 22].

According to the scientists [23, 24], the effectiveness of physical training to form the necessary qualities of specialists depends on the variety of tools used, their scope and direction. The use of physical exercises depends primarily on the nature and peculiarities of the professional activities of law enforcement officers. The professional activities of MLEAs is characterized by stresses and intense psycho-emotional load, pronounced hypodynamic nature, which determines the need to use physical exercises to promote health, restore physical and mental capacity and reduce negative factors (especially stresses and lack of physical activities).

Vigorous muscular activity is a reliable guarantor in the prevention of cardiovascular diseases caused by excessive nervous and mental stress [9, 14]. Numerous studies have shown that physical training significantly improves the mechanisms that regulate vascular tone [13, 25]. The cardiovascular system in a trained person has more stable regulatory mechanisms than in the untrained one, and a sudden significant physical activity in the latter can result in disruption of compensatory capacity and pathological changes in the body [26].

Moreover the results of scientists [3, 5, 27] have shown that the stability of attention, indicators of intellectual abilities and memory of servicemen and law enforcement officers depend on the degree of development of their physical qualities (strength, static endurance of the torso muscles) and the level of general physical fitness. Static endurance of the torso muscles (back and abdomen), ensuring the formation of a muscular "corset" and improving blood supply to the brain, increases the efficiency of intellectual functioning and reduces the risk of occupational diseases.

Our research revealed that health status, morphofunctional status, professionally important psychological qualities, the level of skills in the application of measures of physical coercion and the level of professional qualification of MLEAs have a high degree of dependence on the level of general physical fitness.

In general, among the exercises of general physical training, which have a positive effect on the studied indicators of MLEAs, the most reliable correlation relationships were

found for 1000 m run. This confirms the conclusions of the scientists [12, 16, 17, 20] about the importance of developing and improving overall endurance for law enforcement officers in order to promote their health, improve physical and mental working capacity and efficiency of management environment, as well as prolong their professional longevity. In addition, the correlation analysis allows to suggest that general endurance and strength qualities are the main physical qualities that have a positive effect on health, morphofunctional status, and professionally important psychological qualities of MLEAs, while forming the basis for improving indicators of professional activities, and which should be focused on during conducting physical training sessions with the superior personnel of law enforcement agencies of Ukraine.

CONCLUSIONS

An insufficient level of general physical fitness of managers of law enforcement agencies of all age groups was revealed. The worst level was found among managers of older age groups. Among physical qualities, the worst level was found in the development of endurance.

A reliable relationship between the indicators of health, morphofunctional status, professionally important psychological qualities of MLEAs and the level of their general physical fitness was revealed. The highest correlation coefficients of these indicators were recorded with the level of development of endurance ($r = -0.844 – 0.514$; $p<0.01$) and strength qualities ($r = -0.191 – 0.768$; $p<0.05$-0.01). It was established that the use of general physical training with the predominant use of endurance and strength exercises, taking into account the age of MLEAs is the direction of solving the problem of promoting health, improving indicators of professional activities of MLEAs.

Prospects for further research are the development of methods for recreational exercise with law enforcement officers of older age groups and testing their effectiveness.

REFERENCES

1. Ostapovich V., Barko V., Okhrimenko I. et al. Psychological profile of successful criminal police officer. Inter J Appl Exer Physiol. 2020; 9(3): 120-133.
2. Bondarenko V., Okhrimenko I., Tverdokhvalova I. et al. Formation of the professionally significant skills and competencies of future police officers during studying at higher educational institutions. Revista Românească pentru Educație Multidimensională. 2020; 12(3): 246-267.
3. Barko V., Okhrimenko I., Medvediev V. et al. Professional psychological profile of a modern patrol officer as the basis of efficient official activities. Postmodern Openings. 2020; 11(3): 01-19.
4. Bano B. Job stress among police personnel. International Conference on Economics and Finance Research (IPEDR). 2011; 4: 290-293.
5. Bondarenko V., Okhrimenko I., Piaskovskyi V. et al. Scientific tools for forming professional competence of patrol police officers. International Journal of Evaluation and Research in Education. 2022; 11(2): 687-695. doi: 10.11591/ijere.v11i2.21987.
6. Okhrimenko I., Lyhun N., Pryimak V. et al. 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.
7. Gül Z., Delice M. Police job stress and stress reduction/coping programs: the effects on the relationship with spouses. Turkish Journal of Police Studies. 2011; 3(13): 19-38.
8. Plat M.J., Frings-Dresen M.H., Sluiter J.K. A systematic review of job-specific workers' health surveillance activities for fire-fighting, ambulance, police and military personnel. Int Arch Occup Environ Health. 2011; 84(8): 839-857. doi:10.1007/s00420-011-0614-y.
9. Okhrimenko I., Lyhun N. Stress prevention and management during the quarantine. BRAIN. Broad Research in Artificial Intelligence and Neuroscience. 2020; 11(2): 157-164.
10. Galbraith N, Boyda D, McFeeters D, Galbraith V. Patterns of occupational stress in police contact and dispatch personnel: Implications for physical and psychological health. Int Arch Occup Environ Health. 2021; 94(2): 231-241. doi:10.1007/s00420-020-01562-1
11. Prontenko K., Bondarenko V., Bezpaliy S. et al. Physical training as the basis of professional activities of patrol policemen. Balt J Health Phys Activ. 2020; 12(1): 41-53. doi: 10.29359/BJHPA.12.1.05.
12. 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.
13. Schilling R., Herrmann C., Ludyga S. et al. Does cardiorespiratory fitness buffer stress reactivity and stress recovery in police officers? A real-life study. Front Psychiatry. 2020; 11: 594. doi:10.3389/fpsyt.2020.00594.
14. Tyagi A., Dhar R.L. Factors affecting health of the police officials: Mediating role of job stress. Policing: An Int J of Police Strategies and Management. 2014; 37(3): 649-664.
15. 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.

16. Štefan L., Kasović M., Culej M. Normative values for health-related physical fitness in first-year police officers. J Strength Cond Res. 2020. doi:10.1519/JSC.0000000000003853.
17. Kyslenko D., Prontenko K., Bondarenko V. et al. Development of the physical qualities of future specialists in protective activities due to the use of the kettlebell sport during studies. J Phys Educ Sport. 2017; 17(2): 789-794. doi: 10.7752/jpes.2017.02120.
18. Plisko V., Doroshenko T., Minenok A. et al. Informational indicators of functional capacities of the body for teaching cadets from higher military educational institutions power types of sports. J Phys Educ Sport. 2018; 18 (2): 1050-1054. doi: 10.7752/jpes.2018.s2156.
19. Sanna A., Garbarino S. Fitness for working in a policeman being treated for OSAS. Med Lav. 2019; 110(5): 398-402. doi: 10.23749/mdl.v110i5.8778.
20. 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.
21. Okhrimenko I., Lyakhova N., Horoshko V. et al. Means of psychophysiological indicators improvement of future law enforcement officers in the process of their speciality training. Wiad. Lek. 2022; 75 (4): 871-876. doi: 10.36740/WLek202204122.
22. Marins E.F., David G.B., Del Vecchio F.B. Characterization of the physical fitness of police officers: A systematic review. J Strength Cond Res. 2019; 33(10): 2860-2874. doi:10.1519/JSC.0000000000003177.
23. Lockie R.G., Orr R.M., Dawes J.J. Slowing the path of time: Age-related and normative fitness testing data for police officers from a health and wellness program. J Strength Cond Res. 2022; 36(3): 747-756. doi:10.1519/JSC.0000000000004197.
24. Santa-Rosa F.A., Shimojo G.L., Dias D.S. et al. Impact of an active lifestyle on heart rate variability and oxidative stress markers in offspring of hypertensives. Sci Rep. 2020; 10(1): 12439. doi:10.1038/s41598-020-69104-w.
25. 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.
26. Okhrimenko I., Pavlyk O., Tomenko O. et al. Dynamics of indicators of cadets' physical development and functional status during pentathlon. Int J Hum Mov and Sports Sci. 2021; 9(4): 814-823. doi: 10.13189/saj.2021.090428.
27. Kleygrewe L., Oudejans R.R.D., Koedijk M., Hutter R.I.V. Police training in practice: Organization and delivery according to European law enforcement agencies. Front Psychol. 2022; 12: 798067. doi:10.3389/fpsyg.2021.798067.

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

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Received: 25.08.2022
Accepted: 20.12.2022

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

STRUCTURAL PECULIARITIES OF RATS' TESTES DEVELOPMENT AFTER INTRODUCTION OF FEMALE HORMONES DURING PREGNANCY

DOI: 10.36740/WLek202302107

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ABSTRACT

The aim: To define regularities of testicular construction of the rats' offspring at 1-90 days of postnatal life after the introduction of female sex hormones to pregnant rats during the second and third periods of pregnancy

Materials and methods: The study was conducted on the testes of white laboratory rats' offspring during three months of life. Pregnant rats were exposed to intravaginal injection of Utrozhestan during the second and third periods of pregnancy. histological methods were used. Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J).

Results: Administration of female sex hormones to pregnant female rats leads to a reducing of the relative area, occupied by the convoluted seminiferous tubules with lumen, and increasing in relative area, occupied by extracellular matrix, starting from the 30th and up to the 90th observation day in the offsprings' testes. During the third month after birth, in experimental group a decreasing of the testicles' spermatids differentiation degree is determined

Conclusions: During the study, the following results and conclusions were obtained: decreasing of the relative area, occupied by convoluted seminiferous tubules, increasing in relative area, occupied by extracellular matrix, also decreasing Leydig cells relative amount and a delaying of spermatid differentiation process after exposing to female sex hormones during pregnancy, especially during third period, can lead to disruption of spermatogenesis and spermiogenesis in the future.

KEY WORDS: testes, female sex hormones, Utrozhestan, Leydig cells, spermatids

Wiad Lek. 2023;76(2):292-296

INRTODUCTION

Nowadays the number of infertile couples in Ukraine increases [1] and according to statistics, this index is about 19%, while the particle of male infertility reaches 50% [2]. This fact is confirmed by WHO documents which underline that the reason of infertility is a man in half number of the cases [3]. In modern conditions, the male reproductive system is strongly influenced by various harmful factors associated with urbanization, environmental pollution, the use of chemical synthesis products [4]. In addition, one of the male infertility reasons lays in extreme sensitivity and susceptibility of the male reproductive system to the effects of various exogenous and endogenous factors that affect it during the fetal period. The negative effect of progesterone application during the prenatal period on the process of spermatogenesis has been experimentally proven [5]. This situation leads to the fact that newborn males who were breastfed were exposed to these hormones, which in turn led to disorders in puberty [6].

Nowadays, evidence the issue of morphogenesis and reactivity peculiarities of the male reproductive glands after changing hormonal balance in the mother – placenta – fetus system remains controversial [7] and look for further clarification and further study both in clinical practice and in experimental conditions.

THE AIM

The aim was to define peculiarities of testicular structure of the rats' offspring in postnatal life after the intravaginal introduction of female sex hormones during the second and the third periods of pregnancy.

MATERIALS AND METHODS

The object of the study is testes of 152 white laboratory rats. In the experiment, four groups of animals were analyzed: the first one includes intact 38 rats, the second one – control (38 rats) offspring animals, which were

exposed to intravaginal introduction of saline solution during the second and the third pregnancy periods; the third group is formed by offspring of rats (38 animals), which were exposed to intravaginal introduction of Utrozhestan during the second pregnancy period (from 8th to 14th day), and the fourth one *38 animals) – offspring of rats, which were exposed to intravaginal introduction of Utrozhestan during the third pregnancy period (from 15th to 21st days). The choice of Utrozhestan in experiment is explained by the simplicity of its use and the prevalence of its use in modern clinical practice. An experimental model of the effect of female sex hormones on the development of the fetus testes was created for the intravaginal injection of utrozestan and saline solution. Pregnant females were introducted with the natural medication of progesterone – «Utrozhestan» with a special injector for intravaginal administration throughout the second and the third weeks of pregnancy. A dose of Utrozhestan was calculated depending on the average weight of the mature female rat and drawn up 100 mg. Newborns were obtained from rats with a dated pregnancy established by vaginal smears stained with methylene blue; the presence of sperm in smears was the evidence of the 0 day of pregnancy. Rats were born full term at days 21-22 after conception. The testes were examined at the 1st, 5th, 14th, 30th, 45th, 60th and 90th days of postnatal life. Supporting and withdrawal of animals from experiment was carried out in accordance with the requirements of the European Commission Directive (86/609/EEC), Law of Ukraine № 1759-VI (15.12.2009) On the Protection of Animals from Cruelty.

For histological analysis and morphometric examination, sections were stained with hematoxylin and eosin. In histological samples of testes (×280), a unit area occupied by the convoluted tubules with and without lumen, the extracellular matrix of the interstitium were studied. The size of the external diameter of the convoluted seminiferous tubules was studied. Under a microscope Granum L 60 with oil immersion technique (×700) the relative cells amount at the testicle interstitium were counted: Leydig cells, fibroblasts, fibrocytes. The degree indicator of spermatids differentiation (DISD) – the ratio of the average number of spermatids per unit area of the section of the convoluted seminiferous tubules at VIa and IXa stages of spermatogenesis by classification Leblond C.P. was determined [8]. Five tubules in stages VIa and IXa of spermatogenesis were examined in cross sections of the testes. The external diameter of the tubule (D) and the diameter of the lumen (d) were measured. Then, in the unit area (1000μm), which includes the entire thickness of the spermatogenic cells, the number of spermatids (P) was counted. For each tubule under study, this coution was performed in four fields of view. The calculation of degree indicator of spermatids differentiation (DISD) was made according to the patent (№ 35451A UA, 2001).

Analysis of the obtained results was conducted by means of statistical methods with the use of computer license program «Statistica for Windows 13» (StatSoft Inc., № JPZ804I382130ARCN10-J). The statistical significance of the obtained differences of indicators in the comparison groups was evaluated using the Mann-Whitney U test and considered to be significant at $p<0,05$, that is generally accepted for biological and medical researches. The numerical data of the obtained results are presented as M ± m (arithmetic mean ± standard error of the mean) [9].

RESULTS

The lumen in the convoluted seminiferous tubules appeared at the 5th day of life, and its diameter tends to increase, reaching maximum values for puberty with a simultaneous decrease in the relative area occupied by the convoluted tubules without lumen, the diameter of the convoluted seminiferous tubules with lumen of pubescent rats is much larger [10].

During the period from the 1st to the 14th day of life, the same growth rate of the convoluted seminiferous tubules diameter of the testes in animals of all observed groups was found. However, after the introduction of female sex hormones in the fetal period, in offspring's testes a probable lag of growth rate of convoluted seminiferous tubules was revealed, especially after the 30th day of life. There is a likely decrease in the relative area of the convoluted seminiferous tubules with lumen in experimental rats, compared to control (56,01 ± 0,81 % and 55,23 ± 0,29 % in experimental groups instead of 61,89 ± 1,04 % in a control) and an increase in the relative area occupied by the fibers and the extracellular matrix of the interstitium (15,81 ± 0,41 % and 16,02 ± 0,45 % in experiment and 14,32 ± 0,28 % in control), from the 30th day of life up to the end of the observation period.

Testicular interstituim of intact and control rats was showed an increasing in the relative number of Leydig cells (especially at the 45th – 33,40 ± 0,75% and the 60th day – 30,70 ± 3,08%). The relative fibroblasts amount decreases with increasing observation period. The relative fibrocytes index gradually increases with increasing life expectancy, reaching a maximum by the 30th day (32,02 ± 4,60%). The results obtained are partially consistent with those of other studies [11, 3].

The relative number of Leydig cells after injection of Utrozhestan to females during pregnancy initially

Table I. The Dynamics of Spermatids Differentiation Degree of the Rats' offspring Testes at the Second and Third Months of Life (M ± m)

Group of Observation	Age of Animals (Day of Life)	
	60th	90th
Intact	0,92±0,08	0,98±0,02
Control	0,94±0,03	0,97±0,03
Experimental	0,89±0,03*	0,91±0,01*
Experimental	0,86±0,02*	0,89±0,02*

Notes:

1. Groups of animals: 3 experimental – offspring of rats, which were exposed to intravaginal injection of Utrozhestan during the second pregnancy period (from 8th to 14th day), 4 experimental – offspring of rats, which were exposed to intravaginal injection of Utrozhestan during the third pregnancy period (from 15th to 21st days);
2. the symbol * means that the result is statistically significant comparison with control group, $p \leq 0,05$.

outweighs their number in intact and control animals and begins to decrease significantly from the 30th day (18,80 ± 2,30% and 20,15 ± 3,08% in experimental animals instead of 30,70 ± 3,08% in the control group at the 60th day; 16,41 ± 2,30% and 18,17 ± 3,08% to 28,03 ± 3,08% appropriately at the 90th day). This is confirmed by previously obtained data on the reduction of the testicles absolute mass and facts of the relationship between low testicular mass and decreased glandular epithelium cells index [7].

The results also show a significant lag in the relative fibroblasts number in the initial lifespan and an increase in this indicator from the 45th to the 90th day of life. The relative fibrocytes number in experimental animals, unlike controls, increases with life expectancy (35,16 ± 2,30% and 37,34 ± 3,08% in the experimental groups versus 30,91 ± 3,05% in the control group at the 14 day; 39,53 ± 2,30% and 37,63 ± 3.80% in the experimental groups versus 29,14 ± 2,31% in the control group at the 60th day).

To calculate the spermatids differentiation degree were selected rats' testes samples from the sixtieth up to the ninetieth days after birth, that is, mature males. During this period of life, it is possible to determine all stages of spermatogenesis in the rats' testicular convoluted seminiferous tubules. Mature germ cells develop from spermatids. This period of their development is called spermiohistogenesis. Degree indicator of spermatids differentiation (DISD) in rats of intact and control groups at the 60th day of postnatal life was equal to 0,92 ± 0,08 and 0,94 ± 0,03, and at the 90th – 0,98 ± 0,02 and 0,97 ± 0,03. DISD is very sensitive to the effects of various factors, in particular, according to these researchers, to the effects of toxic agents (epoxy compounds). During exploration was also found a decreasing of DISD in offspring after the Utrozhestan introduction in the fetal period. The results obtained indicate a delay in the process of differentiation of spermatids after exposing to female sex hormones during pregnancy, especially during third period. At the 60th day in the third experimental group DISD was 0,89 ± 0,03, in the fourth – 0,86 ± 0,02, at the 90th day – 0,91 ± 0,01 and 0,89 ± 0,02 accordingly (Table I).

DISCUSSION

Consequently, the results indicate a delay in the offspring's spermatids differentiation process after exposing to female sex hormones during pregnancy, especially during its third period, and impaired spermato- and spermiogenesis in the future which coincides with the data of other researchers [12, 13].

Thus, during the VIa – IXa stages, the most important cytodifferentiation stages consistently take place, they include the essential and irreversible transformation of such cell organelles as the cell center and the Golgi complex, whose derivatives (tail compartment, acrosome) subsequently provide the two most important process functions – sperm motility and fertilization process. In addition, at stage IXa, the intercellular interactions between Sertoli cells and sperm cells are significantly altered – the previous generation of mature sperm loses its connection with Sertoli cells and moves into the lumen of the testicular tubule [14]. This establishes a closer morphofunctional relationship between the supporting cells and the new generation of differentiating spermatids. The previously obtained results are confirmed by determining the degree of differentiation of spermatids of adult rats, which is probably lower in animals of experimental groups compared with intact and control rats and indicates a delay in spermatogenesis in animals of these groups.

This is confirmed by the obtained data on the decrease of the total sperm count, viable sperm, motile sperm, also sperm membrane was severely altered. After the exposure to progesterone during prenatal period, in male mice the levels of serum sexual hormones was changed: especially, the level of serum testosterone

was decreased against of an increase the level of FSH and LH. This experiment showed that prenatal introduction of progesterone caused significant reduction in the number of spermatozoa and increase in the lumen of seminiferous tubule. There is an opinion that the impairment of male reproduction in mice exposed to progesterone during embryonic development could be mediated through the inhibition of testosterone production [15]. Lue Y. and Wang C. described the effect of synthetic progestins such as levonorgestrel (LNG) in combination with testosterone (T) in male contraceptive clinical trials. The aim was to study the effects of this combination progesterone with testosteron on spermatogenesis in adult rodents. Therefore, according to the results of research, combination LNG + T induced germ cell apoptosis of seminiferous epithelial against the background of testicular hormonal deprivation [16].

That is, assessing the status of sperm cells in stages VIa - IXa under conditions of exposure to female sex hormones (Utrozhestan), possible to evaluate the cellular and intercellular mechanisms of impaired spermatogenesis, in particular the process of spermatids differentiation, especially considering the increased sensitivity of the final stages of spermatogenesis to the effects of exogenous and endogenous factors.

The established effect of Utrozhestan in the second and third periods of pregnancy on the reproductive organs of males after birth is a specific effect characteristic of hormonal drugs. It should be specified in more detail in the instructions for use of the drug and taken into account when prescribing it to pregnant women who are carrying a male fetus.

CONCLUSIONS

1. In the offspring of rats after the introduction of female sex hormones in the second and third periods of pregnancy, compared with the control there is a decrease in the relative area of convoluted seminiferous tubules with lumen (56,01 ± 0,81% and 55,23 ± 0,29 % in the experimental groups versus 61,89 ± 1,04% in the control group, $p \leq 0,05$) and an increase in the area occupied by the extracellular matrix of the interstitium (15,81 ± 0,41% and 16,02 ± 0,45 % in the experiment and 14,32 ± 0,28% in the control, $p \leq 0,05$). The found changes are more pronounced for the offspring of animals, which were exposed to intravaginal injection of Utrozestan during the third pregnancy period.
2. In the testicle interstitium of experimental animals, an increase in the relative number of Leydig cells by the 14th day and a significant decrease in their number from the 30th day, especially pronounced at the 60th and 90th day of the life postnatal period were found (18,80 ± 2,30% and 20,15 ± 3,08% in the experimental groups versus 30,70 ± 3,08% in the control group at 60th day; 16,41 ± 2,30% and 18,17 ± 3,08% versus 28,03 ± 3,08%, additionally, at the 90th day, $p \leq 0,05$).
3. The index of spermatids differentiation of adult rats obtained after intravaginal injection of female sex hormones to pregnant females is significantly lower compared to control and intact rats (at the 60th day of life – 0,89 ± 0,01 and 0,86 ± 0,01 in the experiment and 0,94 ± 0,02 in control; at 90th day – 0,91 ± 0,01 and 0,89 ± 0,01 and 0,97 ± 0,02, additionally).

Prospects for further research are to investigate the effect of female sex hormones on the spermiogenesis of mature man offspring of rates.

REFERENCES

1. Tymchenko O.I., Mykytenko D.O., Koba O.P., Lynchak O.V. The level of infertility in the regions according to the MHU. Medical perspectives. 2014;19(3): 105–111.
2. Szkodziak P., Wozniak S., Czuczwar P. Infertility in the light of new scientific reports – focus on male factor. Ann. Agric. Environ. Med. 2016; 23(2): 227–230. doi: 10.5604 / 12321966.1203.
3. Esteves S.C. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination Int. Braz. J. Urol. 2014; 40(4): 443–453. doi: 10.1590 / S1677-5538.IBJU.2014.04.02.
4. Lomeiko O.O., Kolesnyk Yu.M., Avramenko N.V. Male infertility in a large industrial city: the role of chemical, physical and emotional factors. Pathologia. 2018; 15 (1): 114–121. doi: 10.14739/2310-1237. 2018.1.129334.
5. Knez J. Endocrine-disrupting chemicals and male reproductive health. Reprod Biomed. 2013. 26: 440-448. doi: 10.1016/j. rbmo.2013.02.005.
6. Storgaard L., Bonde J.P., Olsen J. Male reproductive disorders in humans and prenatal indicators of estrogen exposure. A review of published epidemiological studies. Reprod. Toxicol. 2006; 21: 4-15. doi: 10.1016 / j.reprotox.2005.05.006.
7. Nold C., Maubert M., Anton L. et al. Prevention of preterm birth by progestational agents: what are the molecular mechanisms? Am J Obstet Gynecol. 2013; 208(3): 223.1–7. doi: 10.1016/j.ajog.2013.01.020.
8. Leblond C.P. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Aun. N. J. Acad. Sci. 1953; 55: 548–573. doi: 10.1111 / j.1749-6632.1952.tb26576.x.
9. Lang T.A., Sesik M. How to describe statistics in medicine. Annotated guide for authors, editors and reviewers. M.: Practical medicine. 2016, 480p.

10. Spas`ka A.M. Histostructure and blood supply of the testes in normal. Vistnyk morfologii. 2011; 179(1): 73—75.
11. Ahmed S.I., Elsheikh A.S., Attia G.A., Ali T.O. Prenatal progesterone exposure of male rats induces morphometric and histological changes in testes. Asian Pacific J Reprod. 2016; 5: 204-209. doi: 10.1016 / j.apjr.2016.04.015.
12. Ahmed S.I., Mohamed A.M., Al-Aed S.Z. Gestational exposure to synthetic steroid hormones decreases sperm count and quality in Wistar rats. Int J Endocrinol. 2020; 1: 1-6. doi: 10.1155 / 2020/1814867.
13. Ahmed S.I., Ali T.O., Elsheikh A.S. Ultrastructure of the testes of rats born from females treated with hydroxyprogesterone hexanoate. Asia Pacific Journal of Reproduction. 2016; 3: 510-513. doi: 10.1016 / j.apjr.2016.10.010.
14. Nakata X. Morphology of seminiferous tubules of mice. Anat. Sci. Int. 2019; 94 (1): 1-10. doi: 10.1007 / s12565-018-0455-9.
15. Harini C., Sainath S.B., Reddy P.S. Recovery of suppressed male reproduction in mice exposed to progesterone during embryonic development by testosterone.Reproduction. 2009; 137(3): 439-448.
16. Lue Y., Wang C., Lydon J.P. et al. Functional role of progestin and the progesterone receptor in the suppression of spermatogenesis in rodents. Andrology. 2013;1(2): 308-17. doi: 10.1111/j.2047-2927.2012.00047.x.

This work is a part of a project «Reactivity of newborn organs after influence of antigens and factors of different nature in the prenatal period» (2013-2019, state registration 0115U003875) funded by Zaporizhia State Medical University.

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

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Received: 18.04.2022
Accepted: 20.12.2022

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

OCCURRENCE OF AMINOGLYCOSIDES RESISTANCE GENES ACC(6)-IB AND ACC(3)-II AMONG GRAM-NEGATIVE ISOLATES CAUSING URINARY TRACT INFECTION IN PEDIATRIC PATIENTS, NAJAF, IRAQ

DOI: 10.36740/WLek202302108

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ABSTRACT

The aim: The aim of the study was to detect the antimicrobial susceptibility patterns and frequency of aminoglycosides resistance genes of Gram-negative bacteria isolated from pediatric patient with UTI.

Materials and methods: The study has been performed with a total of 500 urine specimens collected from pediatric patients under the age of 18 year suspected with UTI, admitted to hospitals in Al-Najaf province/Iraq during the period from November 2018 to March 2019.

Results: A total of 500 urine specimens had been tested, 120 (24%) had significant bacteriuria, while there 380 (76%) had non-significant bacteriuria. Escherichia coli represent about 70 (68.2%) followed by followed by 23 (22.5%) K. pneumoniae, 5 (4.9%) P. aeruginosa, 2 (1.9%) Proteus spp., 1 (0.9%) Enterobacter spp. and 1 (0.9%) Oligella uratolytic. The antimicrobial susceptibility profile of 102 Gram-negative isolates, revealed that 59 (58%) were multidrug resistant (MDR) and 38(37%) were extensive drug resistant (XDR). The PCR results of aminoglycosides resistance showing that 23 (74.1%) Gram-negative isolates had acc(6′)-Ib gene and 12 (38.7%) Gram-negative isolates acc(3′)-II gene.

Conclusions: A high frequency of multi-drug resistance and extensive-drug resistance of isolates were recognized, and an alarming percentage of aminoglycosides resistance to acc(6′)-Ib and acc(3′)-II.

KEY WORDS: aminoglycosides resistance genes, gram-negative, urinary tract infection, pediatric patients

Wiad Lek. 2023;76(2):297-304

INTRODUCTION

Urinary tract infection (UTI) is a public health problem during childhood and is one of the common sources of infections that can cause systemic illness in children and infants [1]. It can cause significant morbidity and considerable mortality, if UTIs are not diagnosed and treated early; it may lead to occurs many complications like end-stage renal failure, renal scarring, and hypertension [2, 3]. Urinary tract infection (UTI) is widely spread and it occurs in all ages from the neonates to the elderly age group [4]. UTI is the third most common infection after gastrointestinal and respiratory infections, which affects about 150 to 200 million people each year worldwide [5]. The prevalence rate of UTI among children is about 8% of girls and 2% of boys [6]. Pediatric urological communities are now confronting an increase in the antibiotics resistance of urinary pathogens, due to excessive use and abuse of antibiotics [7]. The increase of antibiotics resistance can lead to the occurrence of multidrug resistance (MDR) and treatment failure of infection everywhere in the world that considered a series of world problems [5]. UTI caused by bacteria resistance may lead to occur many problems like complications of UTI and inappropriate to treat the infection [8]. The rates of antibiotics resistance can be different according to the country and geographic area [9]. A piece of the updated information in a specific location may aid clinicians in choosing the appropriate antibiotic for empirical treatment [10].

Aminoglycosides had commonly used among children, infants, and neonates because of their activity against a wide range of gram-negative species. Aminoglycosides could be used as an alternative to carbapenem in UTI caused by ESBLs producing isolates [11]. The rate of UTI caused by ESBL producing gram-negative [11, 12], isolates required carbapenem antibiotics has been increasing in children [13].

THE AIM

The aim of the study was to detect the antimicrobial susceptibility patterns and frequency of aminoglycosides resistance genes of Gram-negative bacteria isolated from pediatric patient with UTI.

MATERIALS AND METHODS

A cross-sectional study was conducted during the period from November 2018 to March 2019 in main hospitals (Al-Zahra Teaching and Al-Sadr hospital) in Al-Najaf province, Iraq. A total of 500 urine specimens were collected from children (less than 18 years) that suspected symptomatic UTI, that attending to these hospitals, and exclusion criteria in the current study includes: pediatric patients have a chronic disease, taken antibiotics before culture, and appear mixed growth in culture.

BACTERIAL ISOLATES AND IDENTIFICATIONS

All urine specimens were collected in a sterile container by midstream, urine bag, and catheter methods had based on age and physical status of patients. Then, each urine specimen has labeled and transported directly to a microbiology laboratory for processing and divided into two portions. One portion was for the microscopically examination, for the presence of pus cells, casts, red blood cells, and others. The second portion was streaked on the blood (Himedia, India), MacConkey (Himedia, India) and Chromogenic media (Orientation, CHROMagar, France) by using a calibrated wire loop (0.001 ml). Then, after incubation at 37°C for 24 hours, if the growth of colonies more than 105 CFU/ml it was considered as significant growth (bacteriuria), and these positive culture were further identified by the morphological characteristic pattern of routine standard biochemical reaction [14] and confirmed by VITEK2 compact system (BioMerieux, France).

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibiotic susceptibility was achieved by the used test disk diffusion according to CLSI [15]. E. coli ATCC 25922 was used as the reference strain for quality control of the antibiotics tested. The following antibiotics disks were used in the study includes: ampicillin (10 µg), amoxicillin (10 µg), piperacillin (100 µg), augmentin (30 µg), cefixime (5 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepem (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), cephalexin (30 µg), cephamandol (30 µg), trimethoprime sulphamethazole (5 µg), and nitrofurantoin (300 µg), and identification of multidrug resistant (MDR) and extensively drug resistant (XDR) of the gram-negative isolates were according to the guide line of CDC and ECDC [16].

MOLECULAR CHARACTERIZATION OF AMINOGLYCOSIDES RESISTANCE GENES

DNA has extracted from all resistance isolates, according to the protocol kit of the manufactured company (Favorgen, Taiwan), by using NanoDrop spectrophotometer (Eppendorf) to determine quality and quantity of DNA. The presence of acc(6')-Ib and acc(3)-II aminoglycosides resistance genes in gram-negative isolates were surveyed by the amplification of gene specific primers (Table I). The PCR were prepared in total volume 25 µl, including 12.5 µl of master mix (Promega, USA), 25 pmol of each primer, 5 µl DNA template and then the volume was completed 25 µl with nuclease free water. Then, all PCR products were analyzed by gel electrophoresis with 1.5% agarose, and stained with red safe dye. Finally, the electrophoresis results had been identified by using gel documentation system (Biometra, Germany).

STATISTICAL ANALYSIS

In the present study, statistical analysis was performed by using a chi-square test calculator to determine the p-value for the data and using the SPSS program (version 21, IBM, USA).

RESULTS

DEMOGRAPHICS OF PATIENTS

The results exhibited out of 500 urine specimens were cultured, 120 (24%) specimens were positive for the presence of significant bacteriuria (shown ≥ 105 cu/ml), while 380 (76%) had non-significant bacteriuria. Out of these 120 bacterial isolates, there 102 (85%) of the isolates were gram-negative and 18 (15%) of isolates were gram-positive. The results showed from that of 120 (24%) significant bacteriuria, there 92 (76.7%) urine specimens belonged to girls and 28 (23.3%) urine specimens were belong to boys, and a significant bacteriuria in relation to gender was found to be statistically significant (P value = 0.01). The age of children patients in this study was ranged from 1month to 18 years and the mean aged of children

Table I. Specific primers and PCR conditions used in the present study.

Gene name	Primer name	Sequences (5 to 3)	PCR Condition	Product size (bp)
acc(3)-II	acc(3)-I I-F	ATATCGCGATGCATACGCGG	(95/1 min, 55/1 min, 72/5 min) 30 cycles	877
	acc(3)-I I-R	GACGGCCTCTAACCGGAAGG		
acc(6)-Ib	acc(6)-Ib-cr-F	TTGCGATGCTCTATGAGTGGCTA	(94/45 sec, 53/45 sec, 72/45 sec) 34 cycles	482
	acc(6)-Ib-cr-R	CTCGAATGCCTGGCGTGTTT		

Table II. Socio-demographic indicators among pediatric patients with UTI.

	Category	Total (n=500)	Positive significant bacteriuria (n= 120)	Non growth (n= 380)	P-value
The gender	Male	163 (32.5%)	28 (23.3%)	135 (38%)	0.01
	Female	337 (67.5%)	92 (76.7%)	245 (64.5%)	
Age groups	1 month -1 year	73 (14.6%)	11 (9.1%)	62 (22.6%)	0.06
	2 -5 years	122 (24.4%)	24 (20%)	98 (25.7%)	
	6-11 years	130 (26%)	35 (29.1%)	98 (25.7%)	
	12-18 years	175 (35%)	50 (41.6%)	122 (32%)	
Hosptiliazationes	Outpatient	370 (74%)	100 (83.3%)	270 (71%)	0.007
	Inpatient	130 (26%)	20 (16.6%)	110 (29%)	
Pyuria (5 WBC/HPF)	>5 WBC/HPF	420 (84%)	96 (80%)	324 (85.2%)	0.17
	<5 WBC/HPF	80 (16%)	24 (20%)	56 (14.7%)	

Table III. Antibiotic susceptibility test for Gram-negative bacteria isolated from patients with significant bacteriuria (n=102).

Antibiotic disk	No.(%) of isolates exhibited		
	Resistant	Intermediate	Susceptible
Ampicillin	102 (100%)	0 (0%)	0 (0%)
Amoxicillin	94 (92%)	3 (3%)	5 (5%)
Piperacillin	94 (92%)	3 (3%)	5 (5%)
Augmentin	89 (87%)	1 (1%)	12 (12.5%)
Cefotaxime	102 (100%)	0 (0%)	0 (0%)
Ceftriaxone	83 (81%)	3 (3%)	16 (16%)
Ceftazidime	63 (61.5%)	2 (2%)	37 (36%)
Cefepem	72 (70.5%)	23 (22.5%)	5 (5%)
Cefixime	79 (77.5%)	3 (3%)	20 (19.5%)
Cephalexin	102 (100%)	0 (0%)	0 (0%)
Cephamandol	96 (94%)	2 (2%)	4 (4%)
Imipenem	2 (2%)	4 (4%)	96 (94%)
Meropenem	2 (2%)	2 (2%)	98 (96%)
Ciprofloxacin	42 (43 %)	4 (4%)	56 (55%)
Amikacin	11 (10.5%)	19 (18.5%)	72 (70.5%)
Gentamicin	30 (29.5%)	1 (1%)	71 (69.5%)
Nitrofurantoin	24 (23.5%)	9 (9%)	69 (67.5%)
Trimethoprim Sulfamethoxazole	80 (78.5%)	1 (1%)	21 (20.5%)

was 9.67 years, and they were categorized according to WHO into four groups: 1 month - 1 year (infant), 2-5 years (young child), 6-11 years (child) and 12-18 years old (adolescents). The results showed that the most significant bacteriuria was found in the age group 12-18 year (41.6%), followed by in the age group 6-11 year (29.1%), 2-5 year (20%) and less significant bacteriuria was found in age group 1 month - 1year (9.1%) and the result showed that there was a non-significant association between age groups of children and significant bacteriuria (p-value 0.06). Isolates were obtained at a relatively high percentage 100 (83.3%)

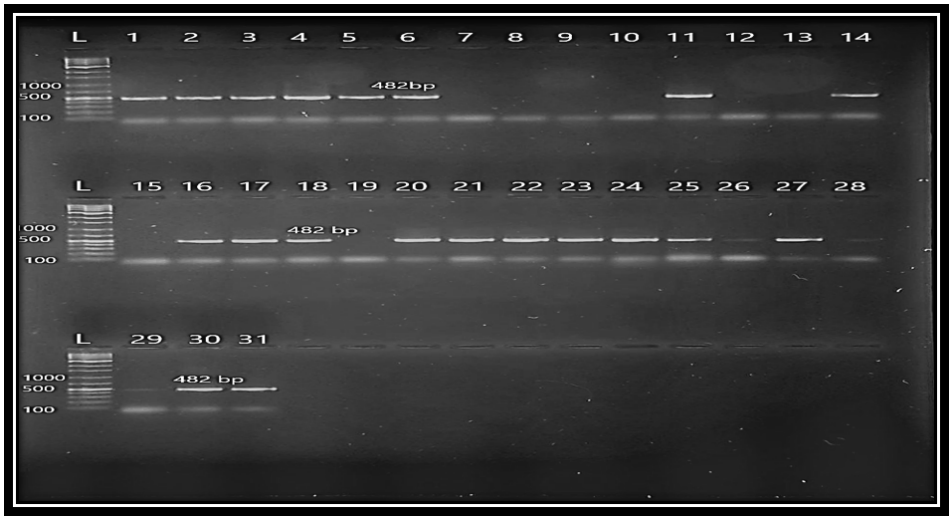

Fig. 1. In a Monoplex PCR agarose gel, amplified products from extracted DNA of Gram-negative isolates and amplified with primers and the primer genes acc(6)-Ib were amplified. The electrophoresis was performed at 70 volt for 1.30 hr with using DNA ladder size (plus 1000), lanes (1, 2, 3, 4, 5, 6,) show positive results at 482 bp.

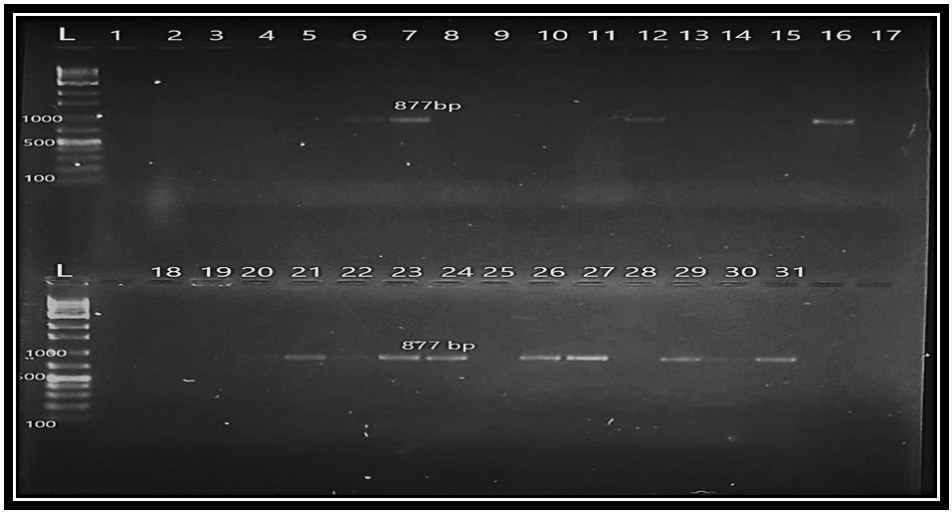

Fig. 2. In a Monoplex PCR agarose gel, amplified products from extracted DNA of Gram-negative isolates and amplified with primers and the primer genes acc(3)-II were amplified. The electrophoresis was performed at 70 volt for 1.30 hr with using DNA ladder size (plus 1000), lanes (7, 12, 16) show positive results at 877 bp.

Table IV. Distribution of aminoglycoside genes among Gram-negative isolates.

Gram-negative isolates resistance to amikacin and gentamicin	Aminoglycosides genes	
	acc(6)-Ib N.(%)	acc(3)-II N.(%)
E. coli (n= 22)	16 (72.7%)	9 (40.9%)
K. pneumonia (n=6)	4 (66.6%)	2 (33.3%)
Proteus spp (n=2)	2 (100%)	0 (0%)
O. uratolytic (n=1)	1 (100%)	0 (0%)
Total of isolates (n=31)	23 (74.1%)	12 (38.7%)

outpatients, while only there 20 (16.6%) inpatients and showed there was a significant association in the hospital status of the children patient with UTI (p-value=0.007) (Table II). Furthermore, 420 (84%) of urine specimens showed pyuria (>five WBC/HPF), and among these, 96 (80%) give culture positive, while 80 (16%) total urine specimens showed no pyuria (<five WBC/HPF), but among these, 24 (20%) give positive culture result and this deference was non-significant (p-value=0.17) and these sociodemographic of pediatric patients (Table II).

IDENTIFICATION OF UROPATHOGEN ISOLATES

Among 102 of gram-negative isolates from urine specimens with significant bacteriuria, 70 (68.2%) isolates were recognized as *E. coli* followed by 23 (22.5%) *K. pneumoniae*, 5 (4.9%) *P. aeruginosa*, 2 (1.9%) *Proteus spp*., 1 (0.9%) *Enterobacter spp*. and 1 (0.9%) *Oligella uratolytic*.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility test was being conducted on 102 gram-negative isolates and used about 18

disks of antibiotics. According to CLSI [15], the results showed there was a high resistance for penicillin groups and most generation of the cephalosporin, while there a moderate resistance rate for fluoroquinolones antibiotic (ciprofloxacin) and low resistance for aminoglycosides antibiotics (amikacin and gentamicin). The results showed that most of the isolates were susceptible to carbapenem antibiotics and are most effective against bacterial isolates (Table III). According to the resistance profile, 59 (58%) were MDR isolates resistant to at least one antimicrobial agent for three different group, 38 (37%) were XDR, the isolates were resistant to at least one agent in all but two or fewer antimicrobial categories but two or fewer, and 5 (5.1%) were non-MDR.

MOLECULAR DETECTION OF AMINOGLYCOSIDE RESISTANCE GENES

Out of 102 Gram-negative isolates, 31 of isolates were resistances to gentamicin and amikacin, all resistance isolates were screened for the presence of acc(6)-Ib and acc(3)-II by Monoplex PCR. The results revealed that 23/31 (74.15%) of isolates carried acc(6)-Ib genes (Fig 1) and 12/31 (38.7%) of isolates carried acc(3)-II genes (Fig 2). The results also revealed that acc(6)-Ib was found among 16/22 (72.7%) of *E. coli* isolates and 4/6 (66.6%) of *K. pneumonia* isolates, while acc(3)-II was found among 9/22 (40.9%) of *E. coli* isolates and 2/6 (33.3%) of *K. pneumoniae* isolates (Table IV).

DISCUSSION

Urinary tract infection (UTI) is a public health problem and is considered one of the infections that cause systemic diseases in children and infants. UTI affects every year about 150 to 200 million people and considered the third most common infection after gastrointestinal and respiratory infections [5]. The dissemination of UTI is evolving among countries due to environmental conditions, lifestyle, poor hygiene, and malnutrition [17]. The spectrum of causative agents causing UTI in hospitals and communities had been continuously changing over the years. Among the 102 Gram-negative isolates indicated in the current investigation, six different species of bacteria were found to be *E. coli* (68.2%) of the isolates, followed by *K. pneumoniae* (22.5%), *P. aeruginosa* (4.9%), *Proteus spp*. (1.9%), *Enterobacter spp*. (0.9%) and *Oligella uratolytic* (0.9%). In the current investigation, it was found that *E. coli* was the most common, followed by *K. pneumoniae, P. aeruginosa*. These results were in agreement with other studies that was done in Iran, Turkey and Pakistan, which reported that the frequency of *E. coli* was 71.6% and *K. pneumoniae* – 10%, *E. coli* – 64.2% and *K. pneumoniae* – 15%, *E. coli* – 65%, and *K.pneumonia* – 25% respectively [18, 19]. *Escherichia coli* was the most common among Gram-negative isolates causing UTI, and this may be due to having certain virulence factors, like fimbriae and hemolysin production, which help for their attachment at the uroepithelial surface that increase the risk for infection [20]. The results of the current study indicated that there was a high resistant rate of Gram-negative isolates to penicillin groups, including ampicillin 100%, amoxicillin 92% and piperacillin 92%, these findings corresponds with a previous study, conducted in Al-Najaf [21]. This resistance may be attributed due to the use of this antibiotic as first-line of treatment; hence, bacteria have developed resistance to this antibiotic [22]. This study indicated that Gram-negative isolates exhibited a degree of diversity of the levels of resistance for cephalosporin including cefotaxime 100%, cephalexin 100%, ceftriaxone 81%, cephamandol 94%, cefepime 70.5% and cefixime 77%, except ceftazidime, that showed moderate resistances reaching to 61.5%, and this results were similar with previous studies was done in Iran and Bangladesh [2, 23]. Resistance to the third-generations cephalosporins was mostly occurred by mutations in the general β-lactamases class A, a form of CTX-M, TEM, and SHV β-lactamases [24]. The results of the present study were demonstrated that imipenem and meropenem are the most effective antibiotics against bacterial isolates, the isolates were susceptible to these antibiotics, except two isolates were resistant and this result is similar to most studies, that done in many countries [2, 5]. This study showed a low resistance rate to aminoglycosides antibiotics, including amikacin 11% and gentamicin 30%, this finding was conformity to the study done in Iran [2] and also comparable with study by Ranjbar et al. in Iran [25]. Resistances of aminoglycosides come from the various mechanisms, and most of these are secretions of aminoglycoside-modifying enzymes (AMEs). These enzymes are divided into three classes: aminoglycosides acetyltransferase (AAC), aminoglycosides phosphotranase (APHs) and aminoglycosides (ANT) [25-27]. The genes encoding these enzymes were often located on mobile elements (plasmid, transposons, and integron) enabling the rapid spreading of the genes in various bacterial [28]. In the current investigation, 31 isolates were resistant to gentamicin and amikacin, then screened by PCR to determine the prevalence of acc(3')-II and acc(6')-Ib among Gram-negative isolates. The results showed that 74.1% were acc(6')-Ib gene and 38.7%

had acc(3)-II gene. The present result was consistent with the previous study, done in Iraq, indicated that the occurrence of acc(6')-Ib was 68% in clinical isolates of K. pneumoniae [29]. As well, a study conducted in Iran by Ghotaslou et al., [30] showed that the prevalence rate of acc(3')-II among Gram-negative isolates is about 23.6% and this result in accordance with our findings. The present result was disagreement with studies done in other countries, such as Saudi Arabia, and Korea reported that the prevalence rate of acc(6')-Ib among isolates of 43%, 22% and 22.3%, respectively [31, 32]. Hence, the current results are in contrast with a study, done by Ho. P. et al. [33], who found that the frequency of acc(6')-Ib was12.2% and acc(3)-II was 91% in E. coli isolated from blood culture. A Study in Egypt was done by Abo-State M. et al. [34] who found that the prevalence rate of acc(3')-II was 40% and acc(6)-Ib was 30%. Furthermore, the current result was low compared to other studies done in Iran, that revealed the occurrence of acc(3')-II were 72.2%, 58% and acc(6')-Ib were 80.7%,71% respectively in K. pneumoniae isolates [35, 36]. In Gram-negative bacteria, the main resistance to amikacin is often involved with acc(6')-Ib and resistance to gentamicin with AME genes: acc(3')-I, acc(3')-II and acc(3')-III [37]. The prevalence of respective genes has been growing over time in several geographical patterns, according to review studies and the prevalence of these resistance genes, which need regular attention and determination.

CONCLUSIONS

A high frequency of multi-drug resistance and extensive-drug resistance of isolates were recognized, and an alarming percentage of aminoglycosides resistance to acc(6')-Ib and acc(3')-II.

REFERENCES

1. Ma J.F., Shortliffe L.M.D. Urinary tract infection in children: etiology and epidemiology. Urol Clin North Am. 2004; 31(3): 517-26. doi: 10.1016/j.ucl.2004.04.016.
2. Pouladfar G., Basiratnia M., Anvarinejad M. et al. The antibiotic susceptibility patterns of Uropathogens among children with urinary tract infection in Shiraz. Medicine (Baltimore). 2017; 96(37): e7834. doi: 10.1097/MD.0000000000007834.
3. Montini G., Tullus K., Hewitt I. Febrile urinary tract infections in children. N Engl J Med. 2011; 365(3): 239-50. doi: 10.1056/NEJMra1007755.
4. Yasmeen B.H.N., Islam S., Islam S. et al. Prevalence of urinary tract infection, its causative agents and antibiotic sensitivity pattern: A study in Northern International Medical College Hospital, Dhaka. North Int Med Coll J. 2015; 7(1): 105-9. doi: 10.3329/nimcj.v7i1.25704.
5. Sharmin S., Alamgir F., Saleh A.A. Antimicrobial sensitivity pattern of Uropathogens in children. Bangladesh J Med Microbiol. 2009; 03(01): 18-22.
6. Lalhmangaihzuali F.E., Zarzoliana Z.V., Laldinmawii G. Antibiotic resistance pattern of Uropathogens in urinary tract infections in children at State Referral Hospital, Falkawn, Mizoram, India. Int J Contemp Pediatr. 2018; 5(6): 2108. doi: 10.18203/2349-3291.ijcp20184182.
7. Ayelign B., Abebe B., Shibeshi A. et al. Bacterial isolates and their antimicrobial susceptibility patterns among pediatric patients with urinary tract infections. Turk J Urol. 2018; 44(1): 62-69. doi: 10.5152/tud.2017.33678.
8. Lee Y.K., Lee H., Kim J.M. et al. The antibiotic resistance pattern of gram-negative bacteria in children younger than 24 months with a urinary tract infection: a retrospective single-center study over 15 consecutive years. Child Kidney Dis. 2015; 19(2): 148-53. doi:10.3339/jkspn.2019.23.1.22.
9. Mathai D., Jones R.N., Pfaller M.A. et al. Epidemiology and frequency of resistance among pathogens causing urinary tract infections in 1,510 hospitalized patients: a report from the SENTRY Antimicrobial Surveillance Program (North America). Diagn Microbiol Infect Dis. 2001; 40(3): 129-36. doi: 10.1016/s0732-8893(01)00254-1.
10. Gunduz S., Altun H.U. Antibiotic resistance patterns of urinary tract pathogens in Turkish children. Glob Health Res Policy. 2018; 3: 10. doi: 10.1186/s41256-018-0063-1.
11. Craig W.A. Optimizing aminoglycoside use. Crit Care Clin. 2011; 27(1): 107-21. doi: 10.1016/j.ccc.2010.11.006.
12. Han S.B., Lee S.C., Lee S.Y. et al. Aminoglycoside therapy for childhood urinary tract infection due to extended-spectrum β-lactamase-producing Escherichia coli or Klebsiella pneumoniae. BMC Infect Dis. 2015; 15: 414. doi: 10.1186/s12879-015-1153-z.
13. Fan N., Chen H., Chen Ch.O.L. et al. Rise of community-onset urinary tract infection caused by extended-spectrum b-lactamase-producing Escherichia coli in children. J Microbiol Immunol Infect. 2014; 47(5): 399-40. doi: 10.1016/j.jmii.2013.05.006.
14. Vandepitte J., Verhaegen J., Engbaek K. et al. Basic laboratory procedures in clinical bacteriology. World Health Organization. 2003.
15. Performance standards for antimicrobial susceptibility testing. 28th ed. Clinical and Laboratory Standards Institute. Wayne, PA. 2018 (CLSI supplement M100).
16. Magiorakos A.P., Srinivasan A., Carey R.B. et al. Multidrug-resistant, extensively drug resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012; 18(3): 268-81. doi: 10.1111/j.1469-0691.2011.03570.x.

17. Shrestha A., Manandhar S., Pokharel P. et al. Prevalence of extended spectrum beta-Lactamase (ESBL) producing multidrug resistance gram-negative isolates causing urinary tract infection. EC Microbiol. 2016; 4: 749-55. doi: 10.1186/s13756-015-0085-0.
18. Abrar S., Ain N.U., Liaqat H. et al. Distribution of bla CTX-M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrob Resist Infect Control. 2019; 8(1): 80.
19. Rezaee M.A., Abdinia B. Etiology and antimicrobial susceptibility pattern of pathogenic bacteria in children subjected to UTI: A referral hospital-based study in Northwest of Iran. Medicine (Baltimore). 2015; 94(39): e1606. doi: 10.1097/MD.0000000000001606.
20. Das R.N., Chandrashekhar T.S., Joshi H.S. et al. Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med J. 2006; 47(4): 281-5.
21. Al-jebouri M.M., Mdish S.A. Antibiotic Resistance Pattern of Bacteria Isolated from Patients of Urinary Tract Infections in Iraq. Open J Urol. 2013; 3(5): 124-31. doi: 10.4236/oju.2013.32024.
22. Kariuki S., Revathi G., Corkill J. et al. Escherichia coli from community-acquired urinary tract infections resistant to Fluoroquinolones and extended-spectrum beta-lactams. J Infect Dev Ctries. 2007; 1(03): 257-62.
23. Nazme N.I., Al Amin A., Jalil F. et al. Bacteriological profile of urinary tract infection in children of a tertiary care hospital. Bangladesh J Child Heal. 2017; 41(2): 77-83. doi: 10.3329/bjch.v41i2.36102.
24. Paterson D.L., Bonomo R.A. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005; 18(4): 657-86. doi: 10.1128/CMR.18.4.657-686.2005.
25. Duffa Y.M., Kitila K.T., Gebretsadik D.M., Bitew A. Prevalence and Antimicrobial Susceptibility of Bacterial Uropathogens Isolated from Pediatric Patients at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia. Int J Microbiol. 2018; 8492309. doi: 10.1155/2018/8492309.
26. Liang C., Xing B., Yang X. et al. Molecular epidemiology of aminoglycosides resistance on Klebsiella pneumonia in a hospital in China. Int J Clin Exp Med. 2015; 8(1): 1381-5.
27. Soleimani N., Aganj M., Ali L. et al. Frequency distribution of genes encoding aminoglycoside modifying enzymes in Uropathogenic E. coli isolated from Iranian hospital. BMC Res Notes. 2014; 7(1): 842.
28. Mir A.R., Bashir Y., Dar F.A., Sekhar M. Identification of genes coding aminoglycoside modifying enzymes in E. coli of UTI patients in India. Scientific World Journal. 2016; 1875865. doi: 10.1155/2016/1875865.
29. Mustafa M.S., Abdullah R.M. Antimicrobial susceptibility and Molecular detection of acc (6')-Ib and acc (6')-II genes among Klebsiella pneumoniae isolates collected from patients. J Pharm Sci Res. 2018; 10(5): 1048-52.
30. Ghotaslou R., Sefidan Y.F., Akhi M.T. et al. Dissemination of genes encoding aminoglycoside-modifying enzymes and arm A among Enterobacteriaceae isolates in Northwest Iran. Microb Drug Resist. 2017; 23(7): 826-32. doi: 10.1089/mdr.2016.0224.
31. Alyamani E.J., Khiyami A.M., Booq R.Y. et al. The occurrence of ESBL-producing Escherichia coli carrying aminoglycoside resistance genes in urinary tract infections in Saudi Arabia. Ann Clin Microbiol Antimicrob. 2017; 16: 1. doi: 10.1186/s12941-016-0177-6.
32. Kim H.C., Jang J-H., Kim H. et al. Multiplex PCR for Simultaneous Detection of Aminoglycoside Resistance Genes in Escherichia coli and Klebsiella pneumoniae. Korean J Clin Lab Sci. 2012; 44(3): 155-65.
33. Ho P-L., Leung L-M., Chow K-H. et al. Prevalence of aminoglycoside modifying enzyme and 16S ribosomal RNA methylase genes among aminoglycoside-resistant Escherichia coli isolates. J Microbiol Immunol Infect. 2016; 49(1): 123-6. doi: 10.1016/j.jmii.2014.08.012.
34. Abo-State M.A.M., Saleh YE-S., Ghareeb H.M. Prevalence and sequence of aminoglycosides modifying enzymes genes among E. coli and Klebsiella species isolated from Egyptian hospitals. J Radiat Res Appl Sci. 2018; 11(4): 408-15. doi: 10.1186/s40348-022-00134-2.
35. Lotfollahi L., Samadi N., Hosainzadegan H., Qomi M.A. Prevalence of aac (3')-IIa and aac (6')-Ib genes incidence involved in aminoglycoside resistance in Klebsiella pneumoniae isolated from clinical samples in Urmia Hospitals, Iran. Am J Pharm Res. 2015; 5(5): 326-34.
36. Harir Foroush M., Shokoohizadeh L., Mirzaee M. Prevalence of Genes Encoding Aminoglycoside Modifying Enzymes in Clinical Isolates of Klebsiella Pneumoniae in the Hospitals of Borujerd. Int J Med Lab. 2018; 5(1): 35-41.
37. Ramirez M.S., Tolmasky M.E. Aminoglycoside modifying enzymes. Drug Resist Updat. 2010; 13(6): 151-71. doi: 10.1016/j.drup.2010.08.003.

This research was supported by Department of Microbiology, College of Medicine, University of Kufa as a part of its research activity.

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

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Received: 23.09.2022
Accepted: 09.02.2023

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

ORIGINAL ARTICLE

MORPHOMETRIC AND MORPHOLOGICAL ASSESSMENT OF CORONARY ARTERIES WITH INTRAVASCULAR ULTRASOUND

DOI: 10.36740/WLek202302109

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ABSTRACT

The aim: To perform a morphometric assessment of the coronary arteries ostia by intravascular ultrasound with morphological evaluation among the Ukrainian population.

Materials and methods: Intravascular images of the ostia of the right (48%) and left (52%) coronary arteries with the minimum diameter, maximum diameter, mean diameter and lumen area were analyzed. An intravascular ultrasound procedure was performed before percutaneous intervention.

Results: A total of 25 IVUS examinations were collected from patients of both sexes and the same ages: 61,27±10,24 for males and females 68±5,83 (p=0.64). The left coronary artery (LCA) ostium assessment was performed in 13 (52%) cases: 8 men and 5 women (32% and 20%, respectively). The right coronary artery (RCA) ostium assessment was performed in 12 (48%) cases: 7 men and 5 women (28% and 20%, respectively). The maximal diameter of the coronary artery ostia was higher in men (5.95±0.66mm) than in women (4.82±0.34mm) ($p<0.0001$). In men, the maximal diameter of the RCA was higher than in the LCA (6.4±0.40mm and 5.56±0.60mm, respectively). The same differences were found in the mean diameter and lumen area ($p<0.05$). In women, the minimum diameter, mean diameter, maximum diameter and lumen area of the RCA were higher than LCA but without statistically significant differences. The anatomic precondition explains the observed changes in echogenicity.

Conclusions: IVUS analysis shows significantly higher parameters of the minimum diameter, mean diameter, maximum diameter and lumen area in men than in women among the Ukrainian population. Therefore, morphological evaluation is crucial in the interpretation of intracoronary images.

KEY WORDS: anatomy, intravascular ultrasound, IVUS, coronary vessels, intravascular ultrasonography

Wiad Lek. 2023;76(2):305-310

INTRODUCTION

Intravascular ultrasound (IVUS) is a modern method of visualizing the coronary arteries from the lumenal surface of the vessel [1]. The cross-sections of the intracoronary images make possible the measuring of the coronary artery size and assessing the structure of the vessel [2], which is extremely important in the context of cardiovascular diseases.

The uniqueness of ultrasound is based on the fact that different layers of the coronary artery wall reflect ultrasound waves differently, anatomically preconditioned. Therefore, understanding the anatomical aspect of the vessel structure is the key to interpreting the intracoronary images. Even though IVUS is widely used in the leading clinics [3], for the majority, it is a less accessible procedure [4]. Unfortunately, IVUS remains an innovative procedure for much medical staff and requires further explanation of coronary vessel morphology.

The coronary artery size is variable and impacts the coronary stent implantation and optimization of the results of coronary revascularization [5, 6]. To our knowledge, there is no data on the size of coronary arteries ostia among the Ukrainian population using IVUS.

THE AIM

To perform a morphometric and morphological assessment of the coronary arteries ostia by intravascular ultrasound among the Ukrainian population.

MATERIALS AND METHODS

Study design and patient selection. Patients from the Lviv Regional Clinical Hospital and the Ukrainian-Polish Heart Center «Lviv» (Lviv, Ukraine) were involved in the study. Inclusion criteria: patients who underwent IVUS of the right coronary artery (RCA) or left coronary artery (LCA) before percutaneous interventions; informed consent of the patients/legal guardians on collecting the clinical data and samples, according to the Decla-

ration of Helsinki. Exclusion criteria: patients after the coronary artery bypass grafting, hemodynamically unstable patients, and patients who underwent IVUS of the branches of the coronary arteries without assessment of the coronary artery ostia. The Local Bioethics Commission approved the study.

IVUS images of 25 patients with the following gender distribution were obtained: 15 men and 10 women. The patient's selection was based on the Guideline for percutaneous coronary intervention according to the American College of Cardiology Foundation, the American Heart Association, and the Society for Cardiovascular Angiography and Interventions [7]. In 13 cases, the indication for IVUS was angiographically indeterminate left main CAD (Class IIa, Level of Evidence: B). In 8 cases, the indication for IVUS was non-stem lesion of the left coronary artery with angiographically intermediate stenosis (50-70% stenosis) (Class IIb, Level of Evidence: B). In this research, it was the lesion of the right coronary artery. IVUS was performed without any complications.

The assessment was performed on an angiograph Siemens Artis Zee Floor Eco (Munich, Germany) and IVUS Philips Volcano (Brussels, Belgium). Access through the radial artery under local anaesthesia with 2% lidocaine solution (Lekhim-Kharkiv, Kharkiv, Ukraine). The introducer Radiofocus Terumo (Fujinomiya, Japan) was introduced along with the conductor, followed by heparin (Novopharm-Biosynthesis, Novograd-Volynskyi, Ukraine). Coronary angiography was performed with an Impulse Boston Scientific diagnostic catheter (Boston, USA). Intravascular imaging was performed using the conduction-directing catheter Launcher Medtronic (Dublin, Ireland). BMU Universal II Abbott (Abbott Park, Illinois, USA) conductor was placed in the respective segment of the coronary vessel. Intravascular ultrasound was performed using the catheter Volcano Eagle Eye Platinum Philips (Brussels, Belgium). The contrast material was Ultravist 470 (Bayer, Germany). We used the cross-sections of the coronary ostia to automatically measure the minimum diameter, mean diameter, maximum diameter and lumen area (LA), which means the media-to-media dimension of the coronary artery. Image analysis was performed using the corresponding software on an IVUS Philips Volcano (Brussels, Belgium). The results of the examination were reviewed separately by two independent interventional cardiologists.

We performed statistical analysis using R version 4.0.5 software (R Core Team, 2021). Student's t-test was used to compare average values. A value of $p<0.05$ was considered to be statistically significant. Data is presented in M±SD.

RESULTS

A total of 25 IVUS examinations were collected from patients of both sexes and the same ages: 61,27±10,24 for males and females 68±5,83 ($p=0.64$). The left coronary artery ostium assessment was performed in 13 (52%) cases: 8 men and 5 women (32% and 20%, respectively). The right coronary artery ostium assessment was performed in 12 (48%) cases: 7 men and 5 women (28% and 20%, respectively).

There is a statistically significant difference between men and women in the minimum diameter, mean diameter, maximum diameter, and lumen area. All morphometric parameters are significantly higher in men. Clinical characteristics of patients and differences between the morphometric parameters in the male and female groups are shown in Table I.

In men, the maximal diameter of the RCA was higher than in the LCA (6.4±0.40mm and 5.56±0.60mm, respectively). The same differences were found in the mean diameter and lumen area ($p<0.05$). In women, the minimum diameter, mean diameter, maximum diameter and lumen area of the RCA were higher than LCA but without statistically significant differences. Morphometric parameters of the left and right coronary arteries in men and women are presented in Table II (Fig. 1).

Intergroup comparison of the LCA showed statistically significantly higher parameters in maximal diameter ($p=0.003$) and mean diameter ($p=0.03$) in men than in women. In addition, all morphometric parameters of the RCA in men were higher than in women ($p<0.001$)

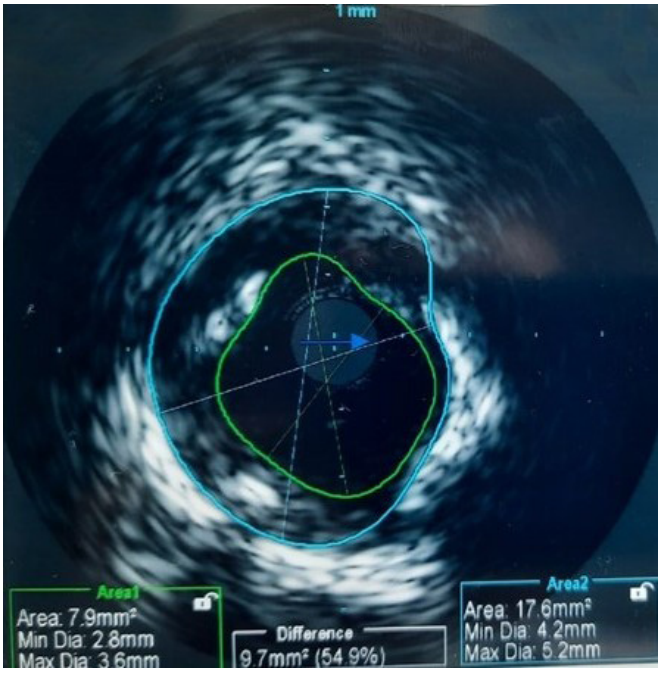

Fig. 1. An IVUS image of the right coronary ostium demonstrates a minimal lumen area (green circle) and lumen area (blue circle) of 17.6 mm2 with a minimal diameter 4.2 mm and a maximal diameter 5.2 mm.

Table I. Clinical characteristics of the patients who underwent IVUS. Values are presented in mean ± standard deviation

Parameters	Males (n=15)	Females (n=10)	p
Age (years)	61,27±10,24	68±5,83	0.64
Height (cm)	1,73±0.05	1,61±30	0.0001
Weight (kg)	84.33±9.62	79,73±13,33	0.008
Body mass index (kg/m^2)	28,25±2.90	30,96±6,53	0.38
Lumen area, mm^2	23.16±6.03	17.31±2.12	0.002*
Minimal diameter, mm	4.79±0.92	4.02±0.4	0.010*
Maximal diameter, mm	5.95±0.66	4.82±0.34	0.00001*
Mean diameter, mm	5.37±0.74	4.41±0.33	0.0002*

*P-value of <0.05 was considered significant

Table II. Morphometric parameters of the left coronary artery (LCA) and right coronary artery (RCA) in men and women. Values are presented in mean ± standard deviation

Parameters	LCA	RCA	p (t) (LCA:RCA)
	M±SD	M±SD	M±SD
	Male patients		
Lumen area, mm^2	20.43±6.92	26.29±2.74	0.043*
Minimal diameter, mm	4.56±1.16	5.04±0.48	0.30
Maximal diameter, mm	5.56±0.6	6.4±0.4	0.005*
Mean diameter, mm	5.06±0.86	5.72±0.4	0.067*
	Female patients		
Lumen area, mm^2	16.35±2.51	18.28±1.2	0.17
Minimal diameter, mm	3.84±0.51	4.2±0.12	0.18
Maximal diameter, mm	4.7±0.25	4.94±0.4	0.30
Mean diameter, mm	4.25±0.33	4.57±0.25	0.14

*P-value of <0.05 was considered significant

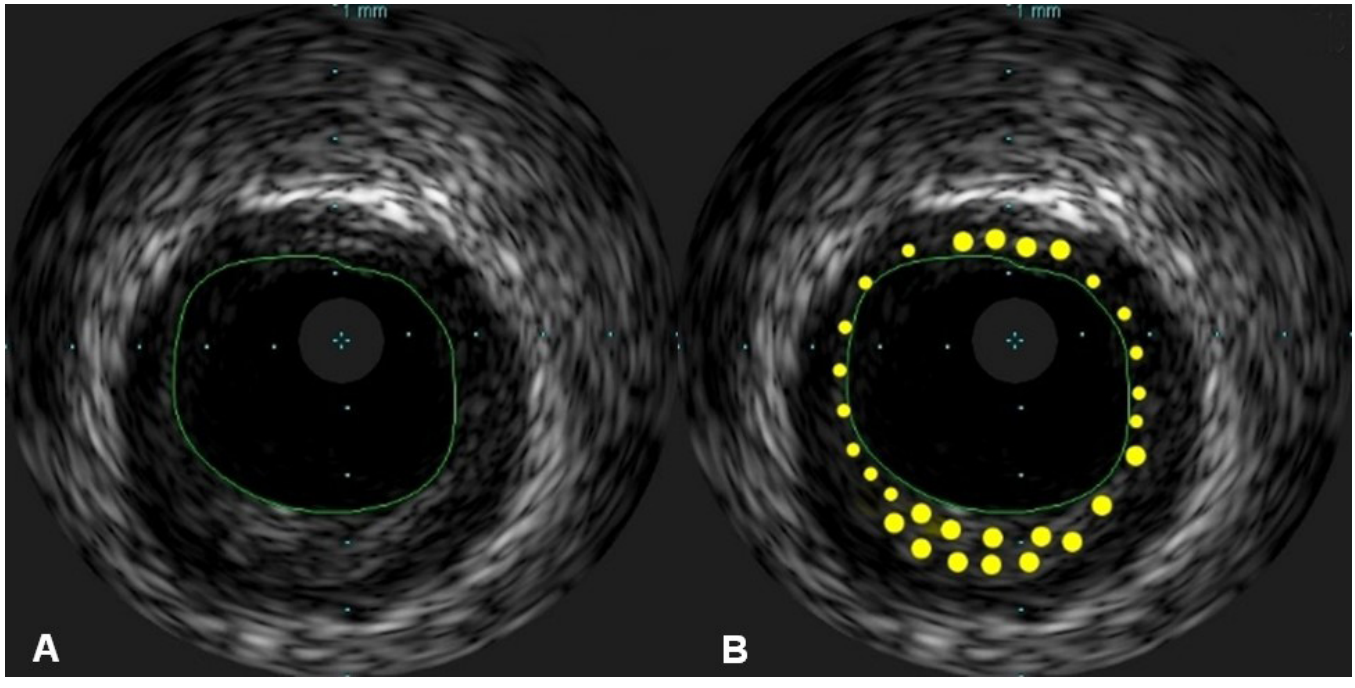

Fig. 2. (A) An IVUS image of the coronary ostium. The grey circle in the middle points to the location of the ultrasound imaging catheter. The lumen is shown as a dark area (green circle). (B) Corresponding intravascular ultrasound image of the coronary artery with depicted intima (yellow circle).

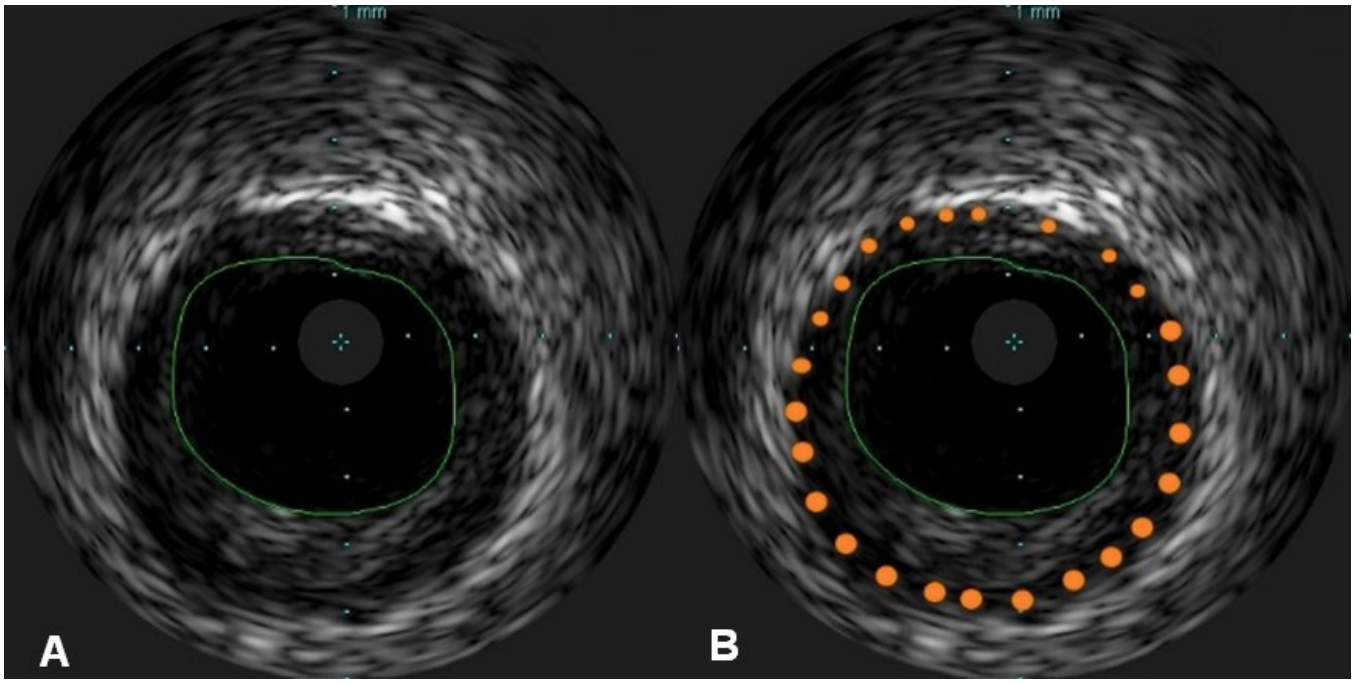

Fig. 3. (A) An IVUS image of the coronary ostium. The grey circle in the middle points a location of ultrasound imaging catheter. The lumen is showed as a dark area (green circle). (B) Corresponding intravascular ultrasound image of coronary artery with depicted media (orange circle).

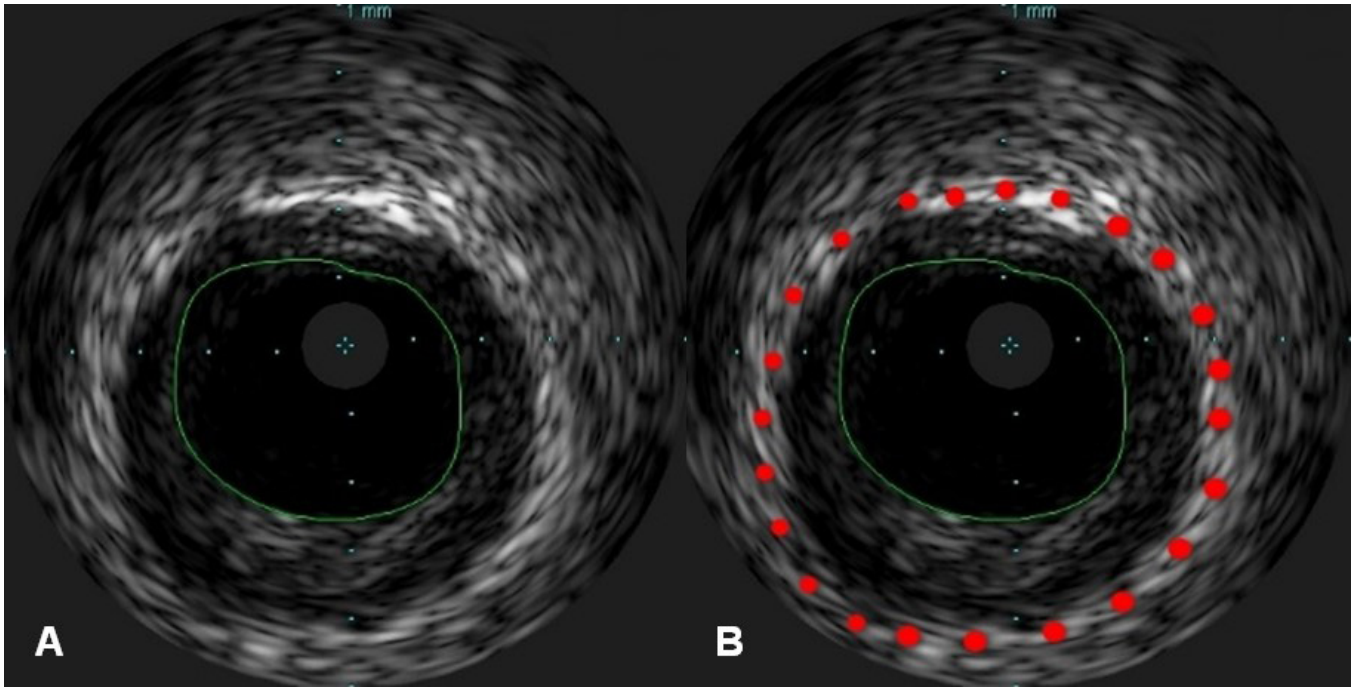

Fig. 4. (A) An IVUS image of the coronary ostium. The grey circle in the middle points to the location of the ultrasound imaging catheter. The lumen is shown as a dark area (green circle). (B) Corresponding intravascular ultrasound image of the coronary artery with depicted adventitia (red circle).

Morphological evaluation of the intravascular imaging showed that the intima reflects ultrasound waves producing a uniform concentric echo, which is visualized in the form of a light ring. The reason is the parallel location of the endotheliocytes to the lumenal edge of the vessel (Fig. 2). Even though the coronary arteries are vessels of elastic type, the media contains a significant number of smooth myocytes, which do not reflect ultrasound waves. Thus, ultrasound waves pass through the media and have a dark colour in the image. It allows us to distinguish the media easily (Fig. 3). The longitudinal orientation of the fibers in adventitia contributes to the intense reflection of ultrasound waves and a bright stripe on the image (Fig. 4). It also occurs in the case of atherosclerotic plaques calcification (Fig. 5). The adventitia, unlike intima, does not have the form of a concentric ring. Anatomically, it is due to the ability of the

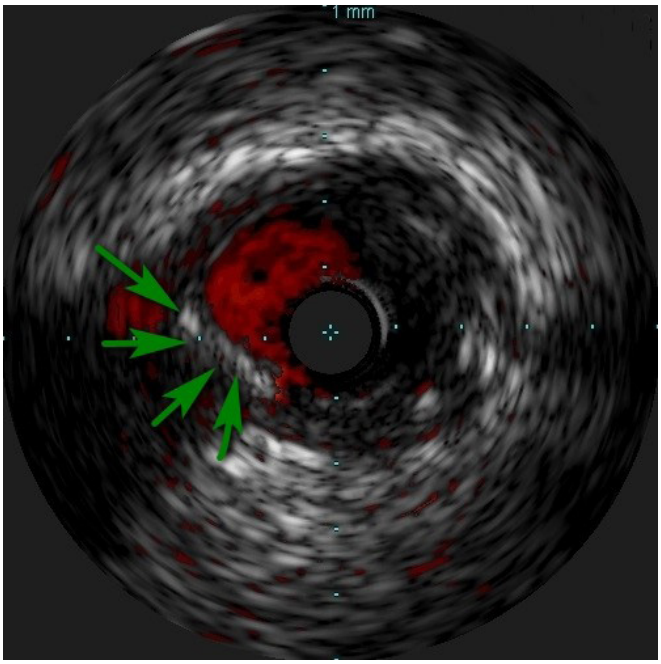

Fig. 5. An IVUS image of the coronary ostium shows an atherosclerotic plaque with calcification (green arrows) and acoustic shadowing.

adventitia to affect the diameter of the coronary artery due to the parallel orientation of the collagen fibers and the loose consistency of connective tissue.

DISCUSSION

he current study shows that coronary artery ostia diameters and luminal areas assessed by intravascular ultrasound were significantly higher in men than in women among the Ukrainian population. Overall, in men, the maximal diameter, the mean diameter and the lumen area of the RCA were higher than in the LCA. In contrast, we did not prove the differences between all morphometric parameters of RCA and LCA in women. In addition, this study found higher all morphometric parameters of the RCA in men than in women, but only the maximal and mean diameter of the LCA. Finally, this study allowed the performing of a morphological evaluation of the coronary artery ostia.

Taking into account that the proximal segments of the coronary arteries are affected in most cases [8], we analyzed the coronary ostia. The diameter of the vessel's lumen correlates with the development of major cardiac events [1, 9]. The data obtained are consonant with the statements made by other authors that coronary artery diameter is higher in men than in women [9-10]. Despite previous studies showing larger diameters of the LCA [3, 9, 10], our results showed higher parameters of the RCA. It could be explained by different factors, from clinical parameters of selected patients to assessing tools and modalities. There are no consolidated registries or publications of coronary ostia diameters among the Ukrainian population assessed by IVUS, which makes this research valuable in further investigations.

The limited availability of IVUS [3] prompted us to add a morphological evaluation. In the presented study, the observed changes in echogenicity are explained by the anatomic precondition. In our opinion, understanding the morphological aspect of the vessel structure is crucial in interpreting intracoronary images. Moreover, it helps to explicate conditions that contribute to the morphological changes of the vascular wall [2, 3, 11].

The provided study has limitations. It was a single-centre, retrospective study. The small sample size was due to the precise guidelines for IVUS use, given several aspects of the availability of IVUS [3, 7]. IVUS is like new technology in Ukraine, which might impact the use frequency. In addition, further research would be welcomed to increase the sample size to generalize the results.

CONCLUSIONS

We conclude that IVUS analysis shows significantly higher parameters of the minimum diameter, mean diameter, maximum diameter and lumen area in men than women in the Ukrainian population. The difference in parameters of the right coronary artery and the left coronary artery varies between gender groups.

REFERENCES

1. Matsushita K., Hibi K., Okada K. et al. Comparison between instantaneous wave-free ratio versus morphometric assessments by intracoronary imaging. Heart Vessels. 2019;34(6):926–35.
2. Peng C., Wu H., Kim S. et al. Recent Advances in Transducers for Intravascular Ultrasound (IVUS) Imaging. Sensors. 2021;21:3540.
3. Ramadan R., Boden W.E., Kinlay S. Management of left main coronary artery disease. J Am Heart Assoc. 2018;7(7):e008151.
4. Koskinas K.C., Nakamura M., Räber L. et al. Current use of intracoronary imaging in interventional practice – results of a European Association of Percutaneous Cardiovascular Interventions (EAPCI) and Japanese Association of Cardiovascular Interventions and Therapeutics (CVIT) clinical practice sur. Circ J. 2018;82(5):1360–8.
5. Beshley D., Dudek D., Wojdyla R. et al. Innovative cardiology and cardiac surgery in Lviv. Proc Shevchenko Sci Soc Med Sci. 2020;62(2):143–9.
6. Shinohara H., Kodera S., Ninomiya K. et al. Automatic detection of vessel structure by deep learning using intravascular ultrasound images of the coronary arteries. Plos one. 2021;16(8): e0255577.

7. Levine G.N., Bates E.R., Blankenship J.C. et al. 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention. Catheter Cardiovasc Interv. 2013;82(4):E266–355.
8. Goel P.K., Liladhar Vora P., Kumar Sahu A., Khanna R. Left main coronary artery diameter - A correlation between intravascular ultrasound and quantitative coronary angiography. Indian Heart J. 2021;73(5):660-3. doi: 10.1016/j.ihj.2021.09.009.
9. Zhou F.F., Liu Y.H., Ge P.C. et al. Coronary artery diameter is inversely associated with the severity of coronary lesions in patients undergoing coronary angiography. Cell Physiol Biochem. 2017;43(3):1247-57. doi: 10.1159/000481765.
10. Reddy S., Kumar S., Kashyap J.R. et al. Coronary artery size in North Indian population - Intravascular ultrasound-based study. Indian Heart J. 2019;71(5):412-7. doi: 10.1016/j.ihj.2019.10.005.
11. Räber L., Mintz G.S., Koskinas K.C. et al. Clinical use of intracoronary imaging. Part 1: guidance and optimization of coronary interventions. An expert consensus document of the European Association of Percutaneous Cardiovascular Interventions. Eur Heart J. 2018;39(35):3281–300.

We thank the Armed Forces of Ukraine for ensuring our security while preparing this manuscript.

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Conflict of interest:
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Received: 23.03.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

IMPACT OF STATINS ON THE CLINICAL COURSE OF COVID-19-ASSOCIATED PNEUMONIA IN UNVACCINATED PATIENTS WITH ARTERIAL HYPERTENSION

DOI: 10.36740/WLek202302110

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ABSTRACT

The aim: To assess the impact of statins on the severity and lethality rate in hypertensive patients with COVID-19-associated pneumonia.

Materials and methods: 106 unvaccinated hypertensive patients were enrolled in the study. 29 (27.4%) patients took statins.

Results: Statins were not associated with reduced risks of lethality (relative risk (RR), 0.24; [95%CI, 0.03–1.79], p=0.16), decline in oxygen saturation < 92% during the inpatient stay (RR, 0.70 [95%CI, 0.39-1.28], p=0.25) and need for supplemental oxygen (RR, 0.84; [95%CI, 0.51–1.37], p=0.48). There was no significant difference in the median length of in-hospital stay between the patients taking statins (14.0 [10.0–15.0] days) and patients, which didn't take statins (13.0 [9.0–18.0] days) (p=0.76). However, subgroup analysis showed that statins reduced the risk of decline in oxygen saturation <92% in patients aged 65 years and older with body mass index ≥ 25.0 kg/m2 (RR, 0.33 [95%CI, 0.11–0.92], p=0.03).

Conclusions: Statins didn't affect the severity and lethality rate in hypertensive patients with COVID-19-associated pneumonia. Subgroup analysis showed that statin use was associated with a decrease in morbidity of patients aged 65 years and older with BMI ≥25.0 kg/m2 hospitalized for COVID-19-associated pneumonia.

KEY WORDS: hypertension, pneumonia, statins, COVID-19, lethality, severity

Wiad Lek. 2023;76(2):311-319

INTRODUCTION

Despite mass vaccination, coronavirus disease 2019 (COVID-19) remains a major public health problem worldwide [1]. Comorbidities including arterial hypertension (AH) are more prevalent in older patients and may significantly worsen the clinical course of COVID-19 [2, 3]. Also, AH is more prevalent in patients with lethal outcomes [4].

Statins remain first-line lipid-lowering medicines. However, they have multiple pleiotropic effects: prevention of left ventricular fibrosis and hypertrophy, inhibition of myocardial apoptosis, endothelial nitric oxide synthase activation, reduction of plasminogen activator inhibitor-1 expression, reduction of endothelin-1 synthesis, increase in peroxisome proliferator-activated receptor-α and γ expression, prevention of T-cell activation, macrophage grows inhibition, decrease in the level of proinflammatory cytokines (IL-1β, IL-6, IL-8 cyclooxygenase-2, TNF-α), inhibition of platelet reactivity and thromboxane A_2 biosynthesis, decrease in C-reactive protein (CRP) level [5]. The protective effects of statins in the COVID-19 course may be explained by their anti-inflammatory, antioxidant, antithrombotic, and immune-modulatory properties [6]. Except for high cholesterol levels, statins are generally recommended for secondary prevention in most patients surviving cardiovascular events such as myocardial infarction and stroke [7, 8].

Despite the statin prescription, patients often discontinue the treatment with these medications. According to the study performed in Taiwan, persistence in statin treatment was 86, 67, 50, and 25% at 6-month, 1-, 2-, and 7-year, respectively [9]. In patients with myocardial infarction, good adherence is present only in 40.9% for statins during 1 year of follow-up. [10].

So, the investigation of statin impact on the clinical course of COVID-19 complicated by pneumonia is actual.

THE AIM

The study aimed to assess the impact of statins on the severity and lethality rate in hypertensive patients with COVID-19-associated pneumonia.

MATERIALS AND METHODS

106 unvaccinated hypertensive patients with COVID-19-associated pneumonia hospitalized during the period of March-June 2021 were enrolled in the study. Hypertensive patients were divided into two groups: the main group, which included 29 patients on statin therapy, and the control group, which included 77 patients who didn't take statins.

Coronavirus SARS-CoV-2 as an etiological factor of pneumonia was confirmed with either PCR or ELISA test with the assessment of IgM level. Pneumonia was confirmed with either chest X-ray or chest computed tomography.

The diagnosis of AH was established in accordance with the criteria of the 2018 European Society of Cardiology guideline [11]. Cardiovascular risk was assessed in accordance with the criteria of the 2021 European Society of Cardiology guideline [12]. Pneumonia severity was assessed in accordance with the CURB65 score. Patients' symptoms were assessed using the questionnaire CAP-Sym.

Inclusion criterion:

- Pneumonia associated with COVID-19 confirmed laboratorically, which leads to hospital admission.

Exclusion criteria:

- Pregnancy;
- Age <18 years;
- Moderate and severe cognitive decline;
- Acute myocardial infarction;
- Acute phase of ischemic stroke, transient ischemic attack;
- Acute phase of intracerebral haemorrhage;
- Active cancer;
- Active gastrointestinal bleeding;
- Stage 5 chronic kidney disease.

A consent form was signed by each prospective participant before recruitment into the study. All procedures in the study met bioethical standards according to the Helsinki Declaration.

Statistical processing of the data was performed using the software Statistica 10 and MS Excel. Shapiro-Wilk test was performed to establish the type of distribution. Descriptive statistics for data with the normal distribution are presented as the mean with standard deviation (Mean±SD). The median and interquartile range (Me [Q1–Q3]) was calculated for data with the abnormal distribution. Categorical data are presented as count and percentage. The T-test was used for the comparison of two variables with the normal distribution. Mann-Whitney U test was performed to compare variables with the abnormal distribution. Fisher's exact test was used for the comparison of two categorical variables. Also, the relative risk with 95% confidential interval was calculated and forest plots were built. The null hypothesis suggested that there is no statistical significance. If the p-value was < 0.05 the null hypothesis was rejected; if the p-value was ≥ 0.05 the null hypothesis was accepted, which is common in biomedical studies.

RESULTS

Characteristics of patients of both groups are shown in table I. Patients' age, body mass index, gender composition, history of AH, and chest computer tomography extent of COVID-19 at hospital admission were similar in both groups. Prior myocardial infarction was more prevalent in patients of the main group (patients who took statins). Other comorbidities were of similar prev-

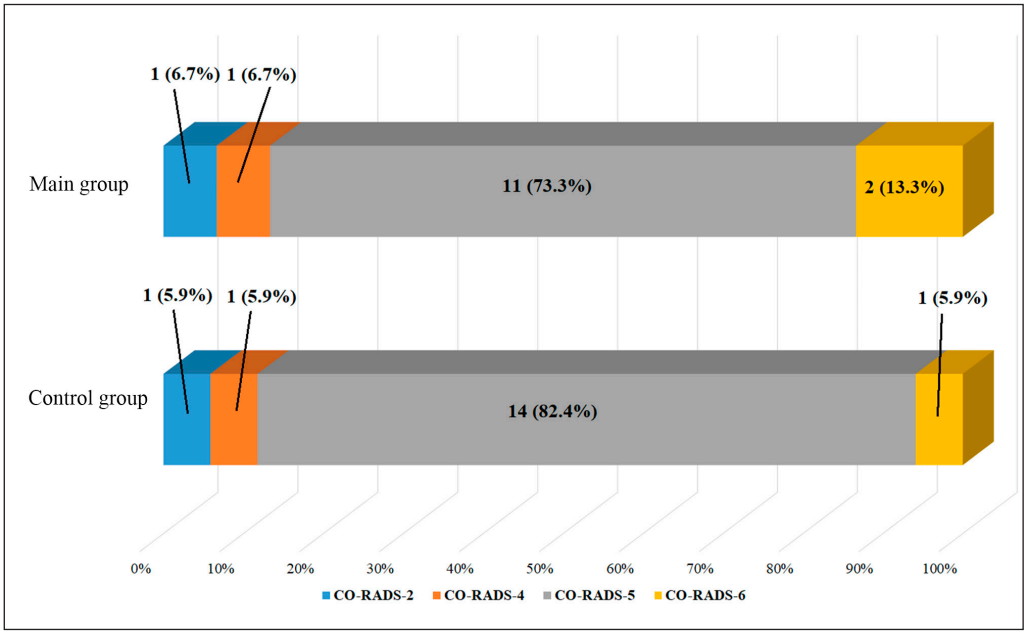

Fig. 1. Lung damage assessed in accordance with the CO-RADS categories

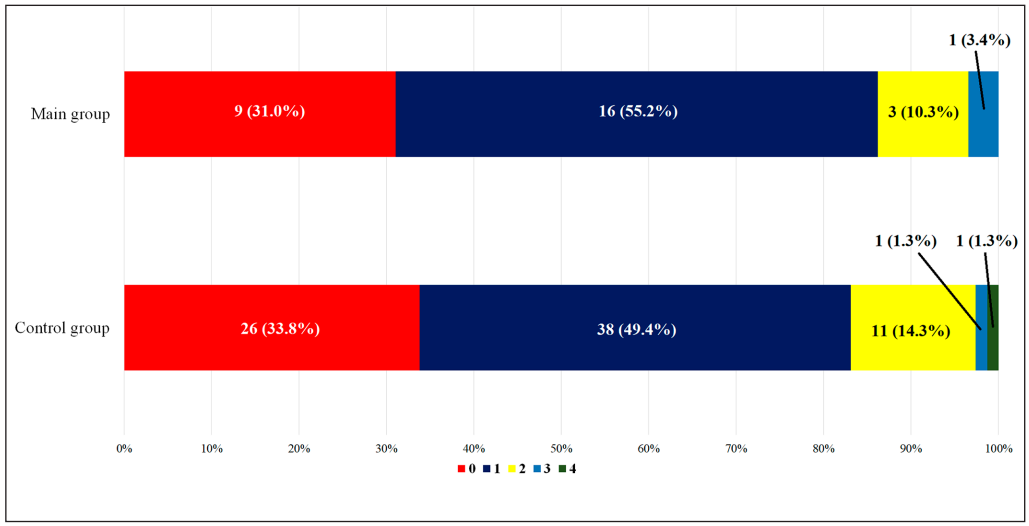

Fig. 2. Pneumonia severity assessment in accordance with the CURB65 score

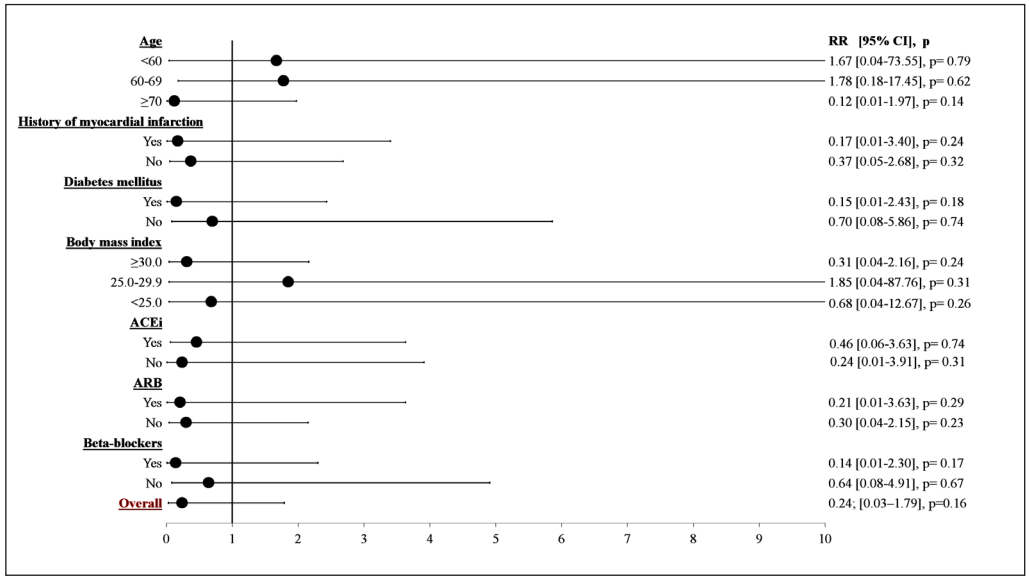

Fig. 3. Forest plot comparing the relative risk of death between the main group and the control group

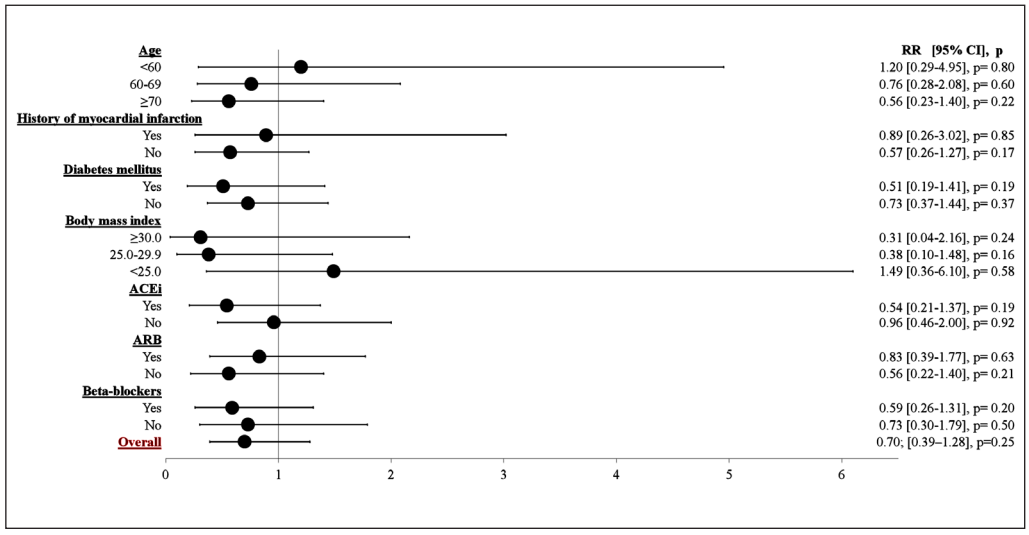

Fig. 4. Forest plot comparing the relative risk of decline in oxygen saturation <92% during hospital stay between the main group and the control group

alence in both groups. Laboratory parameters were similar in patients of both groups.

At the moment of hospitalization, 73 (94.8%) patients in the main group and 25 (86.2%) patients in the control group had bilateral pneumonia (p=0.21), in other patients the pneumonia was unilateral. Lung damage was assessed according to the CO-RADS (COVID-19 Reporting and Data System) categories (Fig. 1).

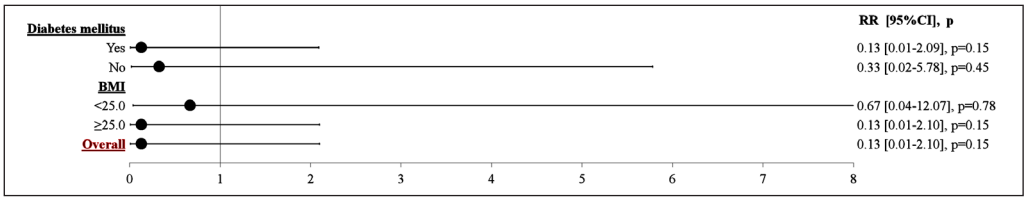

Fig. 5. Forest plot comparing the relative risk of death between the main group and the control group in patients aged 65 years and older

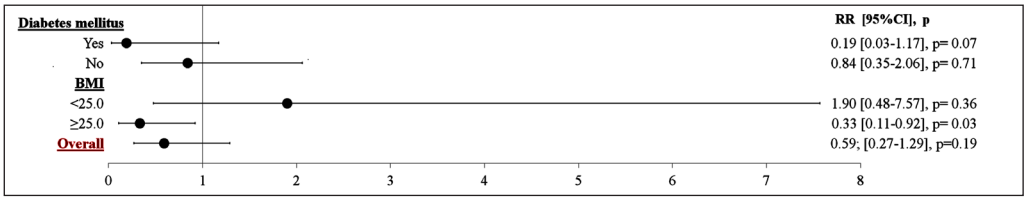

Fig. 6. Forest plot comparing the relative risk of decline in oxygen saturation <92% during total hospital stay between the main group and the control group in patients aged 65 years and older

There was no significant difference in pneumonia severity according to the CURB65 score between both groups at the moment of hospital admission (p=0.98) (Fig. 2).

Grade 1 AH was diagnosed in 1 (3.4%) patient, grade 2 was diagnosed in 20 (69.0%) patients, and grade 3 was diagnosed in 8 (27.6%) patients of the main group. In the control group, 1 (1.3%) patient had grade 1 AH, 51 (66.2%) patients were diagnosed with grade 2 AH, and 25 (32.5%) patients had grade 3 AH.

Cardiovascular risk was high in 2 (6.9%) patients and very high in 27 (93.1%) patients of the main group. Cardiovascular risk was high in 4 (5.2%) patients and very high in 73 (94.8%) patients of the control group. So, cardiovascular risk was similar in patients of both groups (p=0.66).

There was no significant difference according to the CAP-Sym questionnaire between the main group (28.5±3.6 points) and the control group (27.0±2.7 points) at the moment of hospital admission (p=0.49).

Beta-blockers were more frequently prescribed in patients of the main group (p=0.04) (table II). There were no significant differences in other received drugs in patients of both groups. This may be explained by the fact that prior myocardial infarction was more common in the patients of the main group.

Complaints of olfactory disorders were significantly more common in the control group than in the main group at the moment of hospital discharge (p=0.03). There were no significant differences in other complaints (cough, general weakness, breathlessness, palpitations, leg muscle pain, back pain, headache, dizziness, sleep disorders, taste disorders, olfactory disorders, sore throat, nasal congestion, rhinorrhea, diarrhoea, and stomachache) between these groups (Table III).

In the main group, 9 (31.0%) patients were taking atorvastatin, 19 (65.5%) patients were taking rosuvastatin and 1 (3.4%) patient was taking simvastatin. High-intensity statin therapy was used in 12 (41.4%) patients of the main group.

There was no significant difference in the median length of in-hospital stay between the main group (14.0 [10.0–15.0] days) and the control group (13.0 [9.0–18.0] days) (p=0.76).

12 (11.3%) of all enrolled patients with AH died. The mean age of the non-survivors was higher (74.3±4.5 years vs. 67.6±1.7 years, p=0.02). 1 (3.4%) patient died in the main group and 11 (14.3%) patients died in the control group (relative risk (RR), 0.24; [95% confidence interval (CI), 0.03–1.79], p=0.16).

CPAP (Continuous Positive Airway Pressure) was used in 1 (3.4%) patient of the main group (the patient died) and in 9 (11.7%) patients of the control group. Among patients in the control group on the CPAP therapy, 8 (88.9%) patients died, and 1 (11.1%) patient survived. Non-invasive mechanical ventilation was used in 5 (6.5%) patients of the control group; none of them survived. Non-invasive mechanical ventilation was not used in the patients of the main group.

Supplemental oxygen was used in 12 (41.4%) patients of the main group and in 38 (49.4%) patients of the control group (RR, 0.84; [95%CI, 0.51–1.37], p=0.48). Among survivors, median minimal oxygen saturation for total in-patient time was 94.0 [90.5–95.0] % in the main group and 93.0 [90.0–95.0] % in the control group (p=0.55). Decline in oxygen saturation <92% was observed in 9 (31.0%) patients of the main group and in 34 (44.2%) patients of the control group (RR, 0.70; [95%CI, 0.39–1.28], p=0.25).

History of prior myocardial infarction was associated with neither excessive lethality (RR, 0.65; [95%CI, 0.09–4.63], p=0.43) nor decline in oxygen saturation <92% (RR, 1.26; [95%CI, 0.45–3.48], p=0.66).

1 (3.4%) patient in the main group and 9 (11.7%) patients in the control group were transferred to the intensive care unit (ICU) (p=0.28). The patient from the main group died. Among patients from the control group transferred to the ICU, 8 (88.9%) patients died.

Statins didn't show statistical significance in the reduction of lethality rate (RR, 0.24 [0.03–1.79], p=0.16)

Table I. Characteristics and comorbidities of enrolled patients

Parameter	Main group	Control group	p
Characteristics			
Age, years‡	67.5±11.8	68.7±2.0	0.53
Body mass index, kg/m^2 §	26.9 [24.9–32.0]	27.1 [24.2–32.2]	0.91
Male gender†	14 (48.3%)	27 (35.1%)	0.26
History of AH, years§	10.0 [5.0–19.0]	15.0 [7.0–20.0]	0.19
Chest computer tomography extent of COVID-19§	25.0 [15.0–35.0]	20.0 [15.0–30.0]	0.33
Comorbidities			
History of myocardial infarction†	9 (31.0%)	4 (5.2%)	<0.001
Diabetes mellitus †	10 (34.5%)	24 (31.2%)	0.81
History of stroke†	2 (6.9%)	7 (9.1%)	1.0
Atrial fibrillation†	3 (10.3%)	8 (10.4%)	1.0
Heart failure with mid-range ejection fraction†	2 (6.9%)	3 (3.9%)	0.61
Hypothyroidism†	3 (10.3%)	3 (3.9%)	0.34
Laboratory tests at the moment of hospital admission			
Erythrocytes, 10^{12}/L§	4.6 [4.1–4.7]	4.6 [4.2–5.0]	0.37
Haemoglobin, g/L§	128.0 [117.8–138.5]	131.0 [123.5–142.5]	0.23
Leucocytes, 10^9/L§	6.9 [5.2–8.5]	6.2 [4.4–8.4]	0.37
Band neutrophils, %§	4.0 [2.0–6.3]	5.0 [3.0–8.0]	0.19
Segmented neutrophils, %§	71.0 [65.0–75.0]	73.5 [65.0–78.0]	0.37
Lymphocytes, %§	19.0 [15.0-25.5]	17.0 [12.0-22.0]	0.34
Platelets, 10^9/L§	208.5 [148.0-279.0]	188.0 [149.8–242.8]	0.55
Erythrocyte sedimentation rate, mm/h‡	32.4±14.8	32.2±16.3	0.97
Fasting glucose, mmol/L§	5.7 [5.2–7.8]	6.3 [5.4–9.1]	0.31
Total protein, g/L§	68.5 [64.4–78.1]	68.2 [64.0–73.5]	0.76
Total bilirubin, µmol/L§	11.1 [8.7–14.8]	10.1 [8.1–12.4]	0.07
Aspartate aminotransferase, IU/L§	25.8 [19.4–38.9]	29.4 [21.3–38.5]	0.53
Alanine aminotransferase, IU/L§	29.2 [19.9–38.4]	26.0 [18.9–38.4]	0.71
Creatinine, µmol/L§	98.6 [88.9–112.3]	96.0 [86.1–111.0]	0.73
Blood urea nitrogen, mmol/L§	6.2 [5.0–7.4]	6.0 [4.9–7.8]	0.96
Fibrinogen, g/L‡	5.1±1.2	5.3±1.4	0.60
Specific markers at the moment of hospital admission			
Ferritin, ng/mL§	298.5 [136.5–545.0]	359 [205.0–602.5]	0.45
Soluble interleukin-2 receptors, ng/mL§	5.4 [4.1–7.0]	6.3 [4.6–8.4]	0.20
Interleukin-6, pg/mL§	37.8 [7.0–76.2]	44.6 [20.6–99.8]	0.20

‡ Data presented as mean with standard deviation (Mean ± SD)
§ Data presented as median with interquartile range (Me [Q1–Q3])
† Data presented as count and percentage

and the number of patients with an oxygen saturation decline below 92% during a total hospital stay (RR, 0.70 [95%CI, 0.39-1.28], p=0.25) among all enrolled patients (Fig. 3, 4).

However, subgroup analysis showed that statins reduced the risk of decline in oxygen saturation <92% during a hospital stay in patients aged 65 years and older with BMI ≥ 25.0 kg/m^2 by 67.3% (RR, 0.33 [95%CI, 0.11–0.92], p=0.03) (Fig. 6).

Among survivors, soluble interleukin-2 receptor levels at the moment of hospital discharge were lower in patients of the main group than in patients of the control group (p=0.03) (Table IV). Levels of ferritin and interleukin-6 were similar in both groups.

Survived patients of the main group scored 17.6±2.7 points and ones of the control group scored 15.8±1.9 points according to the CAP-Sym questionnaire; there was no significant difference between these values (p=0.40).

Table II. The most common medication taken by patients of both groups

Medications	Main group	Control group	p
Corticosteroids	29 (100.0%)	77 (100.0%)	1.0
Anticoagulants	29 (100.0%)	77 (100.0%)	1.0
ACE inhibitors	18 (62.1%)	42 (54.5%)	0.52
Angiotensin receptor blockers	11 (37.9%)	22 (28.6%)	0.36
Diuretics	14 (48.3%)	31 (40.3%)	0.51
Beta-blockers	15 (51.7%)	23 (29.9%)	0.04
Calcium channel blockers	9 (31.0%)	29 (37.7%)	0.65
Mineralocorticoid receptors antagonists	4 (13.8%)	4 (5.2%)	0.21
Tocilizumab	0 (0.0%)	1 (1.3%)	1,0

Table III. Comparison of common complaints in patients of the main and the control groups

Complaints	At the moment of admission			At the moment of discharge		
	Main group	Control group	p	Main group, survivors	Control group, survivors	p
Cough	26 (89.7%)	61 (79.2%)	0.27	24 (85.7%)	46 (69.7%)	0.13
General weakness	24 (82.8%)	68 (88.3%)	0.52	25 (89.3%)	59 (89.4%)	1.0
Breathlessness	21 (72.4%)	46 (59.7%)	0.26	20 (71.4%)	35 (53.0%)	0.11
Palpitations	14 (48.3%)	32 (41.6%)	0.66	6 (21.4%)	6 (9.1%)	0.17
Leg muscle pain	14 (48.3%)	36 (46.8%)	1.0	7 (25.0%)	15 (22.7%)	0.80
Back pain	11 (37.9%)	27 (35.1%)	0.82	3 (10.7%)	16 (24.2%)	0.17
Headache	10 (34.5%)	22 (28.6%)	0.64	6 (21.4%)	5 (7.6%)	0.08
Dizziness	5 (17.2%)	26 (33.8%)	0.15	1 (3.6%)	4 (6.1%)	1.0
Sleep disorders	18 (62.1%)	49 (63.6%)	1.0	7 (25.0%)	17 (25.8%)	1.0
Taste disorders	6 (20.7%)	17 (22.1%)	1.0	1 (3.6%)	1 (1.5%)	0.51
Olfactory disorders	3 (10.3%)	18 (23.4%)	0.18	0 (0.0%)	11 (16.7%)	*0.03*
Sore throat	11 (37.9%)	35 (45.5%)	0.52	0 (0.0%)	1 (1.5%)	1.0
Nasal congestion	7 (24.1%)	11 (14.3%)	0.25	0 (0.0%)	0 (0.0%)	1.0
Rhinorrhea	6 (20.7%)	15 (19.5%)	1.0	0 (0.0%)	0 (0.0%)	1.0
Diarrhoea	6 (20.7%)	6 (7.8%)	0.08	0 (0.0%)	3 (4.5%)	0.55
Stomachache	6 (20.7%)	6 (7.8%)	0.08	0 (0.0%)	1 (1.5%)	1.0

Table IV. Levels of specific markers at the moment of hospital discharge

Parameter	Main group	Control group	p
Ferritin, ng/mL	391.0 [244.5–511.8]	347.5 [211.0–529.0]	0.58
Soluble interleukin-2 receptors, ng/mL	3.4 [2.6–4.2]	4.7 [3.3–6.8]	0.03
Interleukin-6, pg/mL	5.6 [3.4–7.1]	9.0 [2.0–24.9]	0.36

DISCUSSION

Our study showed that statin failed to reduce a lethality rate and decline in oxygen saturation below 92% during the inpatient stay. However, subgroup analysis demonstrated that statins reduce the risk of and decline in oxygen saturation below 92% during the inpatient stay in patients aged 65 years and older and with elevated BMI (≥25.0 kg/m^2). Also, soluble interleukin-2 receptor levels were lower in patients who took statins.

Multiple studies showed a reduced mortality rate associated with statin intake in patients with COVID-19 [13–16]. Meta-analysis performed by Zein A.F.M.Z. et al. demonstrated that statins use is associated with decreased mortality (RR, 0.72 [95%CI, 0.55–0.95], p<0.001); the association of statin and mortality was not affected by age, gender, diabetes, AH [17]. However, some authors noted that statin use is not associated with a decrease in mortality rate [18–20]. Moreover, Diaz-Arocutipa C. et al. showed that only chronic use of statins significantly reduced mortality according to the adjusted models [21]. Also, statin use was associated with a lower risk for death in hospitalized patients

(hazard ratio (HR), 0.86 [95%CI, 0.79–0.95]) [22]. Kow C.S. showed that statin use is associated with a lower risk of all-cause mortality (odds ratio (OR), 0.63 [95%CI, 0.58–0.84]), and the composite endpoint of severe illness (OR, 0.80 [95%CI, 0.73–0.88]) [23].

Memel Z.N. reported that statin use was associated with improvements in mortality for patients >65 years, but not for patients 65 years or younger [13]. This study supports our findings that only in patients aged 65 years and older and with elevated BMI (≥25.0 kg/m^2), statin use was associated with a decrease in the disease severity.

There is evidence that soluble interleukin-2 receptor levels are predictors of mortality in COVID-19 patients [24] Soluble interleukin-2 receptors are involved in the T-cell immune response regulation and are, therefore, suggested to play a major role in COVID-19 progression [25]. Duration of the disease is positively associated with serum level of soluble interleukin-2 receptors [26]. Kaya H. et al. reported that soluble interleukin-2 receptor levels on admission and sequential monitoring of soluble interleukin-2 receptor levels reflect COVID-19 severity [27]. Association between sIL-2R and disease severity in COVID-19 patients is known [28].

19.8% of all hospitalized hypertensive patients complains of olfactory dysfunction at the moment of hospital admission in our study. According to the meta-analysis made by Aanand P. et al., the incidence of olfactory dysfunction in the included studies ranged from 9.2% to 82.0% with an average rate of 30.2% [29]. Saniasiaya J. et al. reported that the prevalence of olfactory disorders is 47.85% [30]. The olfactory dysfunction assessed by objective olfactory testing is more prevalent than that assessed by self-reporting [31]. Olfactory disorders at the moment of hospital discharge were significantly less common in patients taking statins. Sustentacular cells of the olfactory epithelium with high expression of ACE2 protein may be potentially targeted by SARS-CoV-2 [32]. Also, the virus was found in olfactory neurons [33]. SARS-CoV-2 persistence and local inflammation in the olfactory epithelium may account for long-lasting olfactory disorders [34]. Anti-inflammatory, anti-oxidative, and anti-excitotoxic properties of statins may be associated with neuroprotective effects in neurodegenerative diseases and ischemic stroke [35]. Olfactory cleft and olfactory bulb abnormalities are found in SARS-CoV-2-related anosmia [36]. Thus, the positive effects of statins may be explained by their neuroprotective properties. In addition, statins were effective in the recovery of the olfactory function after 3-methylindole injection in a mouse model [37]. Therefore, statins may have a protective effect on the olfactory function in patients with COVID-19. Khani E. et al. suggested that statins may be potential medicines for the treatment of olfactory disorders induced by COVID-19 [38]. However, there are no published researches demonstrating the effects of statins on olfactory disorders.

Poor adherence to statins in patients with very high cardiovascular risk is a global problem. Khalaf K. et al. reported that low adherence to statins was associated with increased mortality risk in the patients who survived acute myocardial infarction [39]. In our study, 4 patients with prior myocardial infarction didn't take statins and belonged to the control group because of poor adherence, even though statins were recommended for these patients.

The results of our study should be extrapolated carefully because of several limitations. First, the number of enrolled patients. Second, all patients included in the study were not vaccinated against COVID-19. Third, all patients were hospitalized for COVID-19-associated pneumonia, therefore, none of the patients had a mild clinical course.

Further perspectives include an assessment of statin effects on the clinical course of COVID-19 in overweight patients, studying soluble interleukin-2 receptor levels in patients receiving statins, and checking statin's impact on olfactory disorders.

CONCLUSIONS

Statins didn't affect the severity and lethality rate in hypertensive patients with COVID-19-associated pneumonia. Subgroup analysis showed that statin use was associated with a decrease in morbidity of patients aged 65 years and older with BMI ≥25.0 kg/m^2 hospitalized for COVID-19-associated pneumonia.

REFERENCES

1. Hamed S.M., Elkhatib W.F., Khairalla A.S. et al. Global dynamics of SARS-CoV-2 clades and their relation to COVID-19 epidemiology. Sci Rep. 2021;11:8435. doi:10.1038/s41598-021-87713-x.
2. Ejaz H., Alsrhani A., Zafar A. et al. COVID-19 and comorbidities: Deleterious impact on infected patients. J Infect Public Health. 2020;13:1833–1839. doi:10.1016/j.jiph.2020.07.014.
3. Sanyaolu A., Okorie C., Marinkovic A. et al. Comorbidity and its Impact on Patients with COVID-19. SN Compr Clin Med. 2020;2:1069–76. doi:10.1007/s42399-020-00363-4.
4. Gold M.S., Sehayek D., Gabrielli S. et al. COVID-19 and comorbidities: a systematic review and meta-analysis. Postgrad Med. 2020;132:749–55. doi:10.1080/00325481.2020.1786964.

5. Oesterle A., Laufs U., Liao J.K. Pleiotropic effects of statins on the cardiovascular system. Circ Res. 2017;120:229–43. doi:10.1161/CIRCRESAHA.116.308537.
6. Kouhpeikar H., Khosaravizade Tabasi H., Khazir Z. et al. Statin use in COVID-19 hospitalized patients and outcomes: A retrospective study. Front Cardiovasc Med. 2022;9:820260. doi:10.3389/fcvm.2022.820260.
7. Kleindorfer D.O., Towfighi A., Chaturvedi S. et al. 2021 guideline for the prevention of stroke in patients with stroke and transient ischemic attack: A guideline from the American heart association/American stroke association. Stroke. 2021;52:e364–467. doi:10.1161/STR.0000000000000375.
8. Collet J-P., Thiele H., Barbato E. et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur Heart J. 2021;42:1289–367. doi:10.1093/eurheartj/ehaa575.
9. Chen S-T., Huang S-T., Shau W-Y. et al. Long-term statin adherence in patients after hospital discharge for new onset of atherosclerotic cardiovascular disease: a population-based study of real world prescriptions in Taiwan. BMC Cardiovasc Disord. 2019;19:62. doi:10.1186/s12872-019-1032-4.
10. Pietrzykowski Ł., Michalski P., Kosobucka A. et al. Medication adherence and its determinants in patients after myocardial infarction. Sci Rep. 2020;10:12028. doi:10.1038/s41598-020-68915-1.
11. Williams B., Mancia G., Spiering W. et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018;39:3021–104. doi:10.1093/eurheartj/ehy339.
12. Visseren F.L.J., Mach F., Smulders Y.M. et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021;42:3227–337. doi:10.1093/eurheartj/ehab484.
13. Memel Z.N., Lee J.J., Foulkes A.S. et al. Association of statins and 28-day mortality rates in patients hospitalized with severe acute respiratory syndrome Coronavirus 2 infection. J Infect Dis. 2022;225:19–29. doi:10.1093/infdis/jiab539.
14. Diaz-Arocutipa C., Melgar-Talavera B., Alvarado-Yarasca Á. et al. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. Int J Infect Dis. 2021;110:374–81. doi:10.1016/j.ijid.2021.08.004.
15. Umakanthan S., Senthil S., John S. et al. The Effect of Statins on Clinical Outcome Among Hospitalized Patients With COVID-19: A Multi-Centric Cohort Study. Front Pharmacol. 2022;13:742273. doi: 10.3389/fphar.2022.742273.
16. Kollias A., Kyriakoulis K.G., Kyriakoulis I.G. et al. Statin use and mortality in COVID-19 patients: Updated systematic review and meta-analysis. Atherosclerosis. 2021;330:114–21. doi:10.1016/j.atherosclerosis.2021.06.911.
17. Zein A.F.M.Z., Sulistiyana C.S., Khasanah U. et al. Statin and mortality in COVID-19: a systematic review and meta-analysis of pooled adjusted effect estimates from propensity-matched cohorts. Postgrad Med J. 2022;98(1161):503-508. doi: 10.1136/postgradmedj-2021-140409.
18. Ayeh S.K., Abbey E.J., Khalifa B.A.A. et al. Statins use and COVID-19 outcomes in hospitalized patients. PLoS One. 2021;16:e0256899. doi:10.1371/journal.pone.0256899.
19. Peymani P., Dehesh T., Aligolighasemabadi F. et al. Statins in patients with COVID-19: a retrospective cohort study in Iranian COVID-19 patients. Transl Med Commun. 2021;6:3. doi:10.1186/s41231-021-00082-5.
20. El-Solh A.A., Lawson Y., El-Solh D.A. All-cause mortality in COVID-19 patients receiving statin therapy: analysis of veterans affairs database cohort study. Intern Emerg Med. 2022;17(3):685-94. doi: 10.1007/s11739-021-02848-z.
21. Diaz-Arocutipa C., Melgar-Talavera B., Alvarado-Yarasca Á. et al. Statins reduce mortality in patients with COVID-19: an updated meta-analysis of 147 824 patients. Int J Infect Dis. 2021;110:374-81. doi: 10.1016/j.ijid.2021.
22. Santosa A., Franzén S., Nåtman J. et al. Protective effects of statins on COVID-19 risk, severity and fatal outcome: a nationwide Swedish cohort study. Sci Rep. 2022;12(1):12047. doi: 10.1038/s41598-022-16357-2.
23. Kow C.S., Hasan S.S. The Association Between the Use of Statins and Clinical Outcomes in Patients with COVID-19: A Systematic Review and Meta-analysis. Am J Cardiovasc Drugs. 2022;22(2):167-81. doi: 10.1007/s40256-021-00490-w.
24. Jang H.J., Leem A.Y., Chung K.S. et al. Soluble IL-2R Levels Predict in-Hospital Mortality in COVID-19 Patients with Respiratory Failure. J Clin Med. 2021;10(18):4242. doi: 10.3390/jcm10184242.
25. Damoiseaux J. The IL-2—IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor. Clin. Immunol. 2020;218:108515. doi: 10.1016/j.clim.2020.108515.
26. Ma A., Zhang L., Ye X. et al. High Levels of Circulating IL-8 and Soluble IL-2R Are Associated With Prolonged Illness in Patients With Severe COVID-19. Front. Immunol. 2021;12:626235. doi: 10.3389/fimmu.2021.626235.
27. Kaya H., Kaji M., Usuda D. Soluble interleukin-2 receptor levels on admission associated with mortality in coronavirus disease 2019. International Journal of Infectious Diseases. 2021;105:522–4. doi: 10.1016/j.ijid.2021.03.011.
28. Zhang Y., Wang X., Li X. et al. Potential contribution of increased soluble IL-2R to lymphopenia in COVID-19 patients. Cell Mol Immunol. 2020;17(8):878-80. doi: 10.1038/s41423-020-0484-x.
29. Aanand P., Angral S., Varshney S. et al. Incidence of anosmia among Covid 19 patients in India. Indian J Otolaryngol Head Neck Surg. 2021:1–10. doi:10.1007/s12070-021-02641-6.
30. Saniasiaya J., Islam M.A., Abdullah B. Prevalence of olfactory dysfunction in Coronavirus disease 2019 (COVID-19): A meta-analysis of 27,492 patients. Laryngoscope. 2021;131:865–78. doi:10.1002/lary.29286.

31. Lechien J.R., Cabaraux P., Chiesa-Estomba C.M. et al. Objective olfactory evaluation of self-reported loss of smell in a case series of 86 COVID-19 patients. Head Neck. 2020;42:1583–90. doi:10.1002/hed.26279.
32. Fodoulian L., Tuberosa J., Rossier D. et al. SARS-CoV-2 receptors and entry genes are expressed in the human olfactory neuroepithelium and brain. IScience. 2020;23:101839. doi:10.1016/j.isci.2020.101839.
33. Lemprière S. SARS-CoV-2 detected in olfactory neurons. Nat Rev Neurol. 2021;17:63. doi:10.1038/s41582-020-00449-6.
34. de Melo G.D., Lazarini F., Levallois S. et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. Sci Transl Med. 2021;13:eabf8396. doi:10.1126/scitranslmed.abf8396.
35. Saeedi Saravi S.S., Saeedi Saravi S.S., Arefidoust A. et al. The beneficial effects of HMG-CoA reductase inhibitors in the processes of neurodegeneration. Metab Brain Dis. 2017;32:949–65. doi:10.1007/s11011-017-0021-5.
36. Kandemirli S.G., Altundag A., Yildirim D. et al. Olfactory bulb MRI and paranasal sinus CT findings in persistent COVID-19 anosmia. Acad Radiol. 2021;28:28–35. doi:10.1016/j.acra.2020.10.006.
37. Kim H.Y., Kim J.H., Dhong H-J. et al. Effects of statins on the recovery of olfactory function in a 3-methylindole-induced anosmia mouse model. Am J Rhinol Allergy. 2012;26:e81-4. doi:10.2500/ajra.2012.26.3719.
38. Khani E., Khiali S., Beheshtirouy S., Entezari-Maleki T. Potential pharmacologic treatments for COVID-19 smell and taste loss: A comprehensive review. Eur J Pharmacol. 2021;912:174582. doi: 10.1016/j.ejphar.2021.174582.
39. Khalaf K., Johnell K., Austin P.C. et al. Low adherence to statin treatment during the 1st year after an acute myocardial infarction is associated with increased 2nd-year mortality risk-an inverse probability of treatment weighted study on 54 872 patients. Eur Heart J Cardiovasc Pharmacother. 2021;7:141–7. doi:10.1093/ehjcvp/pvaa010.

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

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Received: 07.06.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

GENETIC FEATURES OF CHILDREN WITH IDIOPATHIC SHORT STATURE

DOI: 10.36740/WLek202302111

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ABSTRACT

The aim: To investigate the clinical and genetic characteristics of children with idiopathic short stature, taking into account the polymorphism of the vitamin D receptor (VDR) BsmI gene.

Materials and methods: Eighteen children diagnosed with of idiopathic short stature who were treated at the State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine» were examined. The following values were taken into account: the patient's sex and age, anthropometric data, vitamin D level in the blood (excluding summer months of patient recruitment), bone age, basal growth hormone (GH) level and its level after stimulation tests (clonidine, insulin), IGF-1 levels, blood levels of total and ionized calcium and VDR gene polymorphism.

Results: The A allele carriers of the polymorphic locus BsmI (rs1544410) of the VDR gene are significantly associated with the risk of developing idiopathic short stature OR = 4.47 (95% CI 2.11-9.48; p <0.05). The risk of idiopathic short stature is significantly higher OR = 9.33 (95% CI 3.09-28.16; p <0.05) in children with the presence of the G/A genotype. Vitamin D deficiency (43.83 ± 6.47 nmol/l) was found in children with the BsmI polymorphic variant G/G VDR, and vitamin D insufficiency was found in children with BsmI polymorphic variants G/A and A/A VDR (58.14 ± 20.05 and 51.58 ± 22.84 nmol/l, respectively).

Conclusions: The data obtained regarding the polymorphic locus BsmI (rs1544410) of the of VDR gene cannot exclude it's the involvement in the pathogenesis of idiopathic short stature.

KEY WORDS: children, polymorphism, idiopathic short stature, VDR gene

Wiad Lek. 2023;76(2):320-325

INTRODUCTION

Low growth of the child is associated with many factors, including growth hormone deficiency, neurosecretory disorder of the hormonal axis of somatotropic hormone (GH), intrauterine growth retardation, low idiopathic growth, hypothyroidism and malnutrition. Among them, idiopathic short stature (ISS) is the most common diagnosis, accounting for 60-80% of children with growth retardation [1]. The diagnosis of idiopathic short stature is based on the following features: short but otherwise healthy children, an alteration of growth (height<-2 SD) – the child's height is more than two standard deviation score rates below the mean for age and sex (less than the 3rd percentile), body length and weight at birth are normal, absence of systemic and endocrine diseases, normal magnetic resonance imaging scans, there are no nutritional deficiencies and chromosomal abnormalities. ISS is characterized by proportionally short stature for no apparent reason [2], with a normal response of growth hormone during stimulation tests (>10 ng/ml).

The causes of ISS are diverse and complex, including mutation in the GH receptor gene, a short deletion of the homeobox gene, the dysfunction of GH secretion and structural abnormalities of this hormone [3]. ISS not only affects the growth of children, but also easy leads to abnormal bone development, nervous system dysfunction and increased incidence of chronic cardiopulmonary disease and has a great impact on the children's quality of life in the future [4].

Studies have shown that over 95% of active serum vitamin D is 25-hydroxy vitamin D [25-(OH)D], and serum 25-(OH)D is the most abundant form of serum vitamin D, followed by 1.25-$(OH)_2$D. Although serum 1.25-$(OH)_2$D has the highest biological activity, the half-life of this indicator is short and difficult to quantify. 25-(OH)D is a stable indicator, it is easy to study in blood serum. Currently, it is considered an indicator that reflects the level of vitamin D in the body. Thanks to it, the metabolism of calcium and phosphorus is regulated in the human body, calcium reabsorption is activated in the intestine, as well as calcium deposition in the bones [5].

IGF-1, a single-chain polypeptide, is primarily produced by the liver, the skeletal muscle, and many other tissues in response to GH stimulation. It promotes the growth and differentiation of bones and tissues, as well as the production of muscle mass, thus providing an important regulatory effect on the growth of the body. IGF-1 is closely related to GH levels. Synthesis and secretion of IGF-1 are regulated by GH levels, while IGF-1 levels can generate a negative feedback to regulate the pituitary growth hormone secretion. At the same time, IGF-1 also plays a key role in slowing down the growth of glucose and fat metabolism and metabolic syndrome [6, 7].

THE AIM

The aim was to study the clinical and genetic characteristics of children with idiopathic short stature, taking into account the polymorphism of the *VDR* BsmI gene.

MATERIALS AND METHODS

Eighteen children were examined with diagnosis of idiopathic short stature, who were treated at the State Institution «V.P.Komisarenko Institute of Endocrinology and Metabolism of NAMS of Ukraine».

Patient's sex and age, anthropometric data, vitamin D level in the blood (patients were recruited from September to May), bone age, GH level after stimulation tests (clonidine, insulin), IGF-1 levels, blood levels of total and ionized calcium were analyzed. The mean age of children (14 boys, 4 girls), who were included in the study, was 10.46 ± 3.43 years. The mean growth retardation was minus 2.12 (±0.50) SDS. At the time of examination, all the patients were euthyroid. The study included patients who had not received calcium and vitamin D drugs for 6 months.

The serum 25-hydroxycalciferol (25(OH)D) level was determined by immune-chemiluminescent method. The GH and IGF-1 levels were studied using radioimmunoassay and enzyme immunoassay methods. The control group consisted of 112 healthy children and adolescents aged 9 to 18 (mean age 8.24 ± 3.83 years) [8].

According to the classification, adopted by the International Institute of Medicine (2011), the vitamin D deficiency in children and adults is considered when serum 25(OH)D levels are below 20 ng/ml or 50 nmol/l. The serum 25(OH)D level from 21 ng/ml to 29 ng/ml (from 50.1 to 74.9 nmol/l) should be considered as the vitamin D insufficiency. The normal level of vitamin D is equal to the serum 25(OH)D concentration above 30 ng/ml (more than 75.0 nmol/l).

The determination of the BsmI (rs1544410) polymorphism in the *VDR* gene was performed using the polymerase chain reaction (PCR) method, followed by analysis of the length of the restriction fragments upon their detection by agarose gel electrophoresis.

For genotyping, the venous blood was collected under sterile conditions in 2.7 ml monovetes with potassium salt of ethylenediaminetetraacetic acid ("Sarstedt", Germany) which served as an anticoagulant. First, DNA was eliminated from the peripheral blood using a commercial Quick-DNA$_{TM}$ Mini-prep Plus Kit (manufactured by Zymo Research, USA).

The genes studied were amplified using specific primers (manufactured by Metabion, Germany) and the commercial Dream Taq Green PCR Master Mix (manufactured by Thermo Scientific, USA).The tubes with the final amplification mixture were transferred to the Flex Cycler BU amplifier (Analytic Jena, Germany) to provide the appropriate temperature regime.

The amplification products of DNA fragments (amplicons) of the *VDR* gene were subjected to hydrolytic cleavage by restriction endonuclease BsmI (manufactured by Thermo Scientific, USA), respectively. Separate mixtures were prepared for restriction analysis, and transferred to pre-labeled tubes, and then amplicons were added.

The fragment limiting reaction for the BsmI G/A (rs1544410) of the *VDR* gene was performed according to the manufacturer's recommendations in a solid-state microthermostat at 37°C for 16 hours.

The process was stopped by increasing the temperature to 65°C for 20 minutes. The state of the restriction fragments of the *VDR* gene was analyzed by a 3% agarose gel (agarose from firm Cleaver Scientific, UK), with the addition of ethidium bromide, a marker of molecular weight "Gene Ruler 50 bp DNA Ladder" ("Thermo Scientific", USA) and subsequent visualization using a transilluminator stained with ethidium bromide by computer program "Vitran".

Amplifiers of the BsmI G/A (rs1544410) of the VDR gene were subjected to hydrolytic cleavage in the presence of the 5'-GAATGCN -3'restriction site, resulting in the restricts (the restriction formations) with a molecular mass a 644 bp and 179 bp – GG genotype.

The restriction site disappeared with nucleotide replacement from G to A, if the size of the amplified DNA fragments remained unchanged after interaction with the restriction nuclease (823 bp), then the AA genotype was recorded. Accordingly, all three types of fragments: 823, 644 and 179 p.p.in the heterozygous genotype (GA) were simultaneously observed.

Statistical processing of the study results was performed using Microsoft Excel statistical programs. Laboratory parameters were presented in the form of arithmetic data (mean (M ± m).

The strength of association of the analyzed signs was determined using the value of the odds ratio (OR), which was carried out according to the standard formula.

A confidence interval (CI) was calculated for OR at 95% significance level. If the odds ratio was less than 1, then the risk decreased, if = 1, then there was no risk, if more than 1, then the risk was present (Fletcher et al., 1998). All data were analyzed by nonparametric methods of variational statistics using a computer program MedCalc (2006).

The study was conducted in accordance with the basic principles of bioethics of the Council of Europe Convention on Human Rights and Biomedicine (the 4th of April1997), the World Health Association Helsinki Declaration on Ethical Principles for Conducting Medical Research with the Participation of People (1964-2013).Commission on Biomedical Ethics of the State Institution "V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine" did not reveal violations of moral and legal norms during the study. Informed consent was obtained from participants and their parents.

RESULTS

Vitamin D receptor plays a key role in bone mineralization, intestinal calcium absorption and cell differentiation [9]. The VDR gene is located on chromosome 12q13.1 and consists of a 5 'promoter encoding exons and a 3' regulatory region.

Acting through its own receptor, the hormone-active form of vitamin D – 1.25$(OH)_2$D can cause many effects that affect various biological processes in the body.

Functional studies of each polymorphism in the *VDR* gene are ongoing, and several studies have shown how the VDR polymorphisms have been associated with adult height [9-14]. Most of them studied the single-nucleotide polymorphisms (SNPs) of VDRs on coding exons and in the 3'-regulatory region, where the polymorphic locus BsmI (rs1544410) of the *VDR* gene is located.

Analysis of the distribution of allele and genotype frequencies of the polymorphic locus BsmI (rs1544410) of the VDR gene in the group of patients with ISS and in the control sample [8] was statistically significant (Table I).

The allele frequency of the VDR BsmI polymorphism was 44,4% for the G allele (n = 16) and55,6% for the A allele (n = 20). The study found that the Allele Carriers of the BsmI (rs1544410) polymorphic locus of the vitamin D receptor gene (rs11568820) was significantly associated with a risk of developing idiopathic short stature OR=4.47 (95%CI 2.11-9.48; p<0.05).

It was also shown that the risk of idiopathic short stature was significantly high in the presence of the G/A genotype, OR=9.33 (95%CI 3.09-28.16; p<0.05); also in G/G genotype variant, the risk of idiopathic short stature was increased but not significantly4.13 (95%CI 0.63-26.65; p=0,14); in the A/A genotype variant, the risk of idiopathic short stature was significantly decreased OR=0.07 (95%CI 0.02-0.25; p<0.001).

Mean values of height, weight, height SDS, serum 25(OH)D in the studied group (18 patients) were 123.49±19.62 cm, 26.96±11.11 kg, -2.25±0.85, 48.86±16.71nmol/l, respectively, level of total calcium – 2.40±0.12 mmol/l, serum phosphorus – 1.43±0.11 mmol/l (Table II).

Idiopathic short stature was significantly lower in children with polymorphic G/A variant of genotype compared to G/G and A/A genotypes in children.

The low level of vitamin D was found in all the children with idiopathic short stature regardless of the BsmIrs1544410 polymorphic locus of the vitamin D receptor gene. Vitamin D deficiency (43.83±6.47 nmol/l) was observed in children with the BsmI polymorphic variant of G/G VDR and vitamin D insufficiency (58.14±20.05 and 51.58±22.84 nmol/l, respectively) – in children with the BsmI polymorphic variants of G/A and A/A VDR.

Basal level of GH was normal in all the patients examined. The GH level after the clonidine stimulation test was within the normal limits.

Growth SDS (Standard Deviation Score) was significantly lower in the group of children with polymorphic variant A / A(-2.61±0.38) compared to variants of the BsmI (rs1544410) polymorphic locus of the VDR gene G/A (-1.92±0.45) and G/G (-2.39±0.02).

IGF-1 in all surveyed was within the norm, however, the lowest rate was in patients with polymorphic variant G/G VDR BsmI (108.50±12.02 ng/ml). Normal levels of total and ionized calcium, and phosphorus in serum were found in all the patients examined.

DISCUSSION

An active form of vitamin D is the regulator of calcium metabolism, regulator of calcium absorption in the intestine as well as a direct factor which affects the metabolism and modeling of bone tissue.

The *VDR* gene is consisted of a 5 'promoter (from exon 1a to f), encoding exons (exon 2-9), and a 3' regulatory region (3'-untranslated region) in chromosome 12q13.1. In each nucleotide of the *VDR* gene, polymorphism can occur by chance. As a result of the polymorphism of the *VDR* gene, labeled as genetic variants which occurs in at least 1% of the population, it affects the level of VDR protein in target organs and causes numerous diseases.

Table I. Distribution of allele and genotype frequencies of the polymorphic locus BsmI (rs1544410) of vitamin D receptor gene in the group of patients with idiopathic short stature and in the control sample

Groups and number of individuals (n)	Allele frequencies, %		χ 2; df = 1	Genotype frequencies, %			χ 2; df = 2
	G	A		G/A	G/G	A/A	
Population sample (112)*	34 (15.2)	190 (84.8)	17.1 p<0.001	28 (25.0)	3 (2.7)	81 (72.3)	9.45 p=0.003
Patients with idiopathic short stature	16 (44.4)	20 (55.6)		12 (66.7)	2 (11.1)	4 (22.2)	

Note: Hardy-Weinberg equilibrium for BsmI (P = 0.14)
* – data from source 8.

Table II. Analysis of growth indicators and some serum biochemical parameters in children with idiopathic short stature depending on the polymorphism of the VDR gene

Values	Genotype		
	BsmI (rs1544410), n = 18		
	G/A	G/G	A/A
Growth SDS	-1.92±0.45	-2.39±0.02*	-2.61±0.38**
Basal GH level	1.02±1.34	0.84±0.81	0.11±0.04
GH level after stimulation test with clonidine, ng / ml	14.49±4.49	14.70±2.83	15.38±5.58
25(OH)D, nmol/l	58.14±20.05	43.83±6.47	51.58±22.84
IGF-1, ng / ml	141.48±64.40	108.50±12.02	151.58±66,74
The total calcium, mmol/l	2.42±0.05	2.37±0.02	2.46±0.04
Calcium ionized, mmol/l	1.27±0.17	1.21±0.02	1.24±0.06
Serum phosphorus, mmol / l	1.45±0.17	1.48±0.04	1.39±0.11

Note: * - the significance level between values of the BsmI (rs1544410) GA and GG genotypes (p<0.05); ** - the significance level between the values of BsmI (rs1544410) GA and AA genotypes (p<0.05).

Polymorphisms of Bsm I, Apa I and Taq I in the 3 'untranslated region are associated with the regulation of RNA stability and half-life and lead to a better response to vitamin D in target tissues [15]. Polymorphism also regulates osteocalcin, the hormone that promotes the insulin secretion by the pancreas and is a biochemical marker of bone formation [16]. Several studies have examined the association between the above *VDR* gene polymorphisms and growth [9-10, 13-14, 17]. Barchitta M et al. [18] studied the association between Bsm I polymorphism of the VDR gene and growth in full-term newborns of Caucasian male.

In a study by Wang W. et al. [19] children with the A/G genotype showed a higher growth rate than those with the G/G genotype, which can be seen in our study and may serve as a short-term marker of growth potential.

The greatest interest in the study of the vitamin D functions is primarily explained by its active participation in calcium homeostasis, cyst formations and the regulation of the bone mineral density. Vitamin D binds to a specific steroid receptor encoded by the *VDR* gene. This gene acts as a transcription factor and influences the expression activity of numerous target genes. An in-depth study of the *VDR* gene made it possible to identify polymorphic variants which can lead to structural or functional changes in protein expression. Therefore, these options may be potential clinical and diagnostic markers of musculoskeletal pathology. However, studies of the *VDR* gene are ongoing, and a number of studies have shown conflicting data on the distribution of genotype frequencies of various loci of this gene [20-21], which creates the basis for further work in this area.

The level of vitamin D in our study was the lowest in the group of children with the G/G genotype, and the highest one – in children with the heterozygous genotype (G/A), which is consistent with the study by Marozik P.[22].

CONCLUSIONS

The allele frequency of the VDR BsmI polymorphism was 44,4% for the G allele (n = 16) and 55,6% for the A allele (n = 20). G allele carriers of the *BsmI* (rs1544410) polymorphic locus of the VDR gene (rs11568820) was significantly associated with the risk of developing idiopathic short stature OR=4.47 (95%CI 2.11-9.48; p<0.05).

It was also shown that the risk of idiopathic short stature was significantly high OR=9.33 (95%CI 3.09-28.16; p<0.05) in the presence of the G/G genotype.

The vitamin D deficiency (43.83±6.47 nmol/l) was detected in children with the BsmI polymorphic variant

of G/G VDR and vitamin D insufficiency was detected in children with the BsmI polymorphic variant of G/A and A/A VDR (58.14±20.05 and 51,58±22,84 nmol/l, respectively).

The data obtained regarding the polymorphic locus BsmI (rs1544410) of the of VDR gene cannot exclude it's the involvement in the pathogenesis of idiopathic short stature.

REFERENCES

1. Atalay A., McCord M. Characteristics of failure to thrive in a referral population. Clinical Pediatrics. 2012; 51(3): 219-225. doi: 10.1177/0009922811421001.
2. Inzaghi E., Reiter E., Cianfarani S. The challenge of defining and investigating the causes of idiopathic short stature and finding an effective therapy. Hormone Research in Paediatrics. 2019; 92(2): 71-83. doi: 10.1159/000502901.
3. Li J., Zhang X., Xie S. et al. Analysis of the Influence of High-Dose rhGH Therapy on Serum Vitamin D and IGF-1 Levels in School-Age Children with Idiopathic Short Stature. Evid Based Complement Alternat Med. 2021. doi: 10.1155/2021/5776487.
4. Chi Z., Tan S., Li W. et al. In vitro cytotoxicity of decabrominated diphenyl ether (PBDE-209) to human red blood cells (hRBCs). Chemosphere. 2017; 180: 312-316. doi: 10.1016/j.chemosphere.2017.04.032.
5. Wang W., Luo X.P., Cai L.X. et al. Relationship between vitamin D receptor (VDR) polymorphisms and the efficacy of recombinant human growth hormone (rhGH) treatment in children with idiopathic short stature. Genetics and Molecular Research. 2015; 14(3): 10507-10514. doi: 10.4238/2015.september.8.12.
6. Wang D.D., Sun M., Wang X., Cheng Y.Y. Changes in serum levels of IGF-1, ghrelin and nesfatin-1 and clinical significance after treatment with recombinant human growth hormone in children with idiopathic short stature. Journal of Biological Regulators and Homeostatic Agents. 2019; 33(6): 1759-1763. doi: 10.23812/19-231-L.
7. Choi S.K., Park M.S., Song J.K. et al. Association of polymorphisms in the vitamin D receptor promoter with idiopathic short stature. J Korean Med Sci. 2013; 28(9): 1329-1333. doi: 10.3346/jkms.2013.28.9.1329.
8. Wang Y., Cui Z.Q., Luo T.B., Liu L. Correlations of VDR and VDBP genetic polymorphisms with susceptibility to adolescent idiopathic scoliosis and efficacy of brace treatment. Genomics. 2016; 108(5-6): 194-200. doi: 10.1016/j.ygeno.2016.11.004.
9. van der Sluis I.M., de Muinck Keizer-Schrama S.M., Krenning E.P. et al. Vitamin D receptor gene polymorphism predicts height and bone size, rather than bone density in children and young adults. Calcif Tissue Int. 2003; 73(4): 332-338. doi: 10.1007/s00223-002-2130-2.
10. Xiong D.H., Xu F.H., Liu P.Y. et al. Vitamin D receptor gene polymorphisms are linked to and associated with adult height. J Med Genet. 2005; 42(3): 228-234. doi: 10.1136/jmg.2004.024083.
11. Dempfle A., Wudy S.A., Saar K. et al. Evidence for involvement of the vitamin D receptor gene in idiopathic short stature via a genome-wide linkage study and subsequent association studies. Hum Mol Genet. 2006; 15(18): 2772-2783. doi: 10.1093/hmg/ddl218.
12. d'Alésio A., Garabédian M., Sabatier J.P. et al. Two single-nucleotide polymorphisms in the human vitamin D receptor promoter change protein-DNA complex formation and are associated with height and vitamin D status in adolescent girls. Hum Mol Genet. 2005; 14(22): 3539-3548. doi: 10.1093/hmg/ddi382.
13. Fang Y., van Meurs J.B., Rivadeneira F. et al. Vitamin D receptor gene haplotype is associated with body height and bone size. J Clin Endocrinol Metab. 2007; 92(4): 1491-1501. doi: 10.1210/jc.2006-1134.
14. Jorde R., Svartberg J., Joakimsen R.M., Grimnes G. Associations between polymorphisms related to calcium metabolism and human height: the Tromsø Study. Ann Hum Genet. 2012; 76(3): 200-210. doi: 10.1111/j.1469-1809.2012.00703.x.
15. Wimalawansa S.J. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol. 2018; 175: 177-189. doi: 10.1016/j.jsbmb.2016.09.017.
16. Contreras-Bolívar V., García-Fontana B., García-Fontana C., Muñoz-Torres M. Mechanisms Involved in the Relationship between Vitamin D and Insulin Resistance: Impact on Clinical Practice. Nutrients. 2021; 13(10): 3491. doi: 10.3390/nu13103491.
17. Bao L., Chen M., Lei Y. et al. Association between vitamin D receptor BsmI polymorphism and bone mineral density in pediatric patients: A meta-analysis and systematic review of observational studies. Medicine (Baltimore). 2017; 96(17): e6718. doi: 10.1097/MD.0000000000006718.
18. Barchitta M., Maugeri A., La Rosa M.C. et al. Single Nucleotide Polymorphisms in Vitamin D Receptor Gene Affect Birth Weight and the Risk of Preterm Birth: Results From the "Mamma & Bambino" Cohort and A Meta-Analysis. Nutrients. 2018; 10(9): 1172. doi: 10.3390/nu10091172.
19. Wang W., Luo X.P., Cai L.X. et al. Relationship between vitamin D receptor (VDR) polymorphisms and the efficacy of recombinant human growth hormone (rhGH) treatment in children with idiopathic short stature. Genet Mol Res. 2015; 14(3): 10507-10514. doi: 10.4238/2015.
20. Zhang L., Yin X., Wang J. et al. Associations between VDR Gene Polymorphisms and Osteoporosis Risk and Bone Mineral Density in Postmenopausal Women: A systematic review and Meta-Analysis. Sci Rep. 2018; 8(1): 981. doi: 10.1038/s41598-017-18670-7.
21. Wang S., Ai Z., Song M. et al. The association between vitamin D receptor FokI gene polymorphism and osteoporosis in postmenopausal women: a meta-analysis. Climacteric. 2021; 24(1): 74-79. doi: 10.1080/13697137.2020.1775806.

22. Marozik P., Rudenka A., Kobets K., Rudenka E. Vitamin D Status, Bone Mineral Density, and VDR Gene Polymorphism in a Cohort of Belarusian Postmenopausal Women. Nutrients. 2021; 13(3): 837. doi: 10.3390/nu13030837.

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Received: 08.02.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

USE OF INFLIXIMAB TO ATTENUATE CEREBRAL APOPTOSIS INDUCED BY CEREBRAL ISCHEMIA/REPERFUSION IN MALE RATS

DOI: 10.36740/WLek202302112

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ABSTRACT

The aim: The purpose of the research was to study the role of infliximab global cerebral ischemia-reperfusion injury.

Materials and methods: The rats were split into five groups: Sham group; Control group: occlusion of the common carotid artery for 60 minutes, and subsequently reperfusion for an hour without receiving any medication; Vehicle group: as the control group, but 72 hours before to the ischemia, they were given the medication 0.9 NaCl intraperitoneally (i.p); Treated group-1: as the control group, plus 3 mg/kg of IFX intraperitoneally (i.p) 72 hours prior to ischemia; Treated group-2: as the control group, plus 7 mg/kg of IFX intraperitoneally (i.p) 72 hours prior to ischemia.

Results: Pre-treatment with IFX significantly reduced the percentage of infarct area, but in the IFX (7 mg/kg) group, the infarct area was smaller than at the low dose. The ischemia group had a significant elevated of TNF-α and caspase-3 while a significant lowered in CAT and SOD levels. The pre-treatment with IFX, the TNF-α and caspase-3 levels lowered significantly, furthermore, significantly increased CAT and SOD levels activity ($P \leq 0.05$) as compared with IR group. Among effective groups, I/R+IFX (7mg/kg) group more effective in lowering TNF-α and caspase than I/R+IFX (3mg/kg) group.

Conclusions: Infliximab has neuroprotective effective due to its powerful TNF-α blocker and limit ROS release and cell death signaling which protects the neurons from injury during cerebral ischemia reperfusion.

KEY WORDS: infliximab, apoptosis, ischemia-reperfusion injury, TNF-α

Wiad Lek. 2023;76(2):326-331

INTRODUCTION

Cerebral ischemia-reperfusion injury (CIR) is inability of cerebral tissues to receive enough oxygen and nutrients due to impaired blood flow, followed by the restoration of blood flow, which may cause inflammatory conditions and the production of free radicals, which may worsen the local injury and impair the function [1]. Harmful free radicals may be produced during cerebral ischemia as a result of anaerobic hydrolysis, conversion of ATP to AMP, and other processes. On the other hand, the rise of TNF-α induces NF-kB activation through death domain adapter molecules, which in turn triggers a signaling cascade, releases NF-kB, reaches the nucleus, and finally induces the activation of target genes [2, 3]. As a result, the restoration of the ATP levels during reperfusion enables dynamic Ca^{+2} uptake by the mitochondria and results in the opening of the mitochondrial permeability transition pore (MPTP). Thus, protons and certain molecules associated with apoptosis, such as cytochrome C and capase-3 are thus free to enter the mitochondria. Meanwhile, the influx of ions and water results in enlargement of the mitochondria [4]. Neurological impairment and changes in ethology may be the result from cerebral reperfusion injury, and caspase-3 activation may be related to neuronal apoptosis [5]. TNF-α controls the inflammatory response, cell loss, and proliferation by triggering a number of intracellular pathways. These pathways are directly related to liver regeneration, hepatic damage, and mortality as well as brain injury [6]. TNF-α, ROS inhibitors could be intriguing novel treatments for cerebral ischemia-reperfusion injury in light of current findings. Infliximab is a monoclonal chimeric immunoglobulin G1 (IgG1) TNF-binding blocker [4]. IL-1b, IFN-g, IL-2, TNF-α, IL-6, and IL-17 are among the pro-inflammatory cytokines that it suppresses from being produced [7]. By inhibiting the TNF receptor, which causes lysis of immune cells and cause death in activated macrophages and T cells, and it may also reduce cell infiltration [8].

THE AIM

The purpose of the research was to study the role of infliximab global cerebral ischemia-reperfusion injury.

MATERIALS AND METHODS

ANIMALS

Twenty-five male albino rats (Rattus norvegicus), weighing between 200 and 250 g, were taken from the animal house at the Faculty of Science, Kufa University. The animals were fed a conventional animal food and had enough of water while kept in an animal home that was well-ventilated and maintained at a temperature of 25°C [9]. Guide for the Care and Use of Laboratory Animals were used during the inquiry, and the experiment received approval from the Al-Kufa University` Animal Care and Research Committee. After acclimation for one week, the rats were split into five groups as follows:

1. Sham group: The animals were given general anesthesia without blocking any of their common carotid arteries [10].
2. Control group: The animals had general anesthesia, occlusion of the common carotid artery for 60 minutes, and subsequently reperfusion for an hour without receiving any medication [10].
3. Vehicle group: The animals received the same surgical procedure as the control group, but 72 hours before ischemia, they were given the medication 0.9 NaCl intraperitoneally (i.p) [11].
4. Treated group-1: The animals received the same surgical treatment as the control group, plus 3 mg/kg of IFX intraperitoneally (i.p) 72 hours prior to ischemia [12].
5. Treated group-2: The animals received the same surgical treatment as the control group, plus 7 mg/kg of IFX intraperitoneally (i.p) 72 hours prior to ischemia [13].

INDUCTION OF CEREBRAL ISCHEMIA

In order to induce general anesthesia in the animals, ketamine and xylazine were administered intraperitoneally (i.p) [14]. As soon as the rats lost consciousness, the neck area was incised. The paratracheal muscles were then pulled by sterile artery forceps, and connective tissues were removed using spay force. The common carotid arteries were separated from the vagal nerve and blocked simultaneously on both sides (left and right) for a maximum of 60 minutes. The clamps were then removed and reperfusion started for an additional hour to induce global cerebral ischemia/reperfusion injury [15].

TTC STAIN EVALUATION

Cerebral infarction is evaluated using TTC staining. After 60 minutes of ischemia and 60 minutes of reperfusion, the rats were slaughtered. Brain tissues were sliced into coronal slices, dissolved in 0.2% PBS (w/v), and then stained with triphenyltetrazolium chloride (TTC) stain at 37°C for 30 min on a glass Petri dish with aluminum foil covering to prevent the effect of light on TTC stain were added paraformaldehyde at 10% [3]. Using digital images (digital camera) and image analysis tools, the infarct volume was determined (image J system) [16].

$$I\% = \frac{\text{White Area}}{\text{Total Area}} \times 100\%$$

I =Infarct percentage, white region = the infarction area and the red area = the valid area.

ELISA ASSAY

The levels of TNF-α, CAT, SOD and caspase-3 in cerebral infarction tissues were assessed using ELISA. In a nutshell, the supernatant was obtained by centrifuging rat infarct tissue in PBS buffer with protease inhibitors. The concentrations of TNF-α, CAT, SOD and caspase-3 in cerebral infarction tissues were measured using ELISA, and the precise methods were properly followed by the kit's instructions.

HISTOPATHOLOGICAL STUDY

After reperfusion, brain tissues were preserved in 10% formalin solution and embedded in a paraffin block. Sections of the brain were then cut into 5 µm thick pieces, stained with hematoxylin-eosin (H&E) and examined under a microscope by a professional pathologist. The method of pathological evaluation of this study was considered according to the following criteria [3, 16]:

- Normal (0): Edema, RBC and eosinophilic neurons are absent.
- Slight (1): Edema or eosinophilic neurons are present.
- Moderate (2): edema, eosinophilic neurons, and a few quantities of RBC are present.
- Severe (3): edema, eosinophilic neurons, RBC and necrosis are present.

STATISTIC EVALUATION

The SPSS 26.0 software was used to conduct the statistical analysis. Data are presented as the means standard deviation, and the findings represent are the mean of three independent experiments (SD). One-way analysis of variance (ANOVA) was used to compare experimental and control groups, and then Tukey's test was used to confirm the results. When $P \leq 0.05$, the differences were deemed significant.

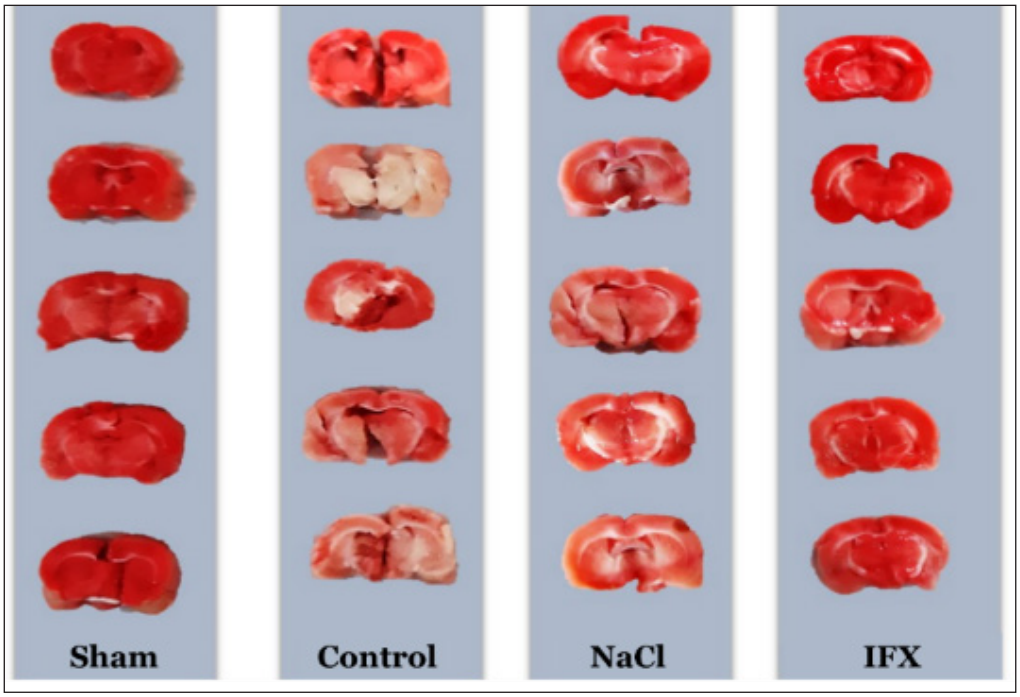

Fig. 1. Photograph of coronal slices of rat brain stained with TTC of all groups. Valid areas were colored red while infarcted areas were white.

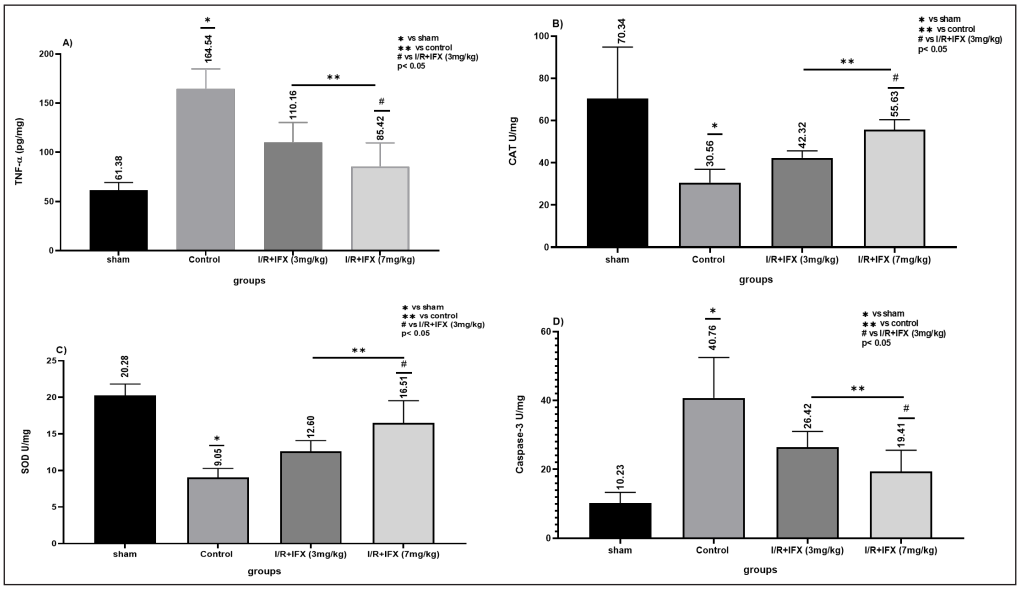

Fig. 2. Rat liver levels in response to infliximab treatment: A: TNF-α (pg/mL), B: CAT (U/mg protein), C: SOD (U/mg protein), and D: Caspase-3 (U/mg).

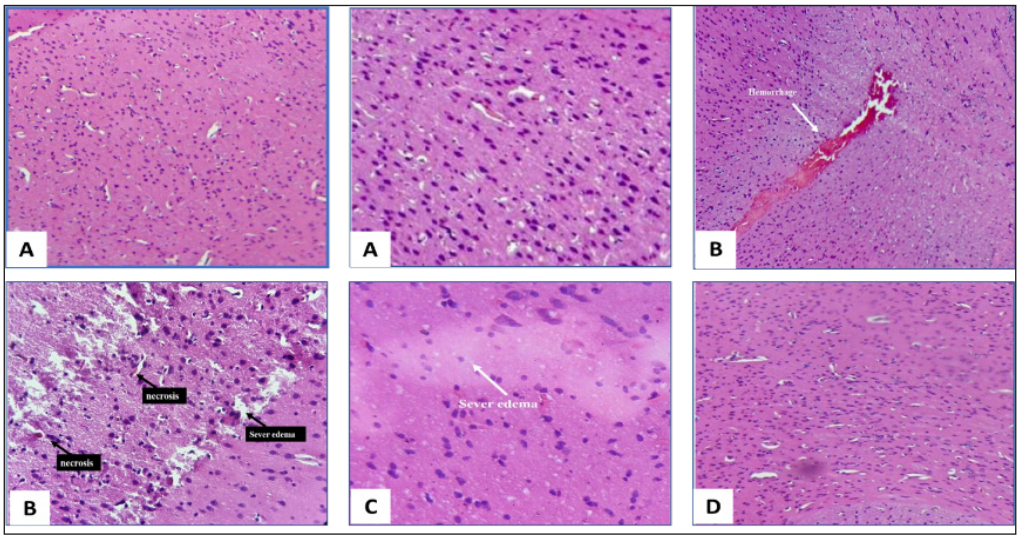

Fig. 3. An examination of the histopathology of the rat brain: (A): sham group, (B): control group - there is a significant amount of ischemic damage, (C): I/R+ IFX (3mg/kg) group, (D): I/R+ IFX (7mg/kg) group.

Table I. Biochemical findings in all study groups (n=5). Data are presented as mean ± SD.

Groups/markers	TNF-α (pg/mL)	CAT (U/mg protein)	SOD (U/mg protein)	Caspase-3 U/mg
Sham	61.38±5.63	70.34±16.68	20.28±1.09[b]	10.23±2.19[c]
Control	164.54±11.0[9a]	30.57±4.51[a]	9.05±0.87[a]	40.76±8.32[a]
I/R+IFX (3mg/kg)	110.16±14.28[b]	42.32±2.3[4b]	12.60±1.05[b]	26.42±3.25[b]
I/R+IFX (7mg/kg)	85.42±17.18[c]	55.63±3.39[c]	16.51±2.15[c]	19.41±4.35[c]

[a] vs sham group $p<0.05$ [b,c] vs I/R groups $p<0.05$

RESULTS

IFX REDUCED THE PERCENTAGE OF INFARCT AREA CAUSED BY IR INJURY

Our results revealed that pre-treatment with IFX significantly ($p < 0.05$) decreased the percentage of infarct area in the IFX (3mg/kg) and IFX (7mg/kg) groups compared to the control group (43.15% 7.72%). The percentage of infarction area between the IFX (7mg/kg) group and the sham group was not significantly different ($p > 0.05$), but the IFX group (7mg/kg) displayed the lowest infarct area (Fig 1).

EFFECT OF IFX ON BRAIN TISSUE LEVELS OF TNF-A, CAT, SOD, AND CASPASE-3

Compared to the sham group, the ischemia group showed a significant elevation ($P\leq0.05$) of TNF-α and caspase-3 while a significant lowering ($P\leq0.05$) in CAT and SOD levels. The pretreatment with IFX, the TNF-α and caspase-3 levels lowered significantly ($P\leq0.05$), furthermore, pretreatment significantly increased CAT and SOD levels activity ($P\leq0.05$) as compared with IR group. Among the effective groups, I/R+IFX (7mg/kg) group more effective in lowering TNF-α and caspase than the I/R+IFX (3mg/kg) group. Table I presents the biochemical findings of all study groups.

IFX REDUCED HISTOPATHOLOGIC SCORE THAT CAUSED BY IR-INJURY

We used a score to compare between the groups according to Abbas Al-Huesini L.M. et al. [16]. Compared to the sham group, histopathological scores in both the control group and the vehicle groupë increased significantly ($p < 0.05$). The histological analysis revealed that pretreatment in rats had considerably less brain tissue damage ($p < 0.05$) than the control group. In comparison to the control group, the IFX (7mg/kg) group demonstrated the least amount of brain tissue damage, followed by the IFX (3mg/kg) group, however, there is significant difference ($p< 0.05$) between IFX (7mg/kg) and IFX (3mg/kg). Figure 2 present the histopathologic findings. An examination of the histopathology of the rat brain is shown in Figure 3.

DISCUSSION

Destruction and death of neurons in brain tissues as a result of an increase in inflammatory and oxidative mediators led to cerebral ischemia-reperfusion injury [17]. Endogenous antioxidants can detoxify free radicals, which are mediators of cellular damage and death [18, 19], however, excessive production of cytokines, such as TNF-α, due to the depletion of these antioxidants in the body, as well as caspase-3, cause cell death [20]. In our study, we found that a minimal percentage of infarct area was shown in the sham group, indicating that there are few necrotic cells in non-ischemic brain slices. We also noted a significant increase in untreated groups after induction of BCCAO when compared to the sham group, indicating an increase of necrotic cells due to downregulation of the Na/K-ATPase pump, increased oxidative stress, and increased inflammatory response, while pre-treatment with IFX. Blood-brain barrier disruption, development of edema and the induction of pyknotic and dark eosinophilic neurons were all effects of I/R [21]. Ischemia led to the production of free radicals, which then brought to mitochondrial and brain damage, according to Chandrashekhar et al. (2010), microscopic analysis of the control group's brain tissue reveals characteristics of neurotic necrosis and blood vessel congestion [15]. In our experiment, we found that a low damage score was shown in the sham group that indicated a basal sore in non-ischemic brain slices. We also noticed that its damage scores increased significantly in the untreated groups after BCCAO induction compared with the sham group, indicating that I/R causes damage to brain tissue. Inhibition of TNF-α has anti-inflammatory and neuroprotective properties. Infarction, edema, oxidative stress, and caspase 3 activation following a stroke were all prevented by pretreatment with a TNF-receptor antagonist. When administered 72 hours prior to ischemia, IFX significantly reduced brain damage scores when compared to untreated groups. Additionally, in the high dose group, the effects more than in the low dose treated groups, revealing that decreasing TNF-α levels also reduced brain damages like hemorrhage and necrosis. This result follows from our hypothesis. In our study, we observed that the lowest levels of TNF-α, capase-3

while minimal level of SOD and CAT were detectable in the sham group, which indicated a basal level in non-ischemic brain slices. We also observed that its level increased significantly in the untreated groups after the induction of BCCAO, in contrast to the sham group, indicating its overexpression. In addition, IFX at high dose group was reduced its levels more than low IFX dose treated groups, which indicated reducing in TNF-α and caspase-3 proteins and reduce in brain inflammation, and an increase levels of SOD and CAT, and this result supposes our suggestion. Administration of IFX 72 hours before ischemia significantly reduced TNF-α, capase-3 levels, and increase levels of SOD and CAT when compared with untreated groups. Inhibition of TNF-α has anti-inflammatory and neuroprotective properties. Infarction, edema, oxidative stress, and caspase 3 activation following a stroke were prevented by pretreatment with a TNF-α receptor antagonist [22]. Inflammation and cellular damage were both markedly increased by the production of the NF-κB protein. Therapy with infliximab and DMF reduces ROS and oxidative stress, respectively, by inhibiting the binding of TNF to its receptor, which was sharply reduced when both drugs were used together. Its protection against I/R-induced pathological changes in the rats' livers may be based on these therapeutic benefits.

CONCLUSIONS

Damage to CIR is caused by increased release of cytokines and signaling of cell death. Given that infliximab is a potent TNF-blocker, both doses prevent cytokine production and protect against cellular damage brought on by cytokine-mediated ROS formation and apoptotic pathways. This leads us to believe that IFX has a neuroprotective effect.

REFERENCES

1. Dorweiler B., Pruefer D., Andrasi T.B. et al. Ischemia-Reperfusion Injury : Pathophysiology and Clinical Implications. Eur J Trauma Emerg Surg. 2007; 33(6): 600-12. doi: 10.1007/s00068-007-7152-z.
2. Singh B., Singh D., Verma V. et al. Angiotensin-converting enzyme 2 as a potential therapeutic target for COVID-19: A review. 2022; 12(2): 215–220.
3. Al-Mudhaffer R.H., Al-Huseini L.M.A., Hassan S.M., Hadi N.R. Bardoxolone Ameliorates Cerebral Ischemia/Reperfusion Injury in Male Rats. Annals of Tropical Medicine and Public Health. 2019; 22: 122-30.
4. Piantadosi C.A., Zhang J. Mitochondrial generation of reactive oxygen species after brain ischemia in the rat. Stroke. 1996; 27(2): 327-32.
5. Liu G., Wang T., Wang T. et al. Effects of apoptosis-related proteins caspase-3, Bax and Bcl-2 on cerebral ischemia rats. Biomed Rep. 2013; 1(6): 861-7.
6. Hadi N., Hassan S., Abbas Al-Huseini L., Al-Mudhafer R. DMF Ameliorating Cerebral Ischemia/Reperfusion Injury in Male Rats. Sys. Rev. Pharm. 2019; 10(1): 206-213. doi: 10.5530/srp.2019.1.36.
7. Hassan S.M., Al Abood R.M., Jawad M.J. et al. DMF Attenuates Ciprofloxacin-Induced Nephropathy in Rats via Nrf2 Pathway. Journal of Pharmaceutical Negative Results. 2022; 13(2): 87-91. doi: 10.47750/pnr.2022.13.02.013.
8. Ebert E.C. Infliximab and the TNF-alpha system. Am J Physiol Gastrointest Liver Physiol. 2009; 296(3): G612-20.
9. Lee Y.T., Laxton V., Lin H.Y. et al. Animal models of atherosclerosis. Biomedical Reports. 2017; 6(3): 259-66.
10. Obadia N., Lessa M.A., Daliry A. et al. Cerebral micro vascular dysfunction in metabolic syndrome is exacerbated by ischemia-reperfusion injury. BMC Neurosci. 2017; 18(1):67. doi: 10.1186/s12868-017-0384-x.
11. Ji Q., Hui K., Zhang L. et al. The effect of hydrogen-rich saline on the brain of rats with transient ischemia. Journal of Surgical Research. 2011; 168(1): e95-e101.
12. Pergel A., Kanter M., Yucel A.F. et al. Anti-inflammatory and antioxidant effects of infliximab in a rat model of intestinal ischemia/reperfusion injury. Toxicol Ind Health. 2012; 28(10): 923-32.
13. Pergel A., Zengin K., Cercel A. et al. The effects of somatostatin and ursodeoxycholic acid in preventing the ischemic injury of the liver following Pringle maneuver in obstructive jaundice-rat model. Hepato-gastroenterology. 2007; 54(73): 229-33.
14. Terao S., Yilmaz G., Stokes K.Y. et al. Blood cell-derived RANTES mediates cerebral micro vascular dysfunction, inflammation and tissue injury following focal ischemia-reperfusion. Stroke; a journal of cerebral circulation. 2008; 39(9): 2560-70.
15. Chandrashekhar V.M., Ranpariya V.L., Ganapaty S. et al. Neuroprotective activity of Matricaria recutita Linn against global model of ischemia in rats. Journal of ethno pharmacology. 2010; 127(3): 645-51.
16. Abbas Al-Huesini L.M., Al-Mudhaffer R.H., Hassan S.M., Hadi N.R. DMF Ameliorating Cerebral Ischemia/Reperfusion Injury in Meal Rats. Systematic Reviews in Pharmacy. 2019; 10(1).
17. Chen X., Zhang X., Wang Y. et al. Inhibition of immunoproteasome reduces infarction volume and attenuates inflammatory reaction in a rat model of ischemic stroke. Cell Death Dis. 2015; 6: e1626.
18. Vepa S., Scribner W.M., Parinandi N.L. et al. Hydrogen peroxide stimulates tyrosine phosphorylation of focal adhesion kinase in vascular endothelial cells. Am J Physiol. 1999; 277(1): L150-8.

19. Singh U., Jialal I. Oxidative stress and atherosclerosis. Pathophysiology. 2006; 13(3): 129-42.
20. Ahmad S., Yousuf S., Ishrat T. et al. Effect of dietary sesame oil as antioxidant on brain hippocampus of rat in focal cerebral ischemia. Life Sci. 2006; 79(20): 1921-8.
21. Garman R.H. Histology of the central nervous system. Toxicological pathology. 2011; 39(1): 22-35.
22. Lin S.Y., Wang Y.Y., Chang C.Y. et al. TNF-α Receptor Inhibitor Alleviates Metabolic and Inflammatory Changes in a Rat Model of Ischemic Stroke. Antioxidants (Basel). 2021; 10(6).

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Received: 23.09.2022
Accepted: 09.02.2023

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

ORIGINAL ARTICLE

HYGIENIC REGULATIONS FOR THE SAFE APPLICATION OF COMBINED PESTICIDES IN THE CEREAL CROPS' CHEMICAL PROTECTION SYSTEM

DOI: 10.36740/WLek202302113

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ABSTRACT

The aim: Hazard characterization and assessment of combined exposure to several pesticides when they simultaneously get to the human body with bakery products.

Materials and methods: Methods of analytical analysis of the range of pesticide active substances, which are registered and used in modern systems of grain crop protection in Ukraine, were used in the study. Normative documents of national legislation on hygienic regulation of pesticides and methodological approaches to assessing the combined effects of pesticide mixtures in food products serve as materials for assessment.

Results: It is established that the total risk of exposure to residual amounts of pesticides in bread products (wheat and rye bread) during its comparable getting into the body was 0.59 for children 2-6 years and 0.36 - for adults, with an allowable value of ≤ 1.0. The combined effect of pesticides per unit of children's body weight is higher, but also lies within acceptable limits. The largest contribution to the overall risk of combined exposure to triazoles is made by flutriafol (38.5-47.0%), which in the future may be the basis for substantiating measures to reduce such exposure and make appropriate management decisions.

Conclusions: Safety of consumption of agricultural products is ensured by strict following hygienic regulations for the pesticide application (application rates, frequency of treatments, pre-harvest intervals), which makes it impossible to accumulate their residual amounts in food. Triazole pesticides, widely used in almost all crop protection systems, pose a potential risk of adverse health effects due to additive or synergistic effects.

KEY WORDS: pesticides, maximum residue levels, combined impact, health risk

Wiad Lek. 2023;76(2):332-338

INTRODUCTION

Agriculture in Ukraine is one of the main sectors of the economy, providing more than 10 per cent of the gross domestic product (GDP). The positive balance of foreign trade in agricultural goods in recent years has reached more than 13.5 billion US dollars and accounts for 40% of the total exports of Ukraine [1, 2]. A prominent place in modern agricultural production and general, the volume of exports is occupied by cereals (wheat, barley); crop areas in Ukraine as of 2019 amounted to 9424.9 thousand hectares [3]. Much attention is paid to modernization to increase crop yield production, accurate technologies in sowing and processing, modern plant protection systems, and new, resistant to diseases and weather conditions hybrids of seed. All this contributes to more efficient management than just the accumulation of land resources [4].

Chemical plant protection products remain the leading factor in increasing agricultural production. Agricultural holdings, farms, and agricultural cooperatives need 38-40 thousand tons of pesticides annually to conduct an efficient process of growing agricultural products [5]. In order to enhance biological action and prevent the development of resistance mechanisms, there is a need to find new active substances. In recent years, the trend of increasing the number of complex mixtures of pesticide formulations containing two or more active substances, the toxic properties of which may be summed up (additive effect) or enhanced (synergistic effect) with a combined impact on man [6-8].

Legal regulation of pesticide used in Ukraine is provided by the existing legal framework. All new preparative forms of pesticides are undergoing state tests for their intended purpose and further use in agricultural production of Ukraine. The criteria for the safety of pesticides for human health are the hygienic regulation of their content in the human environment.

The main standard that minimizes the harmful effects of pesticides on the human body when consuming food is maximum residue level (MRL) - the maximum level of pesticide residues in food, recog-

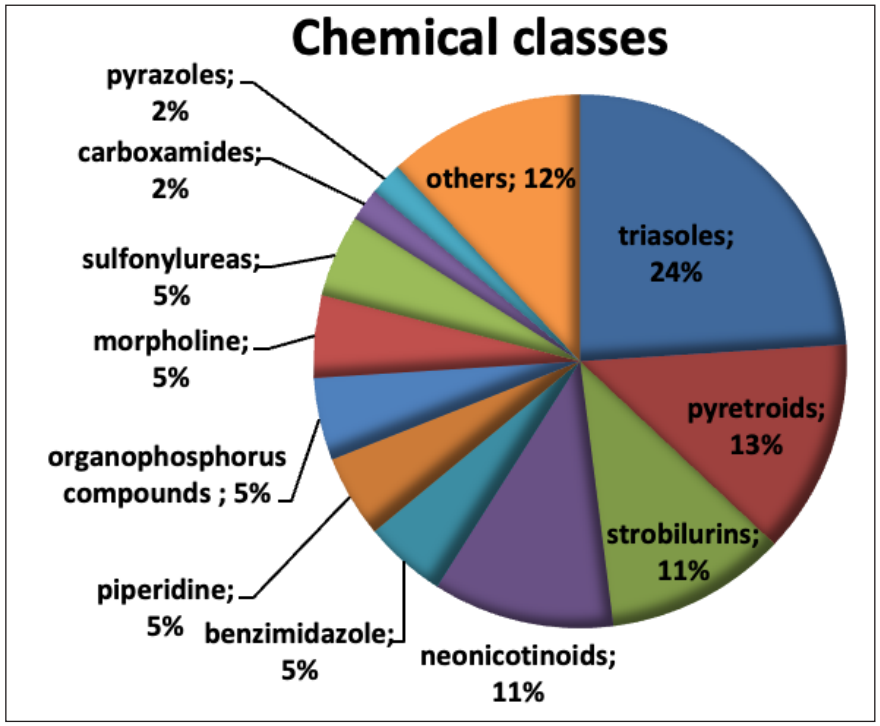

Fig. 1. Distribution of active substances of pesticides by chemical classes
(triazole – 24%, pyrethroid – 13%, strobilurin – 11%, neonicotinoids – 11%, benzimidazole – 5%, piperidine – 5%, organophosphorus compounds – 5%, morpholine – 5%, sulfonylurea – 5%; carboxamides – 2%, pyrazole – 2%, other – 12%)

nized by law as acceptable if properly use of pesticides in accordance with the principles of good agricultural practice. This level of residue should be as low as possible by modern analytical methods and be safe for the health of consumers.

However, a legally established MRL at the state level only applies to one active pesticide substance. It precludes the assessment of the additive or synergistic effect of several pesticides on the human body when they are detected together, even in small concentrations and within established standards. The presence of residues of several pesticides in food products encourages the development of methodological approaches to assessing their cumulative effects and is an important issue in the hygienic regulation of pesticides.

The importance of conducting research to develop a methodology for assessing the cumulative and synergistic effects of pesticides is stated in EU Regulation № 396/2005 of the European Parliament and of the Council on maximum pesticide residue levels [9]. There is a need to consider the cumulative effects of pesticides when setting MRLs when methods become available to assess such impacts.

Currently, there are no scientifically sound approaches to regulating the simultaneous presence of several pesticides in the same food product and clearly defined risk assessment methods that consider all multicomponent mixtures' interactions.

Addressing the combined effects of chemicals, including pesticides, on human health is one of the key initiatives of the EU Chemicals Strategy, adopted by the European Commission on 14 October 2020 under the European Green Course to achieve a non-toxic environment. It is for pesticides that progress has been made in developing a targeted methodology for a better and more accurate way to assess cumulative risk [10].

On the example of the range of active substances of combined preparations used in the system of chemical protection of cereals in Ukraine, we assessed the risk of combined effects of pesticides on human health when they come with bakery products.

THE AIM

The purpose of the research is hazard characterization and assessment of combined exposure to several pesticides when they co-enter the human body with bakery products.

MATERIALS AND METHODS

The objects of the study were the active substances of combined pesticides for various functional purposes (insecticides, fungicides, herbicides) used in Ukraine to protect cereals for various functional purposes [11], and their combined effects on human health when getting into the body with bakery products. The range of pesticides was divided into classes of chemical compounds, determining the nature of toxic effects and dose levels at which no damaging

Table I. Formulas used for risk assessment

Formula	Definition
$E_i = \frac{I_i \times MRL}{M}$	E_i – daily exposure to pesticide residues in bread products, mg / kg bw / day. MRL – maximum residue level of pesticide in cereal grains, mg / kg; I_i – average daily consumption of bakery products (wheat and rye bread), kg / person / day (for an adult - 0.28 kg, children from 2 to 6 years - 0.12 kg) [13]; M – body weight of an adult (60 kg) and children aged 2-6 years (15.6 kg) [14].
$HQ_i = \frac{E_i}{ADI_i}$	HQ_i – hazard quotient for each pesticide active substance, which characterizes the risk associated with the intake of a particular pesticide active substance with bakery products; ADI – acceptable daily intake of the active substance of the pesticide for humans, which characterizes the risk associated with the intake of a pesticide with bakery products; ADI – acceptable daily intake of the pesticide for humans, mg / kg bw / day.
$HI = \sum HQ_i$	HI – hazard index, which is the sum of the hazard quotient of each active substance and characterizes the risk of potential adverse effects from the combined exposure to several active substances when they co-enter the human body with bread products.
$PRF_i = \frac{NOAEL_{ic}}{NOAEL_i}$	RPF_i – the relative potential factor of each active substance from the group of related; $NOAEL_{ic}$ – no observed adverse effect level (dose level of the index compound at which no damaging effects are observed), mg / kg bw; $NOAEL_i$ – no observed adverse effect level (dose level of an individual compound at which no damaging effects are observed), mg / kg bw.
$E_{total} = \sum_{i=1}^{ni} \frac{I_i \times MRL_i}{M} \times PRF_i = \sum_{i=1}^{ni} E_i \times PRF_i$	E_{total} – the total exposure, the combined effect, is estimated by summing the daily exposure of each substance (E_i) multiplied by the corresponding RPF_i.

Table II. Estimation of the combined exposure to active substances of pesticides at their joint intake by organism with bakery products

Active substance	Chemical class*	ADI, mg/kg bw/d	MRL, mg/kg product	E_i, mg/kg bw/d		HQ_i	
				children, 2-6 year	adults	children, 2-6 year	adults
Alpha-cypermethrin	P	0,005	0,01	0,00008	0,00005	0,016	0,010
Azoxystrobin	S	0,03	0,2	0,00154	0,00093	0,051	0,031
Epoxiconazole	T	0,004	0,05	0,00038	0,00023	0,095	0,058
Imidacloprid	N	0,06	0,01	0,00080	0,00050	0,013	0,005
Lambda-cyhalothrin	P	0,003	0,01	0,00008	0,00005	0,027	0,017
Pyraclostrobin	S	0,02	0,2	0,00154	0,00093	0,051	0,031
Propiconazole	T	0,04	0,04	0,00077	0,00047	0,019	0,012
Tebuconazole	T	0,03	0,2	0,00154	0,00093	0,051	0,031
Flutriafol	T	0,01	0,1	0,00077	0,00047	0,077	0,047
Cyproconazole	T	0,002	0,05	0,00038	0,00023	0,190	0,115
Total risk HI:						0,59	0,36

Notes: * P – Pyrethroids; S – Strobilurins; T – Triazoles; N – Neonicotinoids

effects (NOAEL). Theoretical research of normative documents of national legislation was conducted to find reference values for active substances of pesticides and other standards: acceptable daily intake (ADI), maximum residue levels (MRL) in cereals [12], average daily norms (ADN) of bread consumption and rye) [13]. The study also included reports from the European Food Safety Authority (EFSA) and other scientific publications on the toxicological effects of the studied pesticides.

The subject of the study was the exposure levels of pesticide active substances in cereals at the level of MRL and the total potential risk of their combined impact on the health of people of different ages (adults and children aged 2-6 years).

Risk characterization and assessment of combined exposure to pesticides were performed using the formulas listed in Table I.

The estimated total risk of exposure expresses the overall hazard according to the equivalent exposure of the index compound, and therefore can be compared with the ADI of the index compound [16].

Exposures at the level of MRL pesticides in cereals without taking into account the processing factors of agricultural raw materials, which depend on the chemical characteristics of the pesticide, product

Table III. NOAELs of triazoles by hepatic toxicity

Active substance	NOAEL, mg/kg bw/d	Effects	References
Epoxiconazole	0,8 (18-month, mouse)	Hepatotoxicity: increased body liver weight, clinical chemistry, histology, liver cell adenomas and carcinomas in mice	EFSA, 2008 (https://doi.org/10.2903/j.efsa.2008.138r
Propiconazole	3,6 (2-year rat study)	Hepatotoxicity (hypertrophy of hepatocytes, disorders of carbohydrate and fat metabolism)	EFSA, 2017 (https://doi.org/10.2903/j.efsa.2017.4887)
Tebuconazole	3,0 (1-year dog study)	Liver toxicity (rat and mouse), inducer of monooxygenase system, anemia, pathological changes in the adrenal glands, liver tumors in mice of sensitive lines, changes in the lens of the eye (dogs)	EFSA, 2014 (https://doi:10.2903/j.efsa.2014.3485)
Flutriafol	1,0 (2-year rat study)	Liver toxicity (rat and mouse), increased liver weight and histopathology, inducer of monooxygenase system, anemia	EFSA 2010 https://doi.org/10.2903/j.efsa.2010.1868
Cyproconazole	1,0 (2-year rat study); 1,84 (18-month, mouse)	Main target organ of cyproconazole is the liver upon short-term to long-term exposure (increased relative liver weight, increased incidence of hepatocellular hypertrophy, hepatocellular adenomas); probable carcinogen for humans, selective effect on reproductive function	EFSA 2010 https://doi.org/10.2903/j.efsa.2010.1897

Table IV. Relative potential factors and overall risk of combined exposure to triazole pesticides

Active substance	NOAEL, mg/kg bw/d	ADI, mg/kg bw/d	PRF	E_i, mg cyproconazole eq./kg bw/d		% ADI_{IC}	
				children, 2-6 year	adults	children, 2-6 year	adults
Epoxiconazole	0,8	0,004	1,25	0,00048	0,00029		
Propiconazole	3,6	0,04	0,28	0,00022	0,00013		
Tebuconazole	3,0	0,03	0,33	0,00051	0,00031		
Flutriafol	1,0	0,01	1,0	0,00077	0,00047		
Cyproconazole (IC)	1,0	0,002	1,0	0,00038	0,00023		
Total exposure, E_{total}:				0,002	0,001	100,0	50,0

and type of treatment, were used to assess the risk of combined pesticide exposure. For example, the baking of bread process can reduce pesticide residues in the final product, thus reducing the potential adverse effects on humans. Unfortunately, the lack of available information on the distribution of residues between agricultural raw materials and the finished product did not allow us to take into account this processing factor in the risk assessment.

RESULTS

The range of pesticides registered in Ukraine on cereals includes a significant number of mixed preparations of different directions of action, including 5 - herbicides, 33 insecticides and 84 - fungicides. They contain 45 active substances pertaining to various classes of chemical compounds (Fig 1).

The largest number of combinations of active substances in mixed preparations occurs with tebuconazole (27), propiconazole (20), cyproconazole (21), azoxystrobin (17), epoxyconazole (17), epoxiconazole (15), imidacloprid (15), lambda-cyhalothrin (12), flutriafol), alpha-cypermethrin (5), pyraclostrobin (5). This increases the likelihood of their combined use in an integrated cereal protection system and, at the same time, the possibility of their combined effects on human health. The above pesticides were selected to characterize the hazards and assess the combined exposure when they co-enter the human body with bread products (Table II)

The calculated hazard ratios of pesticide residues in bread products for each individual pesticide and in total do not exceed 1.0, which characterizes the impact as acceptable for different age groups.

Further research was aimed at assessing the cumulative effects of substances that are characterized by a similar method/mechanism of biological action and affect the same organ system. Such substances include pesticides from the class of triazoles, grouped in a

cumulative assessment group (CAG) for their ability to have hepatotoxic effects [15]. This group includes epoxiconazole, propiconazole, tebuconazole, flutriafol, cyproconazole, which have a general toxic effect on the body with a predominant hepatotropic effect, and are inducers of the monooxygenase system. The main critical effects of their toxic action and dose levels at which observed to have no damaging effects (NOAEL) are shown in Table III.

To assess the overall total hazard of triazole exposure, we used the concept of relative potential factor (PRF). This indicator is used to determine the potential toxicity of a mixture of substances characterized by a similar mode of action. For this purpose, one pesticide from the group of related was selected as an index compound with a certain RPF, and the RPF of other substances was calculated relative to the index compound. Cyproconazole was selected as the index compound, which is characterized by the most complete toxicological database, including the effect on the liver. For the index compound, PRFs for other substances were calculated (Table IV).

As can be seen from the data in Table 4, according to the equivalent of cyproconazole, the total exposure of residual amounts of triazoles in the consumption of bread products of different age groups is 0.002 mg/kg for children (2-6 years) and 0.001 mg/kg for adults and does not exceed the reference value of the index compound. It was found that the combined effect of pesticides per body weight unit of children is higher, but also lies within acceptable limits.

The contribution of pesticides to the overall total risk of combined exposure to triazoles was ranked: flutriafol - (38.5-47.0)%; tebuconazole - (25.5-31.0)%; epoxiconazole - (24.0-29.0)%; cyproconazole - (19.0-23.0)%; propiconazole - (11.0-13.0)%. The largest contribution to the overall exposure is made by flutriafol.

DISCUSSION

Bakery products are the basic foodstuffs across the globe and the quality of such products includes safety parameters. Safety of bakery products is impossible without control of the grain used for its production. While organic bread and bakery products, like other organic food products, has become more and more popular in recent decades, it still makes minor contribution in general food intake. In terms of organic bread, Germany having the largest market share in Europe, just makes approximately only 8.5 percent of its bread market being made up of organic bread. Germany is followed by France and Italy with the next biggest markets for organic bread [17]. But other bakery products are made from grain grown with pesticide application.

Usually, foods can contain only those pesticides that have been used to protect crops. Therefore, the probability of additive effects of pesticides with different mechanisms of biological action (eg, herbicide, fungicide, insecticide) is quite low. Increased additive action can occur in the case of exposure to one pesticide by different types of exposure (water, food); when using different foods containing residues of the same pesticide; summation of toxic effects when eating foods containing different pesticides, characterized by the same type of biological action [18].

As we found cereal crops in Ukraine are treated with particular types of pesticides. In particular fungicides have leading place increasing the likelihood of their combined use in an integrated cereal protection system and, at the same time, the possibility of their combined effects on human health.

We calculated hazard ratios of pesticide residues in bread products for each individual pesticide in total and found that its do not exceed 1.0, concluding that the impact is acceptable for different age groups.

It was found that among studied fungicides flutriafol topped with the (38.5-47.0) % of the overall total risk of combined exposure, which in the future can be the basis for justifying measures to reduce such impact and make appropriate management decisions.

CONCLUSIONS

1. Research has been conducted to assess the risk to consumer health from the combined effects of pesticides used in cereals. Findings indicate that the total risk of exposure to residual active substances of pesticides in bread products (wheat and rye bread), when combined getting into the body, was 0.59 for children 2-6 years and 0.36 - for adults with a permissible value ≤ 1,0.
2. The conclusion about the acceptable level of total risk for the health of consumers at strict following the hygienic regulations of pesticides application (application rates, frequency of treatments, pre-harvest intervals), as precautionary measures to prevent accumulation of residual quantities of pesticides active substances in foodstuff.
3. Despite the acceptable risk to the population when ingested with bread products, the potential danger of exposure to triazoles should be of concern due to their widespread use on all crops and the possibility of increased adverse health effects through additive or synergistic effects.

REFERENCES

1. Country survey of highly hazardous pesticides in Ukraine. IPEN. 2020. https://ipen.org/documents/country-survey-hhps-ukraine. [date access 03.07.2022]
2. On the approval of the Strategy to promote the attraction of private investments in agriculture for the period until 2023. Official web-portal of the Parliament of Ukraine. 2019. https://zakon.rada.gov.ua/laws/show/595-2019-%D1%80?lang=en#Text [date access 03.07.2022]
3. Sown areas of agricultural crops by their types. State Statistics Service of Ukraine. 2019. http://www.ukrstat.gov.ua/operativ/operativ2019/sg/ppsgk/arh_ppsgk_u.html [date access 03.07.2022]
4. Agroholdings of Ukraine. 2022. https://tripoli.land/agrokholdingi-ukrainy. [date access 03.07.2022]
5. Control of residual quantities of pesticides: Phytosanitary safety of plants. Propozitsiya – The key magazine on agribusiness issues. 2022. https://propozitsiya.com/ua/zaprovadzhennya-yevropeyskogo-kontrolyu-za-pestycydamy-v-ukrayini. [date access 03.07.2022]
6. Cui K., Wu X., Zhang Y. et al. Cumulative risk assessment of dietary exposure to triazole fungicides from 13 daily-consumed foods in China. Environmental Pollution. 2021;286: 117550. doi:10.1016/J.ENVPOL.2021.117550.
7. Vavrinevych O. P. Hygienic estimation of potential combined risk of mixed fungicide harmful effects on workers. Ukrainian Journal of Occupational Health 2015;1: 58–66. doi:10.33573/UJOH2015.01.058.
8. Yastrub T.A., Dontsova D. A. Forecasting the risk of combined effect of avermectin and neonicotinoid insecticides on workers. Ukrainian Journal of Occupational Health. 2021;3: 151–159. doi:10.33573/UJOH2021.03.151.
9. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC (Text with EEA relevance). https://Webarchive.Nationalarchives.Gov.Uk/Eu-Exit/Https://Eur-Lex.Europa.Eu/Legal-Content/EN/TXT/?Uri=CELEX:02005R0396-20201216. [date access 03.07.2022]
10. EFSA-SANTE Action Plan on Cumulative Risk Assessment for pesticides residues. 2008. doi:10.2903/j.efsa.2008.705.
11. Derzhavnyi reiestr pestytsydiv ta ahrokhimikativ, dozvolenykh do vykorystannia v Ukraini. 2021. (in Ukrainian) https://mepr.gov.ua/content/derzhavniy-reestr-pesticidiv-i-agrohimikativ-dozvolenih-do-vikoristannya-v-ukraini-dopovnennya-z-01012017-zgidno-vimog-postanovi-kabinetu-ministriv-ukraini-vid-21112007--1328.html [date access 03.07.2022]
12. Omelchuk. S.T. Pestytsydy : dovidnyk. Kyiv: Interservis. 2019, 904 p. (in Ukrainian)
13. On the approval of sets of food products, sets of non-food products and sets of services for the main social and demographic groups of the population [Pro zatverdzhennia naboriv produktiv kharchuvannia, naboriv neprodovolchykh tovariv ta naboriv posluh dlia osnovnykh sotsialnykh i demohrafichnykh hrup naselennia: postanova Kab. Ministriv Ukrainy vid 11.10.2016. № 780.] 2022. https://www.kmu.gov.ua/npas/249464422 [date access 03.07.2022] (in Ukrainian)
14. Default human factor values for use in exposure assessments for biocidal products (revision of HEEG opinion 17 agreed at the Human Health Working Group III on 12 June 2017. Document history. 2022. https://echa.europa.eu/documents/10162/21664016/recom_14+_default+human_factor_values_biocidal+products_en.pdf/88354d31-8a3a-475a-9c7d-d8ef8088d004. [date access 03.07.2022]
15. Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health. EFSA Journal. 2009;7(9). doi:10.2903/J.EFSA.2009.1167.
16. Combined toxic effects of multiple chemical exposures Published by Vitenskapskomiteen for mattrygghet. Norwegian Scientific Committee for Food Safety. 2008. https://vkm.no/download/18.d44969415d027c43cf1e869/1509708687404/Combined%20toxic%20effects%20of%20multiple%20chemical%20exposures.pdf. [date access 03.07.2022]
17. Prodanchuk N.G., Chmil V.D. The groundness of the use conception of MRL xenobiotics for the sanitary-epidemiological quality control of environment and foodstuffs. 2006. http://medved.kiev.ua/web_journals/arhiv/toxicology/2006/4_2006/index.html [date access 03.07.2022]
18. Prodanchuk N.G., Chmil V.D. The groundness of the use conception of MRL xenobiotics for the sanitary-epidemiological quality control of environment and foodstuffs. 2006. http://medved.kiev.ua/web_journals/arhiv/toxicology/2006/4_2006/index.html [date access 03.07.2022]

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Conflict of interest:

The Authors declare no conflict of interest.

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Received: 30.11.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

SURGICAL APPROACH FOR TREATMENT OF OBSTRUCTIVE JAUNDICE IN PATIENTS OF DIFFERENT AGE GROUPS

DOI: 10.36740/WLek202302114

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ABSTRACT

The aim: Assessment of the effectiveness of using minimally invasive and open methods of bile duct decompression for treatment of obstructive jaundice (OJ) by comparing complications in patients of different age groups.

Materials and methods: We analyzed the results of surgical treatment of 250 patients with OJ. The patients were assigned to two groups: Group I (n = 100) consisting of young and middle-age patients, and Group II (n = 150) consisting of elderly, senile and long-living patients. The average age was 52 ± 6.0 years.

Results: 62 (24.8%) Group I patients and 74 (29.6%) Group II patients were submitted to minimally invasive surgical interventions. 38 (15.2%) Group I patients and 76 (30.4%) Group II patients were submitted to open surgical interventions. Complications after minimally invasive surgery (n = 62) in Group I patients were observed in 2 (3.2%) cases, and in 4 (10.5%) cases after open surgeries (n = 38). Complications following minimally invasive interventions (n = 74) in Group II patients were registered in 5 (6.8%) cases, and in 9 (11.8%) cases following open operations (n = 76). 2 (2.6%) Group II patients died for transmural myocardial infarction.

Conclusions: The use of minimally invasive surgical interventions for treatment of young and middle-aged OJ patients compared to patients of older age groups makes it possible to reduce the frequency of complications by 2.1 times, which is a statistically significant (p <0.05). The frequency of complications after open surgical interventions of bile ducts in patients of different age groups is not statistically significant (p >0.05).

KEY WORDS: endoscopic transpapillary interventions, laparoscopic lithoextraction, biliodigestive anastomoses, external drainage of biliary ducts

Wiad Lek. 2023;76(2):339-345

INTRODUCTION

OJ patients account for one of the most challenging categories of patients with abdominal pathologies with a share in the emergency surgery of 2.6-23.7% [1, 2]. Along with significant advancement in treatment of OJ patients, biliary decompression is often accompanied by development of post-operative liver failure [3, 4]. This complication is called post-decompression liver dysfunction ("rapid biliary decompression syndrome"), which is difficult to treat and lethal in 14-27% of cases [5, 6]. Today, minimally invasive interventions are used along with open methods of bile ducts decompression. Using minimally invasive techniques for treatment of OJ patients has become one of the leading trends in surgery [7, 8]. Biliary surgery today sustains the trend of multi-stage minimally invasive interventions considering the severity of OJ patients' condition [9, 10]. Yet, gaining an experience, surgeons have been increasingly using the one-stage treatment approach [11, 12]. However, the pivotal question of biliary surgery remains a choice of bile ducts decompression technique for patients of different age groups, with different duration of OJ and bilirubin count, burdened with complications and concomitant pathologies [13, 14].

THE AIM

Assessment of the effectiveness of using minimally invasive and open methods of bile duct decompression for treatment of OJ by comparing complications in patients of different age groups.

MATERIALS AND METHODS

In the period from 2002 to 2020, 250 patients, including 146 (58.4%) female and 104 (41.6%) male subjects with OJ of benign and malignant origin underwent treatment at the surgical clinic of the Medical Faculty No.2 of the National Pirogov Memorial Medical University, Vinnytsia. The patients were assigned to age groups according to WHO recommendations. Young, middle-age, elderly, senile, and long-living individuals accounted to 32 (12.8%), 68 (27.2%), 88 (35.2%), 56 (22.4%) and 6 (2.4%) patients, accordingly. The average age was 52 ±

6.0 years. 98 (39.2%) had OJ duration up to 7 days, 62 (24.8%) - 7-14 days, 30 (12.0%) - 14-21 days, 35 (14.0%) - 21-28 days, and 25 (10.0%) – over 28 days. The average duration of OJ was 19 ± 3.5 days.

Benign OJ observed in 210 (84.0%) patients accounted for choledocholithiasis - 149 (59.6%), Myrizzi`s syndrome - 16 (6.4%), stricture of common bile duct (CBD) - 8 (3.2%), stenotic papillitis - 19 (7.6%), chronic fibrous pancreatitis - 10 (4.0%), cyst of the pancreatic head - 5 (2.0%), duodenal ulcer (DU) with penetration into the hepatoduodenal ligament (HDL) - 3 (1.2%) cases. Malignant OJ genesis in 40 (16.0%) patients accounted for the pancreas head cancer - 23 (9.2%), tumor of the major duodenal papilla (MDP) - 5 (2.0%), bile ducts cancer - 8 (3.2%), HDL-invading gallbladder cancer - 2 (0.8%), and other malignancies spreading in porta hepatic - 2 (0.8%).

Study patients were divided into two groups: Group I (young and middle-age patients) consisting of 100 individuals, and Group II (elderly, senile, and long-living patients) inclusive of 150 persons.

The research was conducted in compliance with the major principles of GCP guidelines (1996), Council of Europe Convention on Human Rights and Biomedicine (1997), World Medical Association Declaration of Helsinki on ethical principles for medical research involving human subjects (1964-2000) and Order of Ministry of Health of Ukraine № 281 of November 1, 2000, being approved by the Committee on Bioethics of the National Pirogov Memorial Medical University, Vinnytsia (Minutes No. 30 dated 10.12.2018).

General clinical, laboratory, and instrumental study methods were used for diagnostics. Instrumental research methods included: transabdominal ultrasonography (TUS), endoscopic ultrasonography (EUS), fibrogastroduodenoscopy (FGDS), endoscopic retrograde cholangiopancreatography (ERCP), intraoperative cholangiography (IOC), magnetic resonance imaging (MRI). TUS involving Lodgiq-500 PRO Series GE apparatus was used for screening of gallbladder and bile ducts pathologies in all patients. Pentax-290V fibrogastroduodenoscope was used for FGDS, of OJ patients. 10-20 ml of 30% water-soluble dye was administered for direct contrast of bile ducts in ERCP. IOC was performed by administering 10-20 ml of 30% contrast matter through the bile ducts. If it was impossible to cannulate the MDP, we resorted to EUS using Olympus Exera EU M 60 apparatus. In case of pancreatic diseases, we performed MRI using a Somatom-CR scanner.

The obtained data were statistically processed using descriptive statistic methods involving Microsoft Office Excel 2010 spreadsheet. As quantitative indicators, we calculated sample mean, standard deviation, and mean error. The study was based on the hypothesis of checking statistical significance of the difference between average indicators of complications in Group I and II patients. The pre-estimated normal distribution of the sample data made it possible to use the Student's t-test for their comparison and the assessment of significance at the appropriate threshold of confidence probability P=0.95 (a statistically significant difference is considered statistically reliable at a significance level of at least 0.05 ($p < 0.05$)).

RESULTS

The informativity of TUS in diagnosing the causes of OJ made 160 (64.0%). TUS helped to diagnose bile ducts dilatation and the presence of calculi. EUS was used in case of unsatisfactory TUS results and impossibility of MDP cannulation. The reasons for unsatisfactory TUS results were severe flatulence - 8 (3.2%), obesity - 6 (2.4%), the presence of multiple small calculi in the terminal CBD department - 5 (2.0%), and aerocholia - 3 (1.2%). EUS was also useful for objective assessment of the CBD terminal department and calculi diameter ratio.

All OJ patients were subjected to FGDS, which contributed to assessment of MDP shape and size, and the nature of secreted bile. FGDS diagnosed MDP cancer and HDL-penetrating DU in 5 (2.0%) and 3 (1.2%) patients, accordingly. FDGS was also used to perform a differential diagnostics of OJ between a calculi wedged in the distal CBD and a pathology of the MDP.

ERCP was performed in 85 (34.0%) cases. Choledocholithiasis and stenotic papillitis were diagnosed in 66 (26.4%) and 19 (7.6%) patients, respectively. ERCP failed in 32 (12.8%) cases due to the presence of a concrement in the MDP ampoule, parapapillary diverticulum, and after the Billroth II stomach resection in 20 (8.0%), 10 (4.0%) and 2 (0.8%) cases, accordingly. Post-ERCP complications occurred in 11 (4.4%) patients, including acute pancreatitis in 6 (2.4%), acute cholangitis in 3 (1.2%) and haemorrhage from the MDP in 2 (0.8%) if ERCP was combined with endoscopic papillosphincterotomy (EPST).

60 (24.0%) patients were subjected to IOC. Following open cholecystectomy (OCE), water-soluble contrast matter was administered into bile ducts through the cystic duct stump in 28 (11.2%) and by CBD puncture in 18 (7.2%) cases. During laparoscopic cholecystectomy (LCE), 14 (5.6%) patients were subjected to IOC through the cystic duct. Choledocholithiasis, Myrizzi`s syndrome, CBD stricture, bile ducts cancer, and gallbladder cancer with penetration into HDL were diagnosed in 26 (10.4%), 16 (6.4%), 8 (3.2%), 8 (3.2%), and 2 (0.8%), accordingly. Post-IOC complications were observed in 7

(2.8%) patients, including acute pancreatitis and acute cholangitis in 4 (1.6%) and 3 (1.2%) cases, respectively.

MRI was used for diagnosing pancreas pathologies in 38 (15.2%) patients, including pancreatic head cancer, chronic fibrous pancreatitis, and pancreatic head cyst in 23 (9.2%), 10 (4.0%), 5 (2.0%) cases, accordingly.

OJ patients were subjected to surgical interventions on the top of complex conservative therapy aimed at preventing the development of hepatic insufficiency, which comprised of infusion therapy, hepatoprotectors, glucocorticosteroids, antisecretory therapy, antispasmodics, antibiotics, vitamins, blood-improving therapy, acid-base, protein, and electrolyte balance enhancers. The patients received pre- and post-surgery comprehensive conservative therapy.

136 (54.4%) patients in both groups were submitted to minimally invasive surgical interventions, and 114 (45.6%) – to open surgeries.

62 (24.8%) Group I patients were submitted to minimally invasive surgical interventions. One-stage minimally invasive interventions were used in 24 (9.6%) cases. LCE with lithoextraction involving Fogarty balloon-tipped catheter was used in 15 (6.0%) patients having calculi up to 5 mm positioned below the confluence of the cystic duct into the common hepatic duct. LCE with external CBD drainage was performed in 9 (3.6%) patients with Mirizzi's syndrome type I.

Two-stage minimally invasive interventions were used in 38 (15.2%) patients. At the first stage, endoscopic papillary balloon dilation was performed in 4 (1.6%) patients with single calculi up to 5 mm in size localized in the distal CBD departments, using 4- and 6 mm cylinders pressurized within the range of 4-9 atm. The dilation continued 15-60 seconds. At the first stage, 23 (9.2%) patients with choledocholithiasis were submitted to incomplete EPST (up to 10 mm), which made possible independent discharge of calculi up to 10 mm in diameter. Incomplete EPST was also used in 11 (4.4%) patients with stenotic papillitis. At the second stage, LCE was performed after the elimination of OJ. The second stage was performed on Day 3-10 depending on the severity of patients' condition.

74 (29.6%) Group II patients were submitted to minimally invasive surgical interventions. One-stage minimally invasive interventions were used in 14 (5.6%) cases. Endobiliary transpapillary stenting (7 Fr stent) was used for long-term decompression of bile ducts in 4 (1.6%), 5 (2.0%), and 5 (2.0%) patients with terminal CBD stricture, MDP cancer, and pancreas head cancer, accordingly. The stent was replaced in 3-4 months once inlaid with bile acid salts.

Two-stage minimally invasive interventions were used in 60 (24.0%) patients. Complete EPST (over 10 mm) was performed in 20 (8.0%) patients with choledocholithiasis, which made it possible to perform lithoextraction with a Dormia basket (Olympus FG-22Q, Boston scientific trapezoid RX). Complete EPST was also followed by mechanical lithotripsy involving Olympus BML-201Q lithotripter in 12 (4.8%) patients with 10-20 mm concrements. The fragmented concretions were removed then with a Dormia basket. Complete EPST was also used in 8 (3.2%) patients with stenotic papillitis. Repeated gradual EPSTs were performed as rapid biliary decompression led to a progression of liver failure. 20 (8.0%) patients with signs of purulent cholangitis underwent EPST with a nasobiliary drainage. The presence of drainage made it possible to rehabilitate the bile ducts with antiseptic and antibiotic solutions. At the second stage, LCE was performed after the elimination of OJ. The second stage was performed on Day 5-14 depending on the severity of patients' condition.

38 (15.2%) Group I patients were submitted to open surgical interventions. 8 (3.2%) patients with concrements larger than 20 mm that was impossible to remove transpapillary underwent OCE with choledocholithotomy. In case of obstruction of distal CBD departments, a choledochoduodenoanastomosis was formed using intraoperative technique for prevention of duodenal contents reflux (Ukraine patent No.85986). A 15 mm-long longitudinal choledochotomy and duodenotomy of the crescent-shape in a transverse direction were performed. A "side-to-side" choledochoduodenoanastomosis was formed with a single-row nodal suture. A serous-muscular layer of the duodenum wall was captured by the suture during formation of the anastomosis upper edge. Once the suture is tightened, the crescent flap invaginated into the duodenal cavity, forming a slit-like valve. Post-surgery, the valve prevented rapid decompression of the bile ducts and duodenobiliary reflux. 15 (6.0%) patients with choledocholithiasis underwent areflux choledochoduodenostomy.

Combined areflux hepaticojejunoduodenostomy (Ukraine patent No.112735) was used in 11 (4.4%) patients with CBD stricture and Mirizzi's syndrome type II in 4 (1.6%) and 7 (2.8%) cases, accordingly. The areflux hepaticojejunostomy was formed "side-to-side". To do this, surgeons sutured and pulled up the anterior wall of the jejunum, thus forming a cone. Sub-ligature, the intestinal wall was excised and expanded the point opening with forcepts in the transverse direction. A longitudinal hepaticotomy was performed and the anastomosis between the common hepatic duct and the jejunum with a single-row nodal suture was formed. Once a peristaltic wave passed, the intestinal wall "closed", thus preventing reflux of intestinal contents into the bile ducts and rapid biliary decompression in

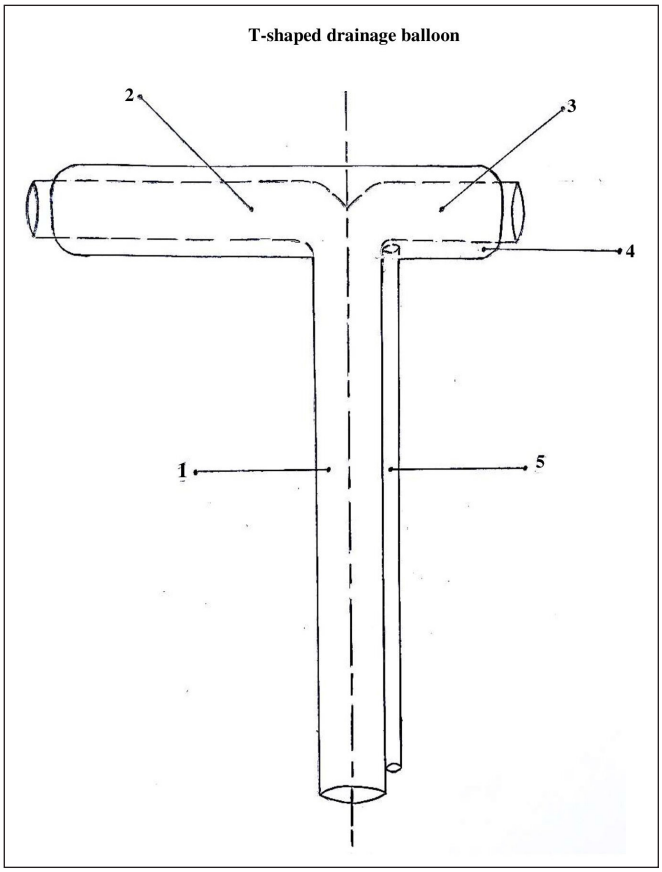

Fig. 1. T-shaped drainage balloon
longitudinal draining tube – 1; long transverse draining tube – 2; short transverse drainage tube – 3; balloon – 4; additional tube – 5

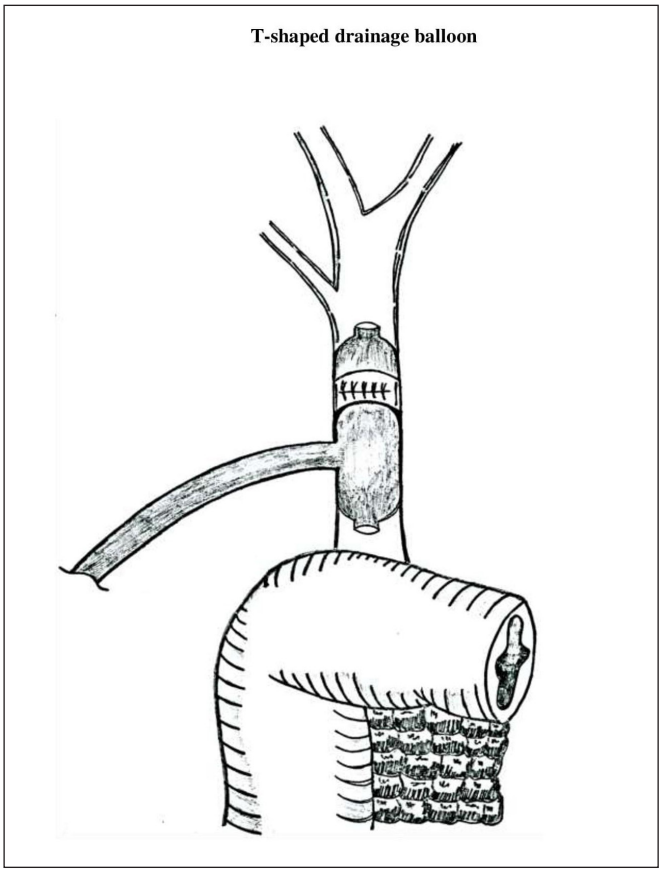

Fig. 2. T-shaped drainage balloon

the postoperative period. The intestinal tube continuity was restored by applying an "end-to-side" interstitial anastomosis at a distance of 30-40 cm from the previously formed biliodigestive anastomosis. To prevent the formation of peptic ulcers and duodenostasis, an additional duodenojejunostomy was formed "side-to-side" between the excluded jejunum segment and the descending duodenum branch. In patients with pancreatic head cancer underwent pancreatoduodenal resection in 4 (1.6%) cases.

76 (30.4%) Group II patients were submitted to open surgical interventions. 18 (7.2%) patients with choledocholithiasis that was impossible concrements to remove transpapillary underwent OCE with choledocholithotomy. To prevent a post-decompression liver dysfunction a probe-obturator for extrahepatic bile ducts was used (Ukraine patent No.104826). Post-OCE, the probe-obturator was introduced into the CBD through the cystic duct stump. The balloons volume increased by injecting saline. In the postoperative period, the balloon-fixator prevented removal of the probe from the cystic duct stump. The balloon-obturator volume was gradually reduced for 7-10 days, which made it possible to perform dosed decompression of the bile ducts.

29 (11.6%) patients with non-tumor OJ genesis underwent areflux choledochoduodenostomy, including choledocholithiasis, chronic fibrous pancreatitis, and pancreatic head cyst in 14 (5.6%), 10 (4.0%), and 5 (2.0%) cases, accordingly. Jurash supraduodenal choledochoduodenoanastomosis was used in 5 (2.0%) patients with pancreatic head cancer. A 15-20 mm anastomosis ensured a longer decompression of bile ducts in case of ascending type of tumor growth. Roux hepaticojejunostomy on the excluded loop of the small intestine was performed in 15 (6.0%) patients with malignant OJ genesis, including 8 (3.2%), 5 (2.0%), and 2 (0.8%) cases of bile ducts cancer, pancreatic head cancer, and gallbladder cancer with penetration into HDL, accordingly. A 15-20 mm hepaticojejunostomy ensured longer decompression of bile ducts in case of subsequent tumor growth. Also, patients with pancreatic head cancer underwent antegrade CBD stenting in 4 (1.6%). 2 (0.8%) patients were subjected to bouginage and stenting of hepaticocholedochus for cancer metastases originating from another location in the liver.

3 (1.2%) patients with DU that penetrated HDL underwent ulcer excision and duodenoplasty. After the separation, a 1/3 duct circle-long defect was formed in the CBD. To prevent forming stricture, a T-shaped drainage balloon was used (Ukraine patent No.104469).

Table I. Complications after minimally invasive surgical interventions

Complications	Group I (n=62)	Group II (n=74)	p
Clipping of the common hepatic duct	1	-	
Failure of cystic duct stump	1	-	
Pancreatic necrosis	-	1	
MDP bleeding	-	2	
Stent obstruction	-	2	
Total	2	5	
%	3,2%	6,8%	p<0.05

Table II. Complications after open surgical interventions

Complications	Group I (n=38)	Group II (n=76)	p
Failure of CBD sutures	1	2	
Failure of cystic duct stump	2	-	
Failure of biliodigestive anastomosis	-	3	
CBD damage	-	1	
External biliary fistula	-	2	
Cholemic bleeding	1	1	
Total	4	9	
%	10.5%	11.8%	p>0.05

T-shaped drainage balloon is a tool made in the form of elastic longitudinal draining tube 1, having a diameter of 5-mm, which is perpendicularly connected to a 2-cm long transverse draining tube 2 and a 1-cm short transverse drainage tube 3, both having a diameter of 4-mm, with a V-shaped recess at the junction of the upper walls of the transverse short and longer draining tubes. The distal end of the tool is equipped with a balloon 4 connected to an additional tube 5, having a diameter of 1-mm (Fig. 1).

Once the CBD defect is sutured, a T-shaped drainage balloon is inserted in a transverse direction through a separate incision in its wall below the lesion, followed by expanding the balloon by saline injection. The balloon positioned at the level of bile duct plasty, thus preventing its narrowing for a long time (Fig. 2). The drainage was easy to remove after reducing the balloon volume due to a V-shaped recess at the junction of the upper walls of the transverse short and longer draining tubes. Balloon dilation was administered for 3 months. No signs of CBD stricture were observed two years post-surgery.

DISCUSSION

Along with significant advancement in treatment of OJ patients, biliary decompression is often accompanied by development of post-operative liver failure ("rapid biliary decompression syndrome") [3-6]. Today, both open and minimally invasive surgical interventions are used in biliary surgery [7, 8]. Minimally invasive interventions are performed both in one and several stages [10-12]. However, in these studies, the criteria for choosing a method of bile ducts decompression in patients of different age groups remain uncertain.

According to the results of the study, no post-decompression liver dysfunction was observed after one-stage minimally invasive surgical interventions. Because, one-stage minimally invasive surgical interventions were performed in patients with hyperbilirubinemia below 200 μmol/l, OJ duration up to 14 days, compensated or subcompensated concomitant pathology, and no concomitant purulent cholangitis and biliary pancreatitis. Two-stage minimally invasive surgical interventions were used for treatment of patients with bilirubin count over 200 μmol/l, OJ duration over 14 days, the presence of comorbidities in the stage of decompensation, complicated by OJ purulent cholangitis and biliary pancreatitis. No significant functional disbalance of the liver was observed following two-stage minimally invasive surgical interventions. Because, two-stage approach involved endoscopic transpapillary interventions at the first stage and LCE at the second stage. The interval between minimally invasive interventions was 3-14 days, which was optimal for the post-decompression period. Open surgical interventions on bile ducts were

used when it was impossible to cope OJ by minimally invasive methods. The formation of areflux biliodigestive anastomoses was associated with a moderate rate of bile duct decompression preconditioned by a valve mechanism. External biliary decompression was associated with a rapid decrease of pressure in bile ducts, which led to post-decompression liver dysfunction and required dosed decompression of the bile ducts with a probe-obturator during 7-10 days and complex conservative therapy.

After minimally invasive surgical interventions, the average duration of hospital stay of Group I and II patients was 5.0 ± 1.2 days and 9.5 ± 1.3 days, accordingly. After open operations, the average length of hospital stay of Group I and II patients was 10.2 ± 1.2 days and 12.3 ± 1.5 days, accordingly.

In the course of the study, an assessment was given of the effectiveness of using minimally invasive and open methods of bile duct decompression for treatment of OJ by comparing complications in patients of different age groups. Complications after minimally invasive surgery (n = 62) were registered in Group I patients in 2 (3.2%) cases: clipping of the common hepatic duct - 1 (1.6%), failure of cystic duct stump - 1 (1.6%). No fatalities were registered. Complications following minimally invasive surgery (n = 74) in Group II patients were diagnosed in 5 (6.8%) cases: pancreatic necrosis - 1 (1.3%), MDP bleeding - 2 (2.7%), and stent obstruction - 2 (2.7%). No lethal cases were registered (Table I).

Complications after open surgery (n = 38) in Group I patients were observed in 4 (10.5%) cases: failure of CBD sutures - 1 (2.6%), failure of cystic duct stump - 2 (5.2%), cholemic bleeding - 1 (2.6%). No fatal cases were registered. Complications following open surgery (n = 76) in Group II patients were diagnosed in 9 (11.8%) cases: CBD damage - 1 (1.3%), external biliary fistula - 2 (2.6%), failure of CBD sutures - 2 (2.6%), failure of biliodigestive anastomosis - 3 (3.9%), and cholemic bleeding - 1 (1.3%). 2 (2.6%) OJ patients died of transmural myocardial infarction (Table II).

Compared to Group II patients, complications in Group I patients were observed 2.1 and 1.1 times less often after minimally invasive and open procedures, accordingly. Thus, these results suggested that the frequency of complications after minimally invasive surgical interventions in Group I patients was statistically significantly lower than the one in Group II patients ($p < 0.05$). It should be noted that no statistically significant difference was found between the frequency of complications after open operations in the patients of I and II study Groups ($p > 0.05$).

CONCLUSIONS

1. The use of minimally invasive surgical interventions for treatment of young and middle-aged OJ patients compared to patients of older age groups makes it possible to reduce the frequency of complications by 2.1 times, which is a statistically significant ($p < 0.05$).
2. The frequency of complications after open surgical interventions of bile ducts in patients of different age groups is not statistically significant ($p > 0.05$). Therefore, should be used open surgical interventions when it was impossible to eliminate OJ by minimally invasive methods in patients of different age groups.

REFERENCES

1. Olsson G., Frozanpor F., Lundell L. et al. Preoperative biliary drainage by plastic or self-expandable metal stents in patients with periampullary tumors: results of a randomized clinical study. Endosc Int Open. 2017; 5(9): E798-E808. doi: 10.1055/s-0043-110565.
2. Sha J., Dong Y., Niu H. A prospective study of risk factors for in-hospital mortality in patients with malignant obstructive jaundice undergoing percutaneous biliary drainage. Medicine (Baltimore). 2019; 98(15): e15131. doi: 10.1097/MD.0000000000015131.
3. Celotti A., Solaini L., Montori G. et al. Preoperative biliary drainage in hilar cholangiocarcinoma: Systematic review and meta-analysis. Eur J Surg Oncol. 2017; 43(9): 1628-1635. doi: 10.1016/j.ejso.2017.04.001.
4. Kanikovskyi O.Y., Karyi Y.V., Babiichuk Y.V. et al. Comparative assessment of bile duct decompression methods in patients with obstructive jaundice of non-tumor genesis. Wiad Lek. 2019; 72(7): 1247-1252.
5. Khoronko Y.V., Korobka V.L., Groshilin V.S. et al. "Rapid" biliary decompression syndrome in obstructive jaundice surgery. Annals of HPB Surgery. 2019; 24(2): 123-129.
6. Liu C., Lu J.W., Du Z.Q. et al. Association of Preoperative Biliary Drainage with Postoperative Morbidity after Pancreaticoduodenectomy. Gastroenterol Res Pract. 2015; 2015: 796893. doi: 10.1155/2015/796893.
7. Wang L., Yu W.F. Obstructive jaundice and perioperative management. Acta Anaesthesiol Taiwan. 2014; 52(1): 22-29. doi: 10.1016/j.aat.2014.03.002.
8. Stark A., Hines O.J. Endoscopic and operative palliation strategies for pancreatic ductal adenocarcinoma. Semin Oncol. 2015; 42(1): 163-176. doi: 10.1053/ j.seminoncol. 2014.12.014.
9. Tang Z., Yang Y., Meng W. et al. Best option for preoperative biliary drainage in Klatskin tumor: A systematic review and meta-analysis. Medicine (Baltimore). 2017; 96(43): e8372. doi: 10.1097/MD.0000000000008372.

10. Nychytaylo M.Y., Dziubanovskyi O.I. Rationale for the timing of laparoscopic cholecystectomy on the basis of the rate of biliary tract decompression in obstructive jaundice caused by cholecystocholedocholithiasis. Hospital Surg. 2019; 4: 73-77.
11. Kagedan D.J., Mosko J.D., Dixon M.E. et al. Changes in preoperative endoscopic and percutaneous bile drainage in patients with periampullary cancer undergoing pancreaticoduodenectomy in Ontario: effect on clinical practice of a randomized trial. Curr Oncol. 2018; 25(5): e430-e435. doi: 10.3747/co.25.4007.
12. Nakai Y., Yamamoto R., Matsuyama M. et al. Multicenter study of endoscopic preoperative biliary drainage for malignant hilar biliaryobstruction: E-POD hilar study. J Gastroenterol Hepatol. 2018; 33(5): 1146-1153. doi: 10.1111/jgh.14050.
13. Ogura T., Takenaka M., Shiomi H. et al. Long-term outcomes of EUS-guided transluminal stent deployment for benign biliary disease: Multicenter clinical experience (with videos). Endosc Ultrasound. 2019; 8(6): 398-403. doi: 10.4103/eus.eus_45_19.
14. Yu H., Wu S., Yu X. et al. Single-incision laparoscopic biliary bypass for malignant obstructive jaundice. J Gastrointest Surg. 2015; 19(6): 1132-1138. doi: 10.1007/s11605-015-2777-4.

The article is a part of complex scientific research work at Surgery Department of the Medical Faculty No.2 of the National Pirogov Memorial Medical University, Vinnytsia, "Evaluation of the effectiveness of minimally invasive techniques and the use of various energy sources in the treatment of diseases of the gastrointestinal tract", state registration number 0120U101673.

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

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Received: 14.04.2022
Accepted: 18.01.2023

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

ORIGINAL ARTICLE

RISK FACTORS ASSOCIATED WITH CANCER PATHOLOGY

DOI: 10.36740/WLek202302115

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ABSTRACT

The aim of the study is comparative assessment of the main risks of the global burden of cancer in the total burden of death.

Materials and methods: A comparative assessment of the main risks of the global burden of cancer within the overall burden of deaths was carried out based on the data of the Global Burden of Disease Study (GBD), data from the Center for Medical Statistics of the Ministry of Health of Ukraine, the National Cancer Registry of Ukraine. The methods of comparative analysis, systematic approach and system analysis, bibliosemantic and medical-statistical methods were used.

Results: Higher attributable risk of death in most nosological forms of cancer among the population of Ukraine (bronchial, tracheal and lung cancer, laryngeal, pharyngeal, lip and esophageal cancer) have been observed. Behavioral factors at the level of Ukraine, compared to the world level, are characterized by significantly higher rates of attributable risk with regard to tobacco smoking (cancer of the larynx, pharynx, lower lip, esophagus) and alcohol consumption (pharynx, liver, lower lip). Environmental and occupational factors in Ukraine do not exceed the global exposure rates, and are lower for some cancer nosologies, namely bronchial, tracheal, lung and laryngeal cancer. Unlike global trends, metabolic factors prevail among the mortality risks of patients with liver, esophageal, uterine and kidney cancer in Ukraine.

Conclusions: Behavioral, occupational, environmental and metabolic risk factors for cancer mortality have high attributable risk. Behavioral risk factors for cancer mortality have the most pronounced impact both globally and in Ukraine, and notably, for the majority of nosological forms of cancer in Ukraine mortality risks are higher compared to the global data.

KEY WORDS: behavioral risk factors, occupational risk factors, environmental risk factors, metabolic risk factors, cancer pathology

Wiad Lek. 2023;76(2):346-351

INTRODUCTION

In Ukraine, as well as globally, cancer pathology ranks second among the causes of death of the adult population [1, 2]. The burden of cancer remains an important public health issue. Risk factors, whose impact on the development of cancer pathology can be modified, have a major effect on life expectancy adjusted for cancer-related disability [3]. Currently, a major impact on the development of cancer is attributed to modifiable risk factors, which constitute the majority among all factors associated with the development of this disease [4]. Moreover, the latter should be taken into account when developing a strategy to reduce premature mortality from non-communicable diseases, including malignant tumors. In order to effectively overcome the growing risk factors for cancer both in Ukraine and globally, comprehensive efforts are needed, which would include both therapeutic and preventive measures to reduce the combined effect of these factors on the development of tumors. Despite the fact that there are types of malignant tumors that are difficult to prevent, it is important to minimize the impact of risk factors on the development of cancer pathology. It should also be noted that primary prevention of cancer development is the most cost-effective and efficient strategy, which should be combined with broad comprehensive measures to reduce the impact of factors on the development of cancer, including developed secondary prevention strategies, screening programs and ensuring effective capacity for diagnosis and treatment of cancer patients. Thanks to existing studies of the global burden of disease and risk factors, it is possible to quantify the burden of cancer associated with modifiable risk factors and compare national and global rates using both mortality and disability-adjusted life years [5 - 8]. The study of global burden of disease, injury, and risk factors (GBD) is the only study to date that quantifies the burden of cancer associated with a broad set of modifiable risk factors for all countries, across age groups, and by sex [2]. GBD 2019, the latest iteration of the GBD study, provides an estimate of the global burden of cancer attributable to risk factors [9, 10]. The International Agency for Research on Cancer Observatory provides estimates of global, regional, and national risk-attributable

cancer burden for a subset of potentially modifiable risk factors (e.g., obesity, alcohol consumption, infections, and ultraviolet radiation), but these estimates are not provided together in a comprehensive way over time, and some potentially modifiable risk factors are not assessed as part of this effort. Studies of the global burden of cancer have identified a list of behavioral, metabolic, environmental and occupational factors associated with both mortality and disability-adjusted life years, providing new insights into the burden of cancer. Globally, a significant share of cancer deaths and years of healthy life lost due to cancer is attributable to modifiable risk factors, with behavioral factors accounting for the largest burden.

The concept of Global Burden of Disease Study (GBD), which is a global program for estimating disability and mortality from major diseases, injuries and risk factors, has been introduced since 1990 to provide information on the state of health of the population and to develop basic health strategies for disease prevention.

THE AIM

The aim of the present study was to carry out a comparative assessment of the main risks of the global burden of cancer within the overall burden of death.

MATERIALS AND METHODS

A comparative assessment of the main risks of the global burden of cancer within the overall burden of deaths was carried out based on the data of the Global Burden of Disease Study (GBD), which is a global program for assessing disability and mortality from major diseases, injuries and risk factors, data from the Center for Medical Statistics of the Ministry of Health of Ukraine, the National Cancer Registry of Ukraine. At the same time, the data on the burden of disease in Ukraine and in the world based on gender and localization of malignant neoplasm were taken into account, attributive risk factors for the development of oncological pathology were studied.

In addition, methods of comparative analysis, systematic approach and system analysis, as well as biblio-semantic and medical-statistical methods were used.

RESULTS

According to the data of the Center for Medical Statistics of the Ministry of Health of Ukraine, in 2019, from among the entire population of Ukraine, 77,481 people died from malignant tumors, which is 198.2 per 100 thousand people.

Studies of the global burden of disease have identified forms of malignant tumors globally that account for more than one percent of the total number of deaths. In particular, according to GBD 2019 data, the most common causes of death for men were: tracheal, bronchial and lung cancer, colon and rectal cancer, stomach, prostate, esophageal and liver cancer. Moreover, at the global level, mortality from the above forms of cancer – except for colon and rectal cancer mortality—exceeds mortality rates in Ukraine (Figure 1). According to the National Cancer Registry of Ukraine, within the structure of malignant tumor mortality in men in Ukraine, the following are shares of the main nosological forms of malignant tumors: tracheal, bronchial and lung cancer – 16.5%, prostate cancer – 12.9%, colon and rectum cancer – 7.7%, stomach cancer – 7.4%, bladder cancer – 6.9%.

The highest female mortality both globally and in Ukraine is the mortality from breast cancer (Figure 2). The next largest causes of female mortality globally are tracheal, bronchial and lung cancer, colon and rectal cancer, stomach cancer and cervical cancer.

In Ukraine, the share of cancers other than colon, rectal and stomach cancer within the structure of cancer mortality is much lower. In the structure of cancer mortality among the female population of Ukraine, the share of deaths from breast cancer is 23.8%, uterine cancer – 11.1%, colon cancer – 7.5%, cervical cancer – 6.3%, ovarian cancer – 5.8%.

Cancer GBD studies have identified mortality risk factors, which are generally split into three groups: environmental and occupational, behavioral, and metabolic. These risk factors are associated with 44.4% of deaths globally among all cancer deaths. In 2019, the total number of years of healthy life lost due to cancer-related premature death and disabling illness associated with these risk factors was 105 million for both sexes combined, accounting for 42.0% of all cancer-related healthy life lost. GBD estimates that men had 67.5 million (60.8-75.1) cancer-related healthy life years lost due to risk factors, or 48.0% (45.3-51.5), and women had 37.6 million (32.8-43.1), or 34.3% (30.9-38.7).

Behavioral risks are considered to be the leading risk factors associated with cancer deaths (Table I). In particular, behavioral factors in Ukraine were 7.2% more associated with bronchial, tracheal and lung cancer, 14.1% – laryngeal cancer, 22.9% – pharyngeal cancer, 11.9% – lip cancer, 19.5% – esophageal cancer.

Among the behavioral factors most strongly associated with cancer mortality and characterized by significantly higher rates of attributable risk in Ukraine compared to global data, tobacco smoking and alcohol consumption should be highlighted.

Thus, compared to the global data, smoking in Ukraine is 8.5% more associated with lung cancer, 8.3% – esophageal cancer, 13.5% – laryngeal cancer, 20.4% – pharyn-

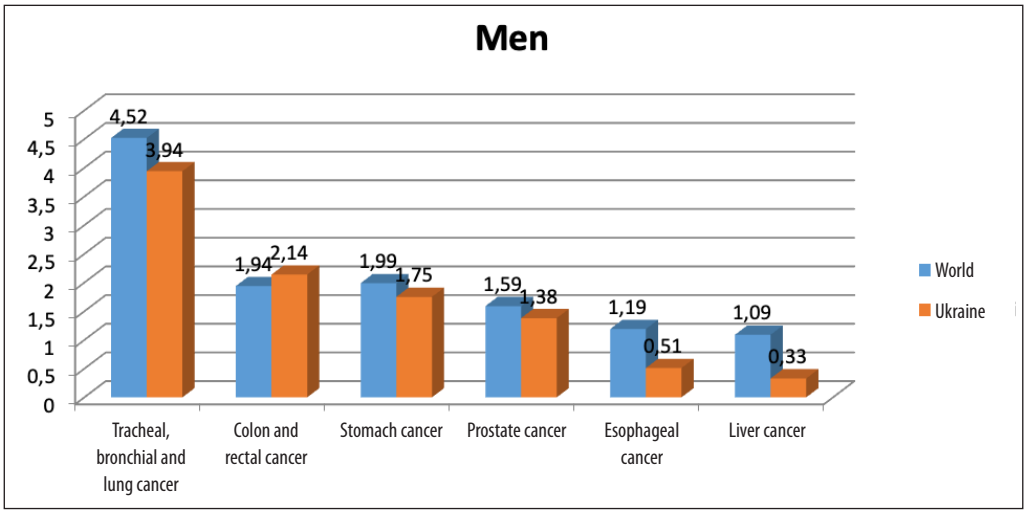

Fig. 1. Share of male mortality from certain types of cancer in the total number of deaths.

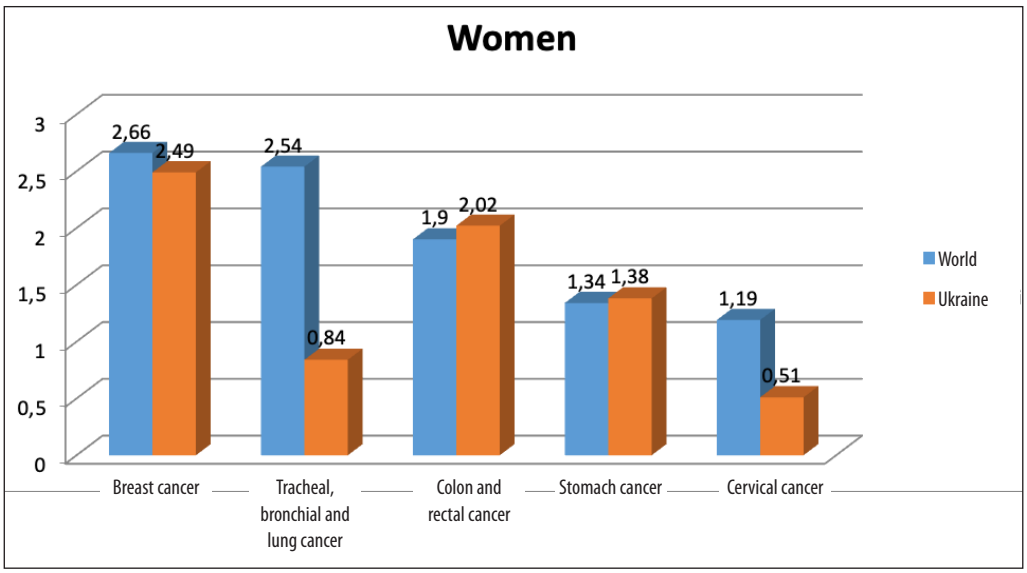

Fig. 2. Share of female mortality from certain types of cancer in the total number of deaths.

geal cancer, 18.0% – cancer of the lower lip (Figure 3).

An important factor associated with the mortality of people with cancer is alcohol consumption. Like tobacco smoking, alcohol consumption also is more strongly associated with mortality of people suffering from various nosological forms of cancer (Figure 4).

he second large group of factors associated with mortality from malignant tumors are environmental and occupational risk factors. Such environmental factors include: contaminated water, poor sanitary conditions, air pollution, environmental pollution, abnormal temperatures, toxic effects of lead. Among occupational risks GBD researchers consider occupational carcinogens, industrial emissions of solid particles, gases and vapors, occupational noise, occupational ergonomic factors.

The data of comparative analysis of the attributable risk of environmental and occupational factors in cancer mortality are presented in Table II.

Overweight, high serum glucose, low-density lipoprotein, high blood pressure, low bone mineral density belongs to metabolic factors, i.e., the third group, and are known risk factors for various chronic diseases and mortality. In most cases, all types of metabolic disorders are combined with each other and their development is based on an increase in the amount of abdominal fat, overweight or obesity. In recent decades, the prevalence of overweight and obesity has been increasing globally, raising concerns about their impact on health. Data of GBD studies on the impact of metabolic factors is presented in Table III.

In general, compared to the global data, metabolic factors in Ukraine are higher among the mortality risks of patients with liver cancer by 6.8%, esophageal cancer – 12.6%, uterine cancer – 15.1%, kidney cancer – 5.6%.

DISCUSSION

The study has observed a significant impact of behavioral factors on the development of cancer pathology. Moreover, in the population of Ukraine, behavioral factors are characterized by higher rates of attributable risk of death

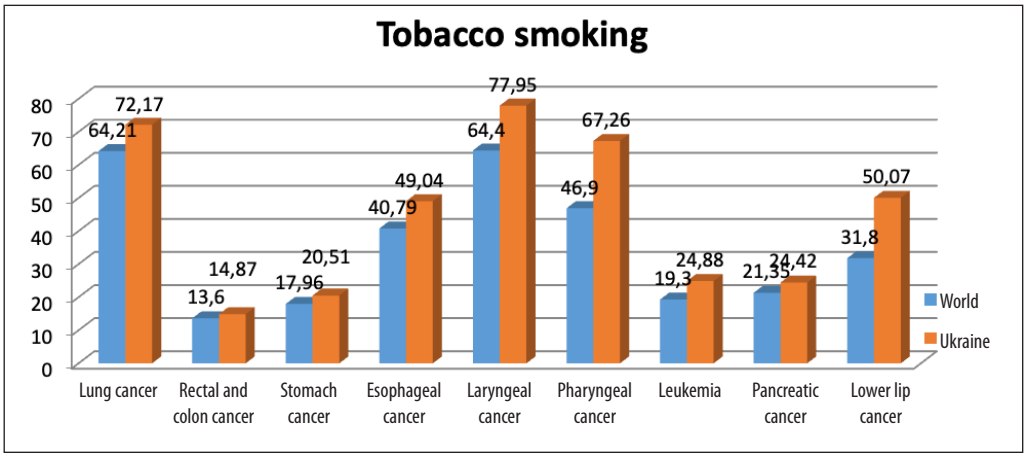

Fig. 3. Indicators of tobacco smoking attributable risk in mortality from certain types of cancer

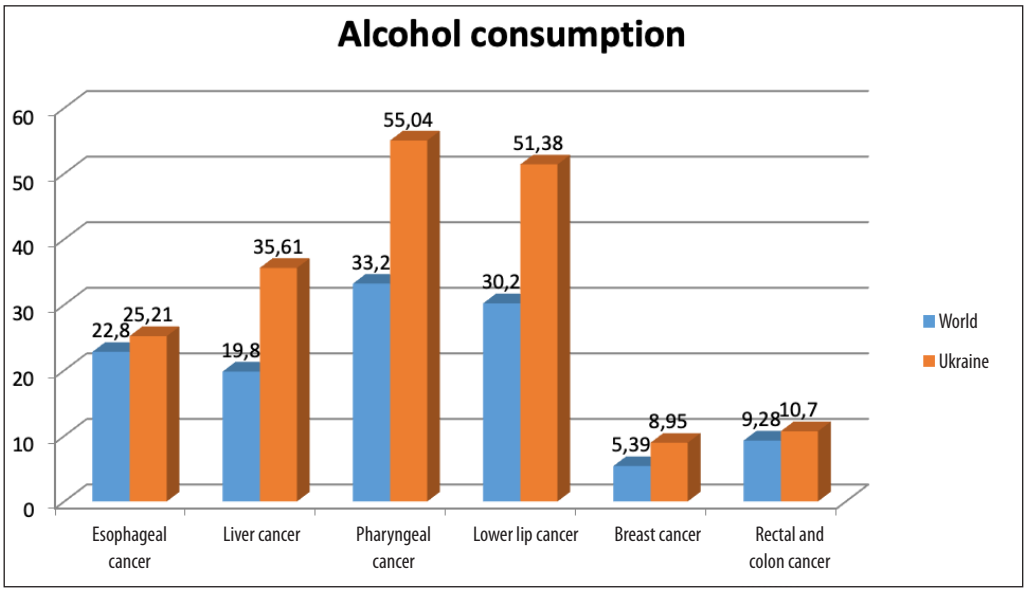

Fig. 4. Indicators of alcohol consumption attributable risk in mortality from certain types of cancer

in most nosological forms of cancer (namely, bronchial, tracheal and lung cancer, laryngeal, pharyngeal, lip and esophageal cancer).

Tobacco smoking has a significant impact on the development of oncological pathology. Thus, recent studies confirm the negative impact of tobacco smoking on public health and indicate an increased risk of developing not only lung cancer but also other cancers in smokers [11]. The age of patients who started smoking, the number of cigarettes and the duration of smoking are important [12]. Reducing smoking may reduce the risk of lung cancer, but the risk remains high [13]. Even when lung cancer is detected, smoking cessation improves the course of the disease, but not all patients want to give up this bad habit. Older people, people diagnosed with smoking-related cancer, people with higher education, and obese people are more likely to quit, while people living below the poverty level are less likely to quit [14].

Combined use of alcohol and tobacco increases the risk of colorectal and prostate cancer. A U-shaped multiplicative association was observed for breast cancer when both alcohol and tobacco were consumed together [15].

Compared to the global data, tobacco smoking in Ukraine is more associated with the risk of lung, esophageal, laryngeal, pharyngeal, and lower lip cancer. The association between alcohol consumption and mortality from liver, pharyngeal and lower lip cancer is especially pronounced in Ukraine.

The numerical data of the indicators show that environmental and occupational factors in Ukraine do not exceed the impact indicators at the global level, and for some nosologies – namely, bronchial, tracheal, lung and laryngeal cancer – cancer pathologies are estimated to be lower.

To date, researchers have confirmed the association between body mass index and the risk of developing adenocarcinoma of the esophagus, colon, rectum, kidney, pancreas, and gallbladder, as well as postmenopausal breast, ovarian and endometrial cancer. GBD researchers attribute about 4% of cancer deaths to high body mass index. Researchers point to strong evidence that being overweight is associated with an increased risk of cancer of at least 13 anatomical areas, including endometrial, esophageal, renal and pancreatic adenocarcinomas; hepatocellular carcinoma; gastric cardia cancer; meningioma; multiple myeloma;

Table I. Data on attributable risk of behavioral factors in cancer mortality

Cancer localization	Impact data at the global level, % (95% CI)	Impact data in Ukraine, % (95% CI)
Bronchial, tracheal and lung cancer	67.82 (65.53-69.95)	75.01 (71.98-77.76)
Laryngeal cancer	69.19 (62.98-74.52)	83.38 (78.67-88.89)
Pharyngeal cancer	61.87 (56.18-66.84)	84.57 (80.05-88.36)
Lip cancer	62.19 (57.50-66.51)	74.09 (68.21-79.06)
Rectal and colon cancer	50.41 (43.64-56.38)	47.52 (39.83-54.48)
Stomach cancer	24.24 (15.68-43.09)	24.51 (17.41-40.63)
Liver cancer	45.81 (39.74-51.30)	69.94 (65.75-73.89)
Esophageal cancer	61.09 (55.37-66.87)	71.43 (64.82-77.28)
Bladder cancer	33.89 (25.88-41.74)	40.79 (31.32-50.02)

Table II. Data of attributable risk of environmental/occupational factors in cancer mortality

Cancer localization	Impact data at the global level, % (95% CI)	Impact data in Ukraine, % (95% CI)
Bronchial, tracheal and lung cancer	33.91 (28.42-39.13)	23.79 (16.59-33.62)
Laryngeal cancer	6.19 (3.92-9.17)	3.75 (1.90-6.05)
Mesothelioma	91.68 (89.70-93.41)	92.45 (87.60-96.43)
Ovarian cancer	3.32 (1.52-5.40)	3.79 (1.18-8.63)

Table III. Data on metabolic factors attributable risk in cancer mortality

Cancer localization	Impact data at the global level, % (95% CI)	Impact data in Ukraine, % (95% CI)
Bronchial, tracheal and lung cancer	8.77 (2.03-19.06)	5.20 (0.99-12.25)
Rectal and colon cancer	16.13 (7.99-26.59)	16.37 (9.35-24.61)
Breast cancer	13.13 (5.91-22.89)	12.15 (5.09-20.69)
Liver cancer	13.47 (5.83-24.62)	20.34 (9.52-34.03)
Esophageal cancer	18.07 (5.78-35.19)	30.66 (8.83-53.82)
Cancer of the pancreas	14.54 (6.40-25.76)	13.25 (6.10-22.4)
Uterine cancer	39.81 (27.64-52.67)	54.87 (40.12-67.92)
Kidney cancer	19.05 (11.09-28.30)	24.77 (15.38-35.36)

colorectal, postmenopausal breast, ovarian, gallbladder and thyroid cancer [16].

It remains evident that at the moment this problem mainly concerns high-income regions, for example, 64% of all cancer cases globally associated with excessive body mass index were observed in North America and Europe. In Ukraine, metabolic factors prevail among the mortality risks in patients with liver, esophageal, uterine and kidney cancer.

CONCLUSIONS

Thus, behavioral, occupational, environmental and metabolic risk factors for cancer mortality have high attributable risk. Behavioral risk factors for cancer mortality have the most pronounced impact both globally and in Ukraine, and notably, for the majority of nosological forms of cancer in Ukraine mortality risks are higher compared to the global data.

REFERENCES

1. Rak v Ukrayini, 2020-2021. Zakhvoryuvanist', smertnist', pokaznyky diyal'nosti [Cancer in Ukraine, 2020-2021. Morbidity, mortality, performance indicators]. Bulletin of the National Chancery Register of Ukraine 2022; 23. Kyiv URL: http://www.ncru.inf.ua/publications/BULL_23/index.htm (UA)
2. Global Burden of Disease (GBD). URL: https://vizhub.healthdata.org/gbd-compare/
3. Kocarnik JM, Compton K, Dean FE et al. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: a systematic analysis for the Global Burden of Disease Study. 2019. JAMA Oncol. 2022; 8: 420-444.
4. Hecht SS Cigarette smoking and lung cancer: chemical mechanisms and approaches to prevention. Lancet Oncol. 2002; 3: 461-469.
5. Evans J, van Donkelaar A, Martin RV et al. Estimates of global mortality attributable to particulate air pollution using satellite imagery. Environ Res. 2013; 120: 33-42.

6. Bianchini F, Kaaks R, Vainio H Overweight, obesity, and cancer risk. Lancet Oncol. 2002; 3: 565-574.
7. Pukkala E, Martinsen JI, Lynge E et al. Occupation and cancer—follow-up of 15 million people in five Nordic countries. Acta Oncol. 2009; 48: 646-790.
8. Takala J Eliminating occupational cancer. Ind Health. 2015; 53: 307-309.
9. Islami F, Goding Sauer A, Miller KD et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin. 2018; 68: 31-54.
10. Poirier AE, Ruan Y, Volesky KD et al. The current and future burden of cancer attributable to modifiable risk factors in Canada: summary of results. Prev Med. 2019; 122: 140-147.
11. Sugawara Y, Tsuji I, Mizoue T et al. Cigarette smoking and cervical cancer risk: an evaluation based on a systematic review and meta-analysis among Japanese women. Japanese journal of clinical oncology. 2019;49(1);77-86.
12. Pierce JP, Shi Y, McMenamin SB et al. Trends in Lung Cancer and Cigarette Smoking: California Compared to the Rest of the United States. Cancer Prev. Res. (Phila). 2019;12(1);3–12.
13. Chang JT, Anic GM, Rostron BL et al. Cigarette smoking reduction and health risks: a systematic review and meta-analysis. Nicotine and Tobacco Research. 2021;23(4);635-642.
14. Talluri R, Domgue JF, Gritz ER, Shete S Assessment of trends in cigarette smoking cessation after cancer diagnosis among US adults, 2000 to 2017. JAMA network open. 2020;3(8);e2012164-e2012164.
15. Viner B, Barberio AM, Haig TR, et al. The individual and combined effects of alcohol consumption and cigarette smoking on site-specific cancer risk in a prospective cohort of 26,607 adults: results from Alberta's Tomorrow Project. Cancer Causes & Control. 2019;30(12);1313-1326.
16. Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism. 2019;92;121-135.

The work is carried out within the framework of the initiative research work of the I. Horbachevsky Ternopil National Medical University (Ternopil, Ukraine) "Risk factors associated with cancer pathology" (state registration number 0120 U104150).

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Conflict of interest:
The Authors declare no conflict of interest

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Received: 19.06.2022
Accepted: 28.01.2023

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

ORIGINAL ARTICLE

FUNCTIONAL STATE OF THE LIVER IN PULMONARY TUBERCULOSIS IN THE DYNAMICS OF PATHOGENETIC TREATMENT WITH A COMPLEX OF AMINO ACIDS

DOI: 10.36740/WLek202302116

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ABSTRACT

The aim: To investigate the influence of prescribing a complex of amino acids in pathogenetic therapy in patients with pulmonary tuberculosis on liver function.

Materials and methods: The study included 50 patients with drug susceptible TB and 50 patients with drug-resistant TB (multidrug-resistant and extensively drug-resistant).

Results: The study included 50 patients with drug susceptible tuberculosis (TB) and 50 patients with drug-resistant TB. When comparing biochemical parameters characterizing liver function in patients with drug-susceptible TB after 1 month of anti-tuberculosis therapy, it was found that patients receiving additional therapy with a complex of amino acids had a lower level of bilirubin, $p<0.05$. After 60 doses, patients receiving additional therapy with amino acids had significantly lower bilirubin levels alanine aminotransferase (ALT) and aspartate aminotransferase (AST), $p<0.05$. When comparing the biochemical parameters characterizing liver function in patients with drug-resistant tuberculosis after a month of anti-tuberculosis therapy, significantly higher protein level was found in the groups of patients receiving additional therapy with amino acids, as well as significantly lower ALT level, AST and creatinine $p<0.05$.

Conclusions: The additional appointment of the complex of amino acids in the pathogenetic therapy of patients with pulmonary tuberculosis makes it possible to reduce the severity of hepatotoxic reactions manifested by the main parameters (AST, ALT, total bilirubin) and to increase the protein-synthetic function of the liver, which allows us to recommend their appointment to improve the tolerance of anti-tuberculosis therapy.

KEY WORDS: tuberculosis, protein-synthetic function of the liver, bilirubin, liver enzymes

Wiad Lek. 2023;76(2):352-359

INTRODUCTION

To achieve the goals of the "End TB" strategy, the treatment effectiveness of drug-susceptible TB should be at least 85%, and for multi-drug resistant TB – at least 75% [1, 2]. However, factors such as treatment interruption due to poor tolerance to anti-tuberculosis therapy, as well as the need to interrupt treatment due to the development of side effects of anti-tuberculosis drugs, reduce the effectiveness of treatment. One of the most important problems in this area is the hepatotoxicity of anti-TB drugs. In the treatment with first-line drugs, the need to interrupt treatment occurs in about 11% of cases [3-5], and in general, the frequency of hepatotoxicity is 5-28% and depends on the chemotherapy regimens used, the initial state of the patient's hepatobiliary system, as well as the criteria for hepatotoxicity used in the clinic (in most reports, an increase in ALT or AST of more than 3 normal limits with symptoms associated with liver damage (abdominal pain, nausea, vomiting, weakness, jaundice) or an asymptomatic 5-fold increase in ALT or AST were used as criteria for drug-induced liver damage) [6, 7].

At the same time, a number of patients often have a transient asymptomatic or symptomatic increase in the level of liver enzymes while taking anti-tuberculosis drugs. Thus, in 20% of patients receiving isoniazid both in monotherapy during chemoprophylaxis and in combination therapy, the level of ALT and/or AST transiently increases [8-10].

2nd-line drugs are also often hepatotoxic [10, 11]. Fluoroquinolones, which are relatively safe drugs and are excreted mainly by the kidneys, nevertheless, can have a hepatotoxic effect associated with a hypersensitivity reaction [12, 13].

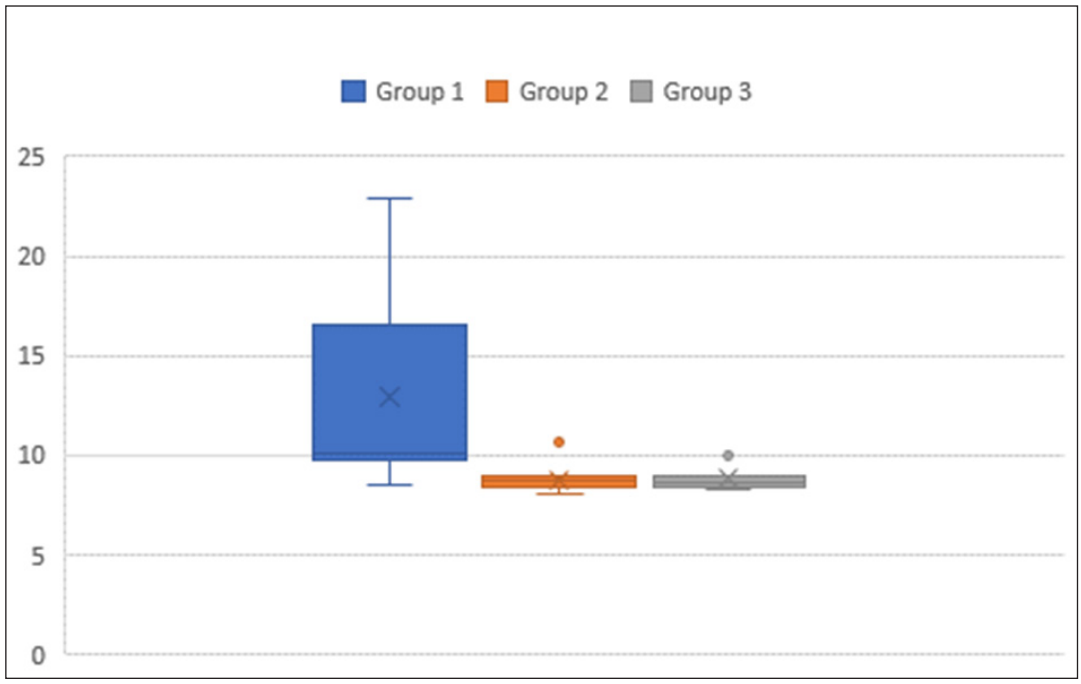

Fig. 1. Comparison of total bilirubin between groups in patients with drug-susceptible tuberculosis after 30 days of therapy.

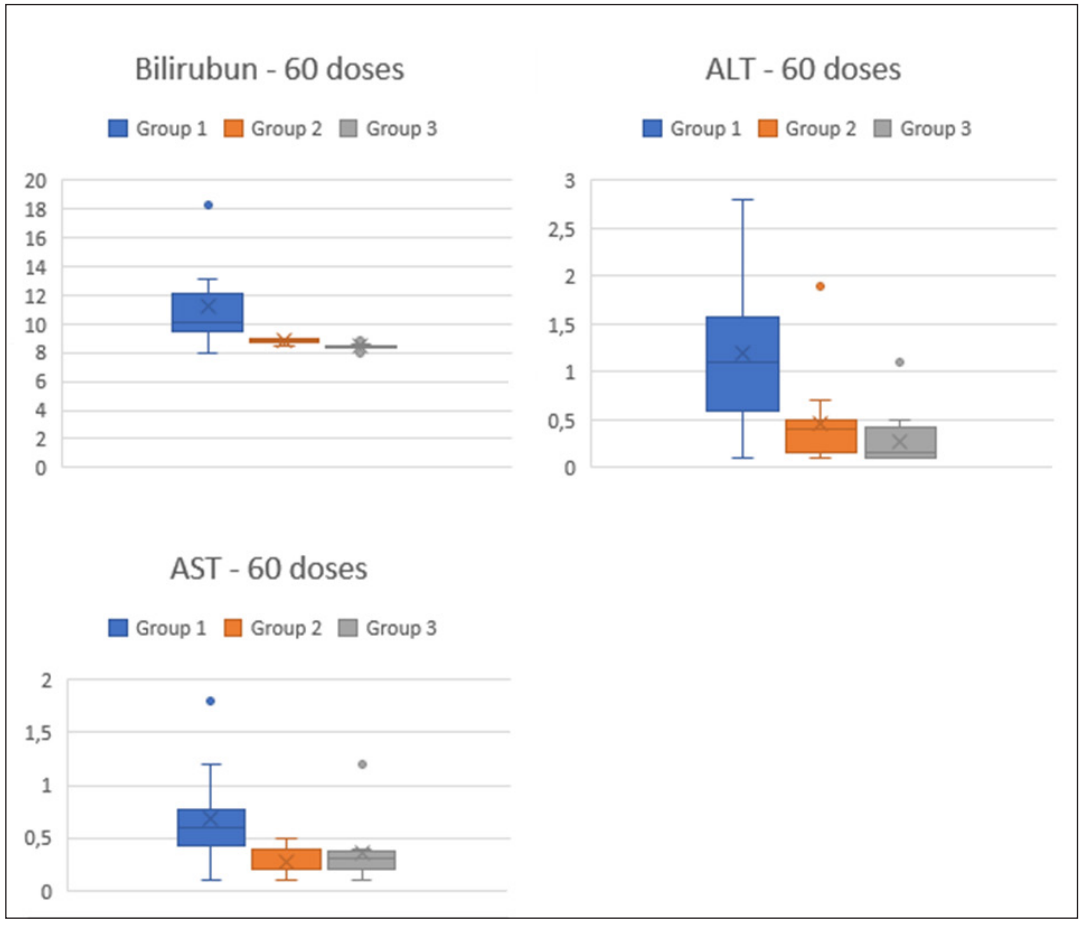

Fig. 2. Comparison of total bilirubin, ALT and AST between groups in patients with drug-susceptible tuberculosis after 60 days of therapy.

Thus, the search for new solutions to pathogenetic therapy in patients with tuberculosis, which will reduce the hepatotoxic effect of anti-tuberculosis drugs and normalize liver function improving the tolerance of anti-tuberculosis therapy, is an urgent issue.

THE AIM

The aim of the study was to investigate the influence of prescribing a complex of amino acids in pathogenetic therapy in patients with pulmonary tuberculosis on liver function.

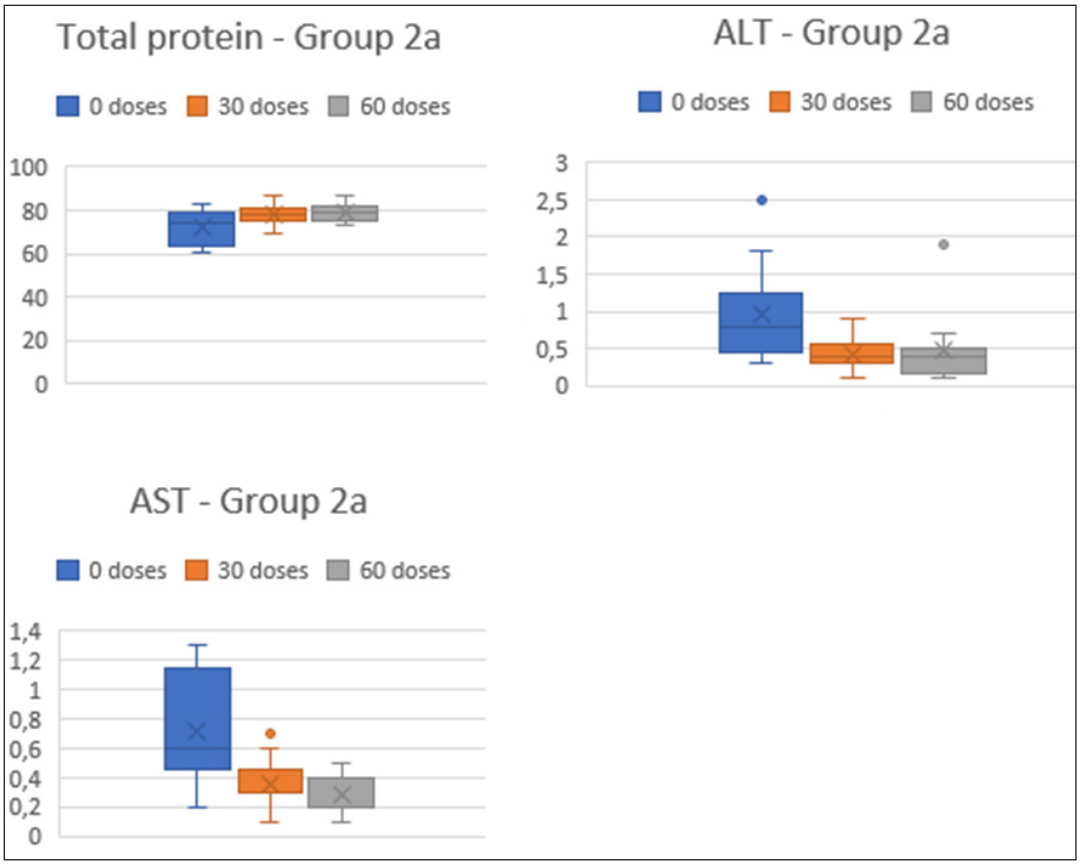

Fig. 3. Dynamics of total protein, ALT and AST in Group 2a of patients with drug-susceptible tuberculosis at 0, 30 and 60 doses of anti-tuberculosis treatment.

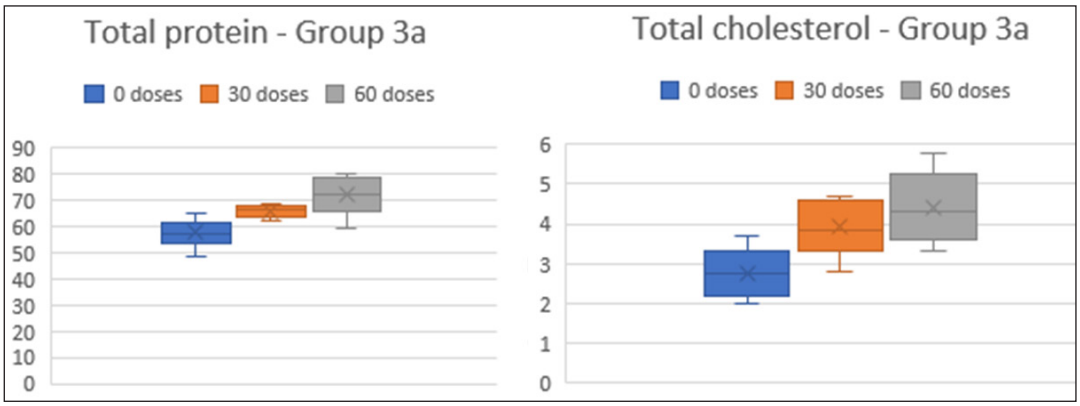

Fig. 4. Dynamics of total protein and total cholesterol in Group 3a patients with drug-susceptible tuberculosis at 0, 30 and 60 doses of anti-tuberculosis treatment.

MATERIALS AND METHODS

The hypothesis that was the basis of the study was that the additional inclusion of a complex of essential amino acids in the treatment of pulmonary tuberculosis improves the detoxification function of the liver, which should be reflected in better liver function tests, and increases the protein synthetic function of the liver, which entails an increase in the level of total protein. The study included 50 patients with drug susceptible TB and 50 patients with drug-resistant TB (multidrug-resistant and extensively drug-resistant). Criteria for including patients in the study were: signing an informed consent of participation in the study; age from 18 to 55 years; pulmonary tuberculosis. Criteria for excluding patients from the study were: age under 18 and over 55 years; tuberculosis/HIV co-infection; pregnancy. The patients were diagnosed, treated and monitored according to current state protocols and WHO guidelines. The patients with drug susceptible TB were divided into 3 groups: Group 1a (n=25) did not receive additional therapy, Group 2a (n=13) received essential amino acids in tablets for 30 days, Group 3a (n=12) received injectable amino acids for 10 days and amino acids in tablets for 20 days. Patients with drug-resistant TB were also divided into 3 groups: Group 1b (n=25) did not receive additional therapy, Group 2b (n=12) received essential amino acids in tablets for 30 days, Group 3b (n=13) received injectable amino acids for 10 days and amino acids in tablets for 20 days. The patients were measured Human-beta-defensine-1 (HBD-1), ALT, AST,

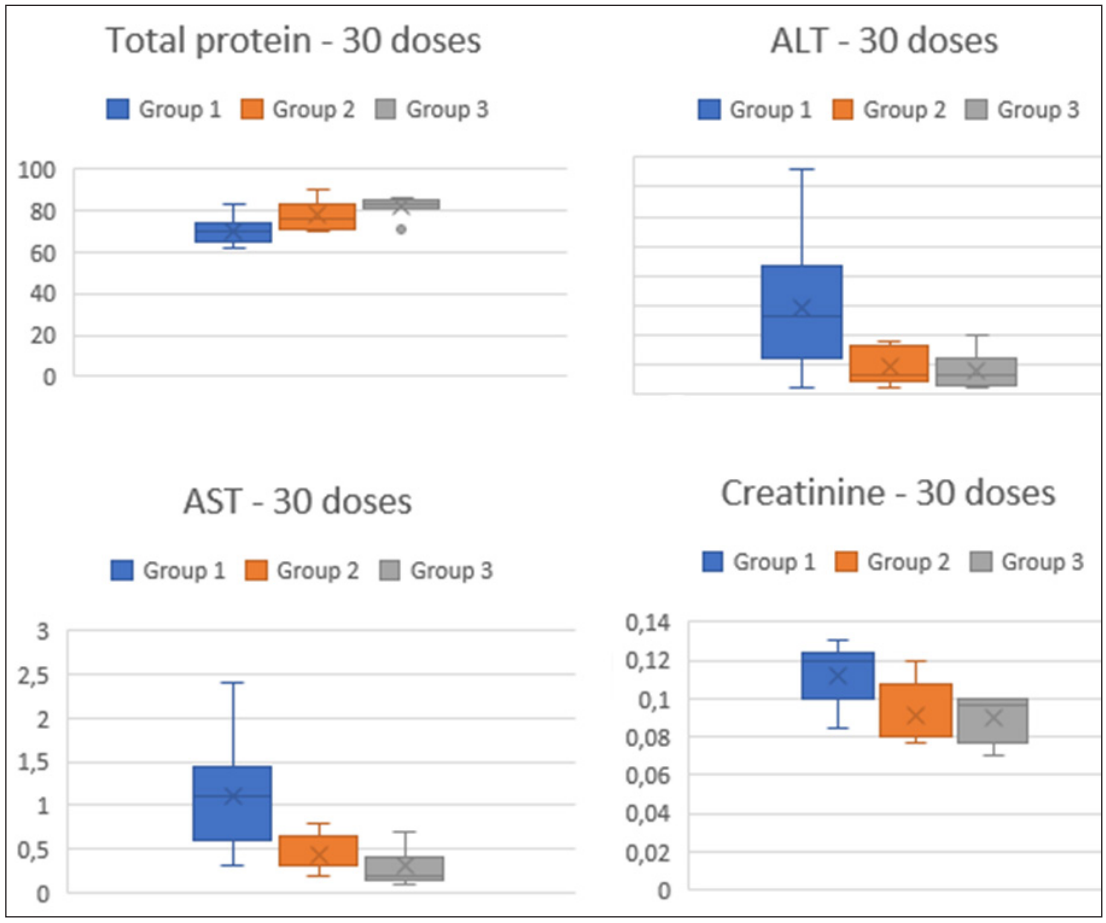

Fig. 5. Comparison of total protein, ALT, AST and creatinine between groups in patients with drug-resistant tuberculosis after 30 doses of anti-tuberculosis therapy.

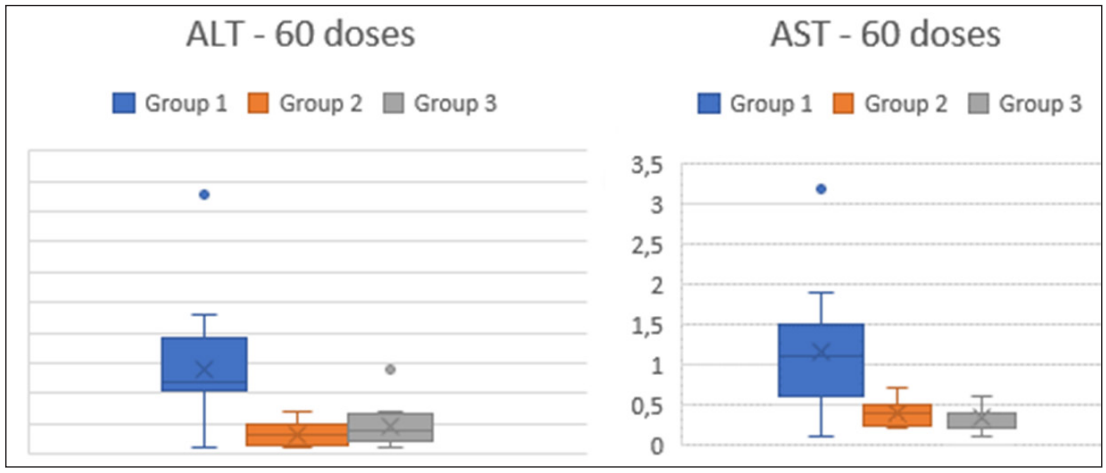

Fig. 6. Comparison of ALT and AST between groups in patients with drug-resistant tuberculosis after 60 days of anti-tuberculosis treatment.

bilirubiny-glutamyltranspeptidase (GGTP), total cholesterol, total protein, urea, creatinine. Statistical data processing was carried out using Statistica 8.0 with the calculation of the Spearman correlation coefficient, the Mann-Whitney test for comparing two independent groups, the Kruskell-Wallis test for comparing multiple independent groups, and the Friedman test for comparing multiple dependent groups.

RESULTS

When comparing biochemical parameters characterizing liver function in patients with drug-susceptible TB after 1 month of anti-tuberculosis therapy, it was found that patients receiving additional therapy with a complex of amino acids had a significantly lower level of bilirubin, which means that the patients without additional appointment of essential amino acids had more pronounced liver damage on the background of anti-tuberculosis treatment (Table I, Figure 1).

After 60 doses, patients receiving additional therapy with amino acids had significantly lower bilirubin, ALT and AST levels (the lowest levels of these parameters were ingroup 2a), which means that prescription of the complex of essential amino acids supports liver detoxification function even

Table I. Comparison of total bilirubin levels in groups of drug-susceptible TB patients after 1 month of treatment

Parameters of blood biochemistry	Group 1a (n=25) - no additional amino acids	Group 2a (n=13) additional amino acids in tablets	Group 3a (n=12) – injectable additional amino acids
Total bilirubin, μmol/L	13.34±2.23*■	8.90±0.23*	8.94±0.37■

* - p = 0,044
■ - p = 0,041

Table II. Comparing of biochemical parameters in groups of patients with drug-susceptible TB after 60 days of treatment

Parameters of blood biochemistry	Group 1a (n=25) - no additional amino acids	Group 2a (n=13) additional amino acids in tablets	Group 3a (n=12) – injectable additional amino acids	p- value
Total bilirubin, μmol/L	11.12±0.86	8.82±0, 06	9.57±0.69	0.0060
ALT, μmol/L	1.17±0.24	0.48±0.17	0.36±0.16	0.0129
AST, μmol/L	0.69±0.14	0.28±0.05	0.38±0.17	0.0194

Table III. Dynamics of biochemical parameters in groups of patients with drug susceptible TB from 0 to 60 doses of treatment

Biochemical parameters	Group 2a (n=13) additional amino acids in tablets			Group 3a (n=12) – injectable additional amino acids		
	Total protein, g/L	ALT, μmol/L	AST, μmol/L	Total protein, g/L	Total cholesterol, mmol/L	Urea, mmol/L
0 doses	72.02±2.28	0.96±0.18	0.72±0.10	57.24±2.91	2.86±0.24	5.98±0.69
30 doses	78.38±1.99	0.43±0.09	0.33±0.08	68.73±3.17	3.86±0.32	4.80±0.56
60 doses	78.86±1.51	0.30±0.07	0.28±0.05	75.76±1.63	4.40±0.38	4.32±0.54
p-value	p=0.043	p=0.002	p=0.014	p=0.037	p = 0.019	p = 0.047

Table IV. Comparing of biochemical parameters in groups of patients with drug-resistant tuberculosis after 1 month of treatment

Biochemical parameters	Group 1b	Group 2b	Group 3b	p-value
Total protein, g/L	70.89±1.31	77.73±2.26	80.62±1.63	p=0.0322
ALT, μmol/L	1.34±0.25	0.44±0.09	0.42±0.11	p=0.0224
AST, μmol/L	1.03±0.13	0.44±0.06	0.32±0.06	p=0.0004
Creatinine, mmol/L	0.111±0.003	0.092±0.005	0.091±0.004	p=0.0070

Table V. Comparing of biochemical parameters in groups of patients with drug-resistant tuberculosis after 2 months of treatment

Biochemical parameters	Group 1b	Group 2b	Group 3b	p-value
ALT, μmol/L	1.28±0.24	0.34±0.09	0.47±0.13	p=0.0044
AST, μmol/L	1.05±0.17	0.43±0.07	0.35±0.05	p=0.0004

30 days after discontinuation of medication and patients without additional treatment with essential amino acids have worse liver function tests (Table II, Figure 2).

Our study of the dynamics of these parameters in Group 2a showed a significant increase in the protein level from; decrease in ALT level; decrease in AST level from 0 doses to 30 doses and then to 60 doses which shows the dynamics of improvement of synthetic and detoxification liver functions in patients who received additional treatment with amino acids in tablet form (Figure 3).

Group 3a also showed a significant increase in total protein from 0 doses to 30 doses and then to 60 doses which shows improvement of synthetic liver function with use of both injectable and tablet forms of essential amino acids. We also found increase of total cholesterol in this group from subnormal to normal values which also emphasizes improvement of synthetic liver function in this patients against the background of additional prescription of essential amino acids. The urea level decreased in this group by 60 doses of treatment which reflects improvement of both liver and renal functions with the use of injectable amino acids (Figure 4).

The dynamics of biochemical parameters in patients with drug susceptible TB who received essential amino acids is shown in Table III.

The study of correlations between HBD-1 and biochemical parameters showed positive correlations with the level of total cholesterol, total protein and GGTP, and also a negative relationship with the level of unconjugated bilirubin, $p<0.05$.

When comparing the biochemical parameters characterizing liver function in patients with drug-resistant

tuberculosis after a month of anti-tuberculosis therapy, significantly higher protein level was found in the groups of patients receiving additional therapy with amino acids, as well as significantly lower ALT and AST levels and creatinine level which shows the same positive effect of essential amino acids in patients with drug resistant TB as in patients with drug susceptible TB (Table IV, Figure5).

At 60 doses patients with drug-resistant tuberculosis receiving additional therapy with a complex of amino acids had significantly lower ALT and AST levels which means better liver detoxification function in them comparing with the patients who did not receive additional treatment with essential amino acids (Table V, Figure 6).

DISCUSSION

Valine, leucine and isoleucine belong to the group of branched-chain amino acids. They stimulate mTOR receptors of hepatocytes, promoting the synthesis of glycogen and various proteins, in particular albumin, and regulate the metabolism of glucose and lipids. Deficiency of these amino acids leads to a decrease in the synthesis of fatty acids by the liver, β-oxidation of fatty acids and an increase in fat mobilization in adipose tissue through the AMP-activated protein kinase pathway [11]. In addition, these amino acids are directly involved in the proliferation and maturation of lymphocytes and dendritic cells. [12]. The modulating effect on lipid metabolism explains the dynamics of the total cholesterol level. Considering that injectable amino acids were received by patients with an initially more severe general state, severe symptoms of intoxication and exhaustion, the increase in cholesterol levels noted in them from subnormal to normal levels is probably due to the anabolic effect of branched-chain amino acids on lipid metabolism. At the same time, the decrease in cholesterol levels in patients with drug-resistant tuberculosis who did not receive additional therapy with amino acids indicates some disturbance of lipid metabolism and depletion of lipid reserves during the tuberculosis process and under the influence of second-line anti-tuberculosis drugs, in particular fluoroquinolones [13-15].

The main function of lysine is participation in protein synthesis. In addition, it is a precursor for the biosynthesis of carnitine and plays an important role in β-oxidation [16].

Methionine is a donor of methyl groups and an activator of enzymes in the processes of transmethylation and transsulfurization. One of the main negative consequences of its insufficiency is a violation of the bile production and the development of cholestasis. [17]. It also prevents fibrosis in the liver. [18].

Threonine is an essential amino acid and is involved in many physiological and biochemical processes, including growth, absorption, digestion, and immune function. To maintain intestinal function, threonine modulates the synthesis and immunity of mucin proteins. Threonine also affects glycine synthesis and protein phosphorylation. Threonine catabolism occurs primarily in the liver via two pathways, the enzymes threonine dehydratase and threonine dehydrogenase [19].

Tryptophan plays an important role in protein synthesis and is a precursor of various biologically active compounds such as serotonin, melatonin, kynurenine, NAD, NADP. Serotonin is a regulator of liver and gastrointestinal tract functions [20].

Phenylalanine is metabolized to tyrosine, which is a precursor of catecholamine neurotransmitters and is also included in the structure of all proteins in the human body.

Based on the functions of amino acids, we can conclude that they all take an important role in the normal functioning of the liver, in particular in its synthetic and detoxification functions, thereby causing a direct or indirect hepatoprotective effect. Both in patients with drug-susceptible and drug-resistant TB, we obtained lower liver function tests, namely ALT, AST and bilirubin, in patients receiving additional therapy with amino acids than in patients without additional pathogenetic therapy. In dynamics, there was a decrease in these parameters in patients with additional therapy with amino acids and an increase in the comparison group. The results obtained are presumably associated with the direct participation of amino acids in metabolic processes supported by the liver, and their hepatoprotective effect.

We also revealed a positive effect of the additional prescription of a complex of amino acids on the protein-synthetic function of the liver, which was manifested by a higher total protein in the blood plasma in patients who received additional amino acids with dynamics to its increase during treatment. The patients without additional amino acids treatment showed dynamics to lowering it.

The positive effect of amino acids has been previously described in patients with infectious diseases, in particular pneumonia [22]. However, the effect of the amino acid complex on the course of pulmonary tuberculosis has not been studied.

Indirectly, these changes in biochemical parameters correlated with the level of HBD-1, a cationic peptide of the immune system that has a direct bactericidal effect on *M. tuberculosis*, and also has chemoattractant properties for immune cells [23]. It was found that the higher the level of protein and total cholesterol, the higher the level

of HBD-1 during treatment. On the other hand, a negative correlation was found between HBD-1 and bilirubin and creatinine, which indicates the need for normal liver and kidney function for the synthesis of cationic peptides of the immune system. The direct correlation between HBD-1 and GGTP, an enzyme involved in the exchange of amino acids by catalyzing the transfer of γ-glutamyl functional groups to amino acids or peptides, deserves special attention. [24].

CONCLUSIONS

The data obtained in the study approved that of prescribing a complex of amino acids in pathogenetic therapy in patients with pulmonary tuberculosis improves synthetic and detoxification liver functions. In patients who additionally received essential amino acids we observed lower levels of total bilirubin, ALT and AST during treatment which showed better detoxification liver function, as well as higher level of total protein which showed better synthetic liver function. Additionally, we observed improvement of lipid metabolism and renal function against the background of prescription of essential amino acids. There were no differences between patients with drug-susceptible and drug-resistant tuberculosis in terms of the studied biochemical parameters at 30 and 60 doses when prescribing additional therapy with a complex of amino acids which allows to recommend pathogenetic treatment with essential amino acids to all the patients with pulmonary tuberculosis

REFERENCES

1. World Health Organization. The End TB Strategy. Global strategy and targets for tuberculosis prevention, care and control after 2015. 2014. http://www.who.int/tb/post2015_TBstrategy.pdf?ua=12014. [date access 22.06.2022].
2. Schaberg T., Rebhan K., Lode H. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. Eur Respir J. 1996;9:2026–30. doi: 10.1183/09031936.96.09102026.
3. Ostapowicz G., Fontana R.J., Schiodt F.V. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002;137:947–54. doi: 10.7326/0003-4819-137-12-200212170-00007.
4. Aithal G.P., Watkins P.B., Andrade R.J. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 2011;89:806–15. doi: 10.1038/clpt.2011.58.
5. Tostmann A., Boeree M.J., Aarnoutse R.E. Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. J Gastroenterol Hepatol. 2008;23:192–202. doi: 10.1111/j.1440-1746.2007.05207.x.
6. Scales M.D., Timbrell J.A. Studies on hydrazine hepatotoxicity. 1. Pathological findings. J Toxicol Environ Health. 1982;10:941–53. doi: 10.1080/15287398209530308.
7. Nakajima A., Fukami T., Kobayashi Y. Human arylacetamidedeacetylase is responsible for deacetylation of rifamycins: rifampicin, rifabutin, and rifapentine. Biochem Pharmacol. 2011;82:1747–56. doi: 10.1016/j.bcp.2011.08.003.
8. Lacroix C., Tranvouez J.L., Hoang T.P. et al. Pharmacokinetics of pyrazinamide and its metabolites in patients with hepatic cirrhotic insufficiency. Arzneimittelforschung. 1990;40:76–9.
9. Todoriko L., Semianiv I., Crisan-Dabija R. et al. Limited proteolysis and oxidative modification of proteins in the hepatocytes of patients with resistant forms of tuberculosis. Pneumologia. 2019;63(3):126-9. doi: 10.2478/pneum-2019-0023.
10. Andrade R.J., Tulkens P.M. Hepatic safety of antibiotics used in primary care. J Antimicrob Chemother. 2011;66:1431–46. doi: 10.1093/jac/dkr159.
11. Bai J., Greene E., Li W. et al. Branched-chain amino acids modulate the expression of hepatic fatty acid metabolism-related genes in female broiler chickens. Mol Nutr Food Res. 2015;59:1171-81. doi: 10.1002/mnfr.201400918.
12. Tajiri K., Shimizu Y. Branched-chain amino acids in liver diseases. World J Gastroenterol 2013;19:7620-9. doi: 10.21037/tgh.2018.07.06.
13. Bensikaddour H., Fa N., Burton I. et al. Characterization of the interactions between fluoroquinolone antibiotics and lipids: a multitechnique approach. Biophysical journal. 2008;94(8):3035–46. doi: 10.1529/biophysj.107.114843.
14. Shvets O.M., Shevchenko O.S., Todoriko L.D. Carbohydrate and lipid metabolic profiles of Tuberculosis patients with bilateral pulmonary Lesions and mycobacteria excretion. Wiadomości Lekarskie. 2020;73(3):1373-6. doi: 10.36740/wlek202007113.
15. Shevchenko O.S., Todoriko L.D., Radzishevska E.B. et al. Dynamics of aldosterone, connective tissue reorganization and glucose level as markers for tuberculosis treatment effectiveness. Archives of the Balkan Medical Union. 2019;54(2):274-80. doi: 10.31688/ABMII.20] 9.54.2.08.
16. Tome D., Bos C. Lysine Requirement through the Human Life Cycle. The Journal of Nutrition. 2007;137(6):1642-5. doi: 10.1093/jn/137.6.1642S.
17. Giudici G.A., Le Grazie C., Di Padova C. The use of ademethionine (SAMe) in the treatment of cholestatic liver disorders: meta-analysis of clinical trials. In: Mato JM, Lieber C, Kaplowitz N, Caballero A, eds. Methionine metabolism: molecular mechanism and clinical implications. Madrid: CSIC Press. 1992, 79p.
18. Martínez-Chantar M.L., García-Trevijano E.R., Latasa M.U. et al. Importance of a deficiency in S-adenosyl-l-methionine synthesis in the pathogenesis of liver injury. Am J Clin Nutr. 2002;76:1177S–82S. doi: 10.1093/ajcn/76/5.1177S.

19. Lee D-Y., Kim E-H. Therapeutic Effects of Amino Acids in Liver Diseases: Current Studies and Future Perspectives. Journal of Cancer Prevention. 2019;24(2):72-8. doi: 10.15430/JCP.2019.24.2.72.
20. Osawa Y., Kanamori H., Seki E. et al. L-tryptophan-mediated enhancement of susceptibility to nonalcoholic fatty liver disease is dependent on the mammalian target of rapamycin. The Journal of biological chemistry. 2011; 286(40): 34800–8. doi: 10.1074/jbc.M111.235473.
21. Kapalka G.M. Nutritional and Herbal Therapies for Children and Adolescents. Academic Press. 2010, 141p. doi: 10.1016/C2009-0-01890-X.
22. Ostrovskyy M.M., Varunkiv O.I., Todoriko L.D. Nitric oxide metabolism in patients with community-acquired pneumonia associated with coronary heart disease and the possibility of its medicamentous management. Wiadomości Lekarskie. 2020;73(3):1707-11. doi: 10.36740/wlek202008122 P 1707-1711.
23. Prasad S.V., Fiedoruk K., Daniluk T. et al. Expression and function of host defence peptides at inflammation sites. International Journal of Molecular Sciences. 2020;21:104. doi: 10.3390/ijms21010104.
24. Whitfield J.B. Gamma glutamyltransferase. Critical Reviews in Clinical Laboratory Sciences. 2008;38 (4):263–355. doi:10.1080/20014091084227.

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

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Received: 24.05.2022
Accepted: 18.01.2023

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

ORIGINAL ARTICLE

EFFECT OF MAGNESIUM L-LACTATE SUPPLEMENT ON BLOOD PRESSURE AND CORRECTED QT INTERVAL IN A SAMPLE OF IRAQI WOMEN WITH METABOLIC SYNDROME

DOI: 10.36740/WLek202302117

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ABSTRACT

The aim: The current study was designed for evaluation the effect of oral magnesium l-lactate supplementation on blood pressure and corrected QT interval in a sample of Iraqi women.

Materials and methods: In this interventional prospective randomized trial, 58 female patients diagnosed with MetS according to the International Diabetic Federation (IDF) criteria and were randomly allocated to receive either placebo or magnesium l-lactate 84 mg, twice daily.

Results: Office blood pressure showed a significant drop in systolic blood pressure (SBP) ($P<0.05$), non-significant decline in diastolic blood pressure (DBP), heart rate (HR), and pulse pressure (PP) ($P>0.05$), while ambulatory blood pressure monitoring (ABPM) recorded a significant reduction in HR in patients on magnesium supplement. Also, there was a significant decline in the SBP ($P<0.05$) and non-significant decline in DBP and PP ($P>0.05$) in patients with masked hypertension on Mg supplement. The changes in corrected QT- interval had no significant effect within Mg group ($P>0.05$).

Conclusions: From above results, one can conclude that oral Mg l-lactate supplement can improve, to a certain extent, blood pressure of women with MetS. Further studies in this aspect may be required.

KEY WORDS: metabolic syndrome, blood pressure, corrected QT, magnesium supplement

Wiad Lek. 2023;76(2):360-369

INTRODUCTION

The term of metabolic syndrome (MetS) was first identified by Haller and Hanefeld in 1975. The crucial components of MetS are obesity, insulin resistance (IR), dyslipidemia, and hypertension (HT). There are approximately 60% of female, ages range from 45 to 49 and 45% of men, ages range from 45 to 49 have MetS in Native Americans according to National Program of Cholesterol Education criteria, and it is considered the highest recorded prevalence worldwide. The MetS is fewer frequent in African-American males but more widespread in Mexican females in the United States. The main risk factors for widening of MetS are physical inactivity, diet rich in fats and carbohydrates, which leads to the two fundamental clinical features of MetS identified by abdominal obesity, and insulin resistance (IR) [1]. The theory of MetS has been developed over the years and obesity is stilled to be a foremost worldwide health problem. The MetS was initially projected as a group of medical risk factors that could be expressed by a common problem - IR. Metabolic syndrome can be associated with broad complications including chronic heart disease (CHD), atrial fibrillation, aortic stenosis, heart failure, ischemic stroke, and possibly veno-thromboembolic disease. Regular exercise is the primary step in treatment of MetS as it increases glucose metabolism in muscle and helps in weight reduction [2]. Patients with MetS who complete the analytical criteria for HT must be treated in accordance with the recommendations of the Seventh Report of the USA Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC7) [3]. Magnesium plays a necessary physiological role in many tasks of the body. This task is achieved by two vital characters, including the ability to make chelates with central ligands and the capacity to compete

with calcium on binding sites presented on proteins and membranes. Magnesium is necessary for the production of nucleic acid and proteins and for specific actions in diverse organs such as the cardiovascular and neuromuscular systems. Over 300 enzymes are dependent on magnesium [4]. When serum magnesium level is <1.8 mg/dL (< 0.74 mmol/L), this indicate for hypomagnesaemia. Most cases of magnesium deficiency are asymptomatic until levels of serum Mg drop below 1.2 mg/dL. Ventricular arrhythmia is one of the most life-threatening consequences of hypomagnesemia [5]. Magnesium deficiency leads to elevation of aldosterone synthesis, production of thromboxane and prostaglandins [6].

THE AIM

This study was designed to evaluate the effect of oral magnesium l-lactate supplementation on blood pressure and corrected QT interval in a sample of Iraqi women.

MATERIALS AND METHODS

This is interventional prospective randomized placebo-controlled trial was carried out on 58 females diagnosed with MetS according to the IDF criteria, with BMI of 30-40 kg/m2, age range from 30 to 60 years, and a waist circumference of > 80 cm, under supervision of professional endocrinologists, from October 2015 to August 2016. The protocol was reviewed and approved by Scientific and Ethics Committee in the College of Pharmacy / University of Al-Mustansiriayah and Alkindy College of Medicine/ University of Baghdad. Verbal consent was taken from each patient, and all participants were advised to take a low carbohydrate and low-fat diet regimen and achieving 60 minutes of aerobic exercise per day during their treatment duration. Certain exclusion criteria were followed to avoid interference with the study design and include: patients with a history of renal impairment, pregnant and lactating women, patients with a newly prescribed and/or added antihypertensive medication (less than one month), patients with a newly prescribed and/or added antidiabetic or lipid lowering agents (less than 2 month), those suffered thyroid disorders, patients on laxatives, diuretics, proton pump inhibitors and alcohols, those on antacids and other preparations or dietary supplements that containing magnesium or calcium in their compositions. Patients were randomly allocated to either placebo or Mg group. From 58 female patients, only 47 completed this study, the remaining 11 were excluded (8 from placebo group and 3 from Mg group) due to poor compliance, violation of the study protocol, or other reasons. Group (A) included 17 females taking placebo capsules twice daily for 8 weeks; while group (B) included 30 females taking Mg L-lactate tablets 84 mg twice daily after meal in a sustained release formula for 8 weeks. Office blood pressure was measured by Walgreens blood pressure monitor after 3-5 minutes rest in the sitting position applying an appropriate cuff size for each patient's arm circumference. Measurement of BP at the upper arm is chosen. The measurement was repeated four times, first reading was excluded, and the arithmetic mean of the three remainder readings was calculated. The measurements of systolic and diastolic blood pressure and heart rate readings were expressed as mm.Hg. and b.p.m, respectively. Pulse pressure (PP) was also calculated from measured DBP and SBP values [PP=SBP-DBP]. Heart rate was also measured [7]. An ambulatory blood pressure monitor was applied on the left arm. None of the patients had work at night. An appropriate cuff size was used (24-32 cm). Daytime value (every 30 minutes) was defined as 6 A.M-6 P.M and nighttime value (every 60 minutes) was defined as 6 P.M-6 A.M according to manufacturer instructions [8]. The records have analyzed by the software of the devise to calculate the requested parameters. For each patient daytime, nighttime, and 24-hour mean SBP, DBP, heart rate measurement were recorded. Twenty four-hours mean pulse pressure also was calculated for each patient. Night blood pressure variability (BPV) was based on intermittent ABPM measurements; BPV is identified by the standard deviation (SD) of the ABP recordings at night [9]. The normal dipping considered when 10%–20% reduction in blood pressure from day to night, and non-dippers are defined by a reduction in BP by less than 10%, while reverse dipping means the reduction of less than 0% [10]. In the present study, patients were classified to four categories to identify BP status according to the International Database on Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) as following [11]:

- Masked hypertensive patients: who had normal office (conventional) BP and elevated BP on ABPM.
- Sustained uncontrolled hypertensive patients: those with elevated BP on both office BP measurement and ABPM.
- Well controlled hypertensive patients: those with normal BP on both measurements of office and ABPM.
- White coat hypertension (WCH): those who expressed elevated BP on office measurement while had normal ABPM.

The readings of corrected QT were measured at baseline and after 8 weeks by using BIOMED electrocardiograph, the measurement was automated.

Table I. Effect of magnesium supplement on office blood pressure readings according to the covariance analysis.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
SBP (mmHg)	Placebo N=17	131.510	130.305±2.330	−1.205±2.330	0.006	↓0.92
	Magnesium N=30		121.787±1.734	−9.723±1.734		↓7.98
DBP (mmHg)	Placebo N=17	79.234	79.079±1.421	−0.155±1.421	0.074	↓0.19
	Magnesium N=30		75.825±1.069	−3.409±1.069		↓4.49
PP (mmHg)	Placebo N=17	52.276	51.030±2.024	−1.246±2.024	0.061	↓2.44
	Magnesium N=30		46.073±1.505	−6.203±1.505		↓13.46
HR (b.p.m)	Placebo N=17	80.351	80.712±1.59	0.360±1.59	0.137	↑0.44
	Magnesium N=30		77.763±1.162	−2.588±1.162		↓3.32

Data expressed by mean ± SEM and percentage of difference

P<0.05 was considered a significant difference between treatment groups at endline

N = number of patients, SEM = standard error of mean, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, HR = heart rate.

Table II. Effect of magnesium supplement on 24 hour ambulatory blood pressure monitoring (ABPM) readings according to covariance analysis.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
24-SBP mmHg	Placebo N= 17	139.96	137.423±2.181	−2.537±2.181	0.493	↓1.84
	Magnesium N= 30		135.511±1.662	−4.449±1.662		↓3.28
24-DBP mmHg	Placebo N= 17	79.934	79.224± 1.230	−0.710±1.230	0.154	↓0.89
	Magnesium N= 30		76.972± 0.940	−2.962±0.940		↓3.84
24-PP mmHg	Placebo N= 17	60.021	58.670±1.472	−1.351±1.472	0.828	↓2.30
	Magnesium N= 30		58.262±1.124	−1.759±1.124		↓3.01
24-HR b.p.m	Placebo N= 17	78.739	80.831±1.205	2.091±1.205	0.004	↑2.58
	Magnesium N= 30		76.168±0.921	−2.571±0.921		↓3.37

Data expressed by mean ± SEM and percentage of difference

P<0.05 was considered a significant difference between treatment groups at endline

N = number of patients, SEM = standard error of mean, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, HR = heart rate

Table III. Effect of magnesium supplement on day ambulatory blood pressure monitoring (ABPM) readings according to analysis of covariance.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Day-SBP (mmHg)	Placebo N= 17	142.347	139.501±2.296	−2.846±2.296	0.451	↓2.04
	Magnesium N=30		137.293±1.755	−5.054±1.755		↓3.68
Day-DBP (mmHg)	Placebo N= 17	82.108	82.110±1.359	0.001±1.359	0.06	↑0.001
	Magnesium N=30		78.797±1.040	−3.311±1.040		↓4.20
Day-PP (mmHg)	Placebo N= 17	60.239	57.586±1.702	−2.653±1.702	0.713	↓4.60
	Magnesium N=30		58.381±1.301	−1.858±1.301		↓3.18
Day-HR (b.p.m)	Placebo N= 17	81.565	83.861±1.336	2.295±1.336	0.002	↑2.73
	Magnesium N=30		78.392±1.021	−3.173±1.021		↓4.04

Data expressed by mean ±SEM and percentage of difference

P<0.05 was considered a significant difference between treatment groups at endline

N= number of patients, SEM= standard error of mean, SBP=systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure, HR= heart rate

STATISTICAL ANALYSIS

Results were presented as mean ± standard error of the mean (SEM) or percent difference. All of the statistical analyses were achieved via the SPSS statistical package version 22.0 (SPSS, Inc.). A two-sample t- test was applied to compare the means of the baseline characteristics between the two groups and then data were analyzed by using the analysis of covariance (ANCOVA) [12]. The significance level for all tests was taken as a P-value of less than 0.05.

Table IV. Effect of magnesium supplement on night ambulatory blood pressure monitoring (ABPM) readings according to analysis of covariance.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Night-SBP (mmHg)	Placebo N= 17	135.714	133.735±2.445	−1.979±2.445	0.703	↓1.47
	Magnesium N= 30		132.535±1.853	−3.179±1.853		↓2.39
Night-DBP (mmHg)	Placebo N= 17	76.195	74.155±1.383	−2.040±1.383	0.873	↓2.75
	Magnesium N= 30		73.875±1.056	−2.320±1.056		↓3.14
Night-PP (mmHg)	Placebo N= 17	59.521	60.265±1.646	0.743±1.646	0.343	↑1.23
	Magnesium N= 30		58.258±1.251	−1.263±1.251		↓2.16
Night-HR (b.p.m)	Placebo N= 17	74.021	75.810±1.334	1.788±1.334	0.042	↑2.35
	Magnesium N= 30		72.284±1.021	−1.737±1.021		↓2.40

Data expressed by mean ± SEM and percentage of difference
P<0.05 was considered a significant difference between treatment groups at endline
N = number of patients, SEM = standard error of mean, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure, HR = heart rate

Table V. Comparison of HT status between office and ABPM in study population.

Type of measurement	HT status		P-value
	Uncontrolled HT	Well controlled HT	
	Baseline		
Office BP	6 (12.8%)	41 (87.2%)	<0.001
ABPM	34 (72.3%)	13 (27.7%)	
	Endline		
Office BP	7 (14.9%)	40 (85.1%)	<0.001
ABPM	33 (70.2%)	14 (29.8%)	

P>0.05 means no significant difference between treatment groups at endline
N = number of patients, HT = hypertension.

RESULTS

1. EFFECT OF MAGNESIUM SUPPLEMENT ON OFFICE BLOOD PRESSURE READINGS

After adjustment of baseline means for placebo and Mg groups according to the analysis of covariance (Table I) it is seen that SBP readings decrease significantly ($p < 0.05$) among patients on magnesium supplements compared with those on placebo after 8 weeks of treatment. The DBP, PP and HR readings decrease but not significantly ($P < 0.05$) among patients on magnesium supplements compared with those on placebo after the same period of treatment.

2. EFFECT OF MAGNESIUM SUPPLEMENT ON ABPM READINGS

A) EFFECT ON 24 HOUR ABPM READINGS

As summarized in table II, there was no significant reduction ($p > 0.05$) in the 24hr-SBP, 24hr-DBP and 24hr-PP readings among patients on magnesium supplements compared with those on placebo after 8 weeks of treatment. Meanwhile, there was a significant reduction ($p < 0.05$) in the 24hr-HR readings among patients on magnesium supplements compared with those on placebo after the same period of treatment.

B) EFFECT ON DAY ABPM READINGS

As illustrated in table III, there was no significant reduction ($p > 0.05$) in the day readings of SBP, DBP and PP among patients on magnesium supplements compared with those patients on placebo after 8 weeks of treatment. Meanwhile, there was a significant reduction ($p < 0.05$) in the day-HR readings among patients on magnesium supplements compared with those on placebo where the levels elevated after the same period of treatment.

C) EFFECT ON NIGHT ABPM READINGS

As shows in table IV, there is no significant reduction ($p > 0.05$) in the night readings of SBP, DBP and PP in patients taking magnesium supplements compared with those patients on placebo after 8 weeks of treatment. Meanwhile the night-HR shows a significant reduction ($p < 0.05$) in the readings among patients on magnesium supplements compared with those on placebo where the readings elevated after the same period of treatment.

Table VI. Effect of magnesium supplement on 24-hrs ABPM- HR depending on cross classification.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Masked HT	Placebo	80.307	82.102±1.509	1.795±1.509	0.015	↑2.18%
	Magnesium	80.307	76.859±1.292	-3.448±1.292		↓4.48%
Uncontrolled HT	Placebo	72.833	73.647±3.457	0.814±3.457	0.514	↑1.10%
	Magnesium	72.833	71.071±1.538	-1.762±1.538		↓2.47%
Well controlled HT	Placebo	82.500	83.821±2.406	1.321±2.406	0.914	↑1.58%
	Magnesium	82.500	83.429±2.406	0.929±2.406		↑1.11%

Data expressed by mean ± SEM and percentage of difference
P<0.05 was considered a significant difference between treatment groups at endline
N = number of patients, SEM = standard error

Table VII. Effect of magnesium supplement on office SBP, office PP, and office HR depending on cross-classification of IDACO.

Table 7A. Effect of magnesium supplement on office SBP depending on cross-classification of IDACO.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Masked HT	Placebo	124.092	123.489±2.952	-0.603±2.952	0.441	↓0.48%
	Magnesium		120.428±2.523	-3.664±2.523		↓3.04%
Uncontrolled HT	Placebo	154.815	161.902±4.379	7.087±4.379	<0.001	↑4.37%
	Magnesium		132.291±1.866	-22.524±1.866		↓17.02%
Well controlled HT	Placebo	117.750	119.554±3.593	1.804±3.593	0.189	↑1.50%
	Magnesium		111.696±3.593	-6.045±3.593		↓5.41%

Table 7B. Effect of magnesium supplement on office PP depending on cross-classification.

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Masked HT	Placebo	47.334	47.911±2.232	0.577±2.232	0.557	1.20%
	Magnesium		45.845±2.614	-1.489±2.614		3.24%
Uncontrolled HT	Placebo	69.661	64.592±12.250	-5.069±12.250	0.310	7.84%
	Magnesium		50.347±5.224	-19.314±5.224		38.36%
Well controlled HT	Placebo	40.087	43.472±2.626	3.385±2.626	0.100	7.78%
	Magnesium		35.028±2.626	-5.059±2.626		14.44%

Table 7C. Effect of magnesium supplement on office HR depending on cross-classification

Parameter	Group	Adjusted baseline mean	Adjusted endline Mean ± SEM	Outcome Mean ± SEM	P-value	% of difference
Masked HT	Placebo	79.519	80.676±1.466	1.157±1.466	0.092	↑1.43%
	Magnesium		77.238±1.250	-2.281±1.250		↓2.95%
Uncontrolled HT	Placebo	79.384	79.646±6.658	0.262±6.658	0.640	↑0.328
	Magnesium		76.155±2.839	-3.229±2.839		↓4.24%
Well controlled HT	Placebo	84.625	81.829±3.479	-2.796±3.479	0.724	↓3.41%
	Magnesium		83.671±3.479	-0.954±3.479		↓1.14%

Data expressed by mean ± SEM and percentage of difference
P<0.05 was considered a significant difference between treatment groups at endline
N = number of patients, SEM = standard error of mean

D) EFFECT ON NIGHT DIPPING AND NIGHT BLOOD PRESSURE VARIABILITY (SD)

After adjustment for baseline according to the analysis of covariance, systolic and diastolic dipping show no significant changes (P >0.05) in patients taking magnesium supplements compared with patients taking placebo after 8 weeks of treatment. There were no significant changes (P >0.05) in systolic SD, diastolic SD and HR-SD readings for patients taking magnesium supplements compared with those taking placebo after 8 weeks of treatment.

3. COMPARISON OF BLOOD PRESSURE STATUS BASED ON BOTH OFFICE AND ABPM MEASUREMENTS

Depending on the office BP measurement, there was no significant difference in BP status between the magnesium and placebo groups before and after the intervention, and similarly, ABPM showed no significant difference in BP status between the pre- and post-intervention treatment groups. According to the cross-classification proposed by IDACO, there was no significant difference in BP status between treatment groups at baseline and endline of the study. When office BP was compared with ABPM in all patients enrolled in this study, HT status showed a significant difference between both measurements (table V).

Magnesium supplementation showed no significant reductions (P>0.05) in 24-hrs ABPM of SBP, DBP and PP in patients with masked HT comparing to other HT status patients. Meanwhile, 24-hrs ABPM of HR showed a significant reduction (P < 0.05) among patient with masked HT after 8 weeks of magnesium supplement (Table VI).

In office BP measurement, SBP showed a significant reduction (P<0.05) among patients with uncontrolled HT compared with other HT status patients after 8 weeks of magnesium supplementation (table VIIA). Office DBP showed no changes. On other hand, office PP readings decrease but not significantly among different HT status patients after 8 weeks of magnesium supplementation (table VIIB). Office HR decreases not significantly (P>0.05) among different HT status patients, although the more reduction was observed in masked HT (table VIIC).

4. EFFECT OF MAGNESIUM SUPPLEMENT ON CORRECTED QT INTERVAL

There were no significant changes in corrected QT intervals (p>0.05) in patients taking magnesium supplements compared with those taking placebo after 8 weeks of treatment.

DISCUSSION

Magnesium ions play a role in regulation of the activities of cardiac and vascular smooth muscle. It regulates the action of numerous plasma membranes and intracellular ion transport pump processes that preserve significant intracellular concentrations of free calcium and sodium in the cytosol [13]. Although magnesium is not directly involved in contraction, it improves vascular smooth muscle and contractility, which is reflected in the effect on calcium ion levels and their availability in vital areas. Magnesium promotes relaxation [14]. Additionally, magnesium can act as a calcium channel blocker (CCB), by directly affecting Ca^{+2} uptake, distribution, and content in vasculature [15]. Depletion of magnesium in vitro and in vivo environments could induce vascular hyperreactivity and frank hypertension [16]. Furthermore, the arterial muscle contracts when a chelating agent that selectively chelates magnesium is added to the medium, at the same time as the arterial muscle relaxes when single that chelates calcium is added. Epidemiological studies have shown a negative relationship between magnesium intake and arterial hypertension. Mechanisms for Mg depletion in hypertensive human include impaired gastrointestinal absorption, compromised cellular Mg handling, and increased urinary Mg losses. In other way, chronic Mg deficiency may promote development of hypertension [17]. The present study found that the office SBP readings was decreased significantly (7.98%) in patients on magnesium supplements compared with those on placebo after 8 weeks of treatment, while the DBP, PP and HR readings decreased but not significantly (4.49%, 13.46% and 3.32% respectively) among patients on magnesium supplements compared with those on placebo after the same period of treatment. Differences in office SBP and DBP values were statistically significant between magnesium and placebo group at the end of a previous study. There were results of the role of magnesium in improving BP irrespective of body weight in metabolic obese normal weight (MONW) patients when magnesium chloride 5% solution was consumed once a day in 24 subjects for 16 weeks, while placebo solution was administered to 23 subjects for the same period [18]. This result is similar to the results of the present study where SBP showed a significant reduction, while it differ compared with DBP result of the present study (P=0.074). This may be attributed to many factors such as sample size, dose and duration of treatment considered in this study. A meta-analysis of 34 randomized controlled trials found that oral Mg supplementation led to a significant reduction in both systolic and diastolic BP (2.00 and 1.78 mm.Hg., respectively), although systolic and diastolic BP responses differed slightly in dose- and duration-dependent manners, respectively. The BP-lowering effects of Mg supplementation were accompanied by elevated serum Mg levels. This result supports a causal antihypertensive effect of Mg supplementation in adults [19]. Cosaro E *et al.* [20] found in their study that supplementation with magnesium pidolate (8.1 mmol twice daily) had no significant effect on office systolic and diastolic BP in 14 normomagneseamic healthy young men had family history of MetS or T2DM after

8 weeks of supplementation with 4 weeks washout period. Lima de Souza et al. evaluated the effect of 400 mg magnesium chloride on IR in 72 women with metabolic syndrome without DM. This study showed a significant reduction in SBP in intervention group (P = 0.004), while the difference between magnesium and placebo group was no significant (P= 0.498). The difference in DBP was also no significant (*P*= 0.964). In this study, the readings of ABPM during 24-hrs, daytime ABPM and nighttime ABPM have the same results since SBP, DBP and PP in all times of measurement decrease not significantly after magnesium supplementation compared with placebo, whereas HR shows a significant reduction in different time readings. One previous study found that no significant effects were observed on mean 24-hrs, mean daytime, and mean nighttime ABPM levels (SBP, DBP, PP, and HR). This agrees with the results of the present study except for HR readings. Hatzisavir LS et al. also studied the effect of oral magnesium supplementation on ABPM in patients with mild hypertension. The number of patients enrolled in the study was 48 persons with mild uncomplicated hypertension. Twenty four of them received magnesium pidolate 600 mg daily for 12 weeks in addition to lifestyle recommendations and the remainder patients were only assessed for lifestyle recommendations There were significant reductions in average 24-hrs SBP and DBP levels in magnesium group compared with control ($P < 0.001$ and $P= 0.002$, respectively). These effects of magnesium supplementation were significant in both daytime and nighttime-ABPM readings [21]. Sacks FM et al. studied the effect of potassium, calcium, and magnesium on blood pressure in 300 women with low Mg intake. Changes in SBP and DBP between magnesium and placebo group were not significant after 12 weeks of the study (P=0.29 and P=0.32, respectively). Vongpatanasin W et al. study showed that there was no significant effect on ABPM readings (P>0.1) in subjects received potassium magnesium citrate (KMgCit) compared with placebo after 4 weeks of supplementation [22]. In dipping status, sleeping systolic BP must be 10% lower than morning systolic BP, and can be measured by 24-hrs ABPM. Nondipping status appears when BP downs by <10% during nighttime [23], occurs in a different situation and diseases, like chronic kidney diseases (CKD) and sympathetic activation. Systolic and diastolic dipping showed no significant changes (↓12.9% and ↓0.43%, respectively) in magnesium group compared with placebo at the end of the present study. The same manner in relation to night variability values, since systolic SD, diastolic SD and HR-SD readings showed no significant changes (↑2.48%, ↑3.14% and ↓0.72, respectively). In certain study, night variability and nocturnal reduction in SBP and DBP (dipping status) did not differ between the Mg and placebo group after 12 and 24 weeks of supplementation with magnesium citrate in overweight and obese subjects. This matches with the results of the present study. Similarly, night HR-variability didn't change significantly in the present study. The sensory stimulation that may happen during cuff inflation may possibly develop the cortical arousal, disturb sleep, and elevating blood pressure as reported by Davies et al. However, the extent to which ABPM can impair nighttime sleep remains unknown. Depending on this result, another study was developed to assess the effect of ABPM on sleep duration, sleep efficiency, and daytime activity. The number of patients enrolled in the study was 103 patients with chronic kidney disease. The results indicated that ABPM was linked with disturbed sleep and decreased physical activity, and this influenced dipping status. Ambulatory BP monitoring may itself motivate non-penetration [24]. Masked hypertension is distinct by normal office BP and high by ambulatory BP. In general, about 15-30% of persons with normal office BP have masked HT. It occurs in accordance with elevated risks of cardiovascular morbidity and mortality when compared to sustained norm tension, which matches the risk of sustained hypertension. The prevalence of masked HT in T2DM patients treated with antihypertensive medications and well controlled office BP was 66.3%, while it found in 13.6% of untreated office normotensive in patients with T1DM [25]. Other studies elucidated that the prevalence of masked HT in T2DM is 30-47.5% in those not treated with antihypertensive agents [26]. Franklin et al. found that from patients who non-diabetic, office normotensive and not on antihypertensive agents, there were 18.8% had masked HT. Meanwhile, 30.5% of patients treated with antihypertensive agents had masked HT [27]. Rodriguez-Moran M et al. and Zhang X et al. reported that a significant reduction in office SBP and DBP readings was observed after magnesium supplementation, whereas no significant effect on office measurements was noticed in Cosaro E et al. study. There was a significant reduction in both office and 24-hrs ABPM readings and this is shown by Kawano Y et al. [28], while Joris PJ et al., noticed that office and ABPM readings didn't change after magnesium interventions. It must be noticed that these studies were different in their study design, inclusion and exclusion criteria, study population enrolled in the study, dose and formulation of magnesium used, and duration of intervention. The review of different studies as shown above, displays a significant reduction in

office DBP and/or SBP as reported in the present study (reduction in SBP), but this significance was not seen in ABPM in most of these studies, in addition to the current study results. To resolve this controversy in results between office BP and ABPM measurements, cross-classification of IDACO was used to differentiate uncontrolled HT from masked HT and well controlled HT from WCH in the current study. This analysis showed no significant reduction in 24-hrs ABPM of SBP, DBP, PP and a significant reduction in HR in masked HT group; therefore, ABPM is needed to clarify the response to magnesium in addition to diagnose masked HT that represent 55.31% of the patients completed follow up of this study. The high prevalence of masked HT in the current study was attributed to the facts that most of patients were diabetics, on antihypertensive medications, and with average eGFR 70.049 ml/min. However, a possible explanation is that antihypertensive drugs and magnesium interaction may counteract effect of Mg, age of patients, and duration of HT may also affect the final results. In this study, office BP measurement showed a significant SBP reduction in uncontrolled HT group and non-significant reduction in other groups, while DBP showed no changes. Pulse pressure displayed no significant reduction in different HT status, and HR measurement also showed no significant reduction in different HT status groups but more apparent in masked HT patients. This small difference could not be explained depending on fact that ABPM is a gold standard of BP measurement. It may be attributed to small sample size and may need elaboration by further research study. The length of the QT interval is inversely proportional to the heart rate. The QT interval is equal to the corrected QT interval once HR is 60 b.p.m [29]. Normal values of QTc intervals are <430 ms for males and <450 ms for females. When QTc interval exceeds 450 ms in males and 470 ms in females, it is considered prolonged. The QT interval prolongation occurs when cardiac repolarization is late. Prolongation in the QTc is not dangerous by itself. However, when QTc exceeds 500 ms, patients may develop Torsade de Pointes (TdP) and cardiac arrest. No dependable guidelines are available to augment magnesium administration in QTc prolongation. However, administration of intravenous magnesium to every patient with a QTc > 500 ms is reasonable [30]. There were no significant changes in the corrected QT interval (↑0.69%) in patients taking magnesium supplements compared with those taking placebo after 8 weeks of treatment in the present study. Another study showed that magnesium sulfate couldn't correct QTc intervals prolonged by overdose of haloperidol in a canine model [31]. This matched with the results of the present study. Intravenous magnesium sulfate (2-4grams IV bolus) reversed TdP and prevented its recurrence in 12 subjects with QTc intervals ranged from 540 to 720 ms who developed TdP. The QTc interval showed no significant changes after treatment [32], as observed in the present study. Naksuk N et al. found in their recent study on 8,498 patients admitted to the Cardiac Care Unit (CCU) at Mayo Clinic Hospital that admission and post admission serum magnesium levels were not associated with QTc interval or sudden cardiac death [33]. One limitation of this study is unselecting of the patients with hypomagnesaemia as inclusion criteria for the study and this may explain the differences in the results when compare with the results of other previous studies. The dose and duration of magnesium l-lactate may be not enough to improve blood pressure and QT interval. The amount of magnesium in the diet of patients must be calculated, genetic and individual variables in Iraqi population also may play a role in the final results and must be studied to evaluate the effect of Mg on BP monitoring and ECG readings. From this study, it can be concluded that magnesium l-lactate in a dose of 84 mg twice a day for 2 months in women with MetS have a significant reduction in office SBP readings and ABPM-HR readings during 24-hours, daytime, and night time measurements. A significant reduction of ABPM-SBP and no significant reduction in ABPM of DBP, PP, and HR were observed in patients with masked HT. Also, no significant change in QTc interval was reported. Many recommendations can be suggested for future work, including further studies with large scale sample and long term duration. The dose used for magnesium L-lactate tablets may be increased, or a different formulation such as magnesium chloride may be used. The effect of magnesium on BP should be studied by ABPM to diagnose masked HT and WCH. Other suggestions may involve studying the comparable effect of magnesium on BP in those patients who administered antihypertensive medications that affect magnesium function (agonist or antagonist effect), using Bpro-wrist ABPM or similar apparatus that don't disturb sleep and may give more reliable results about dipping status. Further studies with more details on the changes in ECG readings such as PR and QRS intervals may also recommended.

CONCLUSIONS

From above results, one can conclude that oral Mg l-lactate supplement can improve, to a certain extent, blood pressure of women with MetS. Further studies in this aspect may be required.

REFERENCES

1. Neill S.O., Driscoll L.O. Metabolic syndrome- a closer look at the growing epidemic and its associated pathologies. Obes Rev. 2015; 16(1):1-12. doi: 10.1111/obr.12229.
2. Estruch R., Martinez-Gonzalez M.A., Corella D. et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med. 2006; 145(1):1-11. doi: 10.7326/0003-4819-145-1-200607040-00004.
3. Dahlof B., Devereux R.B., Kjeldsen S.E. et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomized trial against atenolol. Lancet. 2002; 359(9311):995-1003. doi: 10.1016/S0140-6736(02)08089-3.
4. Ryan M.F. The role of magnesium in clinical biochemistry: an overview. Ann Clin Biochem. 1991; 28 (1):19-26. doi: 10.1177/000456329102800103.
5. Assadi F. Hypomagnesaemia: an evidence-based approach to clinical cases. Iran J Kidney Dis. 2010; 4(1): 13-9.
6. Euser A.G., Cipolla M.J. Magnesium sulfate for the treatment of eclampsia: a brief review. Stroke. 2009; 40(4):1169-75. doi: 10.1161/STROKEAHA.108.527788.
7. Julius S., Palatini P., Kjeldsen S.E. et al. Usefulness of heart rate to predict cardiac events in treated patients with high-risk systemic hypertension. Am J Cardiol. 2012; 109(5):685-92. doi: 10.1016/j.amjcard.2011.10.025.
8. Di Rienzo M., Grassi G., Pedotti A., Mancia G. Continuous vs. intermittent blood pressure measurements in estimating 24-hour average blood pressure. Hypertension. 1983; 5(2):264-9. doi: 10.1161/01.hyp.5.2.264.
9. Mancia G., Fagard R., Narkiewicz K. et al. ESH/ESC Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. 2013; 34(28):2159-219. doi: 10.1093/eurheartj/eht151.
10. Turner J.R., Viera A.J., Shimbo D. Ambulatory Blood Pressure Monitoring in Clinical Practice: A Review. Am J Med. 2015; 128(1):14-20. doi: 10.1016/j.amjmed.2014.07.021.
11. Asayama K., Thijs L., Li Y. et al. Setting thresholds to varying blood pressure monitoring intervals differentially affects risk estimates associated with white-coat and masked hypertension in the population. Hypertension. 2014;64(5):935-42. doi: 10.1161/HYPERTENSIONAHA.114.03614.
12. Committee for Medicinal Products for Human Use (CHMP). Guideline on adjustment for baseline covariates. Clinical Trials. Mult Scler. 2018; 24(11): 1461-1468. doi: 10.1177/1352458517726380.
13. Paolisso G., Barbagallo M. Hypertension, Diabetes Mellitus, and Insulin Resistance the Role of Intracellular Magnesium. Am J Hypertens. 1997; 10(3):346-55. doi: 10.1016/s0895-7061(96)00342-1.
14. Altura B.M., Altura B.T. Magnesium ions and contractions on vascular smooth muscles: relationship to some vascular diseases. Fed Proc. 1981; 40: 2672-2679.
15. Agus Z.A., Kalipourie E., Dukes I., Morad M. Cytosolic magnesium modulates calcium channel activity in mammalian ventricular cells. Am J Physiol. 1989; 256(2):C452-5. doi: 10.1152/ajpcell.1989.256.2.C452.
16. Joffres M.R., Reed D.M., Yano K. Relationship of magnesium intake and dietary factors to blood pressure: The Honolulu Heart Study. Am J Clin Nutr. 1987; 45(2):469-75. doi: 10.1093/ajcn/45.2.469.
17. Belin R.J., He K. Magnesium physiology and pathogenic mechanisms that contribute to the development of the metabolic syndrome. Magnesium Research. 2007; 20(2): 107-29.
18. Rodríguez-Moran M., Guerrero-Romero F. Oral magnesium supplementation improves the metabolic profile of metabolically obese, normal-weight individuals: a randomized double-blind placebo-controlled trial. Arch Med Res. 2014; 45(5):388-93. doi: 10.1016/j.arcmed.2014.05.003.
19. Xi Zhang X., Li Y,. Gobbo L.C.D. et al. Effects of Magnesium Supplementation on Blood Pressure A Meta-Analysis of Randomized Double-Blind Placebo-Controlled Trials. Hypertension. 2016;68(2):324-33. doi: 10.1161/HYPERTENSIONAHA.116.07664.
20. Cosaro E., Bonafini S., Montagnana M. et al. Effects of magnesium supplements on blood pressure, endothelial function and metabolic parameters in healthy young men with a family history of metabolic syndrome. Nutr Metab Cardiovasc Dis. 2014;24(11):1213-20. doi: 10.1016/j.numecd.2014.05.010.
21. Hatzistavri L.S., Sarafidis P.A., Georgianos P.I. et al. Oral magnesium supplementation reduces ambulatory blood pressure in patients with mild hypertension. Am J Hypertens. 2009; 22(10): 1070-5. doi:10.1038/ajh.2009.126.
22. Vongpatanasin W., Peri-Okonny P., Velasco A. et al. Effects of Potassium Magnesium Citrate Supplementation on 24-Hour Ambulatory Blood Pressure and Oxidative Stress Marker in Prehypertensive and Hypertensive Subjects. Am J Cardiol. 2016; 118(6):849-853. doi: 10.1016/j.amjcard.2016.06.041.
23. Head G.A., McGrath B.P., Mihailidou A.S. et al. Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement. J Hypertens. 2012; 30(2):253-66. doi: 10.1097/HJH.0b013e32834de621.
24. Agarwal R., Light R.P. The Effect of Measuring Ambulatory Blood Pressure on Nighttime Sleep and Daytime Activity-Implications for Dipping. Clin J Am Soc Nephrol. 2010; 5: 281-285. doi: 10.2215/CJN.07011009.

25. Rodrigues T.C., Canani L.H., Viatroski R.S. et al. Masked hypertension, nocturnal blood pressure and retinopathy in normotensive patients with type 1 diabetes. Diabetes Res Clin Pract. 2010; 87(2):240-5. doi: 10.1016/j.diabres.2009.10.016.
26. Takeno K., Mita T., Nakayama S. et al. Masked hypertension, endothelial dysfunction, and arterial stiffness in type 2 diabetes mellitus: a pilot study. Am J Hypertens. 2012; 25(2):165-70. doi: 10.1038/ajh.2011.158.
27. Mitsnefes M.M., Pierce C., Flynn J. et al. Can office blood pressure readings predict masked hypertension? Pediatr Nephrol. 2016; 31(1):163-6. doi: 10.1007/s00467-015-3212-5.
28. Kawano Y., Matsuoka H., Takishita S., Omae T. Effects of magnesium supplementation in hypertensive patients: assessment by office, home, and ambulatory blood pressures. Hypertension. 1998; 32(2):260-5. doi: 10.1161/01.hyp.32.2.260.
29. Abriel H., Schlapfer J., Keller D.I. et al. Molecular and clinical determinants of drug-induced long QT syndrome: an iatrogenic channelopathy. Swiss Med Wkly. 2004; 134: 685-94. doi: 2004/47/smw-10532.
30. Utah poison control center. Drug-Induced QT Prolongation. 2005; 7(3).
31. Satoh Y., Sugiyama A., Tamura K., Hashimoto K. Effect of magnesium sulfate on the haloperidol-induced QT prolongation assessed in the canine in vivo model under the monitoring of monophasic action potential. Jpn Circ J. 2000; 64(6):445-51. doi: 10.1253/jcj.64.445.
32. Tzivoni D. et al. Treatment of torsade de pointes with magnesium sulfate. Circulation. 1988; 77(2):392-7. doi: 10.1161/01.cir.77.2.392.
33. Naksuk N., Hu T., Krittanawong C. et al. Association of Serum Magnesium on Mortality in Patients Admitted to the Intensive Cardiac Care Unit. Am J Med. 2017; 130(2):229.e5-229.e13. doi: 10.1016/j.amjmed.2016.08.033.

The authors would like to thank Al-Mustansiryiah University (www. uomustansiryiah.edu.iq), Baghdad- Iraq, for its support in the present work. Special thanks also introduce to the Obesity Research and Therapy Unit/ Alkindy College of Medicine.

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

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Received: 22.09.2022
Accepted: 09.02.2023

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

ORIGINAL ARTICLE

PREDICTORS OF THE DEVELOPMENT OF EMOTIONAL BURNOUT AND THE MOTIVATIONAL COMPONENT OF THE MEDICAL STAFF OF HEALTH CARE INSTITUTIONS IN UKRAINE

DOI: 10.36740/WLek202302118

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ABSTRACT

The aim: To study the stages and diseases of occupational and emotional burnout syndromes and characterize the changes in the psychophysiological state of the medical staff of health care institutions.

Materials and methods: Manifestations and levels of predictors of the development of emotional burnout (PDEB), motivation of medical workers in the Vinnytsia region, and preventive measures of PDEB to improve the motivational component of medical workers. Statistical processing of the research results was performed in the licensed standardized package "Statistica 6.1 for Windows", and included the analysis of the nature of the distribution of characteristics using the Shapiro-Wilk's W test, and the analysis of differences using the Mann-Whitney test. The content analysis of domestic and foreign scientific sources and biblio-semantic and analytical research methods were used in the work. A sociological study was conducted on the dynamics of changes in the psychophysiological state of health of medical staff of psychiatric hospitals and medical staff of general health care facilities (CHP) in the Vinnytsia region, depending on gender and positions.

Results: A survey has been conducted using psychodiagnostic methods of emotional burnout by Boyko V.V. and the adapted approach of Vodopyanova N.E. According to the results of K. Zamfir's method in A. Rean's modification, it was established that external negative motivation exceeds external positive motivation in health care staff, as in male doctors and female doctors from 3,2±0,8 to 2,7±1,0 score and the average medical staff of psychiatric profile in men 3,2±1,8 and 3,0±1,3 and the average medical staff of general profile 3,6±1,0 and 3,2±1,1, respectively, which reflects the negative attitude of the medical staff at the present stage to the implementation of professional activities.

Conclusions: Predictors of the development of emotional burnout are noted in the average female medical workers working in psychiatric institutions: "Stress" - 41.3±19.2 points versus 33.6±22.2 points ($p>0.05$), "Resistance » – 56.6±21.4 points against 40.5±16.6 points ($p<0.05$), "Exhaustion" – 41.5±21.4 points against 39.4±27.4 points ($p>0.05$) compared to men, who may be at risk of transition from a pre-morbid state (mild, moderate form of SPV) to a severe form of chronic psychosomatic or psychovegetative disorder.

KEY WORDS: mental health, emotional burnout, motivation, health professionals, health, prevention

Wiad Lek. 2023;76(2):370-376

INTRODUCTION

The phenomenon of "emotional burnout syndrome" (EBS) or "predictors of the development of emotional burnout" (PDEB) has been studied in the medical field of health for many years from the healthcare professional making an immediate decision, intellectual effort, confidentiality, compassion and respect for the patient and their relatives while maintaining high efficiency in extreme conditions. Working in extreme/stressful conditions has an impact on the health of the health worker and is a risk of occupational disease, but also affects the quality of medical care. European plan for a comfortable stay at the WHO workplace for 2013-2020 introduced several measures/recommendations that will help maintain a balance between personal life and work, value employees, identify early signs of burnout, introduce stress management and improve the provision of quality, highly specialized care to the world's population [1-7].

Lack of motivation of health workers in constant stressful situations in the workplace in the field of health affects the quality of medical services in the field of mental health of the population of Ukraine and the EU [6,7]. In Ukraine, the birth, morbidity, disability, life expectancy, and mortality rates have been steadily deteriorating in recent years, pointing to a deep health crisis and the need to modernize the health care system. Incomplete reform in the field of health care in Ukraine creates conditions that are not able to regulate the processes of motivation of medical staff due to underfunding in this area, leading to a deterioration in the quality of medical care to the population of Ukraine [8].

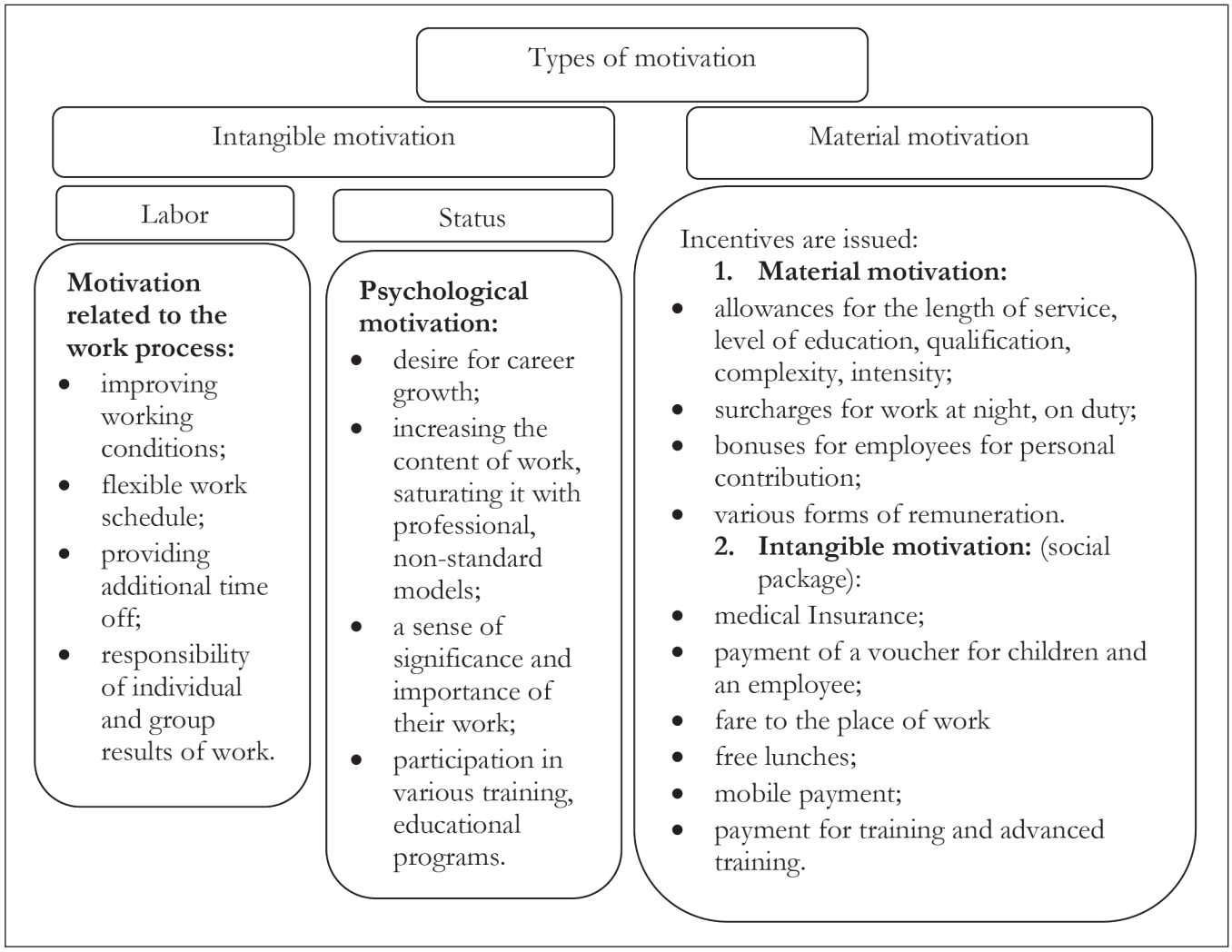

Fig. 1. The scheme of the motivational component of the professional activity of health workers (compiled by the authors of scientific research)

According to Suleiman-Martos N., Zhang S., Azam K., and many scientific studies around the world, the burnout rate among physicians and health care professionals is in the range from 31,4% to 85,8%, but in Ukraine, this figure is higher and ranges from 73,0% to 89,3% [2-4].

The UN has declared the development of health care facilities (HCFs) and the provision of human/medical resources, and the creation of appropriate working conditions for them until 2030 as a priority policy of many countries [5].

The purpose of the work is to determine the manifestations and level of PDEB, the motivation of medical workers in the Vinnytsia region, and the development of preventive measures for PDEB and to improve the motivational component of medical workers.

THE AIM

This study aims to study the stages and diseases of occupational and emotional burnout syndromes and to characterize the changes in the psychophysiological state of the medical staff of health care institutions.

MATERIALS AND METHODS

Our sociological study involved 326 health workers of the Vinnytsia region, of which men – 17,5% and women – 82,5%. The number of psychiatrists who took part in the survey was 23,0%, and the average number of psychiatric and general medical staff (SME) was 77,0%. Experience of professional activity among physicians was 20,4±12,8 years, and among SMP 16,1±11,2 years of the experimental group (psychiatric medical staff) and 22,5± 11,8 years of control group (general medical staff)). The average age among psychiatrists was 44,5±12,7 years, among SMP 38,0±11,4 years of the experimental group (medical staff of psychiatric profile) and 42,1±11,3 years of the control group (general medical staff).

A sociological study was carried out using the method of emotional burnout Boyko V.V. and the adapted method of Vodopyanova N.E., which had evaluated by the following gradations: low, medium, high, and very high level of change (points).

According to the results of K. Zamfir's method in A. Rean's modification, this method allows determining

the relevance of types of motivations: external positive motivation (EPM) - achieving career growth, external negative motivation (ENM) – unpleasant to receive criticism from management.

Statistical processing of the research results was performed in the licensed standardized package «Statistica 6.1 for Windows,» and included the analysis of the nature of the distribution of characteristics using the Shapiro-Wilk's W test and the investigation of differences using the Mann-Whitney test.

RESULTS

An analysis of scientific research and current legislation to study the problem of motivation and burnout syndrome of medical workers shows that these issues have not been resolved at the state level. We conducted a sociological study on the dynamics of changes in the psychophysiological state of health of medical staff of psychiatric hospitals and general staff of health care institutions (CHC) in the Vinnitsa region, depending on age and length of service.

The main factors of professional burnout are individual factors – psychophysiological processes of the human body (temperament, character, traits); factors that affect a person from the outside – working conditions, excessive work demands, communication between colleagues, insufficient conditions for rest or relaxation, psychological comfort in the team, low financial status (low wages), etc. (Table I) (Compiled by the authors on scientific research).

We conducted a sociological study on the dynamics of changes in the psychophysiological state of health of medical staff of psychiatric hospitals and medical staff of health care institutions (HCI) in the Vinnytsia region, depending on age and length of service. A survey has been conducted using psychodiagnostic methods of emotional burnout by Boyko V.V. and the adapted technique of Vodopyanova N.E. Using the practice of Boyko V.V. an assessment of three phases of development of predictors of emotional burnout (DPEB): the phase of stress, resistance, and exhaustion [9]. Furthermore, according to the results of the questionnaire "Professional burnout" (PV), adapted method Vodopyanova NE identified: "emotional exhaustion", which is characterized by loss of energy, the appearance of signs of psychophysiological fatigue, signals of anxiety and depression, anger, aggression, a sense of exhaustion; "Depersonalization", which is characterizing by increased psychological distancing from work, decreased empathy and cynical attitude towards others, patients, pessimistic thoughts about work; "Reduction of professional achievements", which is characterized by negative self-esteem, indifference to the performance of their professional duties and reduced professional efficiency, reduced professional motivation and self-esteem.

According to the results of the psychodiagnostic method of emotional burnout Boyko V.V concerning the phase "Stress" among the average medical staff of psychiatric profile in women is the largest – 41,3±22,2 points compared to men – 33,6±19,2 points ($p < 0,05$) while in the control group on the contrary in men 39,0±23,3 points, and in women 34,8±23,4 points ($p < 0,05$).

In the phase of "Resistance," the highest degree had established in women of the average medical staff of a psychiatric profile - 56,6±21,4 points in comparison with men – 40,5±16,6 points ($p < 0,05$), thus in the control group both among women and among men, indicators steel 50,0±22,9 points against 50,4±22,9 points ($p < 0,05$).

In the phase of "Exhaustion," the highest rates had found in the experimental group among women of the secondary medical staff of psychiatric profile – 41.5±21.4 points against 39.4±27.4 points in men ($p<0.05$), while in the group of average medical personnel of a general medical profile, the indicators were lower and did not differ significantly between men and women (table II).

But among psychiatrists, high rates in the "stress" phase were 40,1±24,7 points for women doctors and 38,5± 22,8 points for men ($p>0,05$). The highest rates of the following phases were lost in male physicians: the "Resistance" phase in male physicians – 54,6±19,7 points, and in female physicians – 52,3±22,0 points ($p>0,05$); phase of "Exhaustion" in male doctors – 42,0±18,7 points, and in female doctors – 34,9±20,0 points respectively ($p>0,05$); In comparison with doctors and nurses in all three phases of STIs, high rates were also found in women on paramedical nursing staff.

However, depending on the gender characteristics, the excess of female workers in all phases was determined: in the phrase "Stress" – 41,3±19,2 points against 33,6±22,2 points ($p>0,05$) , "Resistance" – 56,6±21,4 points against 40,5±16,6 points ($p>0,05$), "Exhaustion" – 41,5±21,4 points against 39,4±27,4 points ($p>0,05$) respectively. Women are most vulnerable to deteriorating psychophysiological conditions and are prone to rapid transition from pre-morbid conditions (mild, moderate forms) to severe chronic psychosomatic or psychovegetative disorders, especially among women paramedics working in psychiatric institutions.

These data had confirmed by the method of the questionnaire "Professional burnout" (OT) according to the adaptation of Vodopyanova N. on emotional exhaustion (Table III).

Emotional exhaustion among women of the average medical staff of psychiatric profile was – 20,3±8,3 points in comparison with women of the average medical staff of the general profile of the CHC – 19,3±8,4 points ($p>0,05$). Among men, this figure was much higher. At men of the average medical staff of the general profile – 20,6±9,9 points against 18,3 ±9,8 points ($p>0,05$) accordingly.

Table I. Stages and symptoms of occupational and emotional burnout syndromes

Syndrome of professional burnout	Syndrome of emotional burnout
Stages of SPB Development The first stage (easy form) The second phase (middle form) The third phase (severe form, chronic psychosomatic/psychovegetative disorders)	Stages of SEB Development The phase «alarm voltage» (harbingers) Phase «resistance» (resistance to increasing stress) Depletion phase
Symptoms psychophysical psychovegetative behavioral	Symptoms emotional deficite emotional alienation personal removal, or depersonalization

Table II. Formation of emotional burnout syndrome (by phases of formation) in health care workers in Vinnytsia region, (M ± SD), points

Phases / Medical staff	Voltage phase «Alarm voltage»	Resistance Phase	Phase «Exhaustion»
Average medical staff of a psychiatric institution, n = 137			
Men (n=9)	33,6±19,2	40,5±16,6	39,4±27,4
Women (n=128)	41,3±22,2*	56,6±21,4*	41,5±21,4*
The average medical staff of the general medical profile of health care institutions, n=101			
Men (n=23)	39,0±23,3	50,4±22,9	38,3±16,8
Women (n=78)	34,8±23,4*	50,0±22,9*	35,7±20,1*

Note.* - the presence of statistically significant (p<0.05 and less) differences compared to the control group

Table III. Formation of emotional burnout syndrome according to the method of «Professional burnout» in health care workers in the Vinnytsia region, (M ± m)

Forms / Medical staff	Emotional Exhaustion	Depersonalization	Professional Success
Paramedics of a psychiatric institution, n=137			
Men (n=9)	18,3±9,8	9,4±5,1	28,8±6,6
Women (n=128)	20,3±8,3*	12,0±5,0	25,6±8,3
The average medical staff of the general medical profile of health care institutions, n=101			
Men (n=23)	20,6±9,9*	14,0±4,8	29,0±7,0
Women (n=78)	19,3±8,4	11,6±4,5	27,5±7,4

Note. * p <0.05 significance of changes in the cohort comparison group

"Depersonalization /Cynicism" is characterized by increased psychological distancing from work, decreased empathy and indifference/cynicism towards others/patients, and pessimistic views on work, which also affect the attitude to their own family. Depersonalization among women again gave high rates, it was noted in women of the secondary medical staff of psychiatric profile – 12,0±5,0 points against 11,6±4,5 points (p>0,05). Professional success was noted in medical staff of general medical profile in men – 29,0±7,0 points and women – 27,5±7,4 points (p>0,05).

According to the results of the method "Motivation of professional activity" by K. Zamfir in the modification of A. Rean allows determining the relevance of types of motivations as 1 – material reward; 2 – the desire for career growth; 3 – the desire not to be criticized by management and colleagues; 4 – the desire to avoid possible punishments or troubles; 5 – focus on prestige, respect from others; 6 – satisfaction from a job well done; 7 – social utility of labor. After the survey, the following scale from 1 to 5 points was used: 1 point – "to a very small extent"; 2 points – "to a fairly small extent"; 3 points – "not big, but not small"; 4 points – "to a large extent"; 5 points – "to a very large extent."

According to the analysis in Table IV, it had been found that male doctors and male SMP psychiatric institution external negative motivation exceeds external positive, which corresponds to the following motivational complex VM> ZPM <ZNM. Thus, in most health workers working in a psychiatric institution, we can observe a decrease in interest in work, a decrease in psychological well-being (lack of purpose in life, negative attitude towards others, lack of desire for career growth, lack of self-perception, indifference to work, etc.), which can negatively affect the quality of medical care and interpersonal relationships in the team and at home.

Table IV. Types of motivation of professional activity in medical workers of psychiatric health care facilities, the average value (points)

Motivation	Doctors		Paramedics (Experimental Group)		Paramedics (control group)	
	Men (n=25)	Women (n=62)	Men (n=9)	Women (n=128)	Men (n=23)	Women (n=78)
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
Internal	3,9±0,9*	3,6±0,9	3,9±1,4	3,3±0,9	3,5±1,0	3,4±0,9
External positive	2,9±0,6	2,7±0,7	3,4±1,5*	2,8±0,9	3,4±0,9	2,9±0,8
External negative	3,2±0,8	2,7±1,0	3,2±1,8	3,0±1,3	3,6±1,0*	3,2±1,1

Note. * - the presence of statistically significant ($p<0.05$ and less) differences compared to the control group

Table V. Social benefits – «Cafeteria», which are using by health workers of the HCF on the principle of choosing «Menu» (compiled by the authors)

«Menu №1»	«Menu № 2»	«Menu № 3»
Payment for food	Payment for gasoline of UAH 500	Payment for training and advanced training
Fare for work	Mobile payment	Payment for travel on public transport
Vacation for a child (in a summer camp or sanatorium)	Vacation for yourself	Vacation for yourself
Payment for a company car	Medical Insurance	Medical Insurance

According to Table IV, women doctors have a motivational complex VM> ZPM = ZNM, which indicates a desire for career growth and the need to receive financial incentives, but they are uncomfortable receiving criticism from management.

According to our research, the highest score was the internal motivation of all health workers in the Vinnytsia region, which was expressed in understanding the usefulness of their work and the desire to engage and improve their professionalism (Table IV).

Another motivational complex is found in men-SMP, who have been working in a psychiatric institution for 7,8±7,5 years and VM>ZPM>ZNM, which emphasizes the predominance of understanding the usefulness of their work, desire to engage and improve their professionalism and strive for punishment growth and for this to receive material rewards, bonuses. They are not afraid to be criticized for their work. In-that is the best motivational complex. The shorter the work experience, the greater the internal positive motivation, the greater the circumstances, and the greater the internal negative motivation and signs of emotional burnout.

Types of motivation (Fig. 1), which we propose to use in the state or municipal CHCs of Ukraine, both tangible and intangible interests of health workers to create an effective motivational mechanism are given in Fig.1.

DISCUSSION

One of the most popular measures of the management system in the EU today is social motivation, the purpose of which is to provide a social package. The social package in the EU countries has the following components: health, work motivation, education, training, social support, recreation, and entertainment. Abroad, the main cost of the social package is up to 50% for various types of insurance, but the main one is health insurance. In Ukraine, the spread of the social collection is typical only for employees of large domestic companies, which are developing rapidly and want to enter the international markets of European countries, and which adhere to the civilized principles of social responsibility for their employees [10].

European medical institutions use a system of motivation such as a "cafeteria" for various types of social benefits, the so-called "menu". This system allows medical staff to independently (with the consent of the head of the HCF) choose the type of compensation for different classes: "Menu №1", "Menu №2", "Menu №3", which will be sufficiently motivated and ready to perform their work (Table V).

The practice of many domestic medical CHPs has shown the imperfection of the motivational mechanism if it does not complement the material incentives or moral (intangible). Optimization of intangible motivation on a corporate basis will facilitate and accelerate the process of implementing effective incentives for their work, as well as increase the likelihood of achieving successful results in the world [11,12].

The introduction of high-quality corporate relations to improve the quality of service will be the basis for the sustainable development of treatment facilities. Therefore, to increase the motivation of professional activity of health professionals as a factor in maintaining their health, the following measures are proposed: to develop and implement various trainings, educational programs, refresher courses to motivate communication in the team; to consider the possibility of exchanging experience in improving the microclimate in the group with other medical HCFs of Ukraine and HCFs of foreign

countries; to introduce the hazard of communication of medical workers in an informal environment (office of psychological relief, participation in sports, cultural events, tourist trips); based on the experience of foreign medical institutions to create self-governing groups (organizations) for independent decision-making on any issues related to the planning of their work and the effective functioning of the medical institution; development of self-government of HCFs with a multi-channel system of their financing, which are not prohibited by law (formation of salaries, bonuses, bonuses, social benefits on the basis of "cafeteria") and adoption (at the local government level) of regulations on intangible and material motivation of professionals local HCFs [13,14].

Thus, the above areas of optimization of motivation and stimulation of health care workers by analogy with European standards are aiming at improving their productivity, career growth, professional development, recognition and approval of colleagues, and management of their professional results, as well as prevention of occupational diseases [15].

Reforming and multifaceted health care economy and multi-channel financing, combining state guarantees with demonopolization and competition; decentralization of public administration, development of self-government of institutions, and independence of health care workers (activities on a contractual basis), provided by the Basic Law of Ukraine, "Fundamentals of Health Care Legislation", and the need to implement European standards of motivation based on changes in psychological state The implementation of intangible and material motivation measures will increase the level of medical services, prevention of occupational diseases of health workers, and thus preserve and strengthen the health of the population and the implementation of the cooperation agreement between Ukraine and the European Union (Association Agreement Chapter 22, "On Public Health", articles № 426, № 427) and gradual integration of Ukraine into European health care networks.

CONCLUSIONS

1. Predictors of the development of emotional burnout are clearly observed in nurses who work in psychiatric institutions: "Stress" – 41,3±19,2 points against 33,6±22,2 points ($p>0,05$), "Resistance" – 56,6±21,4 points against 40,5±16,6 ($p<0,05$), "Exhaustion" – 41,5±21,4 points against 39,4±27,4 points ($p>0,05$) compared to men who may be at risk of transition from a pre-morbid condition , moderate form of STD) in severe chronic psychosomatic or psychovegetative disorders.
2. The motivational component of CHP staff found that external negative motivation exceeds external positive motivation of CHP medical staff, both male and female doctors from 3,2±0,8 points to 2,7±1,0 points, and in the average medical staff psychiatric profile in men 3,2±1,8 points and 3,0±1,3 points and the average medical staff in general profile 3,6±1,0 points and 3,2±1,1 points, respectively, which reflects negative attitude of the medical staff at the present stage to the implementation of professional activities.
3. The less experience (7,8±7,5 years,) the motivational component is as follows (VM>ZPM>ZNM) and has a greater understanding of the usefulness of their work, the desire to engage in and improve their professionalism and strive for career growth and for this to receive material rewards, bonuses.
4. To prevent severe forms of chronic psychosomatic or psychovegetative disorders and low motivational components, it is necessary to constantly monitor medical teams to identify and put a stop to the causes of the formation and conduct preventive measures to minimize them.

REFERENCES

1. Chorna V.V., Makhniuk V.M., Pshuk N.G., Khliestova S. Burnout in mental health professionals and the measures to prevent it. Georgian medical al news. 2021;1(310):113-118.
2. Suleiman-Martos N., Albendin-Garcia L., Gomez-Urguiza J.L. et al. Prevalence and Predictors of Burnout in Midwives: F Systematic Review and Meta-Analysis. Int J Environ Res Public Health. 2020;17(2):E641. doi: 10.3390/ijerph17020641.
3. Zhang S., Wang J., Xie F. et al. A cross-sectional study of job burnout, psychological attachment, and the career calling of Chinese doctors. BMC Health Serv Res. 2020;20(1):193. doi: 10.1186/s12913-020-4996-y.
4. Azam K., Khan A., Alam M.T. Causes and Adverse Impact of Physician Burnout: A Systematic Review. J Coll Physicians Surg Pak. 2017;27(8): 495-501.
5. Ayalew F., Kibwana S., Shawula S. et al. Understanding job satisfaction and motivation among nurses in public health facilities of Ethiopia: a cross-sectional study. BMC Nurs. 2019;18:46. doi: 10.1186/s12912-019-0373-8.
6. Deressa A.T., Zeru G. Work motivation and its effects on organizational performance: the case of nurses in Hawassa public and private hospitals: Mixed method study approach.BMC Res Notes. 2019;12(1):213. doi: 10.1186/s13104-019-4255-7.
7. Deng S., Yang N., Li S., Li H. Doctors' job satisfaction and its relationships with the doctor-patient relationship and work-family conflict in China: a structural equation modeling. Inquiry. 2018;55:46958018790831. doi: 10.1177/0046958018790831.

8. Chorna V.V. Motivation and efficiency of mental health professionals as a predictor of their psychological well-being. Environment and health. 2020; 4 (97): 53-62. doi: 10.32402/dovkil2020.04.053
9. Nikiforova G.S. Workshop on health psychology. G.S. SPb: Peter. 2005, 351p.
10. Barannik L. Social package as an attribute of modern entrepreneurship. Bulletin of Kyiv National University. T. Shevchenko. 2011, 125p.
11. Keovathanak Kh. Are health workers motivated by income? Job motivation of Cambodian primary health workers implementing performance-based financing. Global Health Action. 2016. doi: 10.3402/gha.v9.31068.
11. Filho P.O., Souza M.R., Elias P.E., D'Avila Viana A.L. Physicians' job satisfaction and motivation in a public academic hospital. Human Resources for Health. 2016;14(1):75. doi:10/1186/s12960-016-0169-9.
12. Naumenko G. [From burnout to involvement of medical staff. Strategies for successful creation of an effective organizational culture in a medical institution (practical cases of the Mayo Clinic)]. Psychosomatic Medicine and General Practice, 2020;5(2). doi: 10.26766/pmgp.v5i2.238. Disponível em: https://e-medjournal.com/index.php/psp/article/view/238. Acesso em: 30 mar. 2023.
13. Tatarevskaya M.S., Zbritskaya T.P., Savchenko G.O. Personnel development management: textbook. way. Odesa: Atlant. 2013, 427 p.
14. Gandziuk V.A. Self-assessment of the medical prevention management system in a multidisciplinary health care institution according to functional methods. Bulletin of problems of biology and medicine. 2018;3(145): 350-354. doi: 10/29254/2077-4214-2018-3-145-350-354.
15. Chorna V.V., Shevcchuk A.M. The current state of mental health in the XXI century in the context of health care reform. Europen vector of development of the modern scientific researches: collective monograph/ edited by authors. lst ed.Riga, Latvia: "Baltija Publishing". 2021, 396p. doi: 10.30525/978-9934-26-077-3-1.

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

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Received: 25.01.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

CONDITION OF PERIODONTAL TISSUES ON THE BACKGROUND OF *HELICOBACTER* INVASION: THE CONCEPT OF PATHOGENESIS

DOI: 10.36740/WLek202302119

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ABSTRACT

The aim: To analyze the state of periodontal tissues against the background of *Helicobacter pylori* invasion in dynamics and to propose a possible mechanism of development of inflammatory periodontal diseases in patients with *Helicobacter pylor*i-associated pathology of the gastrointestinal tract.

Materials and methods: We examined 43 patients with *Helicobacter pylori*-associated gastrointestinal pathology and 42 patients of the same age without somatic pathology, including without gastrointestinal pathology associated with *Helicobacter pylori*. Clinical and laboratory research methods (clinical, instrumental, biochemical, histological methods) were used.

Results: Comparing the data of clinical observations and the results of laboratory studies of patients with inflammatory periodontal disease on the background of *Helicobacter pylori*-associated gastrointestinal pathology, obtained in different observation periods, we can assume that basic dental treatment of periodontal disease in such patients undergoing eradication therapy does not provide stable anti-inflammatory, antimicrobial and antioxidant effect, which leads to reduced periods of remission and recurrence of periodontal disease, where oral dysbiosis plays a crucial role.

Conclusions: Comparing the data of clinical observations and the results of laboratory studies of patients with chronic gingivitis on the background of *Helicobacter pylori*-associated gastrointestinal pathology, obtained in different observation periods, we can say that they correlate with each other and suggest that the basic dental treatment of chronic gingivatis on the background of *H.pylori*-associated pathology of the gastrointestinal tract, which is currently undergoing a course of eradication, does not give a stable anti-inflammatory, antimicrobial and antioxidant effect, which leads to recurrence of periodontal disease and shortening remission periods, where oral dysbiosis plays a crucial role.

KEY WORDS: periodontitis, periodontal diseases, periodontal treatment, *Helicobacter pylori*, oral dysbiosis

Wiad Lek. 2023;76(2):377-385

INTRODUCTION

Helicobacter pylori infection is one of the most common chronic infections in the world. According to Hooi J.K.Y. the prevalence of *Helicobacter pylori* was different in different parts of the world. Thus, the highest prevalence of Helicobacter pylori was in Africa and was 70.1%, while in Oceania the prevalence of this infection was the lowest and was only 24.4%. Among some countries, the lowest number of *Helicobacter pylori* (*H.pylori*) infections was found in Switzerland (18.9%), Denmark (22.1%), New Zealand (24.0%), Australia (24.6%) and Sweden. 26.2%), the largest - in countries such as Kazakhstan (79.5%), Pakistan (81.0%), Estonia (82.5%), Portugal (86.4%), Nigeria (87.7 %) [1].

To date, the contamination of the oral mucosa *H.pylori* is considered not only as a factor influencing the development and course of gastroduodenal pathology, but also pathology of the oral cavity [2,3]. The presence of *H.pylori* in the oral cavity leads to a decrease in the activity of lysozyme - an indicator of local nonspecific immunity – due to its inactivation by the bacterium H. PYLORI [4], changes in salivary biochemical parameters, namely lowering pH and increasing salivation rate, associated sialic acid, which causes an increase in viscosity and deterioration of the rheological properties of saliva, resulting in impaired basic salivary functions, leading to the development of major dental diseases [2,5].

THE AIM

To analyze the state of periodontal tissues against the background of *Helicobacter pylori* invasion in dynamics and to propose a possible mechanism of development of inflammatory periodontal diseases in patients with *Helicobacter pylori*-associated pathology of the gastrointestinal tract.

MATERIALS AND METHODS

We observed 43 patients (15 men and 28 women) with *Helicobacter pylori*-associated gastrointestinal pathology. We also examined 42 patients of the same age group (including 17 men and 25 women) without somatic pathology, including without pathology of the gastrointestinal tract (GIT) associated with *H.pylori*.

Helicobacter pylori-associated gastrointestinal pathology included two nosological units - chronic gastritis (CG) (type B gastritis) and chronic gastroduodenitis (CGD), which are the most common diseases of the stomach and duodenum, which most often affect young and middle-aged people.

Verification of gastroduodenal pathology (GDP) was performed on the basis of clinical and instrumental studies: examination of the patient, collection of complaints, life history and medical history and results of instrumental (esophagofibrogastroduodenoscopy) and laboratory studies (histological examination and polymerase chain reaction).

After establishing and confirming the gastroenterological diagnosis, these patients underwent a clinical and laboratory examination of the oral cavity.

Patients collected unstimulated saliva on an empty stomach [7] and calculated the rate of salivation (in ml/min.). The level of markers of inflammation was determined in saliva [7]: malonic dialdehyde (MDA) content [7], elastase activity [8], microbial contamination index – urease activity [9], nonspecific immunity indicator - lysozyme activity [10], antioxidant fermentation activity [7], protein content [11]. The degree of dysbiosis was calculated according to the ratio of urease and lysozyme activity according to A.P. Levitsky [9], and the antioxidant-prooxidant index of API was calculated according to the ratio of catalase activity and MDA content [7].

Hygienic condition of the oral cavity was assessed using the Silness-Loe, Stallard and tartar index. The degree of gingivitis was recorded using the PMA indices in the Parma modification, the Mulemann bleeding index and the Schiller-Pisarev test. To determine the leading clinical symptoms that characterize the severity of the inflammatory-dystrophic process in the periodontal tissues, the depth of the periodontal pocket was determined, and an X-ray examination was performed [12].

In patients with a verified diagnosis of chronic gastritis and gastroduodenitis associated with *H.pylori*, the diagnosis of *H.pylori* infection in the oral cavity was carried out by a molecular biological method - the polymerase chain reaction method, and by a biochemical method - a rapid urease test, since numerous studies by foreign authors have shown that the oral cavity is a permanent reservoir of *H.pylori* bacteria, the latter, when stored in dental plaque, creates a microbial association with periodontopathogens and stimulates their growth, which worsens the course of periodontal diseases [13].

Supra- and subgingival dental plaque collected with a sterile trowel served as biomaterial for the rapid urease test, oral fluid and dental plaque collected in the same way were used for the polymerase chain reaction.

Rapid urease test and PCR diagnostics were performed according to the standard method [14].

All patients were under dispensary observation for 6 months.

When performing statistical processing of the obtained data, we used: calculation of the arithmetic mean and its mean error (M±m); estimation of the probability of the difference obtained

of the results in the compared groups using Student's t-test (t). Statistical processing and analysis of the results are performed using Microsoft Excel license program. A significant difference was considered at a value of $p<0.05$, which is generally accepted for medical and biological research. [15].

ETHICAL ASPECTS

The work complies with the ethical standards of the Declaration of Helsinki by the World Medical Association. A written informed consent was obtained authorizing the publication of the medical history and the results of the examination.

RESULTS

The main complaints of patients with gastrointestinal diseases associated with *H.pylori*, from the oral cavity at the initial reception were bad breath, severe bleeding gums (both when brushing teeth and when eating solid food), swelling and redness of the gums, itchy gums, sore gums when eating, the presence of soft plaque and tartar, some patients complained of dry mouth, periodic rashes on the oral mucosa, which coincided with periods of exacerbation of gastrointestinal diseases.

During the objective examination of the oral cavity, patients were found to have: significant deposits of soft plaque, supragingival and subgingival calculus; pigmented plaque, located circularly in the neck of the tooth (most often the lower front teeth, upper and lower molars); edema clear; enlarged gingival papillae, severe hyperemia with a cyanotic tinge, bleeding gums (sometimes spontaneous), the inflammatory process spread to the gingival papillae and the marginal edge of the gums, in some patients even the alveolar part of the gums was affected; noted violations of the relief of ash papillae (loss of pointed peaks).

The results of the index assessment of the oral cavity in patients with *H.pylori*-associated gastrointestinal

Table I. Index assessment of the condition of the oral cavity in somatically healthy patients and in patients with *Helicobacter pylori*-associated gastrointestinal pathology

Index / Patients' group	Silness-Loe	Stallard	Tartar index	PMA, %	Schiller-Pisarev test	Bleeding index	Periodontal pocket, mm
Patients without somatic pathology (n=42)	1,24±0,28	1,42±0,27	0,86±0,24	23,3±2,03	1,24±0,25	0,95±0,2	1,2±0,2
Patients with Helicobacter pylori-associated gastrointestinal pathology (n=43)	1,72±0,27 p>0,05	1,74±0,35 p>0,05	1,98±0,27 p<0,05	69,9±2,70 p<0,001	2,62±0,37 p<0,001	2,64±0,37 p<0,001	2,6±0,5 p<0,05

Table II. Dynamics of changes in the clinical condition of the oral cavity according to hygienic and periodontal indices in patients with *Helicobacter pylori*-associated gastrointestinal pathology after a course of eradication therapy

Index / Term of definition	Silness-Loe	Stallard	Tartar index	PMA,%	Schiller-Pisarev test	Bleeding index
Before treatment (n=43)	1,72±0,27 p>0,05	1,74±0,35 p>0,05	1,98±0,27 p<0,05	69,9±2,70 p<0,001	2,62±0,37 p<0,001	2,64±0,37 p<0,001
In 1 month (n=34)	0,6±0,21 p<0,1 p1<0,001	0,80±0,22 p<0,05 p1<0,05	0,20±0,08 p<0,05 p1<0,001	38,8±3,40 p<0,001 p1<0,001	1,50±0,50 p>0,05 p1<0,1	1,33±0,27 p>0,05 p1<0,05
In 3 months (n=31)	1,56±0,34 p>0,05 p1<0,05 p2<0,05	1,62±0,32 p<0,05 p1>0,05 p2<0,05	1,10±0,22 p<0,05 p1<0,01 p2<0,05	42,2±4,80 p<0,001 p1<0,001 p2>0,05	1,60±0,56 p>0,05 p1<0,2 p2>0,05	1,67±0,74 p>0,05 p1>0,05 p2>0,05
In 6 months (n=30)	1,89±0,2 p<0,01 p1>0,05 p2<0,001 p3>0,05	1,87±0,21 p<0,05 p1>0,05 p2<0,05 p3>0,05	1,6±0,26 p<0,05 p1>0,05 p2<0,001 p3>0,05	56,6±3,70 p<0,001 p1<0,01 p2<0,01 p3<0,05	2,41±0,35 p<0,01 p1>0,05 p2<0,2 p3>0,05	2,32±0,40 p<0,01 p1>0,05 p2<0,05 p3>0,05

Note: p - in comparison with a group of patients without somatic pathology; p_1 - compared with data before treatment; p_2 - in comparison with the data obtained after 1 month; p_3 - in comparison with the data obtained after 3 months.

Table III. Biochemical parameters of oral fluid of patients with periodontal diseases on the background of *Helicobacter pylori*-associated gastrointestinal pathology

Studied indicator / Accompanying pathology	Salivation rate, ml/min	Protein content, g/l	MDA content, mmol/l	Elastase activity, mk-kat/l	Catalase activity, mkat/l	Index API	Urease activity, mk-kat/l	Lisozyme activity, unit/l	Degree of dysbiosis (DD)
Patients without somatic pathology (n=42)	0,48±0,05	0,65±0,05	0,20±0,02	0,30±0,04	0,30±0,04	13,0±0,2	7±2	78±7	1,0±0,2
Chronic gastritis (n=16)	0,69±0,07 p<0,05	0,75±0,09 p>0,05	0,21±0,01 p<0,05	0,47±0,03 p<0,01	0,27±0,04 p<0,05	8,0±0,4 p<0,05	11±3 p>0,1	27±6 p<0,01	6,8±2,3 p<0,05
Chronic gastro duodenitis (n=27)	0,55±0,06 p>0,3	0,85±0,17 p<0,05	0,26±0,02 p>0,05	0,55±0,07 p<0,01	0,13±0,02 p<0,01	9,2±0,2 p<0,05	36±8 p<0,01	40±4 p<0,05	19,2±3,5 p<0,05

pathology and in somatically healthy patients are presented in Table I.

The data in Table I show that patients with *H.pylori*-associated gastrointestinal pathology have significantly increased values of dental indices, there are significant pathological changes in periodontal tissues compared with patients without somatic pathology. Thus, in patients with gastrointestinal pathology associated

Table IV. Biochemical parameters of oral fluid of patients with HCG on the background of *Helicobacter pylori*-associated gastrointestinal pathology after a course of eradication therapy in the dynamics of observation

Patients group	Chronic gastritis			Chronic gastroduodenitis		
Studied indicator	Before treatment n=16	In 1 months n=13	In 6 months n=11	Before treatment n=27	In 1 months n=21	In 6 months n=19
Positive rapid urease test, %	69	76,9	90,9	96,3	90,5	94,7
Salivation rate, ml/min	0,69± 0,07 p<0,05	0,62± 0,05 p>0,05 p1>0,05	0,67±0,06 p<0,05 p1>0,3 p2>0,05	0,55± 0,06 p>0,3	0,52± 0,03 p>0,3 p1>0,3	0,53±0,04 p>0,3 p1>0,3 p2>0,3
Protein content, g/l	0,75± 0,09 p>0,05	0,61± 0,04 p>0,05 p1>0,05	0,73± 0,07 p>0,3 p1>0,3 p2>0,05	0,85± 0,09 p<0,05	0,63± 0,05 p>0,05 p1<0,05	0,82± 0,14 p<0,3 p1>0,3 p2>0,05
MDA content, mmol/l	0,21± 0,01 p>0,05	0,15± 0,02 p< 0,1 p1<0,05	0,31± 0,02 p<0,01 p1<0,05 p2<0,001	0,26±0,02 p>0,05	0,18± 0,02 p>0,05 p1<0,01	0,38± 0,02 p<0,01 p1<0,05 p2<0,001
Elastase activity, mk-kat/l	0,47±0,03 p<0,01	0,36± 0,05 p>0,05 p1<0,1	0,59± 0,04 p<0,01 p1<0,05 p2<0,01	0,55±0,07 p<0,01	0,40± 0,03 p<0,05 p1<0,05	0,66± 0,05 p<0,01 p1>0,2 p2<0,001
Catalase activity, mkat/l	0,27±0,04 p>0,05	0,34±0,04 p>0,05 p1>0,05	0,22±0,03 p>0,1 p1>0,3 p2<0,05	0,13±0,02 p<0,01	0,24±0,03 p>0,05 p1<0,01	0,11±0,02 p<0,01 p1>0,3 p2<0,001
Index API	8,0±0,4 p<0,01	22,7±1,9 p<0,001 p1<0,001	7,1±0,3 p<0,01 p1<0,1 p2<0,001	9,2±0,2 p<0,01	13,3±1,6 p>0,05 p1<0,05	2,9±0,5 p<0,01 p1<0,1 p2<0,001
Urease activity, mk-kat/l	11±3 p>0,1	6±1 p>0,05 p1>0,05	16±4 p>0,05 p1>0,3 p2<0,05	36±8 p<0,01	14±2 p<0,05 p1<0,05	49±7 p<0,01 p1>0,3 p2<0,001
Lisozyme activity, unit/l	27±6 p<0,001	35±4 p<0,001 p1>0,05	21±5 p<0,001 p1>0,3 p2<0,05	40±4 p<0,05	49±6 p<0,01 p1>0,05	28±4 p<0,01 p1>0,3 p2<0,01
Degree of dysbiosis (DD)	6,8±2,3 p<0,05	1,9±0,4 p<0,001 p1<0,05	8,5±1,7 p<0,01 p1>0,3 p2<0,001	19,2±3,5 p<0,05	3,2±0,9 p<0,05 p1<0,001	19,4± 1,8 p<0,01 p1>0,3 p2<0,001

Note: p - in comparison with patients without somatic pathology; p_1 - in comparison with the indicator before treatment; p_2 - in comparison with the indicator obtained after 1 month

with *H.pylori*, the PMA index was increased 3 times, indicating the presence of inflammation in periodontal tissues - severe gingivitis, the average value of the Schiller-Pisarev test increased 2.1 times, and the bleeding index Mulemann exceeded the group of somatically healthy patients by 2.8 times.

Among patients without somatic pathology, the structure of periodontal tissue morbidity was as follows:

Table V. Prevalence of oral *H.pylori* in patients with chronic catharral gingivitis against the background of *H.pylori*-associated gastrointestinal tract pathology in different observation periods

Diagnostik method	PCR (abs/%)		Urease rapid test (abs/%)			
Observation period	Positive	Negative	+	++	+++	-
Before treatment	10/22 (45,5%)	12/22 (54,5%)	15/43 (34,9%)	16/43 (37,2%)	6/43 (13,95%)	6/43 (13,95%)
After 1 month	6/22 (27,3%)	16/22 (72,7%)	8/34 (23,5%)	18/34 (53,0%)	3/34 (8,8%)	5/34 (14,7%)
After 6 months	8/18 (44,4%)	10/18 (55,5%)	3/30 (10,0%)	21/30 (70,0%)	4/30 (13,3%)	2/30 (6,7%)

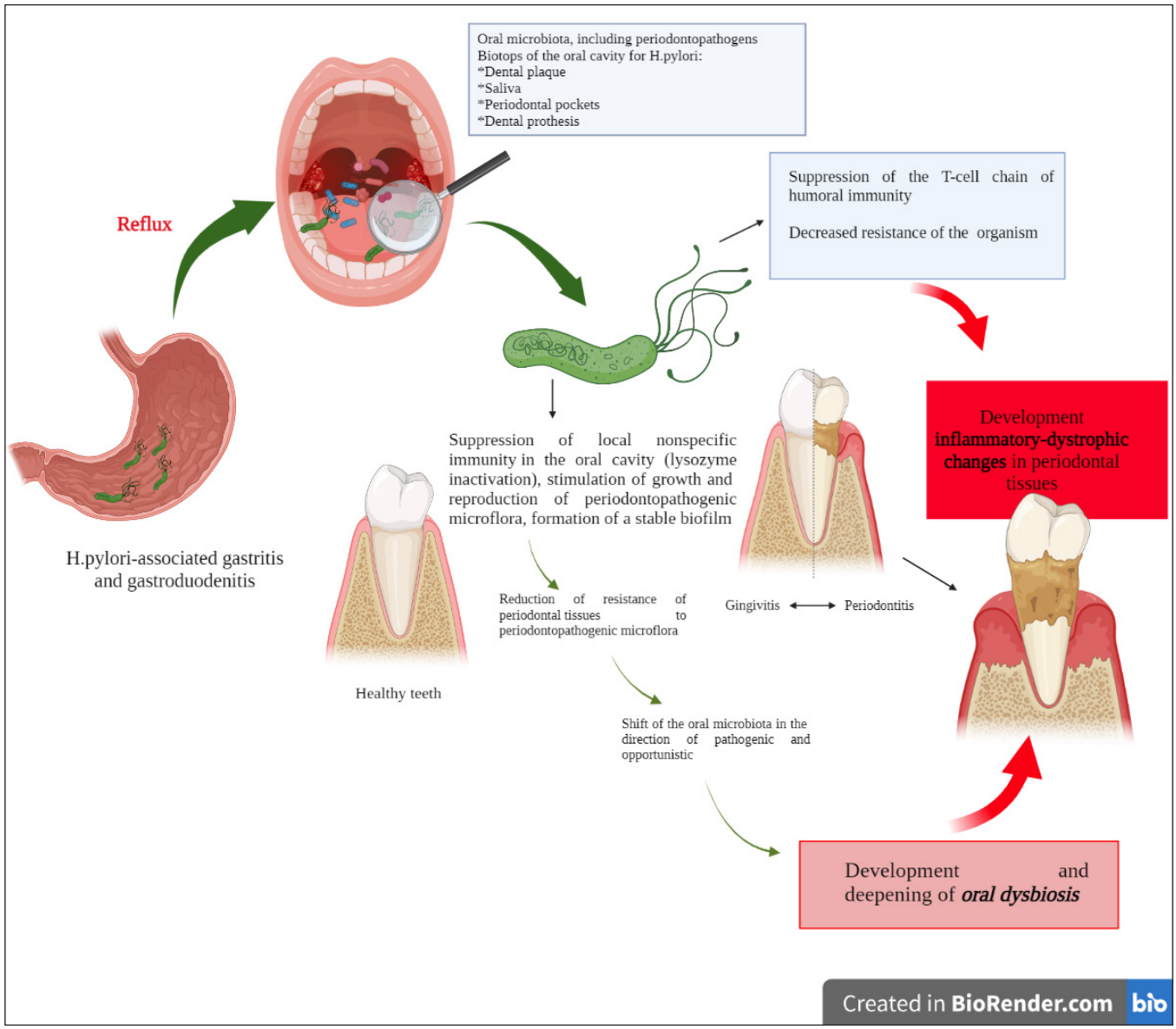

Fig. 1. Pathogenesis of inflammatory periodontal diseases on the background of *Helicobacter Pylori* invasion

47.6% of subjects were diagnosed with chronic catarrhal gingivitis (CCG), 9.5% of patients – chronic generalized periodontitis (CGP) of the initial and first degree, while 42, 9% are healthy. Among patients with *H.pylori*-associated gastrointestinal pathology, CCG was detected in 81.4% of subjects, and in primary and first-degree CGP in 18.6%.

Patients with CCG on the background of *H.pylori*-associated gastrointestinal pathology, were subsequently

under dispensary observation and we performed examinations after 1, 3 and 6 months.

At the end of the course of basic therapy of CCG (according to the protocol) and antihelicobacter therapy (AHBT), patients noted a decrease in symptoms of periodontal tissues: decreased bleeding gums (bleeding gums occurred only during brushing), swelling and redness of the gums, disappeared or decreased from the mouth, there was a metallic taste in the mouth as a manifestation of side effects of AHBT.

On objective examination of the oral cavity, it was noted that the redness and swelling of the gums decreased, and the relief of the gums was partially restored. Examination of the oral cavity after 1 month in 47.1% of patients showed slight deposits of soft plaque, mild hyperemia and swelling of single gingival papillae, in 23.5% of subjects noted improvement in the oral cavity after basic therapy with CCG. At the same time, 29.4% of the subjects observed a slight deterioration in the clinical condition of the oral cavity – slight deposits of soft plaque, gingival redness, swelling of single gingival papillae, in some areas – bleeding gums, clear painless on palpation. At the same time, patients did not complain.

After 3 months, 41.9% of patients complained of bleeding gums, which occurs both during brushing and eating, bad breath, after 6 months, 60% of patients in this group have an increase in subjective symptoms of periodontal tissue, which is confirmed by clinical examination and corresponds to moderate and severe CCG.

The results of the index assessment of the oral cavity of this group of patients, presented in Table II, correlate with the data of the clinical examination.

The condition of periodontal tissues according to periodontal indices changed as follows. After 1 month, the PMA index decreased by 44.5%, after 3 months - an increase in the PMA index by 1.1 times compared to previous data, but it does not reach the initial level, while after 6 months the PMA index increases by 1.5 times compared to post-treatment data, and almost reaches baseline, which correlates with the results of a clinical examination of the oral cavity. A similar trend is observed with the Schiller-Pisarev breakdown, and with the bleeding index.

In addition to the index assessment of the oral cavity, the biochemical parameters of oral fluid in patients with CCG and CGP associated with *H.pylori* were studied, which revealed differences in biochemical parameters in the oral fluid of patients with periodontal disease (PB) (CCG and CGP) on the background of gastrointestinal tract (chronic gastritis and gastroduodenitis) in comparison with patients without somatic pathology.

Tables III and IV below show the results of biochemical analysis of oral fluid before treatment, as well as 1 and 6 months after treatment.

The results of pre-treatment biochemical analysis of oral fluid in patients with combined *H.pylori*-associated gastrointestinal pathology (CG and GD) revealed the following changes in the oral cavity: increased inflammatory processes (in patients with CG and GD increased elastase activity by 56.7% and 83,3%, respectively), lipid peroxidation processes (in patients with CG and CGD, the content of MDA increased by 1.05 and 1.3 times, respectively), a decrease in the activity of the antioxidant defense system (catalase activity in patients with CG and GD is reduced by 10% and 57%, respectively, API index decreased by 38.5% and 29.2%, respectively), decrease in local nonspecific resistance (decrease in lysozyme activity in patients with CG and GD by 2.9 and 1.95 times, respectively), and as a consequence development of oral dysbiosis (diabetes in patients with CG and GD increased by 6.8 and 19 times, respectively), which plays a crucial role in the development and deterioration of inflammatory-dystrophic processes of the oral cavity.

The results of biochemical analysis, conducted after 1 month, indicate that the basic dental treatment in this group of patients helps to reduce inflammatory processes in periodontal tissues. Thus, in patients with *H.pylori*-associated CG and GD there is a decrease in elastase activity by 1.31 and 1.38 times, respectively, and the MDA content - by 1.4 and 1.44 times, respectively.

After 6 months, biochemical analysis showed a significant increase in elastase activity and an increase in the content of MDA in the oral fluid in patients with both CG and GD associated with H.PYLORI. The state of the antioxidant system (AOS) of the oral cavity also changed. Basic therapy of CCG increased catalase activity by 25.9% in patients with CG and by 84.6% in patients with GD; after 6 months there is a significant decrease in catalase activity. In proportion to this, there are changes in the API index. Thus, after 1 month, the API index in patients with concomitant H.PYLORI-associated CG and GD increased by 2.84 and 1.45 times, respectively, indicating an improvement in AOS after baseline therapy. However, after 6 months we see a deterioration of the situation – a significant decrease in the API index.

The state of local nonspecific immunity and the level of microbial contamination were studied on such indicators as lysozyme activity and urease activity. Basic dental treatment reduces urease activity and increases lysozyme activity, but after 6 months of observation we observe the opposite situation, which indicates a shift in the balance of oral microbiota in the direction of pathogenic and opportunistic, which against the background of reduced nonspecific antimicrobial protection in the cavity to the development of oral

dysbiosis, which clearly reflects the degree of dysbiosis (DD), calculated by Levitsky.

All patients with Helicobacter-associated pathology of the gastrointestinal tract and without somatic pathology in the oral cavity were detected with the *H.pylori*, while 22 patients were randomly selected for PCR (due to the high cost of this diagnostic method) with a verified diagnosis of chronic gastritis and gastroduodenitis associated with *H.pylori*.

The results of the identification of *H.pylori* in the oral cavity of patients with gastrointestinal diseases are presented below (Table V).

During the first examination of patients with a confirmed diagnosis of *H.pylori*-associated gastrointestinal tract pathology according to PCR data, the bacterium is identified in the oral cavity in 45.5% of cases.

At the same time, the results of the rapid urease test show that in patients with CG in the oral cavity, *H.pylori* was detected in 69% of cases, in patients with CGD in 96.3% of cases, in general, in this group of patients, *H.pylori* in the oral cavity cavity was detected in 86% (Table IV), while the degree of microbial insemination of the mucous membrane of the oral cavity, determined according to the data of rapid urease test, was different:

- + – in 15 patients;
- ++ – in 16 patients;
- +++ – in 6 patients;
- negative result – in 6 patients.

After the course of eradication therapy, the determination of the *H.pylori* in the oral cavity was carried out after 1 and 6 months.

In the oral cavity 1 month after the AHBT according to PCR, *H.pylori* was detected in 3 patients with CG and in 3 patients with CGD, in a total of 6 patients, which was 27.3%. At the same time, according to the data of rapid urease test in the oral cavity of patients with CG, *H.pylori* was detected in 10 out of 13 cases, which was 76.9%, and among patients with CGD, the prevalence of oral *H.pylori* was 90.5%, which on average for the group was 85.3%.

After 6 months, the results of identification of *H.pylori* in the oral cavity were as follows. According to PCR results, *H.pylori* was detected in 5 patients with CG and in 3 patients with CGD, which was 44.4%. The results of the rapid urease test show that a positive result was obtained in almost 100% of cases.

The obtained results of rapid urease test indicate that the oral cavity is a reservoir of the bacterium *H.pylori*, and, despite the conducted AHBT, it is not possible to achieve complete elimination of the bacterium in different biotopes of the oral cavity, which is confirmed by our studies conducted in dynamics (1 and 6 months after the during the course of antibacterial therapy, the bacterium persists in the oral cavity), and is consistent with literature data [14]. In addition, in the presence of *H.pylori* bacteria in plaque and saliva, it stimulates the growth of periodontopathogenic microflora, which was shown in [13], which subsequently leads to worsening of the course of periodontal diseases, which is consistent with our clinical data.

DISCUSSION

The results of dynamic monitoring of patients with CCG and concomitant *H.pylori*-associated pathology of the gastrointestinal tract indicate that professional oral hygiene, teaching patients the rules of oral hygiene at home, as well as patient motivation contribute to maintaining oral hygiene in the first three months, with some deterioration and almost achieving the level of the initial data after half a year of observations, as evidenced by the indices of Silness-Loe, Stallard and tartar index. The condition of periodontal tissues according to periodontal indices changed as follows. After 1 month, the PMA index decreased by 44.5%, after 3 months there was an increase in the PMA index by 1.1 times compared to the previous data, but does not reach the initial level, after 6 months it increases by 1.5 times compared to the data after treatment, and practically reaches the initial level, which correlates with the results of a clinical examination of the oral cavity. A similar trend is observed with the Schiller-Pisarev test and with the bleeding index.

Biochemical analysis of the oral fluid showed that in patients with inflammatory periodontal diseases against the background of *H.pylori*-associated pathology of the gastrointestinal tract (chronic gastritis and gastroduodenitis), the functional activity of the salivary glands increases, the protein content in the oral fluid increases (which leads to deterioration, which is one of the factors that worsen oral hygiene), increases the activity of inflammatory markers, which indicates an increase in inflammatory processes in response to inflammation in the gastric mucosa and duodenum, the balance in the "antioxidant system-peroxide processes" towards strengthening the latter. the microbial contamination of the oral cavity increases and local nonspecific reactivity decreases, which leads to a shift in the balance of the microflora of the oral cavity towards opportunistic and pathogenic and the development of oral dysbiosis.

All of the above points to the role of the bacterium *Helicobacter pylori* in the development and progression of periodontal diseases as one of the possible mechanisms of the pathogenesis of diseases such as gingivitis and periodontitis. *H.pylori*-associated pathology of the

gastrointestinal tract significantly exacerbates pre-existing oral disorders in chronic catarrhal gingivitis and chronic generalized periodontitis. Oral dysbiosis plays an important role in the pathogenesis of these pathological changes in the oral cavity. Previously, the role of this etiological factor was not taken into account in the pathogenesis of periodontal disease.

The pathogenesis of periodontal diseases that occur against the background of *H.pylori*-associated pathology of the gastrointestinal tract can be represented as follows (Fig 1).

The main mechanism of inflammatory periodontal diseases in patients with *H.pylori*-associated pathology of the gastrointestinal tract is systemic and local exposure to *H.pylori*, which subsequently leads to immune system dysfunction (changes in cellular immunity with a decrease in the content of T-lymphocytes and their autoimmune functions also function), *H.pylori* bacterium inactivates lysozyme, enhances apoptosis of macrophages and impaired antigen presentation), sensitization, stimulates the growth and reproduction of pathogenic periodontal microflora, forms stable microbial associations in the biofilm. In addition, *H.pylori* causes a violation of cell renewal cycles – an increase in proliferative processes on the one hand and an increase in apoptosis on the other. Changes in the human immune system contribute to and provoke pathological changes in the oral cavity, which in turn further leads to a further weakening of immunity and a more severe clinical course of the disease and its transition to the chronic stage. In most cases of chronic inflammatory reactions, there is a pronounced autointoxication of the body, which depresses the immune system [16], conditions are created to reduce the resistance of periodontal tissues to biofilm bacteria, activate periodontopathogens [17] and, as a result, the occurrence of an imbalance of microflora leads to the development of dysbiotic changes of various severity and deepening of inflammatory processes in periodontal tissues.

The substantiation of this link in pathogenesis dictates the need for new approaches to the prevention and pharmacotherapy of this disease.

CONCLUSIONS

Comparing the data of clinical observations and the results of laboratory studies of patients with chronic catarrhal gingivitis on the background of *Helicobacter pylori*-associated gastrointestinal pathology, obtained in different observation periods, we can say that they correlate with each other and suggest that the basic dental treatment of chronic catarrhal gingival patients -associated pathology of the gastrointestinal tract, which is currently undergoing a course of eradication, does not give a stable anti-inflammatory, antimicrobial and antioxidant effect, which leads to recurrence of periodontal disease and shortening remission periods, where oral dysbiosis plays a crucial role.

Therefore, to normalize the oral cavity, eliminate inflammatory changes and dysbiosis, patients with gastrointestinal diseases associated with *Helicobacter pylori*, together with basic therapy of periodontal disease and treatment of the main somatic disease, it is advisable to prescribe correct differentiated schemes for prevention and treatment of oral pathology. prevention of disease recurrence.

REFERENCES

1. Hooi J.K.Y., Lai W.Y., Ng W.K. et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-429. doi:10.1053/j.gastro.2017.04.022.
2. Moseeva M.V., Belova E.V., Vakhrushev Ia.M. Eksp Klin Gastroenterol. 2010;(2):19-21.
3. Wei X., Zhao H.Q., Ma C. et al. The association between chronic periodontitis and oral Helicobacter pylori: A meta-analysis. PloS one. 2019; 14(12):e0225247.
4. Kirillov V.A., Dronova O.B., Bukharin O.V. Faktory persistentsii Helicobacter pylori [Persistence factors of Helicobacter pylori]. Zh Mikrobiol Epidemiol Immunobiol. 2003;(4):8-11.
5. Nomura R., Kadota T., Ogaya Y. et al. Contribution of Streptococcus mutans to Helicobacter pylori colonisation in oral cavity and gastric tissue. Sci Rep. 2020;10(1):12540. doi:10.1038/s41598-020-69368-2.
6. Tereshchenko S.Yu., Olkhovskii I.A. Diagnostica khronicheskoi infektsii Helicobacter pylori u detei [The diagnostic of Helicobacter pylori infection children] Klinicheskaia laboratornaia diagnostika. 2014; 2: 48-53. (in Russian)
7. Levitsky A.P., Denga O.V., Makarenko O.A. et al. Biokhimicheskie markery vospaleniya tkaney rotovoy polosti: metodicheskie rekomendatsii [Biochemical markers of inflammation of oral cavity tissue: method guidelines]. Odessa, KP OGT. 2010, 16p. (in Russian)
8. Levitsky A.P., Stefanov A.V. Metody opredeleniya aktivnosti elastazy i eye ingibitorov: metodicheskie rekomendatsii [The methods of the determination of the activity of elastase and its inhibitors: method guidelines]. Kiev, GFK. 2002, 15p. (in Russian)
9. Levitsky A.P., Makarenko O.A., Selivanskaya I.A. et al. Fermentativnyy metod opredeleniya disbioza polosti rta dlya skrininga pro- i prebiotikov: metodicheskie rekomendatsii [Enzymatic methods for determination of oral dysbiosis for screening pro- and prebiotics: method guidelines]. Kiev, GFC. 2007, 23p. (in Russian)

10. Levitsky A.P. Lizotsym vmesto antibiotikov [Lysozyme instead of antibiotics]. Odessa, KP OGT. 2005, 74p. (in Russian)
11. Lowry O.H., Rosebrough N.J., Farr A.L. et al. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275.
12. Kucevlyak V.F., Lahtin Yu.V. Indeksna ocinka parodontal'noho status [Index assessment of periodontal status]. Sumy, Mriya. 2015, 104p. (in Ukrainian)
13. Hu Z., Zhang Y., Li Z. et al. Effect of Helicobacter pylori infection on chronic periodontitis by the change of microecology and inflammation. Oncotarget. 2016; 7(41): 66700-66712.
14. Uotani T., Graham D.Y. Diagnosis of Helicobacter pylori using the rapid urease test. Annals of translational medicine. 2015; 3(1).
15. Lapach S.N., Chubenko A.V., Babich P.N. Statisticheskiye metody v medico-biologicheskikh issledovaniyakh s ispolzovaniem Excel [Statistical methods in medical and biological research by using Excel]. Kiev, Morion. 2000, 320p. (in Russian).
16. Bohatu S.I. Sochetannaya patologiya: zabolevaniya perodonta I gastroduodenalnoi zony (obzor literatury) [Combined pathology: periodontal and gastroduodenal diseases (literature review)]. Innovations in dentistry. 2017; 3-4 (16): 40-46. (in Russian).
17. Kilmuhametov Yu.H., Batig V.M., Abramchuk I.I. Zabolevaniya parodonta na fone somaticheskih patologii [Periodontal diseases on the background of the somatic pathologies]. Young scientist. 2017;26 (100):57-62. (in Russian).

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

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Received: 26.06.2022
Accepted: 10.01.2023

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

ORIGINAL ARTICLE

HEIGHT AND WEIGHT CHARACTERISTICS OF MILITARY SERVICEMEN OF MOBILIZATION AGE WITH DISEASES OF THE CARDIOVASCULARY SYSTEM

DOI: 10.36740/WLek202302120

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ABSTRACT

The aim establish the height and weight characteristics of servicemen of mobilization age with cardiovascular diseases, the frequency and etiological fraction of excess body weight and obesity in the risk of developing cardiovascular diseases.

Materials and methods: The study included military personnel, exclusively male (n=127), who made up the observation group. The age of the study participants varied from 19 to 64 years, the average age - 43.06±4.07. All study participants were undergoing inpatient examination and treatment with cardiovascular diseases. The material for the study was the results of anthropological examinations and data from primary accounting medical documentation (medical history, primary medical card, evacuation ticket, etc.).

Results: It was established that the prevalence of obesity in the observation group was 26.0%, which is significantly higher compared to the frequency of obesity in the control group of 13.2% ($\chi2=17.02$; $P=0.0003$). It was found that obesity of the III stage degree occurred significantly more often in the experimental group - 3.03% compared to the control group - 0.4%, ($\chi2=5.73$; $P=0.01$). The calculated value of the etiological share (EF of obesity) was equal to 51-66%, which indicates a high contribution of obesity to the development of cardiovascular diseases.

Conclusions: It was established that the prevalence of obesity of various degrees among servicemen with diseases of the cardiovascular system is significantly higher compared to the frequency of obesity in the male population of Ukraine.

KEY WORDS: cardiovascular diseases, anthropological studies, obesity, body mass index, etiological fraction of risk factors

Wiad Lek. 2023;76(2):386-390

INTRODUCTION

The tasks of military personnel are usually carried out in extreme conditions, where there are many factors that require a lot of endurance and physical training. The body of military personnel is forced to adapt in a short period of time to the conditions of military service or to active combat operations, which is associated with high physical loads. The ability of military personnel to quickly adapt to new conditions in the war largely depends on their initial state of health [1].

Good physical condition is the foundation on which a certain level of functioning of various systems and organs, psycho-emotional stability of the body is formed, ensure high work capacity and maintain health [2]. Studies have proven that the better the physical development of troops, the more proportional the ratio between weight and height and the more successful the actions of soldiers during combat activity [3]. In the structure of physical capability of military personnel for successful actions, the most important are: endurance, the ability to resist fatigue during fast and long movements, which cannot be achieved with excess body weight (EBW) and obesity.

Modern society in most countries of the world is characterized by a low physical activity, an irrational diet with an increase in the number of refined products, overeating, consuming a large amount of food at night, constant psychological stress, all of which lead to an increase in the frequency of EBW and obesity among people of all age groups, especially young people [4, 5]. People under the age of 45 who have EBW are 2.1 times more likely to have a high cholesterol level than people with a normal body weight [6, 7]. The presence of EBW at a young age is a factor that determines the high probability of obesity, premature death and disability. At the same time, people with EBW are more likely to have cardiovascular disease (CVD), type II diabetes, problems of the musculoskeletal system, the digestive system, the bronchopulmonary system, and also have psychological difficulties that reduce work capacity and negatively affect the quality of life [8, 9, 10, 11]. On average, EBW reduces life expectancy in men: if it

exceeds 10% of the norm, life expectancy is reduced by 13%, if it is exceeded by 20% - by 25%, if it is exceeded by 30% - by 42%. A number of authors emphasize the importance of timely correction of EBW in young people, as the main factor in preventing the development of obesity, CVD and a number of diseases associated with EBW [10, 12]. For a long time, CVD remain the leading cause of disability and reduced life expectancy, while EBW and obesity pathogenetically contribute to their development and progression.

At the moment, war is ongoing on the territory of Ukraine and military personnel protect our country from the invaders. Therefore, the issue of preserving and maintaining the optimal state of their health is extremely important, in order to ensure the full participation in combat operations of each soldier.

THE AIM

Purpose of the study - to establish the height and weight characteristics of servicemen of mobilization age with CVD, the frequency and etiological fraction of EBW and obesity as a risk factor for the development of CVD.

MATERIALS AND METHODS

The study included military personnel, only male (n=127), who made up the observation group. The age of the study participants varied from 19 to 64 years, the average age - 43.06±4.07. All study participants were undergoing inpatient examination and treatment at the " Amosov National Institute of Cardiovascular Surgery of the National Academy of Medical Sciences of Ukraine" with CVD. The material for the study was the results of anthropological examinations and data from primary medical documentation (medical history, primary medical card, evacuation ticket, etc.).

The participants of the study underwent a clinical and anthropological examination with measurement of height and body weight and subsequent calculation of the body mass index (BMI), which was determined according to formula 1.

$$\text{BMI} = \text{weight (kg)} / \text{height}^2 (\text{m}^2) \quad (1)$$

The interpretation regarding the presence of EBW, obesity with an indication of its degree was evaluated according to the following criteria: less than 18.5 - body weight deficit; 18.5 - 24.9 - normal body weight; 25.0 - 29.9 - EBW; over 30.0 - obesity; 30.0 - 34.9 - first degree obesity; 35.0 - 39.9 - second degree obesity; more than 40.0 - third-degree obesity [13, 14].

Statistical indicators of the male population of Ukraine of the State Statistical Service of Ukraine for the year 2021 were taken as the comparison group [15].

The significance of differences in the frequencies of the studied signs between the observation and control groups at a significance level of 0.05 was determined by the χ^2 test with Yates correction. MS Excel 2010, SPSS Statistics 18.0 software was used.

The assessment of the risk of CVD development in this study was based on fundamental developments [16]. To determine the strength of the association of EBW and obesity with CVD, the relative risk ratio (RR) according to formula 2 was used. The variance of the RR indicator according to formula (3), which is necessary to calculate the 95% confidence interval (CI) for RR according to formula (4):

$$\mathbf{R} = \frac{\mathbf{A_1}}{\mathbf{N_1}} \Big/ \frac{\mathbf{A_0}}{\mathbf{N_0}} \quad (2)$$

RR – relative risk ratio;

$$\boldsymbol{var}[\ln(\boldsymbol{RR})] = \frac{\boldsymbol{N_1} - \boldsymbol{A_1}}{\boldsymbol{N_1} \cdot \boldsymbol{A_1}} + \frac{\boldsymbol{N_0} - \boldsymbol{A_0}}{\boldsymbol{N_0} \cdot \boldsymbol{A_0}} \quad (3)$$

ln(RR) – dispercion (D);

$$\mathbf{D} = \boldsymbol{e}^{\ln(\boldsymbol{RR}) \mp 1{,}96 \cdot \sqrt{\boldsymbol{var}[\boldsymbol{ln}\,(\boldsymbol{RR})]}} \quad (4)$$

$$e \approx 2{,}7128$$

e – natural logorythm base, a
ln – natural logorythm.
Ethiological risk fraction (*RF*) calculated by formula:

$$\boldsymbol{RF} = \mathbf{100} \cdot (\boldsymbol{RR} - \mathbf{1})/\boldsymbol{RR}(\%) \quad (5)$$

The materials used do not violate the principles of bioethics and can be published, all research participants gave their consent and signed an information agreement (excerpt from the protocol No. 2 of the meeting of the Commission on Bioethics of the Amosov NICVS National Academy of Sciences of Ukraine dated July 2, 2022) .

RESULTS AND DISCUSSION

As a result of conducting a clinical and anthropological examination of the study participants, it was established: the average height of servicemen of mobilization age is 179±10.5 cm; average body weight - 88±.8 kg; average BMI - 27.4±3.9. In the observation group, the following were found: 1 person with body weight deficit (0.7%); 41 persons with normal body weight (32.4%); 52 persons with EBW (40.9%); 33 persons with obesity (26.0%), and one person had its extreme degree - obesity of the III degree. (3.03%). It should be noted that

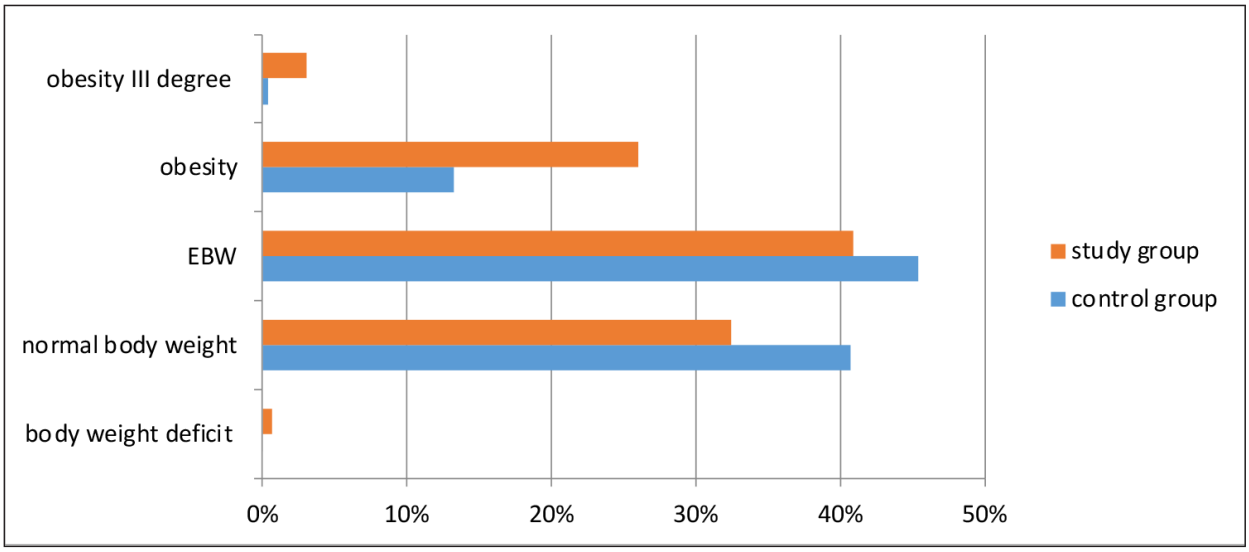

Fig. 1. The frequency of deviations from normal body weight in the group of servicemen with CVD compared to the data of the male population of Ukraine for 2021

Table I. Initial Data

		Exponated		Total	RR (CI)	RF, %	CVD risk
		group № 1	group № 2				
Patients with obesity	Yes	A_1	A_0	A	RR (CI)	Ethiological risk fraction, %	By criteria in table 2
	No	$N_1 - A_1$	$N_0 - A_0$	$N - A$			
Total		N_1	N_0	N			

Table II. Categories of assessment of cause-and-effect relationships

0<RR<1,0	1,1<RR<1,5	1,6<RR<2,0	2,1<RR<3,2	3,3<RR<5,0	RR≥5,1
EF=0	EF<33%	EF=34-50%	EF=51-66%	EF=67-80%	EF=81-100%
«zero»	«low»	«moderate»	«high»	«very high»	«almost total»

Table III. Frequency distribution of obesity in servicemen with CVD

Study groups, n		obesity «+»		obesity «-»		P, χ^2	RR (CI)	RF, %	Risk degree
		n	M±m, %	n	M±m, %				
Observation	127	33	26,0±3,8	94	74,0±3,8	P=0,0003 χ^2=17,02	2,31 (1,52-3,49)	51-66	High risk
Control	22 million 441 thousand	2 million 962 thousand	13,2±0,01	19 million 479 thousand	86,8±0,01				

Table IV. Frequency distribution of obesity of the III degree in the study participants

Study groups, n		obesity «+»		obesity «-»		P, χ^2	RR (CI)	RF, %	Risk degree
		n	M±m, %	n	M±m, %				
Observation	33	1	3,03±1,5	32	96,97±1,5	P=0,01 χ^2=5,73	7,58 (1,10-52,20)	81-100	Almost total risk
Control	2 million 262 thousand	11850	0,4±0,06	2 million 950 thousand	99,6±0,06				

in the group of servicemen with CVD, EBW and obesity were more common than in the control group (Figure 1).

The analysis of the study of the distribution of EBW in the group of servicemen with CVD showed that its prevalence in the control group was higher (45.4%) compared to the observation group (40.9%), but was not characterized by significant differences (P>0.05). When determining the frequency of deviations from

the norm of body weight in servicemen from the CVD, a significantly higher difference in the number of cases of obesity was established - 26.0% in comparison with the corresponding frequency of obesity in the male population of Ukraine, which was 13.2% ($\chi2$=17.02; P=0 .0003). It was also established that obesity of the III degree occurred significantly more often in the experimental group - 3.03% compared to the control group - 0.4%, ($\chi2$=5.73; P=0.01).

The next step of our research was to establish the etiological fraction of obesity in the risk of development of CVD. The methodology for assessing the etiological fraction was based on fundamental developments [16], the calculation of the RR of the development of obesity in the study participants was carried out according to formulas (2; 3; 4; 5). Notation to formulas (2, 3) and initial data for calculating RR, D and CI of obesity development are presented in Table I.

The assessment of cause-and-effect relationships between obesity and the risk of developing of CVD in study participants was carried out according to the categories indicated in Table II.

Therefore, the RR values were determined, which made it possible to establish that obesity increases the risk of developing of CVD by 2.31 times (RR=2.31; 95% CI: 1.52-3.49). And the presence of obesity of the III degree 7.58 times (RR=7.58; 95% CI: 1.10-52.20), data are presented in Tables III, IV.

Thus, the role of obesity and its extreme degree (3rd degree obesity) in the risk of CVD development has been clarified. The determined RF values of the risk of obesity indicate a high (from 51 to 66%) share of the contribution of this factor to the development of CVD. And the RF value for third-degree obesity. indicates that its etiological share is decisive and predisposes to the development of CVD (81–100%).

CONCLUSIONS

It was established that the frequency of EBW in servicemen of mobilization age with CVD (40.9%) is lower than in the male population of Ukraine (45.4%) and is not characterized by reliable differences. The prevalence of obesity among servicemen with CVD is 26.0%, which is significantly higher compared to the prevalence of obesity in the male population of Ukraine of 13.2%, ($\chi2$=17.02; P=0.0003). It was found that obesity of the III degree occurred significantly more often in the experimental group - 3.03% compared to the control group - 0.4%, ($\chi2$=5.73; P=0.01).

Calculated etiological fractions of obesity and its extreme degree (obesity of the IIIrd degree) in the risk of developing CVD. Accordingly, the value of the etiological fraction (RF of obesity) was equal to 51-66%, which indicates its high contribution to the development of CVD. And the value of the etiological share of obesity of the III degree. (RF obesity of the IIIrd degree) equaled 81-100%, this indicates that the etiological fraction of obesity of the IIIrd degree is decisive and condemns the development of CVD.

REFERENCES

1. Aphonin V., Ena M., Pociluyko P. Height and weight characteristics of physical development of cadets of the National Academy of Ground Forces. Young sports science of Ukraine. 2016;2:185-9. (in Ukrainian).
2. Romanchuk S.V., Boyarchuk O.M., Romanchuk B.M. The current state and prospective directions of improvement of physical training in the ground forces. Pedagogy, psychology and medico-biological problems of physical education and sports: scientific journal. 2010;12:6-9. (in Ukrainian).
3. Kostukovich B.E., Karankevich A.I. Theoretical analysis of optimization of professional and applied physical training. Scientific substantiation of physical education, sports training and personnel training in physical culture and sports. 2004:375-376. (in Ukrainian).
4. Ermolenko N.O., Zarudna O.I. Excess body weight and the main factors causing its development. Nursing. 2016;2:38-40. (in Ukrainian).
5. Jang JR, Hidayat K., Chen C-L., et al. Body mass index, waist circumference, and risk of hearing loss: a meta-analysis ans systematic review of observational study. Environ Health Prev Med. 2020; 25 (1): 25. doi:10.1186/s12199-020-00862-9.
6. Gulich M.P. Rational nutrition and a healthy lifestyle are the main factors in maintaining the health of the population. Problems of aging and longevity. 2011;20(2):128-132. (in Ukrainian).
7. Khan MAF, Sohaib M, Jqbal S, et al. Nutritional assessment of servicemen in relation to area of duty and feding habits: a Pakistani prospective. Braz J Biol. 2021;83:e250789. doi:10.1590/1519-6984.250789.
8. Li M, Gong W, Wang S, Li Z. Trends in body mass index, overweight and obesity among adults in the USA, the NHANES from 2003 to 2018:a repeat cross-sectorial survey. BMJ Open. 2022;12(12);e065425. doi:10.1136/bmjopen-2022-065425.
9. Kabootarim M., Asgari S., Mansournia M.A., et al. Different Weight Histories and Risk of Incident Coronary Heart Disease and Stroke: Tehran Lipid and Glucose Study. J Am Heart Assoc. 2018;7(4):e006924. doi:10.1161/JANA.117006924
10. Mitchenko O.I. Obesity as a risk factor for cardiovascular diseases. New medicine. 2006;3:24-29. (in Ukrainian).
11. Vlasenko M.A., Semenuk I.V., Slobodyanuk G.G. Diabetes and obesity - an epidemic of the 21st century: a modern approach to the problem. Ukrainian therapeutic journal. 2011;2:50-5. (in Ukrainian).

12. Bolshakova O.V., Malinovska T.M. Peculiarities of carbohydrate and fat metabolism in various forms of fat deposition in children and adolescents with metabolic syndrome. Pediatrics, obstetrics and gynecology. 2012;5:25-30. (in Ukrainian).
13. National Institutes of Health (NIH), National Heart, Lung, and Blood Institute (NHLBI). The practical guide: identification, evaluation, and treatment of overweight and obesity in adults. Bethesda: National Institutes of Health. 2000, NIH publication 00-4084.
14. Khosla T, Lowe CR. Indices of obesity derived from body weight and height. Br J Prev Soc Med. 1967;21:122-128.
15. Data from the State Statistics Service of Ukraine https://health.fakty.com.ua/ua/napulsi/aktualno/hto-vazhche-choloviky-chy-zhinky-derzhstat-nazvav-serednij-zrist-ta-vagu-ukrayintsiv/amp/ (in Ukrainian).
16. Vitte P.M. Methods of research in the epidemiology of non-infectious diseases (Reference and methodical manual). Kyiv, 2005, p.118. (in Ukrainian).

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

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Received: 12.08.2022
Accepted: 20.01.2023

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

ORIGINAL ARTICLE

MORPHOFUNCTIONAL STATUS OF INSTRUCTORS OF HIGHER EDUCATIONAL INSTITUTIONS DURING THEIR PEDAGOGICAL ACTIVITIES

DOI: 10.36740/WLek202302121

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ABSTRACT

The aim: To research the morphofunctional status of instructors of higher educational institutions of different age groups in the process of their pedagogical activities.

Materials and methods: The research was conducted in the period from 2019 to 2021. The research involved 126 instructor officers (men) of different age groups: under 30 years of age – 21 people, 31-35 years of age – 27 people, 36-40 years of age – 32 people, 41-45 years of age – 27 people, over 45 years of age – 19 people. Morphofunctional status of the instructor officers was assessed by the indicators of their height, body weight, lung vital capacity, wrist dynamometry, heart rate, blood pressure and relevant indices.

Results: It was found that during the study (2019-2020) the Kettle index, vital index, strength index, Robinson index and duration of recovery processes deteriorated among instructor officers of all age groups. However, in instructor officers of 36-40, 41-45 and over 45 years of age most of indices deteriorated reliably ($P < 0.05$). The values of the studied indices are below average or low in most instructors of all age groups, most instructors are overweight.

Conclusions: It was found that the level of morphofunctional status of the instructional staff is insufficient to perform the tasks of their pedagogical activities. Rationally organized health-improving physical training sessions, taking into account the age group, morphofunctional status of instructors and the time of training sessions during the working day can be an effective way to solve this problem.

KEY WORDS: physical training, health, morphofunctional status, instructor officers

Wiad Lek. 2023;76(2):391-397

INTRODUCTION

There is a constant need in society for the training of qualified, highly moral and faithful to the state ideals law enforcement officers [1-4]. Their physical training plays an important role under these circumstances [5-7]. There is a growing need to improve the organization and conduct of physical training sessions with all categories of law enforcement officers in order to improve indicators of their physical fitness, morphofunction status and health [8-10].

The analysis of the work of many scientists [11, 12] has shown that there has been a tendency to reduce the level of physical fitness and health of the population of Ukraine in recent years, and law enforcement officers are no exception. This is especially true of instructional staff of higher educational institutions of Ukraine with specific learning environment (HEI SLE), whose professional pedagogical activities are characterized by a significant reduction in physical activity.

The professional pedagogical activities of instructor officers of HEIs SLE is accompanied by a significant number of negative factors, including: low motor activity, nervous and emotional stress, mental overload and stress, monogony and forced working posture and others [13-16]. The action of negative factors during service activities causes a set of functional disorders in instructors that affect the functions of the cardiovascular system, respiratory system, nervous system,

musculoskeletal system and results in metabolic disorders. The pedagogical activities are characterised by the fact that instructor officers receive almost no physical load, which leads to a significant reduction in energy expenditure, accumulation of excess nutrients in the body and increase of excess body weight [17-20].

THE AIM

The aim is to research the morphofunctional status of instructors of higher educational institutions of different age groups in the process of their pedagogical activities.

The hypothesis of the research is to study the level and dynamics of indicators of the main life support systems of the body (cardiovascular, respiratory and muscular) of the instructor officers of the HEI SLE of different age groups under the influence of negative factors of their professional activity in order to justify the content of health-improving classes with physical exercises taking into account their age group and morphofunctional status.

MATERIALS AND METHODS

The research of indicators of morphofunctional status of the instructional staff was conducted in the period from 2019 to 2021 at National Academy of Internal Affairs (NAIA, Kyiv, Ukraine). The research involved 126 instructor officers (men) from different departments of NAIA. The studied contingent was divided into age groups: under 30 years of age – 21 people, 31-35 years of age – 27 people, 36-40 years of age – 32 people, 41-45 years of age – 27 people, over 45 years of age – 19 people. This distribution of officers is determined by the Order of the Ministry of Internal Affairs of Ukraine dated January 26, 2016 No. 50 "On approval of the Regulation on the organization of official training of employees of the National Police of Ukraine" (https://zakon.rada.gov.ua/laws/show/z0260-16#Text). Research methods: analysis and synthesis of literature, pedagogical observation, medical and biological methods, methods of mathematical statistics.

Morphofunctional status of the instructor officers was assessed by the indicators of their height, body weight, lung vital capacity, wrist dynamometry, Kettle index, vital and strength indices, Robinson index, heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP), time of restoring heart rate to baseline after 20 squats for 30 seconds. The research of the indicators of morphofunctional status of the instructors was conducted at the Central Polyclinic of the Ministry of Internal Affairs of Ukraine (Kyiv) during the medical examination by medical staff in the morning. The following devices were used: electronic scales (TEFAL Atlantis, REF: PP-3019 B6, max 160 kg) with an accuracy of 100 g, height meter (P No. 175, value of the smallest graduation in 1 cm), manual dynamometer (DRP-90; value of the smallest graduation in 2 kgf), electronic spirometer ("SPIROPRO", BTL-08), electronic tonometer ("SANITAS", SBM-07).

The Kettle index (KI) characterizes the features of the body-build of the instructors, defined in g / cm as the

Table I. The assessment of the Kettle index for 30-59 year-old-men (g / cm)

The value of the KI	The indicator of body weight
≥ 540	Adiposity
451-540	Overweight
416-450	Excess weight
400	Good weight
360-399	Average weight
320-359	Insufficient weight
300-319	Extremely insufficient weight
200-299	Exhaustion

Table II. The assessment of indices that characterize the morphofunctional status of instructor officers

The level of indices values	Studied indices				
	KI, g / cm	VI, ml / kg	SI, %	RI, c.u.	TRHRB, s
Low	501 and >	50 and <	60 and <	111 and >	180 and >
Below average	451-500	51-55	61-65	95-110	120-180
Average	450 i <	56-60	66-70	85-94	90-120
Higher than average	-	61-65	71-80	70-84	60-90
High	-	66 and >	81 and >	69 and <	59 and <

Table III. The dynamics of morphofunctional status of instructor officers of different age groups in the process of their pedagogical activities (n=126, Mean±SD)

Years of research	Age groups				
	Under 30 years of age	31-35 years of age	36-40 years of age	41-45 years of age	Over 45 years of age
	n=21	n=27	n=32	n=27	n=19
			KI (g / cm)		
2019	415.35±7.60	433.28±8.69	457.08±6.88	460.68±7.29	463.67±8.46
2020	430.21±10.21	440.48±8.82	469.02±7.03	472.76±7.90	476.15±9.42
2021	434.60±8.77	448.80±8.50	481.84±6.70	483.58±7.68	488.94±9.27
P_{19}-P_{20}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{20}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{19}-P_{21}	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05
			VI (ml / kg)		
2019	56.19±2.06	55.79±1.43	54.81±1.50	54.46±1.14	52.31±1.31
2020	55.65±1.28	54.32±1.47	52.77±1.53	52.26±1.18	51.45±1.45
2021	54.75±1.66	52.79±1.42	50.48±1.49	50.17±1.27	48.02±1.39
P_{19}-P_{20}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{20}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{19}-P_{21}	> 0.05	> 0.05	< 0.05	< 0.05	< 0.05
			SI (%)		
2019	61.94±2.20	60.38±1.43	57.76±1.40	56.08±1.45	55.01±1.44
2020	59.98±2.36	58.37±1.39	55.34±2.12	54.86±1.46	53.67±1.56
2021	59.05±1.96	57.77±1.20	54.42±1.54	52.25±1.49	50.10±1.50
P_{19}-P_{20}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{20}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{19}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05
			RI (c. u.)		
2019	88.36±0.95	91.55±1.37	93.18±1.45	94.30±1.90	95.51±2.41
2020	89.17±1.25	92.01±1.58	94.27±1.76	95.72±1.55	96.36±1.61
2021	89.86±0.85	92.96±1.81	94.97±2.05	96.43±1.32	97.65±1.41
P_{19}-P_{20}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{20}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{19}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
			TRHRB (s)		
2019	110.43±5.62	123.44±5.64	135.56±7.01	137.26±5.40	140.21±8.02
2020	109.81±6.19	126.78±5.43	141.38±7.29	142.44±6.41	147.79±6.95
2021	111.14±5.97	131.11±6.88	146.33±6.72	147.25±5.86	148.03±6.93
P_{19}-P_{20}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{20}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
P_{19}-P_{21}	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Note: P_{19}-P_{20}, P_{20}-P_{21}, P_{19}-P_{21} - the significance of the difference between the officers' indicators in 2019-2020, 2020-2021, 2019-2021 respectively, determined by means of Student's t-test

ratio of their body weight to their height. The assessment of the Kettle index for 30-59 year-old-men was carried out according to Table I and Table II.

The vital index (VI) is an important criterion for the reserve of external respiration functions, defined as the ratio of vital capacity of the lungs to body weight (ml / kg). The strength index (SI) characterizes the state of the muscular system. It is determined by the ratio of the dynamometry of the stronger hand to body weight (%). The Robinson index (RI) characterizes the reserve and economization of cardiovascular functions. Decreasing of the indicator determines the improvement of a par-

ticular system. The Robinson index was determined by the formula: RI = (heart rate · SBP) / 100, where heart rate is the heart rate at rest for 1 minute (beats / min), SBP is systolic arterial blood pressure (millimetre of mercury). The assessment of vital, strength indices, Robinson index and time of restoring heart rate to baseline (TRHRB) was carried out in accordance with Table II.

The methods of mathematical statistics were used to check, systematize and correctly process the received data and present them in tabular form. The significance of the difference between the studied indicators was determined by means of Student's t-test. The significance for all statistical tests was set at $p<0.05$. All statistical analyses were performed with the IBM SPSS Statistics 21 software, adapted to medical and biological researches.

This study followed the regulations of the World Medical Association Declaration of Helsinki – ethical principles for medical research involving human subjects. The procedure for organizing this study was previously agreed with the committee on compliance with Academic Integrity and Ethics of the NAIA. The topic of the study was approved by the Academic Council of the NAIA (No. 12 dated 28.08.2020). Informed consent was received from all participants who took part in this study.

RESULTS

The study of the Kettle index gives the right to conclude that no significant difference between the average indicators of the instructor officers of age groups under 30 years of age and 31-35 years of age was identified during 2019-2021 ($P > 0.05$). In instructors of 36-40, 41-45 and over 45 years of age during 2019-2021 indicators of the Kettle index significantly ($P < 0.05$) worsened by 24.76 g / cm, 22.90 g / cm and 25.27 g / cm respectively (Table III). Comparing the KI in the instructors under 30 years of age and over 45 years of age at the end of the research (2021) it can be noted that the index value is significantly worse by 54.34 g / cm in the instructors of the older age group ($P < 0.05$). The assessment of the KI shows that only the instructors under 30 years of age at the beginning of the research (415.35 g / cm) were characterised by the index at the average level of its fullness for men and corresponded to the indicator of "good weight". The instructors under the age of 30 in 2020 and 2021, as well as the instructors of 31-35 years of age were characterised during the research as those having "excess weight". The KI of the instructors of other age groups indicated "overweight" in the instructional staff of the HEI SLE. The research conducted showed that the pedagogical activities of the instructor officers in the HEI SLE are characterized by a hypodynamic regime and an increase in body weight in the process of their service. This indicates the need to improve the instructional staff's physical fitness in order to stabilize their body weight.

The research of the dynamics of the VI in the process of pedagogical activities of the instructional staff gives the right to state that the indicators of the instructor officers of all age groups tend to decrease, but did not change significantly during the research ($P > 0.05$) in instructors of age groups of under 30 years of age and 31-35 years of age. In instructor officers of 36-40, 41-45 and over 45 years of age during 2019-2021 indicators of the VI index significantly ($P < 0.05$) worsened by 4.33 ml / kg, 4.29 ml / kg та 4.29 ml / kg respectively (Table III).

Comparing the indicators of the VI in the officers of different age groups, it can be noted that in 2021 a significant difference was found only between the values of the instructors under 30 and 40-45 and over 45 years of age in 2019 ($P < 0.05$; $P < 0.01$). The highest indicators of the VI were recorded among the instructor officers under 30 years of age i. e. the value was assessed as "below average" (56.19 ml / kg) at the beginning of the research, and as "below average" (54.75 ml / kg) at the end of the research. The instructors of other age groups had below average level of the VI at the beginning of the research and below average and below (instructor officers over 45 years of age) levels at the end of the research. All this indicates the weakening of the functional capabilities of the respiratory system in the instructional staff, both in the process of their pedagogical activities and with increasing age group.

The analysis of the strength index showed that its value in instructor officers of all groups decreased in the process of their pedagogical activities, but did not differ significantly in all age groups at the beginning and the end of the research ($P > 0.05$) except instructori over 45 years of age. The difference between SI indicators at the beginning and at the end of the research in this group of instructor officers is 4.91 % and is significant ($P < 0.05$) (Table III). The indicators of the SI reduced in the instructor officers over 45 years of age compared to the instructors under 30 years of age by 6.93; 6.31; and 8.95 %, respectively ($P < 0.05$-$P < 0.01$) and were the lowest. Analysing the SI indicators, it can be noted that only the instructors under 30 years of age were characterised in 2019 by its value below average, and in 2020 and 2021 and during the research the level of reserves of muscular system functions was estimated as "low" in all other groups of the instructor officers.

The research of the RI in the instructor officers showed a deterioration of the indicators from 2019 to 2021 in all study groups ($P > 0.05$): the difference between the indicators of 2019 and 2021 for instructor under 30 years of age is 1.50 c. u., 31-35 years of age – 1.41 c u., 36-40

years of age – 1.79 c. u., 41-45 years of age – 2.13 c. u., over 45 years of age – 2.14 c. u. (Table III). The research of the dynamics of the RI indicators showed that the cardiovascular system of the instructor officers deteriorated with increasing instructional experience i. e. the value of the indicator for the officers over 45 years of age was significantly worse than for the instructors under 30 years of age by 7.15 c. u. in 2019, by 7.19 c. u. in 2020 and by 7.79 c. u. in 2021 ($P < 0.01$). The indicators of the functionality of the cardiovascular system of most of the instructional staff were at the "average" and "below average" levels.

The research of the dynamics of TRHRB shows a decrease in the indicator of the instructors of all age groups during the research ($P > 0.05$) (Table III). There was a deterioration of this indicator with the increase of the age group of the instructors, which indicates the weakening of their cardiovascular system in the process of their pedagogical activities. Thus, if in 2019 the average indicator of TRHRB for the officers under 30 years of age was 1 minute 51 seconds, then it made 2 minutes 28 seconds for the instructors 36-40 years of age ($P < 0.001$). The results of the officers 36-40 years of age, 41-45 years of age and over 45 years of age did not differ significantly ($P > 0.05$). The instructors under 30 years of age were characterised as those having the level of functional capabilities of the cardiovascular system in terms of TRHRB values as "average"; the level of the rest of the studied groups was assessed as "below average".

DISCUSSION

The analysis of a large number of literature sources [21-23] shows that the high level of morphofunctional status, health and efficiency of professional activities of law enforcement officers largely depends on the level of their physical fitness, formed in the process of regular physical training sessions. The analysis of the normative documents on the organization of physical training in the HEI SLE allowed to reveal a number of shortcomings that reduce the impact of physical training on physical fitness, morphofunctional status and health of instructor officers, including: insufficient consideration within the guiding documents of the peculiarities of the service activities of the scientific and instructional staff; the main focus during training sessions is on improving the indicators of special physical training and preparation for inspections; the time planned for scheduled physical education training sessions and the lack of clear organization of the training sessions cause a low percentage of attendance by the instructor officers and do not allow to address issues of improving physical fitness and promoting the health of instructional staff in the process of their pedagogical activities; the time allotted for scheduled physical training sessions in the afternoon does not allow conducting classes with sufficient load and density for the effective development of physical qualities; the duration and frequency of training sessions are insufficient to increase the level of physical fitness and maintain the required level of morphofunctional status and health; performance of the tasks of service activities, the functional duties, the orders of commanders in extracurricular time which is allocated for independent physical training sessions; there are not enough factors in the guiding documents that would stimulate an increase in the level of general physical fitness of instructor officers; a large number of appeals to the medical department and a constant increase in the number of instructor officers who belong to the group of medical physical culture due to their state of health.

The analysis of the system of organization of physical training with the instructional staff of higher educational institutions of other law enforcement agencies showed the presence of similar shortcomings [9, 24]. In particular, the instructor officers of theoretical departments in higher military educational institutions are forced to miss scheduled physical training sessions because they are involved in conducting classes with cadets. Some scientists point to the presence of a number of occupational diseases in the instructors of HEIs [25].

Our research revealed that the level of morphofunctional status of the instructional staff of the HEI SLE at the present stage is insufficient to fulfil the instructional tasks and needs to be improved. The experts [5, 7, 21, 22] note that one of the ways to solve the above problem is to improve the physical training of instructor officers in the HEI SLE based on the use of general physical training, taking into account the age group and time for such training sessions during the working day. Our results complement and expand existing scientific data on the level of physical fitness and health of law enforcement officers.

CONCLUSIONS

It was found that during the study (2019-2020) the Kettle index, vital index, strength index, Robinson index and duration of recovery processes deteriorated among instructor officers of all age groups. However, in instructor officers under 35 years of age, the deterioration of most indices turned out to be unreliable ($P > 0.05$). But in instructor officers of 36-40, 41-45 and over 45 years of age most of indices deteriorated reliably ($P < 0.05$). The

most pronounced changes were found in the indicators of Kettle index (22.90-25.27g / cm), vital index (4.29-4.33 ml / kg), strength index (3.34-4.91 %), Robinson index (1.79-2.14 c. u.). This indicates a negative trend in the indicators of morphofunctional status of instructor officers in the process of their pedagogical activities.

It was set that the values of all studied indices in the instructors of older age groups are significantly worse than in the instructors of younger age groups, which also indicates the deterioration of instructors' morphofunctional status. The values of the studied indices are below average or low in most instructors of all age groups; most instructors are overweight. All this confirms the negative impact of factors of professional activities of instructors on the state of their main life support systems of the body: cardiovascular, respiratory and muscular. In our opinion, the main negative factor that caused the deterioration of the indicators of the morphofunctional status of instructors is insufficient motor activity.

It was found that the level of morphofunctional status of the instructional staff of the HEI SLE is insufficient to perform the tasks of their pedagogical activities and needs to be improved. This problem is especially acute for the instructor officers under the age of 40 and older. Rationally organized health-improving physical training sessions, taking into account the age group, morphofunctional status of instructors and the time of training sessions during the working day can be an effective way to solve this problem.

THE PROSPECT OF FURTHER RESEARCH

It is planned to investigate the level and dynamics of the indicators of physical health and mental working capacity of the instructors of the HEI SLE in the process of their pedagogical activities.

REFERENCES

1. Barko V., Okhrimenko I., Medvediev V. et al. Professional psychological profile of a modern patrol officer as the basis of efficient official activities. Postmodern Openings. 2020; 11(3): 01-19.
2. Bondarenko V., Okhrimenko I., Tverdokhvalova I. et al. Formation of the professionally significant skills and competencies of future police officers during studying at higher educational institutions. Revista Românească pentru Educație Multidimensională. 2020; 12(3): 246-267.
3. Okhrimenko I., Lyakhova N., Horoshko V. et al. Means of psychophysiological indicators improvement of future law enforcement officers in the process of their speciality training. Wiad. Lek. 2022; 75 (4): 871-876. doi: 10.36740/WLek202204122.
4. Bondarenko V., Okhrimenko I., Piaskovskyi V. et al. Scientific tools for forming professional competence of patrol police officers. International Journal of Evaluation and Research in Education. 2022; 11(2): 687-695. doi: 10.11591/ijere.v11i2.21987.
5. Grupe D.W., Stoller J.L., Alonso C. et al. The impact of mindfulness training on police officer stress, mental health, and salivary cortisol levels. Front Psychol. 2021; 12: 720753. doi:10.3389/fpsyg.2021.720753.
6. 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.
7. Prontenko K., Bondarenko V., Bezpaliy S. et al. Physical training as the basis of professional activities of patrol policemen. Balt J Health Phys Activ. 2020; 12(1): 41-53. doi: 10.29359/BJHPA.12.1.05.
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. 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.
10. Okhrimenko I., Pavlyk O., Tomenko O. et al. Dynamics of indicators of cadets' physical development and functional status during pentathlon. International Journal of Human Movement and Sports Sciences. 2021; 9(4): 814-823. doi: 10.13189/saj.2021.090428.
11. 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.
12. Shvets D., Yevdokimova O., Okhrimenko I. et al. The new police training system: Psychological aspects. Postmodern Openings. 2020; 11(1): 200-217.
13. Okhrimenko I., Lyhun N., Pryimak V. et al. 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.
14. Schaible L.M. The impact of the police professional identity on burnout. Policing: An International Journal. 2018; 41(1): 129-143. doi: 10.1108/PIJPSM-03-2016-0047.
15. Scheuch K., Haufe E., Seibt R. Teachers' health. Dtsch Arztebl Int. 2015; 112(20): 347-356. doi:10.3238/arztebl.2015.0347.
16. Quattrin R., Ciano R., Saveri E. et al. Burnout in teachers: an Italian survey. Ann Ig. 2010; 22(4): 311-318.
17. Anderson G., Litzenberger R., Plecas D. Physical evidence of police officer stress. Policing: An International Journal. 2002; 25(2): 399-420.
18. Gül Z., Delice M. Police job stress and stress reduction/coping programs: the effects on the relationship with spouses. Turkish Journal of Police Studies. 2011; 3(13): 19-38.

19. Brütting J., Druschke D., Spitzer S., Seibt R. Health status of long-term sick leave and working female teachers in Germany: A cross-sectional study. Int J Occup Med Environ Health. 2018; 31(2): 227-242. doi:10.13075/ijomeh.1896.01115.
20. Silva A.A., Fischer F.M. Teachers' sick leave due to mental and behavioral disorders and return to work. Work. 2012; 41(1): 5815-5818. doi:10.3233/WOR-2012-0961-5815.
21. Grupe D.W., McGehee C., Smith C. et al. Mindfulness training reduces PTSD symptoms and improves stress-related health outcomes in police officers. J Police Crim Psychol. 2021; 36 (1):72-85. doi:10.1007/s11896-019-09351-4.
22. Schilling R., Herrmann C., Ludyga S. et al. Does cardiorespiratory fitness buffer stress reactivity and stress recovery in police officers? A real-life study. Front Psychiatry. 2020; 11: 594. doi:10.3389/fpsyt.2020.00594.
23. Loucks J.S. Educating law enforcement officers about mental illness: nurses as teachers. J Psychosoc Nurs Ment Health Serv. 2013; 51(7): 39-45. doi:10.3928/02793695-20130503-03.
24. Doody C.B., Robertson L., Cox K.M. et al. Pre-deployment programmes for building resilience in military and frontline emergency service personnel. Cochrane Database Syst Rev. 2021; 12(12): CD013242. doi:10.1002/14651858.CD013242.pub2.
25. Peñalba V., McGuire H., Leite J.R. Psychosocial interventions for prevention of psychological disorders in law enforcement officers. Cochrane Database Syst Rev. 2008; (3): CD005601. doi:10.1002/14651858.CD005601.pub2.

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

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Received: 25.08.2022
Accepted: 20.01.2023

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

ORIGINAL ARTICLE

EVALUATION OF THE EFFECTIVENESS OF THE GASTRODUODENITIS PREVENTION PROGRAM IN PATIENTS OF RETIREMENT AGE WITH ESSENTIAL ARTERIAL HYPERTENSION WHO TOOK PART IN THE PROGRAM «AFFORDABLE MEDICINES» UNDER THE IBCR

DOI: 10.36740/WLek202302122

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ABSTRACT

The aim: To assess the effectiveness of the gastroduodenitis prevention program we have developed in patients of retirement age with essential arterial hypertension who participate in the «Affordable Medicines» program.

Materials and methods: A combined (retrospective and prospective) study was conducted, in which 150 patients took part. The main group consisted of 100 patients of retirement age with essential arterial hypertension and gastroduodenitis, which arose against the background of treatment of essential arterial hypertension. The control group consisted of 50 patients of retirement age with essential arterial hypertension without gastroduodenitis. For this category of the population developed a program for the prevention of gastroduodenitis. To assess the effectiveness of this prevention program, an «incremental cost-benefit ratio» (ICBR) is used.

Results: An assessment of the effectiveness of the gastroduodenitis prevention program we developed in patients of retirement age with essential arterial hypertension who participate in the «Affordable Medicines» program.

Conclusions: Identified categories of patients for whom the developed prevention program is effective.

KEY WORDS: essential arterial hypertension, gastroduodenitis, prevention program, clinical – economic assessment, costs – efficiency, costs – benefits

Wiad Lek. 2023;76(2):398-405

INTRODUCTION

For five years, the «Affordable Medicines» program has been operating in Ukraine, implemented by the Government of Ukraine [1]. This program was created for patients with cardiovascular diseases, bronchial asthma and type II diabetes mellitus. It allows you to get free of charge, or with a small surcharge of medicines for the treatment of the above diseases. Free access to treatment often causes abuse of medicines. The presence of such a problem prompted us to study the peculiarities of using this program by patients of retirement age with essential arterial hypertension (EAH) [2-4]. Studying this issue, we drew attention to the increase in exacerbations of gastroduodenitis (GD) in some patients, which was the impetus for the development of a prevention program (PP) for them and an assessment of the effectiveness of this program.

THE AIM

To assess the effectiveness of the gastroduodenitis prevention program developed by us in patients of retirement age with essential arterial hypertension who participate in the «Affordable Medicines» program.

MATERIALS AND METHODS

A combined (retrospective and prospective) study was conducted.

The case group consisted of 100 patients of retirement age with essential hypertension and gastroduodenitis. 80% of patients were selected retrospectively (information was obtained by selecting data from patients' medical records, communication with patients and their family doctors). The duration of hypertension in all patients is different. Patients with the longest duration - 17 years (2000 - 2017). The shortest duration is 3 years (2015 - 2017). Gastroduodenitis in each patient began in a different period of time, but after the onset of hypertension.

20% of patients were selected prospectively. The beginning of inclusion in the study was in 2017. Duration of stay in the study - 3 years.

The control group consisted of 50 patients with essential hypertension who did not have gastroduodenitis. 80% of patients were recruited into the group retrospectively. The duration of hypertension in all patients is different. Patients with the longest duration - 17 years (2000 - 2017). The shortest duration is 3 years (2015 - 2017).

20% - prospectively. The beginning of inclusion in the study - 2017. Duration of stay in the study - 3 years.

Patients were randomly selected for the study. All of them live in the same region of the country.

We have developed a program for the prevention of gastroduodenitis for patients of retirement age with essential hypertension. The proposed prevention program was tested on patients who participated in the prospective part of the study. The effectiveness of individual elements of the program included in the study were tested also on a retrospective group.

To obtain the expected results, the classical theoretical model was adapted (P. Zweifel and W. G. Manning, Handbook), based on the economic theory of premorbid moral hazard (ex ante moral hazard theory).The provisions of the classical economic theory modified during the work concerned the function of utilities from economic variables, features of the clinical process, as well as individual characteristics with a continuous scale of measuring utilities at the stage of model verification (A. A. Akhmedova, O. M. Ocheredko). It was believed that the patient is a rational economic agent who consciously makes a choice in order to achieve the maximum number of utilities per dollar spent. Clinical and economic evaluation of prevention, treatment and rehabilitation programs faces methodological difficulties, in particular, the problem of identifying the form of the model based on economic theory. In this study, a model was developed based on the work of Zweifel & Breyer, with theoretical assumptions derived from classical theories of moral hazard and utility. The balance model describes the equilibrium, i.e. the optimal choice of the scope of the prevention program, and improves the classical cost-effectiveness, cost-utility and cost-benefit analyses.

The use of this approach allows to include in the model important factors that modify the equilibrium, in particular, the individual response of the patient to treatment and to obtain meaningful unbiased conclusions.

Based on the developed theoretical model, the effectiveness of the proposed prevention program was evaluated. The statistical justification for testing the effectiveness of the prevention program belongs to the data analysis techniques under the general name Average treatment effect (ATE).

To evaluate the effectiveness of the proposed prevention program for patients with essential hypertension at risk of gastroduodenitis, a simpler Poisson model with fixed effects was first applied, which includes the entire set of factors in a linear predictor.

To more accurately reproduce the blood pressure effect of the program and to increase the power of the tests, a mixed model with full and reduced sets of predictors was applied to study the robustness of the effects. The mixed model allowed to include and evaluate the effects of design elements.

A powerful modern driver of the implementation of hierarchical mixed models, to which this model structure belongs, is the MCMC (Monte Carlo and Markov chains) algorithms. During the execution of the work, the most developed and powerful Gibbs sampler was chosen.

The analytical software module is written in WinBUGS, which is an abbreviation of the expression Bayesian inference using Gibbs (software). The calculation of model parameters was carried out in the WinBUGS package version 1.4. Preliminary data preparation, as well as the study of convergence in Markov chains was carried out in the environment of the mathematical analytical system R, version 3.1.0 based on the CODA package. All the graphical images are also created in the R environment (GRAPHICS package).

Subsequently, the effectiveness of the program was evaluated by IECR (incremental efficiency-cost ratio) and IBCR (incremental benefit-cost ratio) according to the developed balance model.

RESEARCH METHODS

1. Systematic approach and system analysis. The application of this method allowed to systematically present the problems of gastroduodenitis prevention in patients of retirement age with essential arterial hypertension.
2. Statistical. The use of the statistical method of research allowed to determine the volume of observations, the reliability of the results, to substantiate hypotheses and statistical tests.
3. Epidemiological. This method helped in organizing the study and creating an observation plan and sampling frame.
4. Sociological. The method was applied at the stage of collecting data on individual risk factors and medical events.
5. The method of statistical modeling by MCMC algorithms. It was used to estimate the model parameters.
6. Expert estimates. The use of the method of expert assessments allowed to study the effectiveness of

the proposed program for the prevention of gastroduodenitis in patients of retirement age with essential hypertension.

7. Survival curves. The method allowed to obtain the expected difference in the risk of developing gastroduodenitis due to the appointment of the prevention program.

Information about the patient that we were interested in: age (all patients of retirement age), gender, single/not single (distribution of their savings is different in such patients), working/not working (income only in the form of a pension changes the patient's ability to invest in prevention), served by the social security service/not served (use of social security services limits the right to choose medicines, methods of prevention, etc.), stage and degree of hypertension at the time of onset of the disease and at the time of collection of information (established by the The stage and degree at the time of data collection allow us to see changes in the dynamics), year of diagnosis of hypertension and gastroduodenitis, annual expenditures for the treatment of hypertension and gastroduodenitis (we calculated how many and what drugs were prescribed each day. We looked at the prices for these drugs in our country as of the year when the drugs were prescribed. Prices were taken from the official website of state statistics[4]. Calculating the costs per year, we converted the amount of costs to the average dollar exchange rate in our country in the respective year). We were also interested in: the year of the first hospitalization for hypertension and the number of hospitalizations per year (treatment in hospitals has different schemes than outpatient treatment. This may change the risk of comorbidities), annual reimbursement (determined in the same way as the cost of treatment), smoker/non-smoker, obesity/non-obesity, which drugs were the main ones for hypertension treatment (the type of drug may affect the risk of comorbidities), doctor's note on non-compliance with prescriptions (compliance also affects the effectiveness of treatment and the risk of complications or comorbidities).

Burden of comorbidity by Charlson index (to obtain more reliable data, the Charlson index was used, which allowed to include patients with comorbidities in the study).

We use the term «incremental ratio of benefits-costs» (IB R) as more convenient in our study instead of the used «incremental cost-benefit ratio», respectively, the abbreviation IBCR instead of the usual ICBR. Both options carry the same information. The main for converting IECR «incremental cost-efficiency ratio» into IBCR are prevented by intervention (participation in PP) costs in case of occurrence or exacerbation of the GD. It should be noted that these are expected costs. While in most observed patients-years, these costs were zero (without exacerbation of GD), the expected costs can not be zero. In the case of zero observed values, the expected values were equal to the weighted average GD costs of the same patient in other years, while in the complete absence of exacerbations in the patient during the follow-up period, the expected costs were modeled as group-based on the patient's set of signs. Additional direct costs associated with going to the doctor and individually weighted hospitalization are also taken into account. Compliance played a great role in modeling the expected costs, non-compliance with doctor's prescriptions by patients «hides» real costs in connection with the GD, so the corresponding observed values for incomplete compliance are listed at full costs in connection with the GD. Since the number of possible exacerbations has the Poisson distribution, we used this law to obtain the expected number of prevented exacerbations for each patient-year of follow-up and found the direct costs prevented by the PP as the product of the expected number of exacerbations in the context of patient-years for the expected costs per exacerbation [5-8].

For the convenience of calculations, patients were divided into 6 groups:

Group 0: Patients with AH who do not receive hypotensive therapy (by their own decision) and have/do not have a complaint from the gastrointestinal tract.

Group 1: Patients taking anticoagulants and/or anti-agents who have/do not have a complaint from the gastrointestinal tract.

Group 2: Patients taking NSAIDs who have/do not have a complaint from the gastrointestinal tract.

Group 3: Patients with resistant AH and have/do not have a complaint from the gastrointestinal tract.

Group 4: Patients who take long-term medications that reduce the tone of the lower esophary sphincter and have/do not have a complaint from the gastrointestinal tract.

Group 5: Patients receiving only hypotensive therapy and having/not complaining from the gastrointestinal tract.

RESULTS

As a result of the study in order to determine the clinical and economic efficiency of the program for the prevention of gastroduodenitis developed by the authors in patients of retirement age with essential arterial hypertension, receiving drugs for hypertension under the Government Program, it was allowed to see the categories of persons of retirement age for whom

Table I. Distributed the values of the average annual expected losses C ($) in the event of or exacerbation of the GD

Indicators	Minimum	0,25	Median	Average	0,75	Maximum
C	15	270	349	356	357	2250
-IBCR	0.001	0.419	0.913	1.214	1.713	13.162
log(-IBCR)	-6.861	-0.870	-0.091	-0.312	0.538	2.577

Table II. Assessing the significant effects of predictors on log(-IBCR)

Predictors	β	m	t	p
(Constant)	-2,44267	0,311	7,86	0,0000000
Costs for the treatment of hypertension	-0,00028	0,000	1,70	0,0903058
Age	0,01624	0,004	4,53	0,0000063
Gender	0,33515	0,060	5,60	0,0000000
Lonely/not lonely	-0,64607	0,068	9,54	0,0000000
Stage AH II	-0,35687	0,094	3,79	0,0001567
Stage AH III	-0,73419	0,221	3,32	0,0009400
AH degree	0,37727	0,100	3,78	0,0001654
The fact of hospitalization	0,10174	0,061	1,66	0,0976089
Participation in the reimbursement program	0,00925	0,004	2,50	0,0126191
Income	0,00011	0,000	2,31	0,0212948
Compliance	0,85771	0,056	15,29	0,0000000
Complaints	-0,25194	0,071	3,57	0,0003690
Group 1	-0,15704	0,110	1,42	0,1547417
Group 2	0,13152	0,187	0,70	0,4810536
Group 3	0,40062	0,171	2,34	0,0194467
Group 4	0,52354	0,114	4,60	0,0000047
Group 5	0,51890	0,112	4,65	0,0000037

Standardized Sampling Error = 1.035 on 1326 degrees of freedom
R2 = 0.2759, Adapt. R2 = 0.2667
F (17; 1326) = 29.77, p < 2.2e-16

the developed program is clinically and cost-effective.

Having distributed the values of the average annual expected losses C ($) in the event of or exacerbation of the GD, -IBCR and log(-IBCR) received the data given below (Table I) where C is the average annual expected cost of gastroduodenitis and IBCR and its logarithmic value:

For the formation of the level of IBCR, we identified significant (Table II) without loss of information, as evidenced by the characteristics of the models (the same standardized sample error, multiple correlation coefficient and its adapted to the number of independent parameters of the model option).

As can be seen from the table, with an increase in the cost of treatment of hypertension, the additional effect of the proposed PP decreases, that is, an additional $ 1 spent on the treatment of hypertension leads to a decrease in the incremental ratio of benefits-costs, because the increase in the cost of treating hypertension is usually accompanied by an increase in the number of drugs due to the progression of the disease.

As the patient's age increases, the additional effectiveness of the program also increases.

Significant was the predictor gender. That is, in men, the additional effectiveness of the proposed PP is 33% better, since this group is marked by a worse compliance, which improves with the participation of PP.

In singles, the additional effect of the program is reduced by 64%.

As the AH stage increases, the additional effect of the program also decreases.

Significant in assessing the incremental ratio of benefits-costs were predictors of the degree and fact of hospitalization. According to the data obtained with an increase in the degree of hypertension, the additional effect of PP improves by 37%. And in hospitalized patients, the additional effect of the proposed PP is 10% better.

In hypertensive patients who participated in the reimbursement program, the additional effect of PP increased by 0.9%.

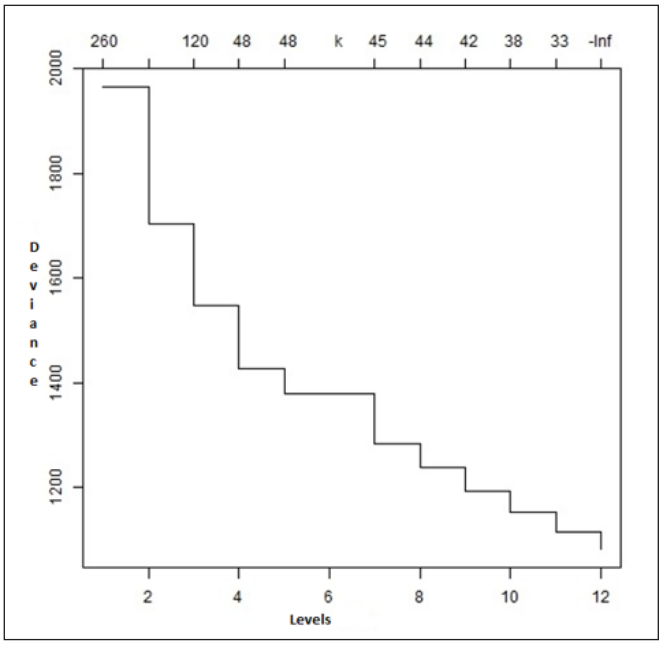

Fig. 1. Changes in deviance with each subsequent level of DT

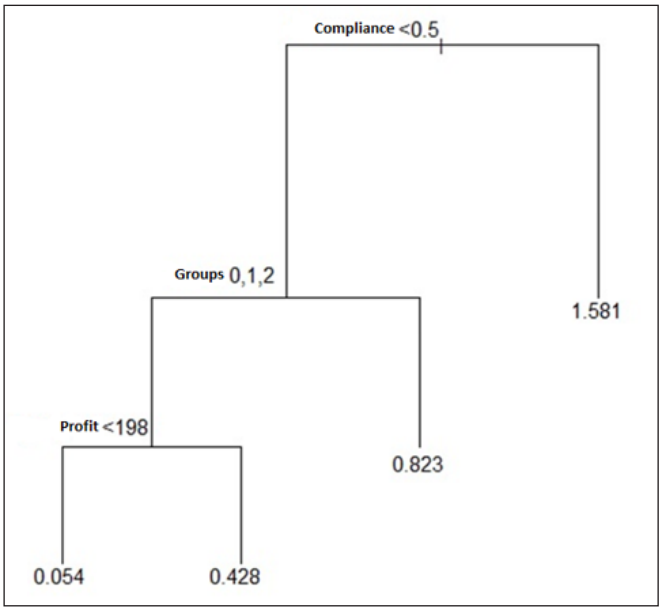

Fig. 2. The tree of determination of targeted groups of patients by IBCR values due to participation in PP

The increase in income naturally showed an increase in additional effect by 0.01%, and high compliance increased the additional effect of the program by 85.7%.

In persons who had complaints from the gastrointestinal tract, the additional effect of the proposed PP was significantly reduced by 25%.

Considering patients according to the groups, it is clear that the additional effect of PP is significantly greater in groups 3, 4, 5 by 40%, 52% and 51%, respectively, compared to the zero group.

Justifying the targeted groups of patients according to IBCR, we separated the situations with IBCR > = 1 (with an additional cost due to participation in PP) from economically irrational with IBCR < 1. According to the analysis of the dynamics of deviances with each subsequent level of the decision tree (DT), we determined the number of levels, namely 4, since it is this value that ensures the constancy of the efficiency factor of DT k, the values of which are postponed on the upper scale (Fig.1.)

The resulting DT is shown in (Fig. 2). Fundamentally forming (the first level) is the compliance factor with split at the level of 0.5. Compliance is an important factor in taxonomization. Low compliance (more than 5 marks on non-compliance with the regimen and doctor's prescriptions for 10 years) causes a drop in the effectiveness of PP.

Dichotomization is carried out in accordance with the value of compliance. The right branch was formed by patients with compliance > 0.5, the left branch - with a value of < 0.5. Such a division shows that the program we developed gives an additional cost in compliance > 0.5. That is, spending $ 1, we get in the weighted average value of $ 0.581 of the prohibited costs by $ 1 additional costs per PP.

Less attractive, which do not need this PP, according to this decision tree, were groups {0, 1, 2} with low compliance. Their low-income combinations are shown to be even more irrational. That is, the costs are greater than the profit, spending $ 18 we have $ 1 profit.

The next step we detailed exactly the right branch of the first level of DR (Fig.2, 3) is patients with good compliance.

Among this contingent of patients, the form-forming feature was marital status: lonely or not lonely. The left branch shows not lonely and is very attractive, especially with a very high compliance. That is, spending $ 1, we have $ 3.02 profit. With less compliance, the cost of treating AH is important.

With costs < $90, the expected profit was $0.88. That is, for one additional dollar spent on PP, the patient of this subgroup saved an average of $ 1.88 by reducing the expected risk (and associated costs) of the occurrence or exacerbation of the GD. Provided that the cost of treatment of AH exceeds $ 90, the expected profit was $ 2.04.

In singles, which are described by the right branch, more important is not compliance, but the cost of treatment of AH.

At high costs, the proposed program gives a good result. Spending $1 we have $0.93 profit. At a lower cost, the result is worse and therefore profit matters for such patients. If the income <100$ PP is not effective. The only condition under which the proposed PP is appropriate was the situation when the profit > $ 1326, then spending $ 1 we have $ 0.82 profit.

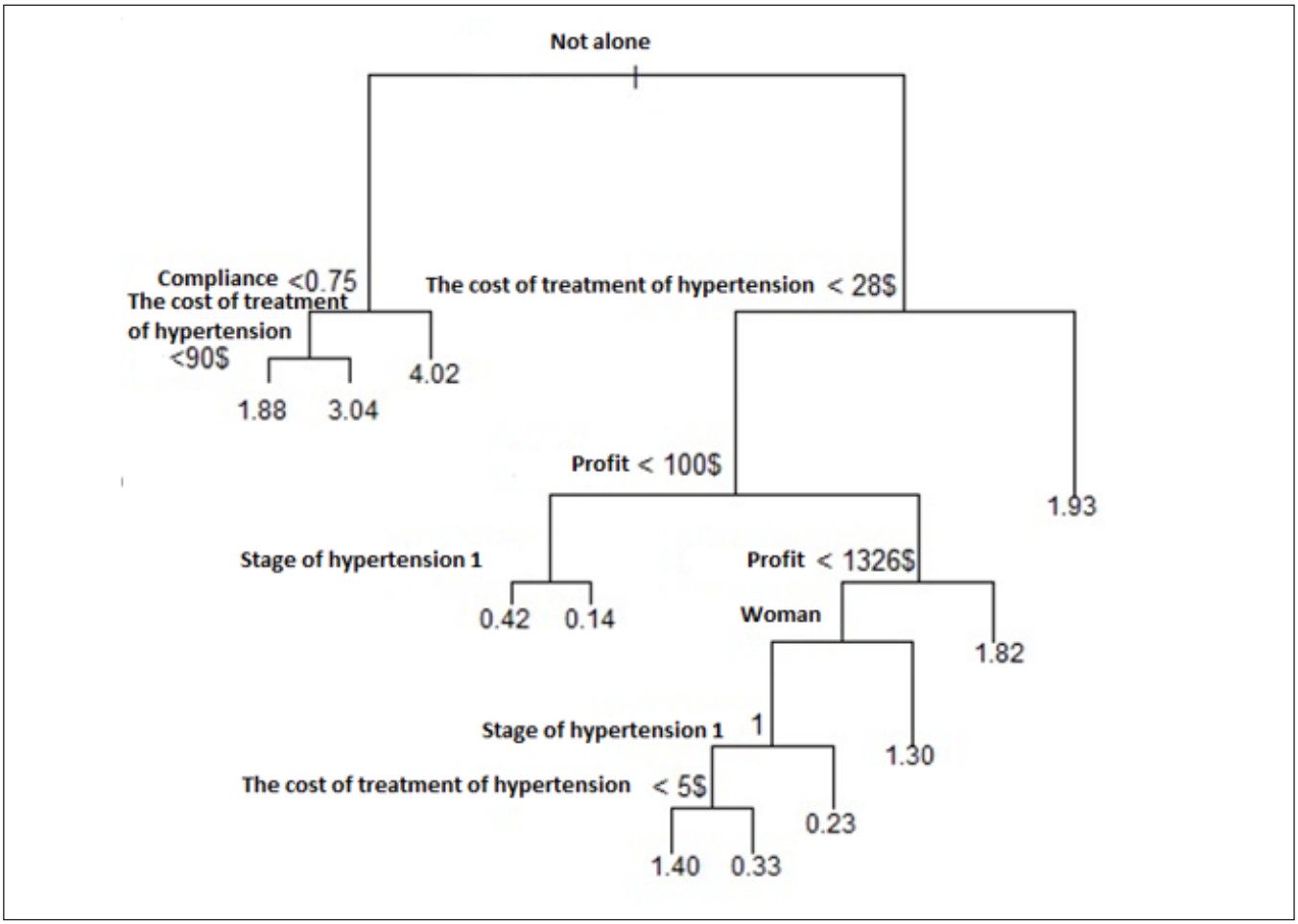

Fig. 3. The tree of determination of targeted groups of patients with high compliance at IBCR value due to participation in PP

With an income of $ 100 to $ 1326 per year, gender matters. For men spending $ 1, we have $ 0.3 profit. If a female patient of PP is appropriate only under the condition of the first stage of hypertension with a small cost for the treatment of AH, namely < $ 5. That is, spending $ 1, we have $ 0.4 profit.

DISCUSSION

While reading the study, questions may arise:

- Is the assumption of equality of seven-elasticities not "pulled by the ears"?
- How to estimate $\Delta\pi_i$ in practice?
- What are the possibilities of practical application of the model beyond our study?

Indeed, the assumption of the equality of the seven elasticities is debatable, but it is supported by the idea that for moderate differences in the expected levels of utilities in both states, the effect of the factors on the quantity of utilities is the same. For example, the relative decline in utilities due to aging, smoking, and deteriorating housing conditions is the same in both states, provided that the severity of the disease is moderate. Strictly speaking in terms of probability, the independence of the factors as moderators of utilities and the fact of the disease (just another factor) is required. This is obvious for most situations. However, it should be recognized that the assumption is violated if a serious disease changes the probability of occurrence of other events (factors) x.

For diseases whose pathogenesis does not involve exacerbations, but only stage progression, it is convenient to use a cohort design using survival models (in particular, frailty models) to obtain estimates of $\Delta\pi_i$ (Gelman A., Carlin J., Stern H.).

If the pathogenesis involves exacerbation, a convenient design is a panel with a convenient modeling of $\Delta\pi_i$ and a panel logistic model.

In this case, multiple exacerbations are possible, so a panel Poisson model (with fixed and mixed effects) is used.

Clinical and economic evaluation of prevention, treatment and rehabilitation programs faces methodological difficulties, in particular, the problem of identifying the form of the model based on economic theory. The model developed is based on the work of Zweifel & Breyer [1, 2], the theoretical assumptions stem from the classical theories of moral hazard and consumer utility. The mod-

el characterizes equilibrium and is based on classical cost-effectiveness, cost-utility and cost-benefit analyses.

The use of this approach allows to reliably screen out factors by which groups differ, in particular, individual patient response to treatment and to obtain meaningful unbiased conclusions. In addition, the researcher can apply sophisticated modern design with elements of nesting, blocking, clustering to increase the power of testing working hypotheses.

The discussion gives grounds for the application of empirical studies devoted to modern health problems.

CONCLUSIONS

1. Additional effectiveness of the proposed prevention program increases with the age of patients.
2. In men, the additional effectiveness of the proposed PP is 33% better.
3. In single persons, the additional effect of the program is reduced by 64%.
4. As the AH stage increases, the additional effect of the program decreases.
5. With the increase in the degree of hypertension, the additional effect of PP improves by 37%.
6. In hospitalized patients, the additional effect of the proposed PP is 10% better.
7. In hypertensive patients who participated in the reimbursement program, the additional effect of PP increased by 0.9%.
8. The increase in income naturally showed an increase in the additional effect by 0.01%, and high compliance increased the additional effect of the program by 85.7%.
9. In persons who had complaints from the gastrointestinal tract, the additional effect of the proposed PP was significantly reduced by 25%.
10. Considering patients according to the groups, it is clear that the additional effect of PP is significantly greater in groups 3, 4, 5 by 40%, 52% and 51%, respectively, compared to the zero group.
11. Low compliance (more than 5 marks on non-compliance with the regimen and doctor's prescriptions for 10 years) causes a drop in the effectiveness of PP.
12. The program we developed gives an additional cost for compliance > 0.5. That is, spending $ 1, we get in the weighted average value of $ 0.581 of preventable costs for $ 1 additional costs for PP.
13. The combination of groups with low compliance and low profits are shown as even more irrational. That is, the costs are greater than the profit, spending $ 18 we have $ 1 profit.
14. The group is not alone in combination with a very high compliance shows that spending $ 1 we have $ 3.02 profit. With less compliance, the cost of treating AH is important. With costs < $90, the expected profit was $0.88. That is, for one additional dollar spent on PP, the patient of this subgroup saved an average of $ 1.88 by reducing the expected risk (and associated costs) of the occurrence or exacerbation of the GD. Provided that the cost of treatment of AH exceeds $ 90, the expected profit was $ 2.04.
15. In singles, compliance is not important, but the cost of treating AH. At high costs, the proposed program gives a good result. Spending $1 we have $0.93 profit. At a lower cost, the result is worse and therefore profit matters for such patients. Provided that the income <100$ PP is not effective. The only condition under which the proposed PP is appropriate was the situation when the profit > $ 1326, then spending $ 1 we have $ 0.82 profit. With an income of $ 100 to $ 1326 per year, gender matters. For men spending $ 1, we have $ 0.3 profit. If a female patient of PP is appropriate only under the condition of the first stage of hypertension with a small cost for the treatment of AH, namely < $ 5. That is, spending $ 1, we have $ 0.4 profit.

REFERENCES

1. WHO. Report «Assessment of the Affordable Medicines Program in Ukraine». 2019. Electronic document. https://www.euro.who.int/__data/assets/pdf_file/0019/400429/52308-WHO-Affordable-Medicines-Programme-Ukraine-UKR_low_V7.pdf [date access 16.07.2022]
2. Klymeniuk V.P., Ocheredko O.M., Chereshnyuk H.S., Aymedova A.A. Improvement of medical care for patients with acute coronary syndrome as a component of medical reform in Vinnytsia region. Ukraine. Health of the nation. 2017, 133p.
3. Annual report on the state of public health, sanitary and epidemiological situation and results of the health care system of Ukraine 2018 / Ministry of Health of Ukraine, State Institution «UISD of the Ministry of Health of Ukraine». Kyiv: IEC «Medinform». 2019, 458 p.
4. State Statistics Service of Ukraine. 2022. http://www.ukrstat.gov.ua/ [date access 16.07.2022]
5. Akhmedova A.A. Evaluation of the effectiveness of the gastropathy prevention program in patients of retirement age with hypertension. The first step in science - 2020: materials of the XVII scientific and practical conference of students and young scientists with international participation, Vinnytsia National Medical University. M.I. Pirogov. 2020, 479 p.

6. Akhmedova A.A., Ocheredko O.M. Adaptation of the theoretical model of premorbid moral risk for the practical study of the economic effectiveness of the primary prevention of polymorbid pathology by cost methods – benefits and costs – efficiency. Health and social dimensions in and out of the academic space: materials of the international conference, Vinnytsia National Medical University. M.I. Pirogov, National University of Kyiv-Mohyla Academy, Maastrih University, Netherlands. 2017, 36p.
7. Ahmedova A.A., Ocheredko O.M. Substantiation of the model of clinical and economic evaluation of the effectiveness of prevention, treatment and dispensary programs for patients with comorbid conditions on the example of patients with high blood pressure and duodenitis. Wiad. Lek. 2018;71(3): 479 – 484.
8. Akhmedova A.A., Ocheredko O.M., Klimenyk V.P. Assessment of the effectiveness of the gastropathy risk reduction program in patients with arterial hypertension. Wiad. Lek. 2019;72(3): 409 – 417.

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

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Received: 08.04.2022
Accepted: 18.01.2023

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

ORIGINAL ARTICLE

ASSOCIATION BETWEEN *H. PYLORI* INFECTION AND IRRITABLE BOWEL SYNDROME

DOI: 10.36740/WLek202302123

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ABSTRACT

The aim: To research the association of *H. pylori* infection in patients with irritable bowel syndrome.

Materials and method: In this case control study, 43 patients with IBS (13 males and 30 females) diagnosed according to Rome IV criteria and other 43 controls where included, the patients and control are matched to the gender and age which ranged from 18 to55 years, the study groups were referred to perform a stool antigen test for *H. pylori*.

Results: Presence of *H. pylori* was compared between patients with IBS and controls using chi-square test. There was significant correlation between the presence of *H. pylori* and IBS, chi-square = 4.09, P-value = 0.043. The odd ratio for patients with *H. pylori* to have IBS was 2.53 (95% confidence interval: 1.02-6.29). There is no significant relationship between type of IBS and presence of *H. pylori*, chi-square = 2.87, P-value = 0.238. There is no significant relationship between the presence of *H. pylori* and age, BMI, gender, occupation, or marital state.

Conclusions: Results of our study had shown an association of *H. pylori* infection in patients with IBS, which may propose a link of this infection in IBS pathophysiology.

KEY WORD: *H. pylori*, Irritable bowel syndrome, diet exposure, stress exposure

Wiad Lek. 2023;76(2):406-414

INTRODUCTION

Irritable bowel syndrome (IBS) is common diagnosed gastrointestinal diseases. IBS, in the absence of any other causative disease, is defined as the presence of abdominal pain or discomfort with altered bowel habits [1]. The prevalence of IBS around the world ranged from 10% to 25% with variation; the highest occurring in South America 21.0%, while in the South Asia 7.0% [2]. In most countries, women had more symptoms of IBS than men, and the diagnostic criteria employed - rates in women are about 1.5 to 3 fold more than those happen in men. IBS develops in many age groups, even in children and elderly, with no difference observed in the frequency of subtypes regarding age: around 50% of patients with IBS developed first symptoms before the age of 35 years [3, 4]. Many articles suggest that lower income is lead to poorer health care outcomes, lower quality of life, and raised life stressors [5], while others suggest that the contrary is right and that being in a higher socioeconomic group during childhood is associated with higher prevalence of IBS [6]. IBS is considered a disorder, with many causes, the following among the probable theory contributing are: gastrointestinal dysmotility, inflammation, visceral hypersensitivity, and altered intestinal micro biota [6]. Diet and stress exposure (including early life events) have palmed as causing factors to this multifactorial disorders [6]. Nutritional factors are a common cause. Many patients consume chemical food (non-allergic) with poorly absorbed, short-chain carbohydrates (fructose, sorbitol and lactose, along with others), together identified as FODMAPs. Their fermentation in the colon causes pain, bloating, flatulence and changed bowel habit, and many patients may experience small bowel bacterial overgrowth (SIBO) leading to symptoms. This "gut dysbiosis" may specify the response to probiotics or the non-absorbable antibiotic like rifaximin, which have been described in trials [7]. Because stress plays as a cause in the development of IBS, the major aspect of the stress response system, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, have been the subject of numerous investigations of IBS. Finally, genetic predisposition and environmental interactions, such as familial susceptibility and psychobiology stressors, have been blamed in the pathogenesis of IBS [8]. Patients with IBS have been observed to disturbances in the brain-gut axis (BGA) including central

and autonomic functions, peripheral factors, peptides, and hormones. Clinical features present with recurrent episodic abdominal pain, altered bowel habits (diarrhea, constipation, or mixed), or other gastrointestinal or extra intestinal manifestations. Abdominal pain: the cardinal symptom is often a pressure sensation that can be mild to severe. The pain may be mild enough to go unnoticed, or it may severely interfere with limited activities of daily living; pain may decrease with defecation. Pain, that is progressive; that awakens the patient from sleep or that is associated by anorexia, malnutrition, or weight loss is no typical for IBS [9]. Altered bowel habits - changes in bowel habits, are the most common clinical feature in IBS. The most common form is constipation alternating with diarrhea, commonly with one of these symptoms occurring. Most patients also complain of a sense of partial evacuationof the bowels, which causes repeated attempts at defecation in a short of time. In many patients, diarrhea may be the predominant feature. Diarrhea, as a result of IBS, usually comprises with small amounts of loose stools. Multiple of patients have stool volumes of less than 200 ml. Diarrhea is preceded by lower abdominal cramps [10]. Other gastrointestinal manifestations, consist of sense of a lumen the throat, acid reflux, satiety nausea, symptoms, no cardiac chest pain, bloating, and flatulence. Furthermore, the patients with IBS have tendency to reflux gas from the distal to the more proximal intestinal part, which clarify the belching. Extraintestinal manifestations include urinary frequency or urgency and fibromyalgia [10]. The diagnosis is based on the presence of specific symptoms in the absence of organic disease [11]. As there is no specific biomarker or test to confirm or rule out a diagnosis of IBS, the Rome IV guidelines outline how the diagnosis of IBS requires certain approach, limited diagnostic tests, and careful follow-up. The presence of recurrent abdominal pain in association with abnormal bowel habits are defining features of IBS [11]. Abdominal bloating and distention are also common present, but are not required to make the diagnosis of IBS. While meeting these criteria serves as a firm basis for reaching a positive diagnosis of IBS, they do not "confirm" IBS or "rule out" other conditions, nor do they capture all dimensions of the patient's condition in order to optimize treatment [12]. Complete blood count, serum biochemistries and thyroid function study, stool examination for ova and parasites, and abdominal imaging studies are not recommended in the routine diagnostic evaluation of IBS [13].

ROME IV GUIDELINES

Recurrent abdominal pain that is occurring at least 1 day per week in the last 3 months, along with two or more of the following criteria:

RELATED TO DEFECATION

- Associated with a change in frequency of stool
- Associated with a change in form of stool.

Criteria achieved for the last 3 months with symptom onset at least 6 months before diagnosis [13]. Management of irritable bowel syndrome primarily by providing psychological and advising dietary measures. Pharmacologic treatment is adjunctive and should be specific to symptoms [14].

PATIENT AND DIETARY ADVICE

Reassurance and explaining the functional nature of the disorder and how to limit the obvious precipitants are first steps of the inpatient diet. UK`s National Institute for Health and Care Excellence (NICE) recommends eating frequently, avoiding trigger foods, and limiting alcohol and caffeine intake. This diet has been found to be as effective as a low-FODMAP diet for the IBS-D [15]. FODMAPs may serve as nutrient for the colonic bacteria and play role in the growth of gram-negative commensal bacteria, which may have role in epithelial damage and subclinical mucosa inflammation. In contrast, a low FODMAP diet reduces IBS symptoms [16]. General lifestyle advice symptoms of irritable bowel syndrome can be lowered by regular exercise [17], which should be in conjunction with dietary advice. The importance of sleep should also be advised as improved quality of sleep has been found as part of management.

PHARMACOLOGICAL TREATMENT

ANTISPASMODICS

Anticholinergic drugs can temporary relieve symptoms such as painful cramps related to intestinal spasm. Many studies show, that postprandial pain is best treated by giving antispasmodics 30 min before meals so that effective blood levels are achieved before the onset of pain [18].

ANTIDIARRHEAL

Peripherally acting opiate-based agents are the initial therapy of choice for IBS-D. Studies have shown increased segmental contractions of the colon, delayed fecal transit, increased anal pressure, and decreased rectal perception with these drugs [18].

ANTIDEPRESSANTS

Antidepressants can significantly work by manipulating visceral hypersensitivity and abnormal central pain

sensitization [19]. Serotonin receptor antagonists have been treated with IBS-D. Serotonin, acting on 5-HT3 receptors, enhances the sensitivity of afferent neurons projecting from the gut. In humans, a 5-HT3 receptor antagonist like aldosterone reduces painful stimulation in IBS [18]. Novel 5-HT4 receptor agonists, such as tegaserod, exhibit prokinetic activity by stimulating peristalsis. In IBS-C, tegaserod accelerate intestinal and ascending colon transit [18]. Patients with intractable symptoms are sometimes treated with tricyclic antidepressants such as amitriptyline or imipramine (10–25 mg orally at night) [14].

MODULATION OF GUT FLORA

Gut dysbiosis may contribute to the pathogenesis of IBS, and this has led to great interest in using antibiotics, prebiotics, and probiotics for the treatment of IBS. The non-absorbable oral antibiotic rifaximin is the most common antibiotic of the treatment of IBS. In a double-blind, placebo-controlled study, patients receiving rifaximin at a dose of 550 mg two times daily for 2 weeks had substantial improvement of many IBS symptoms over placebo [18].

HELICOBACTER PYLORI

H. pylori is a spiral shaped, microaerophilic, gram negative bacterium measuring approximately 3.5 microns in length and 0.5 microns in width [20].

PATHOPHYSIOLOGY

H. pylori is specific to the gastric environment, where it lives within or beneath the gastric mucous layer. It exposes the underlying mucosa to acidic peptic damage by disrupting the mucous layer, liberating enzymes and toxins, and adhering to the gastric epithelium. In addition, the host's immune response to *H. pylori* induces an inflammatory reaction, which further perpetuates tissues injury. Chronic inflammation induced by *H. pylori* leads to chronic gastritis, peptic ulcer disease, while in other cases, gastritis progresses to atrophy, intestinal metaplasia, and finally gastric carcinoma or rarely due to persistent immune stimulation of gastric lymphoid tissue to gastric lymphoma.

EPIDEMIOLOGY

H pylori is the most common chronic bacterial infection in humans, occurring worldwide and in individuals of all ages. Infection is more common and acquired at an earlier age in developing countries compared with industrialized nations, within any age the infection can occur, also affect in black more than white. Infection, once acquired, persists and may or may not cause gastroduodenal disease [21].

ROUTE OF TRANSMISSION

H. pylori is mainly acquired by the fecal-oral, oral-oral or gastro-oral route [21].

CLINICAL FEATURE

Reported symptoms of *H. pylori* infection are relatively non-specific, such as epigastria pain, postprandial fullness, bloating, nausea, and vomiting, signs of acid hyper secretion and delayed gastric emptying [22].

DIAGNOSIS

H. pylori infection diagnosed by noninvasive test such as serological testing for IgG antibodies with sensitivity and specificity of 85% and 79%, respectively. This test is of little value in confirming eradication of the infection, because antibodies persist for many months, if not longer, after eradication [22], and another diagnostic test is urea breath test, which involves drinking 13C¬labeled or 14C-labeled urea, which has a sensitivity and a specificity of 95% but considerable resources and personnel required to perform this test [23]. Infection can also be detected H. pylori fecal antigen test with high negative and positive predictive values with utilizing monoclonal antibody (specificity and a sensitivity of 95%) and it can be used before and after eradication treatment of *H. pylori* [24]. Despite their high sensitivity, both the breath test and the fecal antigen test give false negative results possible if the patient uses proton pump inhibitor (PPI) or with the use of antibiotics or bismuth preparations [25]. *H. pylori* infection can also be detected by invasive endoscopic biopsy method of the gastric mucosa [25].

TREATMENT

No specific drug achieves the optimal eradication rate on its own, and most treatment regimens include two antibiotics in addition to a proton pump inhibitor (PPI) [25]. Triple therapy for *H. pylori* plays a role in eradication and consists of a PPI, amoxicillin 1 g twice daily, and clarithromycin 500 mg twice daily for 7 to 14 days. In patients allergic to penicillin, metronidazole have substituted for amoxicillin. Although triple therapy originally achieved 90% *H. pylori* eradication, however rates have declined under 70% in some areas [25].

THE AIM

The aim of the study is to research the association of *H. pylori* infection in patients with irritable bowel syndrome.

MATERIALS AND METHODS

A prospective case-control study accomplished on a sample of patients and their relatives who attended the outpatient clinic of internal medicine in al Al Sader Medical City in Najaf during the period between January 2019 and January 2020, where 86 people were enrolled in this study. Each of them was informed that he or she is a part of a scientific study and a verbal consent was obtained from each one of them.

The participants were divided into two groups:

Group 1: (The IBS group) which comprised of 43 IBS cases (13 males and 30 females) diagnosed as IBS according to Rome IV criteria and based on a complete history and physical examination. Their age ranged from 18 to 55 years, with a mean age of 33.06 ± 10.35 years and a median age of 32 years. All of them were interviewed and a complete medical history was taken, including patient's age, gender, BMI, drug history, duration of illness, any history of pre-existing medical conditions such as autoimmune diseases, diabetes mellitus, and associated gastrointestinal symptoms like dyspepsia, nausea, vomiting, heartburn, abdominal pain, diarrhea, and any history of peptic ulcer or gastritis.

Group 2: (The control group) which comprised of 43 persons (20 males and 23 females) apparently healthy persons matched cases regarding age and gender. They were evaluated for any gastrointestinal symptoms associated with *H. pylori* infection.

INCLUSION CRITERIA

All IBS cases were selected according to Rome IV criteria, which are currently the criteria for the diagnosis of IBS.

EXCLUSION CRITERIA

These include any worrisome symptoms, which make us to think of a diagnosis other than IBS:

- Anemia,
- Weight loss,
- Rectal bleeding,
- Recent use of antibiotics or PPIs (4 weeks for antibiotics and 2 weeks for PPI),
- Nocturnal symptoms,
- Onset at age after 50 years,
- Abnormal abdominal examination (like mass),
- Family history of ovarian or colorectal cancer.

H. PYLORI STOOL ANTIGEN TEST.

All participants were ordered to submit fresh fecal specimens which were tested for presence of *H. pylori* infection by fecal antigen positivity with rapid test according to manufacturer's instructions (from Wondfo Biotech, Qaradb.v.b.a, Cipal Street 3, B-2440 Geel, and Belgium) (Figure 1).

The *H. pylori* stool antigen rapid test was used because it is non-invasive and rapid. The test was accomplished by taking a fresh fecal sample (within two hours) and then taking a small amount with a stick and putting it in ϕ stool collection tube which contain one ml of sample extraction buffer, followed by vigorous shaking of the stool collection tube, after two minutes the stool collection tube for stool collection was opened by unscrewing the cap, and two drops of the sample were added to the rapid immunoassay strip. The test result can be obtained in 15 minutes.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS®) Software (version 23.0 for Linux®) was used to perform the statistical analysis for this study. Qualitative data are represented as numbers and percentages, while continuous numerical data are represented as mean ± standard deviation. Numerical variables were compared between study groups using Student's t-test (for 2 groups with normally distribute variables), while categorical variables were compared using chi-square test. The data are presented using the appropriate tables and visualized using appropriate figures. P value of< 0.05 was considered statistically significant.

RESULTS

This study included a total of 86 participants, 43 of whom were cases with IBS (diagnosed according to Rome IV criteria) and 43 age- and gender-comparable healthy controls. Age of participants ranged from 18 to 55 years, with a mean age of 33.06 ± 10.35 years and a median age of 32 years. No significant difference was found in age between research group 31.95 ± 10.20 and controls 34.16 ± 10.49, t-test = 0.99, P-value = 0.325. The distribution of age groups by study groups is shown in Figure 2.

Males accounted for 38.4% of epy study participantsб while females accounted for 61.6% of them (Figure 3), and there was no statistically significant difference in gender was observed between cases and controls, chi-square=2.41, P-value=0.121 (table I).

Body-mass index (BMI) of study participants ranged from 18.6 kg/m^2 to 37.0 kg/m^2, with a mean BMI of 27.54 ± 4.28 kg/m^2. There was no significant difference in BMI between cases 27.9 5± 4.51 and controls 27.12 ± 4.04, t-test = 0.90, P-value = 0.370. Figure 4 shows the BMI classes among study participants by study group.

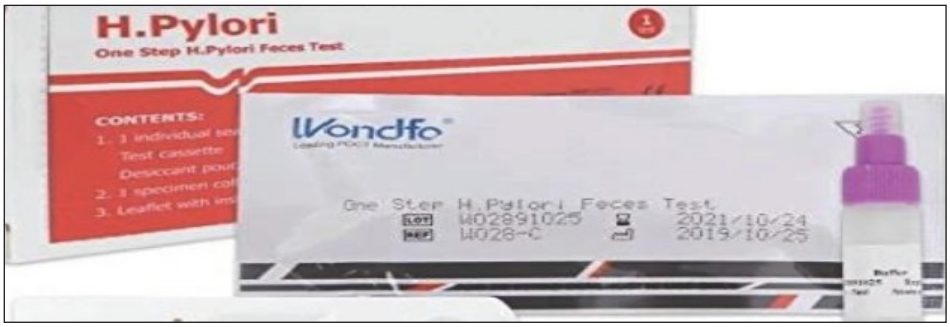

Fig. 1. One Step *H. Pylori* Faces Test

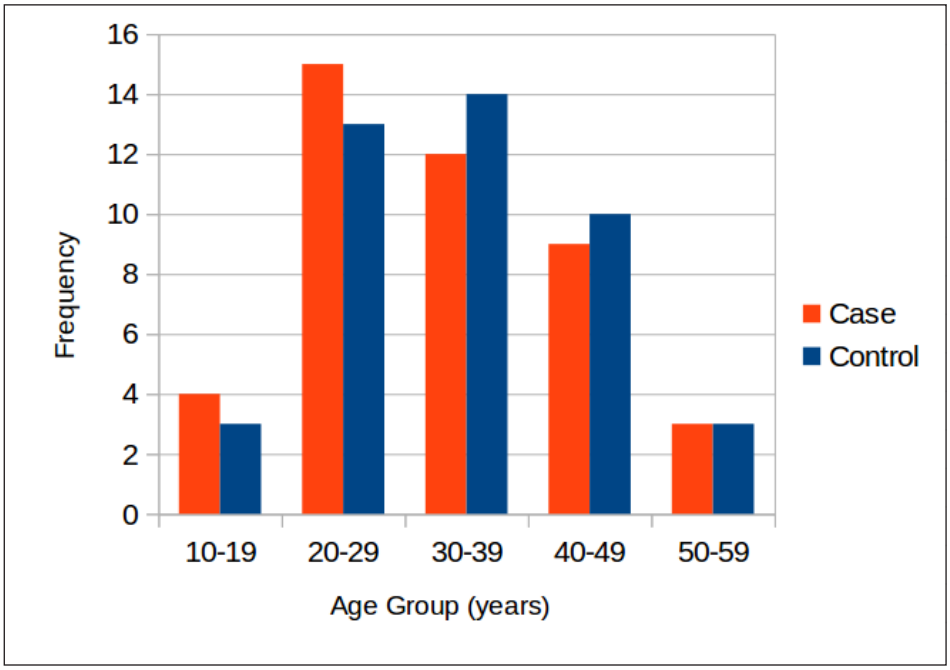

Fig. 2. The distribution of age groups by study groups

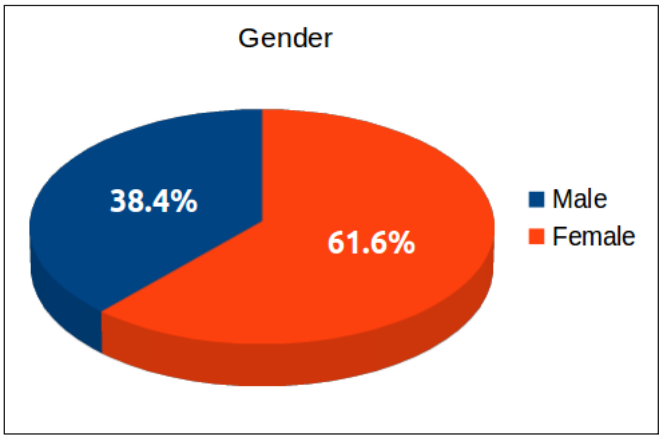

Fig. 3. Gender distribution of study participants.

Demographic characteristics are summarized in table II. There was no significant difference between cases and controls regarding marital status, smoking, residency or occupation (P-value > 0.05), and there is no significant association between the presence of *H. pylori* and age, BMI, gender, occupation, or marital status (tables II, III, IV, V).

The presence of *H. pylori* was compared between IBS cases and controls using a chi-square test. There was significant correlation between the presence of *H. pylori* and IBS, chi-square = 4.09, P-value = 0.043 (Table VII). The odds ratio for *H. pylori* patients to have IBS was 2.53 (95% confidence interval: 1.02-6.29). Among cases, there is no significant relationship between type of IBS and presence of *H. pylori*, chi-square = 2.87, P-value = 0.238 (Table VII).

DISCUSSION

Eighty six persons categorized into 5 age groups, including 43 patients with IBS, with mean age of 33.06 ± 10.35 years, revealed that IBS is more common in age group of 20-39 (with a percentage of 63%) than in other age groups among patients with IBS. This result is almost reminiscent of a study in Lebanon (2017), which mentioned 67% of patients of the same age group [26]. A study in Iran found that the prevalence of IBS is higher at a young age and decreases as the age increases [27]. However, these results of high prevalence of IBS in young patients may be due to emotional and psychological issues, such as stress of exams, losing jobs, unemployment, and life stressors. Regarding gender, the present

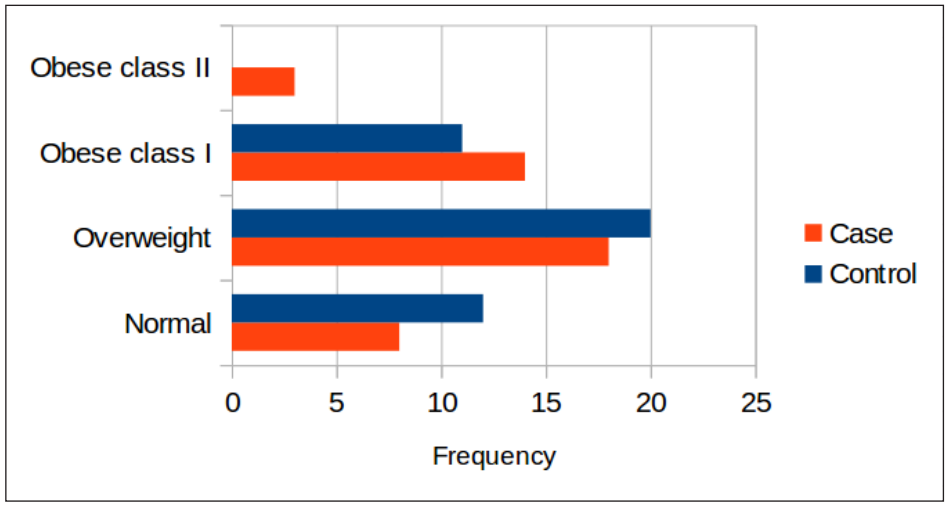

Fig. 4. BMI classes of study participants by study groups.

Table I. Comparison of gender distribution between cases and controls.

Gender	Study Group, No. (%)			P-value
	IBS	Control	Total	
Male	13 (39.39%)	20 (60.61%)	33 (100%)	0.121
Female	30 (56.60%)	23 (43.40%)	53 (100%)	
Total	43 (50.00%)	43 (50.00%)	86 (100%)	
Chi-square = 2.41, P-value = 0.121				

Table II. Demographic characteristics of study participants.

Demographic Characteristics		Study Group, No. (%)			P-value
		IBS (n=43)	Control (n=43)	Total (n=86)	
Marital status	Single	14 (56.00%)	11 (44.00%)	25 (100%)	0.661
	Married	27 (47.37%)	30 (52.63%)	57 (100%)	
	Divorced	1 (33.33%)	2 (66.67%)	3 (100%)	
	Widowed	1 (100%)	-	1 (100%)	
Smoking	Smoker	31 (50.00%)	31 (50.00%)	62 (100%)	1.000
	Non-smoker	12 (50.00%)	12 (50.00%)	24 (100%)	
Residency	Urban	30 (49.18%)	31 (50.82%)	61 (100%)	0.812
	Rural	13 (52.00%)	12 (48.00%)	25 (100%)	
Occupation	Unemployed	3 (30.00%)	7 (70.00%)	10 (100%)	0.084
	Housewife	16 (48.48%)	17 (51.52%)	33 (100%)	
	Employed	9 (56.25%)	7 (43.75%)	16 (100%)	
	Student	13 (72.22%)	5 (27.78%)	18 (100%)	
	Worker	2 (22.22%)	7 (77.78%)	9 (100%)	

study revealed that IBS occurring more in female (69%) than males patients (31%), and our result are approximately higher than those of a research by Ford [28] in United States, which mentioned that 62% of study participants were female and the remaining 38% were male. The reason for this was suggested that women are more superior to men in searching medical care or because of difference in psychological reactions, hormonal changes and clinical features. Regarding BMI, the study discovered that IBS is greater in overweight (BMI 25-29.9 kg/m^2) with percentage of 42% than other subgroups, with the next frequency being in obese (30-34.9 kg/m^2) and a percentage of 32.5%, and this is comparable to a study in China which mentioned 48% overweight and 10% obese in patients. There are many theories that explain the association between obesity and IBS including alteration in small intestinal and colonic transit in obese persons. In this study, about 62% of IBS patients were married, 2% were divorced, and

Table III. Comparison of presence of *H. pylori* with certain characteristics.

Variable		Positive	Negative	Total	P-value
Age (years)		32.23 ± 8.87	33.53 ±11.14	33.06 ± 10.35	0.554
BMI (kg/m^2)		27.32 ± 3.87	27.66 ± 4.52	27.54 ± 4.28	0.725
Gender	Male	15 (45.45%)	18 (54.55%)	33 (100%)	0.152
	Female	16 (30.19%)	37 (69.81%)	53 (100%)	

Table IV. Comparison of presence of *H. pylori* with occupation.

Occupation	Positive, No. (%)	Negative, No. (%)	Total, No. (%)	P-value
Unemployed	6 (60.00%)	4 (40.00%)	10 (100%)	
Housewife	8 (24.24%)	25 (75.76%)	33 (100%)	
Employed	8 (50.00%)	8 (50.00%)	16 (100%)	
Student	8 (44.44%)	10 (55.56%)	18 (100%)	0.066
Worker	1 (11.11%)	8 (88.89%)	9 (100%)	
Total	31 (36.05%)	55 (63.95%)	86 (100%)	

Table V. Comparison of presence of *H. pylori* with marital state.

Marital state	Positive, No. (%)	Negative, No. (%)	Total, No. (%)	P-value
Single	10 (40.00%)	15 (60.00%)	25 (100%)	
Married	19 (33.33%)	38 (66.67%)	57 (100%)	
Divorced	2 (66.67%)	1 (33.33%)	3 (100%)	0.589
Widow	-	1 (100%)	1 (100%)	
Total	31 (36.05%)	55 (63.95%)	86 (100%)	

Table VI. Comparison of presence of *H. pylori* between IBS and controls.

H. pylori	Study Group, No. (%)			P-value
	IBS	Control	Total	
Positive	20 (64.52%)	11 (35.48%)	31 (100%)	
Negative	23 (41.82%)	32 (58.18%)	55 (100%)	0.043*
Total	43 (50.00%)	43 (50.00%)	86 (100%)	
Chi-square = 4.09, P-value = 0.043				

*Significant at $P < 0.05$

Table VII. Comparison of presence of *H. pylori* with type of IBS.

H. pylori	Type of IBS, No. (%)				P-value
	IBS-D	IBS-C	IBS-M	Total	
Positive	7 (35.00%)	8 (40.00%)	5 (25.00%)	20 (100%)	
Negative	4 (17.39%)	8 (34.78%)	11 (47.83%)	23 (100%)	0.238
Total	11 (25.58%)	16 (37.21%)	16 (37.21%)	43 (100%)	
Chi-square = 2.87, P-value = 0.238					

2% were widows, similar to what was mentioned by Khan [29], who expected familial complications to be behind this result. Regarding the residency, the present study revealed that 70% of patients with IBS live in urban areas, and this is higher than was demonstrated by Yan-Fang Zhao [30], which disclosed that urban participants were 52% of patients and rural ones were 48%, and it is clear that IBS has higher prevalence in patients living in urban regions. A possible cause was the higher stressors and more complex life in urban than rural regions. The present study demonstrated that the proportion of patients with IBS who were positive for *H. pylori* infection was approximately 65%, which was slightly higher than the proportion, reported by McDonald et al. in their study conducted in Peru in 2013, which was 57.4% [31], and the proportion of 55.7% reported by El-Badry. in their study conducted in Egypt [32]. However,

the Presence of *H. pylori* infection in the present study was found to be significantly higher among patients with IBS, with a P-value of 0.043. This finding was closely similar to the finding demonstrated by Hasan, Jaafer in Diyala – Iraq, which found a significant correlation between *H. pylori* infection and IBS, with a P-value of 0.044 [33]. This correlation was also consistent with the finding by Yahoob, which concluded that the presence of *H. pylori* is common in patients with IBS-D [34].

CONCLUSIONS

The results of this study revealed significant association of *H. Pylori* infection with IBS, which may propose a link of this infection in IBS pathophysiology.

REFERENCES

1. Occhipinti K., Smith J.W. Irritable bowel syndrome: a review and update. Clin Colon Rectal Surg. 2012; 25(1): 46-52.
2. Husain N., Chaudhry I.B., Jafri F. et al. A population-based study of irritable bowel syndrome in a non-Western population. Neurogastroenterol Motil. 2008; 20(9): 1022-1029.
3. Wilson S., Roberts L., Roalfe A. et al. Prevalence of irritable bowel syndrome: a community survey. Br J Gen Pract. 2004; 54(504): 495-502.
4. Lovell R.M., Ford A.C. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012; 10(7): 712-721. e4.
5. Howell S., Talley N.J., Quine S., Poulton R. The irritable bowel syndrome has origins in the childhood socioeconomic environment. Am J Gastroenterol. 2004; 99(8): 1572-1578.
6. Lee Y.J., Park K.S. Irritable bowel syndrome: Emerging paradigm in pathophysiology. World Journal of Gastroenterology. 2014; 20: 2456-2469.
7. Davidson's Principle and Practice of Medicine 23th. 2018, 907p.
8. Fichna J., Storr M.A. Brain-gut interactions in IBS. Frontiers in Pharmacology. 2012; 3: 1-12.
9. Fukudo S., Kanazawa M. Gene, environment, and brain-gut interactions in irritable bowel syndrome. Journal of Gastroenterology and Hepatology. 2011; 26: 110-115.
10. Camilleri M. Physiological underpinnings of irritable bowel syndrome: Neurohormone mechanisms. Journal of Physiology. 2014; 592: 2967-2980.
11. Owyang C., Dennis L., Kasper M.D. et al. Harrison's principle of internal medicine, 20th edition. McGraw-Hill Education. 2018; 320: 2276-2282.
12. Longstreth G.F., Thompson W.G., Chey W.D. et al. Functional bowel disorders [published correction appears in Gastroenterology]. 2006; 131(2): 1480–1491.
13. Suerbaum S., Michetti P. Helicobacter pylori infection. N Eng l J Med. 2002; 347: 1175.
14. Ernst P.B., Peura D.A., Crowe S.E. The translation of Helicobacter pylori basic research to patient care. Gastroenterology. 2006; 130: 188.
15. Pounder R.E., Ng D. The prevalence of Helicobacter pylori infection in different countries. Aliment Pharmacol Ther. 1995; 9(2): 33.
16. Malaty H.M. Epidemiology of Helicobacter pylori infection. Best Pract Res Clin Gastroenterol. 2007; 21: 205-214.
17. Perez-Perez G.I., Rothenbacher D., Brenner H. Epidemiology of Helicobacter pylori infection. Helicobacter. 2004;9(1): 1-6.
18. Loy C.T., Irwig L.M., Katelaris P.H., Talley N.J. Do commercial serological kits for Helicobacter pylori infection differ in accuracy? A meta-analysis. Am J Gastroenterol. 1996; 91: 1138-1144.
19. Vaira D., Vakil N. Blood, urine, stool breath, money, and Helicobacter pylori. Gut. 2001; 48: 287-289.
20. McColl K.E. Clinical practice. Helicobacter pylori infection. N Engl J Med. 2010; 362: 1597-1604.
21. Gisbert J.P., Pajares J.M. Stool antigen test for the diagnosis of Helicobacter pylori infection: a systematic review. Helicobacter. 2004; 9: 347-368.
22. Chey W.D., Wong B.C. Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am J Gastroenterol. 2007; 102: 1808-1825.
23. Chatila R., Merhi M., Hariri E. et al. Deeb. Irritable bowel syndrome: prevalence, risk factors in an adult Lebanese population. BMC Gastroenterology. 2017; 17: 137.
24. Khademolhosseini F., Mehrabani D., Nejabat M. et al. Irritable bowel syndrome in adults over 35 years in Shiraz, southern Iran: Prevalence and associated factors. J Res Med Sci. 2011; 16: 200-6.
25. Ford A.C., Bercik P., Morgan D.G. et al. Characteristics of functional bowel disorder patients: a cross-sectional survey using the Rome III criteria. Aliment Pharmacol Ther. 2014; 39: 312-321.
26. Ladep N.G., Okeke E.N., Samaila A.A. et al. Irritable bowel syndrome among patients attending General Outpatients' clinics in Jos, Nigeria. Eur J Gastroenterol Hepatol. 2007; 19: 795-9.
27. Zhao Y-F., Guo X-J., Zhang Z-S. et al. Epidemiology of Functional Diarrhea and Comparison with Diarrhea-Predominant Irritable Bowel Syndrome: A Population-Based Survey in China. PLoS ONE. 2012; 7(8): e43749.
28. Pickett-Blakely O. Obesity and Irritable Bowel Syndrome: A Comprehensive Review. Gastroenterology & Hepatology. 2014; 10(7).

29. Han S.H., Lee O.Y., Bae S.C. et al. Prevalence of irritable bowel syndrome in Korea: population-based survey using the Rome II criteria. J Gastroenterol Hepatol. 2006; 21: 1687-1692.
30. McDonald K., Shopinski S., Wilkinson A. et al. Correlation between functional gastrointestinal disorders and gastric mucosa histopathology findings, including Helicobacter pylori infection, in Lima, Peru. Revista de Gastroenterologiadel Peru. 2015; 35(2): 137-140.
31. El-Badry A., Abd El Wahab W., Hamdy D., Aboud A. Blastocysts subtypes isolated from irritable bowel syndrome patients and co-infection with Helicobacter pylori. Parasitology Research. 2017; 117: 127-137.
32. Hasan A., Jaafer A., Athab A. Rate of Helicobacter Pylori Infection among Patients with Irritable Bowel Syndrome. Gulf Medical Journal. 2017; 6(1): 16-21.
33. Yakoob J., Abbas Z., Naz S. et al. Virulence markers of Helicobacter pylori in patients with diarrhea-dominant irritable bowel syndrome. British Journal of Biomedical Science. 2012; 69(1): 6-10.
34. Xiong F., Xiong M., Ma Z. et al. Lack of Association Found between Helicobacter pylori Infection and Diarrhea-Predominant Irritable Bowel Syndrome: A Multicenter Retrospective Study. Gastroenterology Research and Practice. 2016; 3059201. doi: 10.1155/2016/3059201.

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

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Received: 07.09.2022
Accepted: 09.02.2023

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

ORIGINAL ARTICLE

LETHALITY ANALYSIS IN PATIENTS WITH ACUTE SMALL BOWEL OBSTRUCTION. ADVERSE FACTORS AND POSSIBLE WAYS TO ELIMINATE THEM

DOI: 10.36740/WLek202302124

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ABSTRACT

The aim: To determine obstruction factors that can lead to lethal consequences for patients with acute small intestine in order to develop possible directions for their prevention.

Materials and methods: During the retrospective analysis of 30 patients with acute small bowel obstruction, the causes and contributing factors of mortality were determined.

Results: The cause of mortality in the first three days of the postoperative period was the progression of the phenomena of intoxication, which led to the appearance of the syndrome of enteric insufficiency and the development of multiple organ dysfunction. Mortality in the later period was observed due to the decompensation of concomitant diseases that accompanied acute obstruction of the small intestine. In the process of the study it was determined that the possible reasons of the postoperative complications in the studied group of patients, apart from the elderly and senile age of the patients and delayed medical care, according to the results of our observation were: uncorrected hypotension and hypovolemia in the postoperative period, refusal of the intubation of the small intestine and permanent decompression of the gastrointestinal tract, early removal of the nasogastric tube, long-term anemia and hypoproteinemia, prevention of stress ulcers of the gastrointestinal tract in the elderly and senile age, refusal of early use of enteral nutrition and later restoration of motility of the gastrointestinal tract.

Conclusions: Treatment of patients with acute small intestine obstruction requires the selection of a specially designed treatment program (optimal timing of preoperative preparation, selection of the minimum volume) at all stages of surgical care, taking into account the existing concomitant pathology, age and hospitalization period.

KEY WORDS: intestine obstruction, lethality analysis, adverse factors

Wiad Lek. 2023;76(2):415-419

INTRODUCTION

It is known that among all acute surgical diseases of the abdominal cavity, acute small intestine obstruction (ASIO) remains one of the most complex and urgent problems of urgent surgery and is accompanied by high mortality. Intestinal obstruction still remains the most common condition for emergency exploratory laparotomy despite the significant decline in mortality and morbidity since the late 1900 s [1 - 4]. The main reasons for the unsatisfactory treatment results in such cases are the development of various purulent-septic complications, patients' tardiness in seeking medical help, as well as their elderly age [5]. Diagnostic and tactical difficulties mostly arise when dealing with senior patients [6, 7]. This is because some generally accepted principles of acute abdominal surgical pathology treatment may be undesirable for them due to their age and existing concomitant pathologies [8].

THE AIM

The purpose of our study is to identify the factors that can lead be fatal for patients with acute small bowel obstruction in order to develop possible approaches to the prevention of such.

MATERIALS AND METHODS

To determine the negative aspects of how ASIO unfolds, we conducted a retrospective analysis of fatal cases in 30 patients with the pathology in the question. Clinical examination of patients including such conventional methods

Table I. Terms of admission and the age of patients

Term of admission	20-59 years	60-74 years	75-89 years	Total
Up to 6 hours	-	-	-	-
6 -24 hours	1	5	3	9
More than 24 hours	1	10	10	21
Total	2	15	13	30

Table II. Analysis of primary diagnoses during hospitalization (n = 29)

Primary diagnoses	20-59 years	60-74 years	75-89 years	Total
Acute intestinal obstruction	1	10	6	17 (58,62 %)
Strangulated hernia	-	1	2	3 (10,34 %)
Diseases of the liver and pancreas in the acute phase	-	-	2	2 (6,90 %)
Stomach ulcer, perforation		1	2	3 (10,34 %)
Acute gastrointestinal bleeding	1	-	-	1 (3,45 %)
Kidney disease	-	-	1	1 (3,45 %)
Postoperative ventral hernia. Coprostasis.	-	1	-	1 (3,45 %)
Cholelithiasis. Chronic calculous cholecystitis. Gallbladder-duodenal intestinal fistula	-	1	-	1(3,45 %)
Total: *	2	14	13	29

Note* - 1 patient sought medical help on his own

as clinical, laboratory, and instrumental research (X-ray, sonographic examination, electrocardiography, and others) were used to determine the functional state of the patients [9,10], the comorbidity index [8], and the degree of surgical and anesthetic risk according to ASA scale [10 - 14].

Statistical processing of the material was performed using a personal computer and a Microsoft Excel spreadsheet application using the "STATISTICA-10 for Windows®-6, 0" package. Graphs were designed using the programs "Microsoft Excel 7.0". $P < 0,05$ was considered statistically significant.

RESULTS AND DISCUSSION

The causes of ASIO in the examined patients included adhesive peritoneal disease – for 19 (63.34%) patients, strangulation of internal and external hernias – for 10 (33.33%) patients, and in 1 (3.33%) case, the cause was a gallstone. Earlier 20 patients have undergone from 1 through to 4 surgical interventions (appendectomy, cholecystectomy, herniotomy, gastric surgery, gynecological surgery, intestinal obstruction, etc.).

The disease duration for patients with ASIO was, on average, (3.64 ± 0.36) days. The terms of admission of patients in the examined group, depending on their age, are shown in Table I.

The data given in Table 1 shows that 70.0% of patients in all the age groups were admitted to the surgical department later than 24 hours after the symptoms appeared, and mostly these are patients aged 60 years and over. On the first day of the disease, only 30.0% of all patients were hospitalized. Thus, late hospitalization of patients with ASIO is one of the unfavorable factors during the course of the disease.

As a result of the study, it was found that among 30 patients who were admitted to the surgical department, 29 people were hospitalized by the direction of medical workers and only 1 patient came to the hospital on his own to get medical help. At the prehospital stage, the deceased patients were diagnosed with the diseases listed in Table II.

When analyzing the results obtained from patients with an unfavourable course of the disease, we revealed that only 58.62% of the patients admitted to the hospital were diagnosed as having intestinal obstruction, while strangulated hernias of various localization were in 10.34% of cases, and exacerbation of gastric ulcer with potential perforation was found in 10.34% of cases; in turn, exacerbation of liver and pancreatic diseases had happened in 6.90% of cases with one incident of a kidney disease, gastrointestinal bleeding, coprostasis, and gallbladder duodenum intestinal fistula based on calculous cholecystitis. As can be seen from the above data, diagnosing ASIO in patients before their hospitalization posed significant difficulties, especially with regard to the elderly patients. In our opinion, the reasons for establishing false diagnoses at this stage could be both objective and subjective. The objective ones include the following:

1. the short time given for the patients' examination in the emergency department;
2. the absence of laboratory and special examination methods at this stage;

Table III. Distribution of patients depending on the period of acute obstruction of the small intestine and compensation of the functional state of a patient

№	Periods of ASIO	Number of patients	Functional state of a patient	
			Compensation	Decompensation
1	The period of acute intestinal passage disorder („ileus cry")	-	-	-
2	The period of acute disorders of intrahepatic intestinal hemocirculation (imaginary well-being)	14	4	10
3	Period of peritonitis (terminal)	16	-	16
	Total:	30	4	26

Table IV. Distribution of patients in the study group according to the ASA scale

Classification	Definition of Patient Status	Numer of patients
I	Healthy patient	2 (6,67 %)
II	Mild systemic disease	-
III	Severe systemic but not incapacitating diseases	4 (13,34 %)
IV	Severe, incapacitating disease that is a constant threat to life	20 (66,67 %)
V	Moribund patient, not expected to survive within 24 hours with or without the operation	4 (13,34 %)
E	Cases of emergency	30 (100 %)
	Total:	30 (100 %)

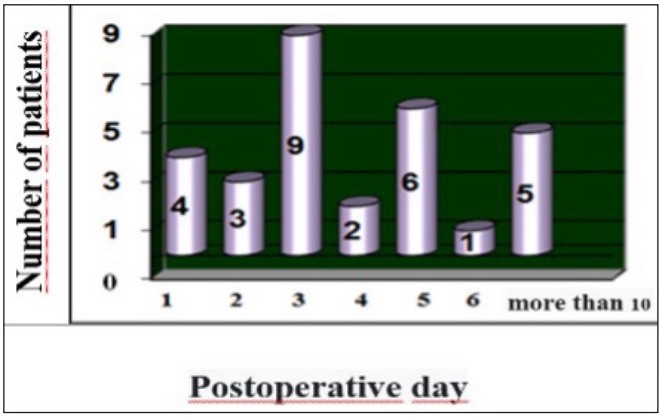

Fig. 1. Terms of mortality of patients with acute small bowel obstruction after surgery in a hospital

3. the impossibility to dynamically observe the patients;
4. atypical disease symptoms in the elderly and the senile;

Subjective failures include:

1. patients' tardiness in seeking medical help;
2. underestimation and superficial interpretation of the data obtained in anamnesis and objective research.

After being admitted to the hospital, all the patients were distributed depending on the stage of the disease and the compensation of patients' functional state (Table III).

None of the deceased patients were hospitalized in a surgical hospital ward during the first period of the disease. In the second period of ASIO, only 46.67% of the patients in the study group were hospitalized. It must me noted that even in the second period of the disease, the functional state of the patients who sought medical help was in the stage of decompensation. In our opinion, this can be explained by the peculiarities of how ASIO unfolds and the decompensation of the existing concomitant diseases.

Doctors, including surgeons, often encounter the problem of comorbidity [8]. At the same time, in the treatment of patients with ASIO, due attention is rarely paid to the presence of a whole spectrum of other diseases and the treatment focuses mainly on the major surgical illness.

A syndromic analysis as well as the formation of a diagnostic and therapeutic concept for patients with ASIO is essential for the calculation of the potential risk.

It was revealed that the number of elderly and senile patients in the study amounted to 93.33%. The importance of this problem lies in the increased number of patients with ASIO accompanied with the diseases of other organs and systems. In such cases, the clinical symptoms of this acute surgical pathology can be confused with those of concomitant diseases or their complications. This can hinder the diagnostic process and thus postpone the choice of treatment tactics [10]. The polymorbidity of today's patients is caused not only by living conditions and environmental peculiarities, but also the tremendous diagnostic capabilities of modern equipment.

In the group of patients under the study, concomitant diseases were observed in 29 (96.67%) cases with a significant predominance of damage in their cardiovascular and respiratory systems (75.90%). The comorbidity index was determined according to Charlson M.E. et al. (1987). The comorbidity index at the age of 59 was 0.5, at the age of 60-74 it was 5.5, and for the patients aged 75-89 it was 6.07. In 76.67% of cases, patients showed from to 2 through 4 concomitant diseases. Such high figures in de-

ceased patients indicate the great influence the identified concomitant diseases have on the patients with ASIO, as the comorbidity increases the severity of the condition and worsens the prognosis.

As part of the preoperative assessment of the condition of the study group of patients, the ASA scale was used (Table IV).

According to the ASA scale, only 20.0 % of patients with favourable prognoses after surgery were assigned to the first three classes. 66.67 % of patients had severe compensated diseases that were a constant threat to their lives, and 13.34% of patients were admitted in such a condition that, according to the ASA scale, they corresponded to classification V and their prognosis, with or without surgery, predetermined death.

For patients with ASIO, presurgical preparation was carried out on average for (40.29 ± 7.28) hours; the operation was performed within 24 hours for 17 (56.67%) patients. The average duration of surgery performance in this group of patients was (2.05 ± 0.37) hours; however, in 46.43% of cases, it was more than 2 hours. In 7 (23.33%) cases, a relaparotomy was performed with the duration of the operation of (2.15 ± 0.42) hours, and for 3 (10.0%) patients, the operation lasting 2.06 ± 0.32 hours was performed for the third time.

Nasogastrointestinal intubation was performed in 17 patients (56.67% cases, and in 2 cases it was performed during relaparotomy), simultaneous intraoperative decompression of the gastrointestinal tract was performed for 2 (6.67%) patients, and the imposition of enterostomy was performed for 3 patients during relaparotomy.

We conducted a study of the duration of treatment of patients with ASIO in a hospital (Figure 1).

In more than half of the operated patients (53.33 %), mortality occurred in the first three days after surgery. The third and fifth days after surgery were the most unfavourable during the postoperative period in patients with ASIO (30 % and 20 %).

The cause of death during the first three days after surgery was the progression of intoxication phenomena, primarily due to functional obstruction of the small intestine, which resulted in the appearance of enteral insufficiency syndrome and the development of multiple organ dysfunction [7,9,15], that in many cases caused decompensation of the existing concomitant pathology. Lethality in the later period was observed due to the decompensation of concomitant diseases that accompanied ASIO. The cause of patients' death in 24 (80.0%) cases was determined as multiple organ failure; in 5 (16.67%), it was acute cardiovascular insufficiency, and in one case (3.33%), it was a pulmonary embolism.

During the investigation, it was determined that the possible causes of the complicated course of the postoperative period in the study group of patients, except the elderly and senile patients, and late medical care seeking, were the following:

- uncorrected hypotension and hypovolemia in the postoperative period;
- failure of small intestine intubation and constant decompression of the digestive canal [5,10];
- early removal of the nasogastrointestinal probe;
- prolonged anaemia and hypoproteinemia;
- lack of prevention of stress ulcers of the digestive canal in the elderly and senile age;
- failure of early use of enteral nutrition;
- the later restoration of the motility of the digestive tract.

CONCLUSIONS

1. Treatment of patients with acute small bowel obstruction requires the choice of a specially developed treatment program (algorithm) at all stages of surgical care, taking into account the existing concomitant pathology.
2. Optimization of surgical care for patients with acute small bowel obstruction should meet the optimal timing of preosurgical preparation, choosing the minimum amount of surgical care provided during surgery.
3. When treating patients with ASIO, it is necessary to use effective methods of decompression of the digestive canal, early restoration of motility of the gastrointestinal tract, with the use of enteral nutrition.

REFERENCES

1. Catena F, De Simone B, Coccolini F, Di Saverio S, Sartelli M, Ansaloni L. Bowel obstruction: a narrative review for all physicians. World J Emerg Surg. 2019 Apr 29;14:20. doi: 10.1186/s13017-019-0240-7. PMID: 31168315; PMCID: PMC6489175.
2. Amara Y, Leppaniemi A, Catena F, et al. Diagnosis and management of small bowel obstruction in virgin abdomen: a WSES position paper. World J Emerg Surg. 2021 Jul 3;16(1):36. doi: 10.1186/s13017-021-00379-8. PMID: 34217331; PMCID: PMC8254282.
3. Kanat B. H., Eröz E., Saçli A., et al. Surgical Recovery of Intestinal Obstructions: Pre- and Postoperative Care and How Could it Be Prevented?. In: Sozen, S., editor. Surgical Recovery [Internet]. London: IntechOpen; 2020 [cited 2022 Jul 17]. Available from: https://www.intechopen.com/chapters/72437 doi: 10.5772/intechopen.92748
4. Taniguchi K, Iida R, Watanabe T, et al. Ileo-ileal knot: a rare case of acute strangulated intestinal obstruction. Nagoya J Med Sci. 2017 Feb;79(1):109-113. doi: 10.18999/nagjms.79.1.109. PMID: 28303069; PMCID: PMC5346628

5. Mukhin A.S. Otdel'nov L.A., Chukhin O.V., Taranov M.V. Analiz letal'nykh iskhodov u bol'nykh s ostroy kishechnoy neprokhodimost'yu neopukholevoy etiologii. Materialy ÍÍ s"yezda khirurgov PFO. 27-28 sentyabrya 2018:172-174 https://www.volgmed.ru/uploads/files/2018-10/90150-sbornik_tezisov_2018.pdf (in Russian)
6. Perrone G., Giuffrida M., Papagni V., Management of Acute Large Bowel Obstruction in Elderly Patients. In: Latifi, R., Catena, F., Coccolini, F. (eds) Emergency General Surgery in Geriatrics . Hot Topics in Acute Care Surgery and Trauma. Springer, Cham. 2021. https://doi.org/10.1007/978-3-030-62215-2_21
7. Koşar MN, Görgülü Ö. Incidence and mortality results of intestinal obstruction in geriatric and adult patients: 10 years retrospective analysis. Turk J Surg. 2021 Dec 31;37(4):363-370. doi: 10.47717/turkjsurg.2021.5177. PMID: 35677479; PMCID: PMC9130947.
8. Görgülü Ö, Koşar MN. The effects of comorbidity factors on the prognosis in geriatric sepsis patients in the intensive care unit. Medicine Science 2021; 10(1): 31-5. doi: https://doi.org/10.5455/medscience.2020.07.133
9. Zabolotskikh Igor, Trembach Nikita. High perioperative risk patients: two approaches to stratification. Review. Alexander Saltanov Intensive Care Herald. 2019:34-46. doi: 10.21320/1818-474X-2019-4-34-46.
10. Dziubanovskyi IY, Benedykt VV, Danchak VY, Prodan AM. Acute obstruction of the small intestine . Controversial and unresolved issues of the digestive decompression in the light of successes and failures of surgical treatment. Hospital surgery 2020;(1):130-6. Available at: https://ojs.tdmu.edu.ua/index.php/surgery/article/view/10750
11. Sarsenbayeva G.I., Tursynbekova A.E. Modern approaches to the assessment of comorbidity in patients. Cardiosomatics. 2019;10(1):19-23. https://cyberleninka.ru/article/n/sovremennye-podhody-k-otsenke-komorbidnosti-u-patsientov
12. Dziubanovskyi IY, Benedykt VV, Prodan AM . Ways of improving the results of treatment of patients with acute ob-struction of the small intestine after its resection. Scientific and practical journal Art of medicine 2018;4(8):61 -65. Available from: https://art-of-medicine.ifnmu.edu.ua/index.php/aom/article/view/41
13. Mayhew D, Mendonca V, Murthy BVS. A review of ASA physical status - historical perspectives and modern developments. Anaesthesia. 2019;74(3):373-379. doi: 10.1111/anae.14569. Epub 2019 Jan 15. PMID: 30648259.
14. Hurwitz EE, Simon M, Vinta SR, et al. Adding Examples to the ASA-Physical Status Classification Improves Correct Assignment to Patients. Anesthesiology. 2017;126(4):614-622. doi: 10.1097/ALN.0000000000001541. PMID: 28212203.
15. Fevang BT, Fevang J, Stangeland L, et al. Complications and death after surgical treatment of small bowel obstruction: A 35-year institutional experience. Ann Surg. 2000;231(4):529-37. doi: 10.1097/00000658-200004000-00012. PMID: 10749614; PMCID: PMC1421029.

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Conflict of interest:
The Authors declare no conflict of interest

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Received: 02.06.2022
Accepted: 19.01.2023

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

ORIGINAL ARTICLE

DIAGNOSTIC UTILITY OF IMMUNOHISTOCHEMICAL EXPRESSION OF KI-67, P63 AND AMACR IN PROSTATE INTRAEPITHELIAL NEOPLASIA

DOI: 10.36740/WLek202302125

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ABSTRACT

The aim: To determine malignant transformation and progression ability of high grade and low grade prostate intraepithelial neoplasia with the help of immunohistochemical method.

Materials and methods: The results of examination of 93 patients with PIN (50 patients with high grade PIN and 43 patients with low grade PIN) were assessed comparatively using immunohistochemical markers. Semiquantitative method was used to evaluate **Ki**-67, **p**63 and AMACR tissue expression with four grades from „+" to „++++" or from 1 to 4 points: '+' – low reaction, '++' – poor reaction, '+++' – moderate reaction and '++++" – intense reaction.

Results: There were statistically significant differences in immunohistochemical expression rates between HGPIN and LGPIN. Patients with HGPIN had higher Ki-67 and AMACR expression rate and lower p63 expression rate than patients with LGPIN. Intense and moderate Ki-67 expression was detected in HGPIN more often, in 24 % and 11 % respectively. Low and moderate AMACR expression was determined in HGPIN more often, in 28 % and 5 % respectively. Low and not evident p63 expression was observed in HGPIN more often, in 36 % and 8 % respectively.

Conclusions: HGPIN has common morphological peculiarities with prostate adenocarcinoma. Immunohistochemical detection of Ki-67, p63 and AMACR is aimed to differentiate among patients with PIN a group of high malignant transformation risk.

KEY WORDS: prostate intraepithelial neoplasia, immunohistochemical markers

Wiad Lek. 2023;76(2):420-426

INTRODUCTION

Prostate cancer (PC) is among the most common malignancies diagnosed in men [1]. Premalignant" conditions of PC lie on a spectrum between the classification of benign and malignant. Researcher who first described this concept over a century ago recognized that histologically, these lesions comprise morphologically abnormal cells that do not meet the strict requirements for the diagnosis of malignancy [2]. Frequently, premalignant lesions including high-grade prostatic intraepithelial neoplasia (HGPIN) and low-grade prostatic intraepithelial neoplasia (LGPIN) are diagnosed upon prostatic biopsy. PIN is defined as a preinvasive, neoplastic proliferation of secretory cells within preexisting ducts and acini, and is commonly seen in association with PCa [3]. PIN has been proposed to arise with progressive morphologic abnormalities along a continuum from normal epithelium, to low-grade PIN to high-grade PIN and then to invasive carcinoma [4]. These lesions often have a propensity with time to develop into malignant lesions, and some may be appropriately "screened" and dealt with before this change occurs. Although both HGPIN and LGPIN have both been described by many as "premalignant" lesions with malignant potential, others have argued that this may not entirely be accurate [5]. Ultimately, contemporary evidence regarding HGPIN develop malignancy is limited, and institutions report varied results. The formal distinction between premalignant and malignant lesions in the prostate as such remains somewhat unclear [6].

A large number of molecular alterations have been identified in high-grade PIN lesions, many of which are also found in adenocarcinoma implying that PIN is molecularly intermediate between normal and carcinoma [7, 8]. Biological features of precancerous states, their capability for invasion and metastasis depend on balance of growth factors and inhibitors. Molecular markers reflect proliferation, apoptosis, angiogenesis processes which are determined by receptor spectrum of investigated tissues. Immunohistochemical methods are essential for specific visualization of different cells in tissue, growth factors and their receptors, hormones [9]. Alpha-*methyl*-acyl-CoA racemase (AMACR) is a ferment

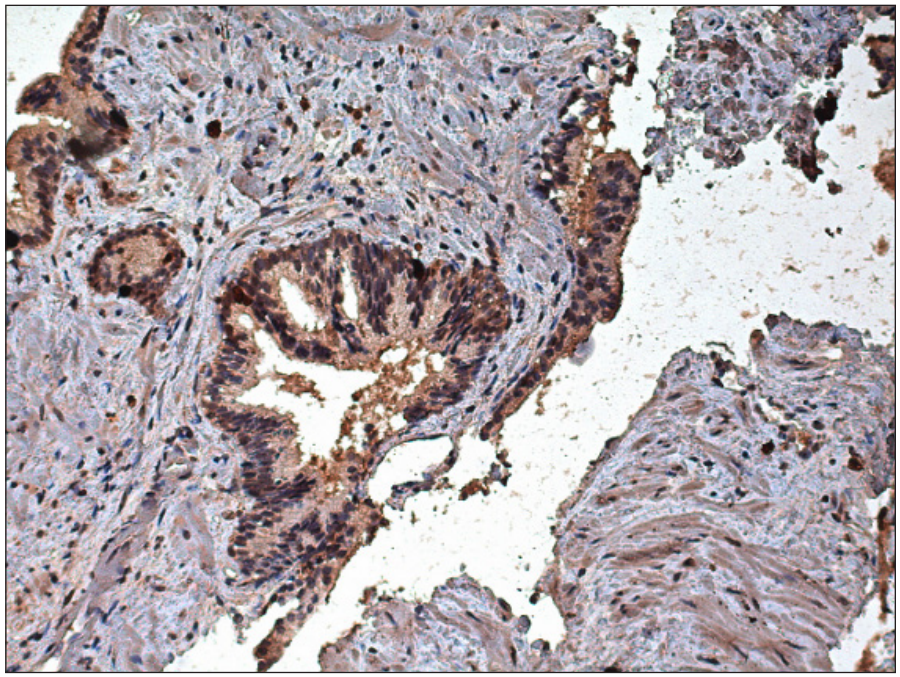

Fig. 1. HGPIN, moderate Ki-67 expression at 200x magnification.

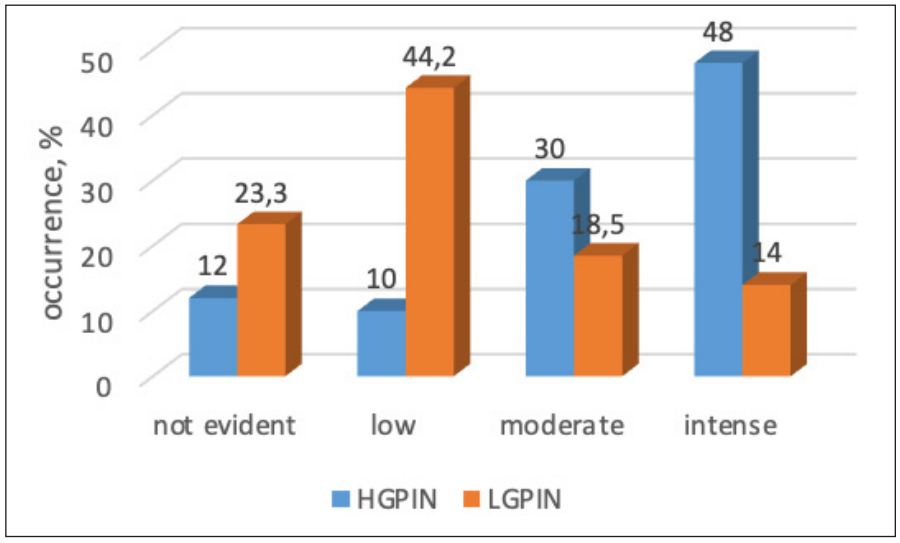

Fig. 2. Ki-67 expression in high grade and low grade PIN

that enhances free radical processes and DNA damage in cell [10]. p63 expresses by basal epithelium layer of prostate tissue exceptionally and is a marker of tumour invasion [11]. Ki-67 belongs to regulative proteins and is used as universal proliferation marker for assessment of malignant growth. Complex use of immunohistochemical markers which reflect different features of cancerogenesis is aimed to determine malignisation potential in every individual case and to detect group of patients with high risk [12-14].

THE AIM

The aim was to define immunohistochemical peculiarities of prostate intepithelial neoplasia in aspect of malignant transformation and progression ability.

MATERIALS AND METHODS

We studied 93 patients from 56 to 76 years old (mean age 67±2,3 y.o.) with PIN who underwent transrectal multifocal prostate biopsy or transurethral prostate resection. With the help of immunohistochemical methods were studied molecular biological features of PIN, especially its ability for malignisation and progression. Expression of proliferation marker Ki-67, marker of invasive growth p63 and marker of malignant progression AMACR was investigated. Immunohistochemical reaction assessment was based on coloration intensity and immunopositive cells distribution. Semiquantitative method was used to evaluate Ki-67, p63 and AMACR tissue expression with four grades from „+" to „++++" or from 1 to 4 points: '+' – low reaction, '++' – poor reaction, '+++' – moderate reaction and '++++" – intense

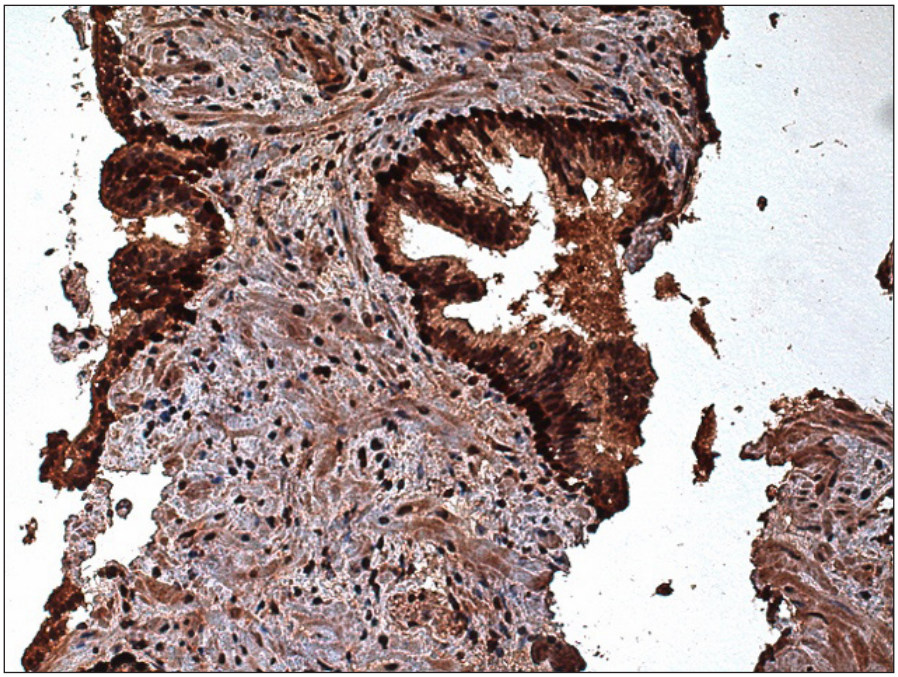

Fig. 3. LGPIN, intense p63 expression at 200x magnification

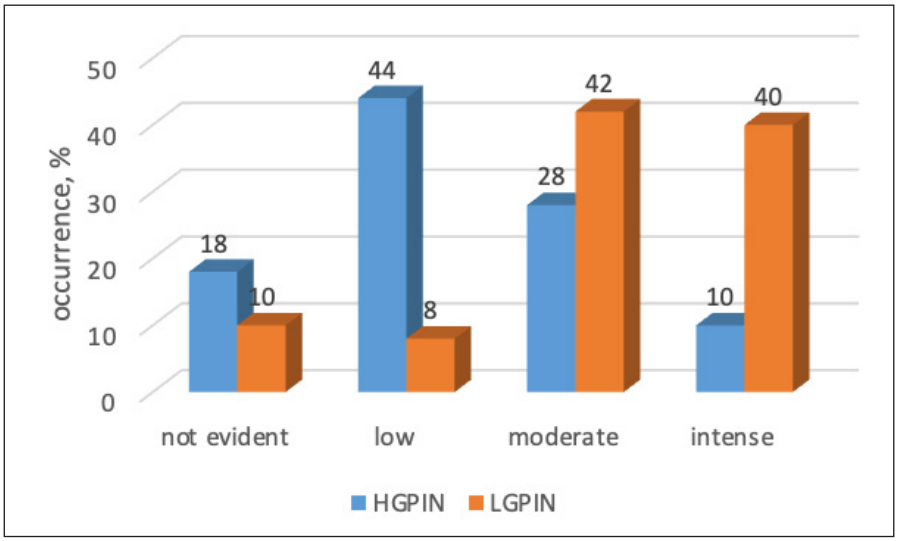

Fig. 4. p63 expression in high grade and low grade PIN

reaction. Complex immunohistochemical investigation with studiing of Ki-67, p63 and AMACR expression was performed 93 patients. Among them were 50 patients with high grade PIN and 43 patients with low grade PIN.

Statistical processing of data was performed with the help of descriptive and variative statistics. The significance of received results and comparison between groups was evaluated using non-parametric Mann Whitney u test criteria. At assessment of the differences between the groups, the value $p < 0.05$ was considered significant.

RESULTS

Analysis of Ki-67, p63 and AMACR expression regarding grade of PIN was conducted. Analysis of Ki-67 expression showed, that in HGPIN group 24 (48 %) of patients had intense expression "++++", 15 (30 %) of patients had moderate expression "+++" (Fig. 1), in 6 (12 %) cases Ki-67 expression was not evident "+" and in 5 (10 %) patients was detected low expression Ki-67 level.

In patients with LGPIN mainly was determined low "++" Ki-67 expression – in 19 (44,2 %), not evident "+" Ki-67 expression - in 10 (23,3 %) cases, moderate "+++" Ki-67 expression – in 8 (18,5 %) patients and intense "++++" Ki-67 expression – in 6 (14 %) patients.

Comparing expression Ki-67 levels in patients with high grade and low grade PIN should be noticed that generally patients with HGPIN had higher Ki-67 values. In LGPIN low Ki-67 expression was detected in 34 % and not evident Ki-67 expression in 11 % more often than in HGPIN. Conversely, patients with HGPIN had in

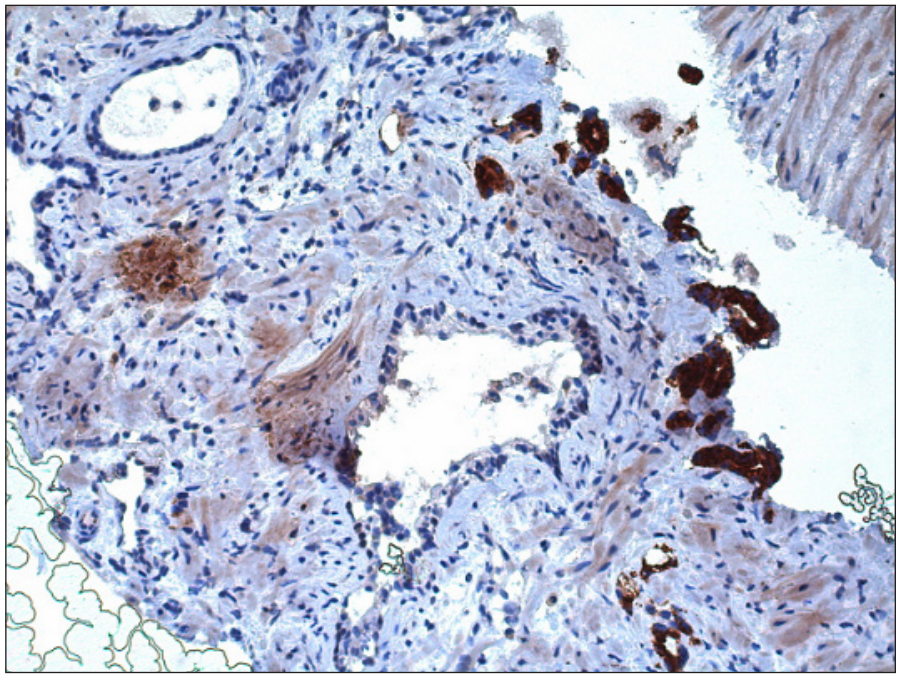

Fig. 5. HGPIN, low AMACR expression at 200x magnification

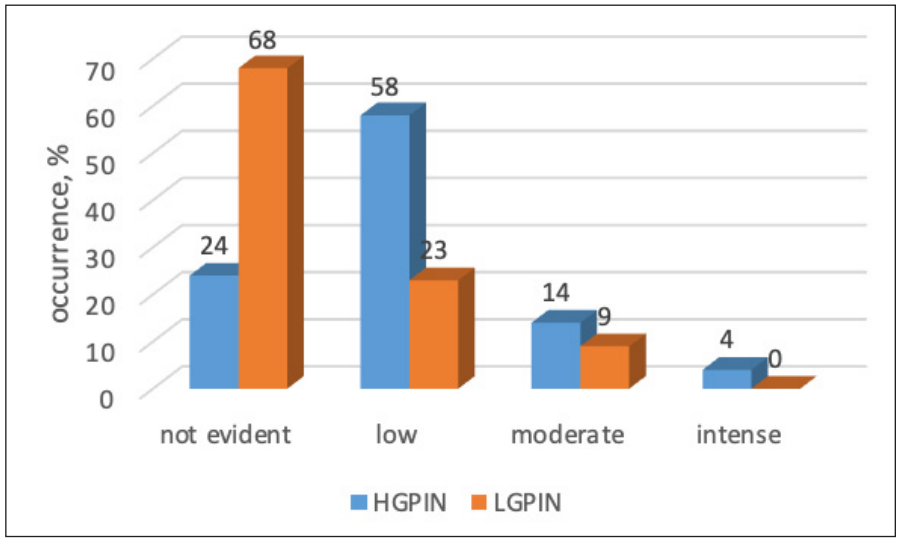

Fig. 6. AMACR expression in high grade and low grade PIN

24 % more often intense and in 11 % moderate Ki-67 expression than patients with LGPIN (p<0,05). (fig 2).

One of the investigation tasks was to determine the meaning of marker p63 in PIN diagnostics, especially in assessment of precancerous diseases invasive potential.

It was determined that patients with HGPIN had in 22 (44 %) of cases low '++' p63 expression. Moderate '+++' p63 expression level was detected in 14 (28 %) patients, not evident '+' p63 expression was in 9 (18 %) of cases and in 5 (10 %) patients intense p63 expression was diagnosed.

In patients with LGPIN mainly was determined moderate p63 expression, namely in 21 (42 %) of cases and intense '++++' expression – in 20 (40 %) patients (fig. 3), not evident p63 '+' expression was observed in 5 (10 %) of patients, low '++' p63 level – in 4 (8 %) cases.

Analysing the differences in p63 expression levels in patients with high grade and low grade PIN should be noticed that generally patients with LGPIN had higher p63 values. In LGPIN intence p63 expression was detected in 30 % and moderate p63 expression in 14 % more often than in HGPIN. Conversely, patients with HGPIN had in 36 % more often low and in 8 % not evident p63 expression than patients with LGPIN (p<0,05). (fig. 4).

Analysis of AMACR immunohistochemical expression showed that in HGPIN group 29 (58 %) of patients had low expression "++" (fig. 5), 12 (24 %) of patients had not evident expression "+", in 7 (14 %) cases AMACR expression was moderate "+++" and in 2 patients was detected intense AMACR expression level.

The following data were established in patients with LGPIN regarding AMACR expression: not evident ex-

Table I. Rates of Ki-67, p63 and AMACR expression in patients with HGPIN and LGPIN.

PIN grade	Ki-67				p63				AMACR			
	+	++	+++	++++	+	++	+++	++++	+	++	+++	++++
HGPIN rate, %	12	10	30	48	18	44	28	10	24	58	14	4
LGPIN rate, %	23,3	44,2	18,5	14	10	8	42	40	68	23	9	-

pression – in 29 (68 %) cases, low expression – in 10 (23 %) patients, moderate expression – in 4 (9 %) patients. There were no cases with intense AMACR expression detected in patients with LGPIN.

Comparing AMACR expression levels in patients with high grade and low grade PIN should be noticed that generally patients with HGPIN had higher AMACR values. In LGPIN not evident AMACR expression was detected in 44 % more often than in HGPIN. Also there were no cases of intense AMACR expression in patients with LGPIN. Conversely, patients with HGPIN had in 28 % more often low and in 5 % moderate AMACR expression than patients with LGPIN ($p<0,05$). (fig 6).

Thus, studying of immunohistochemical expression rate of markers Ki-67, AMACR and p63 determined statistically significant differences between high grade and low grade PIN. Patients with HGPIN were characterized by higher rates of Ki-67 and AMACR and lower rates of p63 comparing with those of LGPIN (table I). Obtained data confirm common morphological features of HGPIN and PC, higher ability of HGPIN to malignant transformation comparatively with LGPIN.

DISCUSSION

In last decade investigations were made to determine molecular factors that could help to detect a group of high malignization risk in patients with PIN. Our data correlate with investigation of Allina D et al., according to latest data of genetic, proteomic and morphological analysis of HGPIN is the most likely precursor of adenocarcinoma [15]. This is precondition for high PC detection in patients with PIN during follow up. The use of immunohistochemical methods in prostate pathology is essential for precise diagnostics of PC. By Popov S. at all immunohistochemical analysis of biopsy material using monoclonal antibodies to p63, high-molecular cytokeratins of clone 34βE12 and AMACR for suspected prostate cancer specifies the micromorphological picture and demasks prostate cancer, increases the detectability of prostate cancer; thirdly, it reduces the occurrence of false-negative results [13]. To the opinion of Trabzonlu et al, a better understanding of the early stages of prostate cancer initiation, potentially arising from precursor lesions, may fuel development of powerful approaches for prostate cancer prevention or interception. In case of HGPIN rearrangements of molecular markers TMPRSS2–ERG result in the overexpression of the oncogenic transcription factor ERG and represent an early event in prostate cancer progression [16]. Haffner et al. suggest that ERG-positive HGPIN is closely associated with ERG-positive carcinoma as a result of the strong tendency for ERG-positive HGPIN to invade. This finding might explain previous observations suggesting that patients with ERG-positive HGPIN lesions more often show carcinoma on repeat biopsy, and add to our understanding of the order of molecular and morphological events and complex clonal relationships that can occur during the development and progression of primary prostate cancers [17].

Grivas N. et al. explored the role of angiogenesis as a prognostic factor in progression of premalignant prostate states. The authors compared angiogenesis in benign prostatic hyperplasia, HGPIN and prostate cancer. Vascular endothelial growth factor (VEGF) is considered as the most potent mediator of physiologic and pathologic angiogenesis and lymphangiogenesis. The aim of our study was to record and compare the immunohistochemical expression of VEGF, VEGFR-1, VEGFR-2 and CD105 in PC and HGPIN. The authors came to the conclusion that angiogenesis was significantly higher in PC when compared to HGPIN, while there was no difference between benign prostate hyperplasia and HGPIN [18]. According to data of Dareen A et al stromal caveolin-1 expression was significantly higher in benign prostate hyperplasia cases (88%) than HGPIN (25%) and PC cases (15%) respectively. This finding confirmied that an absent stromal ceveolin-1 directly correlates with progression of prostate cancer. Caveolin-1 plays an important role in prostatic carcinogenesis and metastasis. Stromal expression of caveolin-1 in carcinoma is reduced in relation to PIN [19].

In patients with PIN detected patterns influence on observation and treatment choise, namely demonstrate the necessity of additional examination of patients with PIN and of prescription of treatment to prevent malignant transformation of precancerous prostate pathology in these patients.

CONCLUSIONS

1. Ki-67 proliferation marker expression rate is higher in patients with HGPIN than with LGPIN. In HGPIN

intense Ki-67 expression was detected in 24 % and moderate Ki-67 expression in 11 % more often than in LGPIN.
2. p63 invasion marker expression is higher in LGPIN. Intense p63 expression was determined in 30 % and moderate p63 expression in 14 % more often in LGPIN comparing with HGPIN.
3. AMACR progression marker expression is higher in patients with HGPIN comparing with LGPIN. Low AMACR expression was observed in 44 % more often in LGPIN than in HGPIN. There were no cases with intense AMACR expression in patients with LGPIN detected.
4. Expression rates of proliferation, invasion and progression markers in patients with HGPIN has common features with prostate adenocarcinoma, what is a sign of great HGPIN malignisation potential. Patients with HGPIN and high rates of Ki-67, AMACR and low rate of p63 belong to group of risk and need detailed examination and observation.

ABBREVIATIONS

AMACR alpha-*methyl*-acyl-CoA racemase
HGPIN high grade prostate intraepithelial neoplasia
LGPIN low grade prostate intraepithelial neoplasia
PC prostate cancer
PIN prostate intraepithelial neoplasia

REFERENCES

1. Sung H., Ferlay J., Siegel R. Global cancer statistic 2020. CA Cancer J Clin. 2021; 71: 209-249.
2. Gasparrini S., Cimadamore A. Pathology and molecular updates in tumors of the prostate: towards a personalized approach. Expert Rev Mol Diagn. 2017; 17(8): 781-789. doi: 10.1080/14737159.2017.1341314.
3. Bostwick D.G., Montironi R. Prostatic intraepithelial neoplasia and the origins of prostatic carcinoma. Pathol Res Pract. 1995; 191: 828-832.
4. Bostwick D.G., Cheng L. Neoplasms of the prostate. Urologic surgical pathology. Third edition: Saunders. 2014, 410 p.
5. Kudryavtsev G., Kudryavtseva L., Mikhaleva L. Immunohistochemical study of Ki-67, p53 and Notch1 expressions in prostate cancer of different grades. Arkh Patol. 2020; 82(5):42-49. doi: 10.17116/patol20208205142.
6. Manning T., Christidis D., Perera M. Atypical Small Acinar Proliferation and High Grade Prostatic Intraepithelial Neoplasia in the Era of Multi-Parametric MRI: a Contemporary Review. Urology. 2017; 107: 5-10.
7. Magi-Galuzzi C. Prostate cancer: diagnostic criteria and role of immunohistochemistry. Mod Pathol. 2018; 31: 12-21. doi: 10.1038/modpathol.2017.139.
8. Giunta E., Annaratone L., Bollito E. Molecular Characterization of Prostate Cancers in the Precision Medicine Era. 2021; 13 (19): 4772. doi: 10.3390/cancers13194771.
9. Diffalha S., Shaar M., Barkan G.A. Immunohistochemistry in the workup of prostate biopsies: Frequency, variation and appropriateness of use among pathologists practicing at an academic center. Ann Diagn Pathol. 2017; 27: 34-42.
10. Shapovalova M., Davydova J., Henzler C. Exploiting the transcriptional specificity of the alpha-methylacyl-CoA racemase AMACR promoter for the molecular imaging of prostate cancer. Oncotarget. 2018; 9: 36693-36704.
11. Berlin A., Castro-Mesta J., Rodriguez-Romo L. Prognostic role of Ki-67 score in localized prostate cancer: A systematic review and meta-analysis. Urol Oncol. 2017; 35: 499-506.
12. Rathod S., Jaiswal D., Bindu R. Diagnostic utility of triple antibody (AMACR, HMWCK and P63) stain in prostate neoplasm. J Family Med Prim Care. 2019; 28: 2651-2655.
13. Popov S., Guseynov R., Skryabin O. Immunohistochemical analysis as a method of increasing the detection of prostate cancer at primary biopsy. 2019; 6: 41-49.
14. Mandel P., Wenzel M., Hoeh B. Immunohistochemistry for Prostate Biopsy-Impact on Histological Prostate Cancer Diagnoses and Clinical Decision Making. 2021; 28 (3): 2123-2133. doi: 10.3390/curroncol28030197.
15. Allina D., Andreeva Y., Zavalishina L. High-grade prostatic intraepithelial neoplasia: state-of-the-art. Arkh Patol. 2015;77(1):69-74. doi: 10.17116/patol2015771692015.
16. Trabzonlu L., Kulac I., Zheng Q. Molecular Pathology of High-Grade Prostatic Intraepithelial Neoplasia: Challenges and Opportunities. Cold Spring Harb Perspect Med. 2019; 9(4): 1-19.
17. Haffner M., Weier C., Xu M. Molecular evidence that invasive adenocarcinoma can mimic prostatic intraepithelial neoplasia (PIN) and intraductal carcinoma through retrograde glandular colonization. J Pathol. 2016; 238(1): 31–41.
18. Grivas N., Goussia A., Stefanou D. Microvascular density and immunohistochemical expression of VEGF, VEGFR-1 and VEGFR-2 in benign prostatic hyperplasia, high-grade prostate intraepithelial neoplasia and prostate cancer. Cent European J Urol. 2016; 69(1): 63–71.
19. Dareen A., Duaa S. Prognostic significance of epithelial/stromal caveolin-1 expression in prostatic hyperplasia, high grade prostatic intraepithelial hyperplasia and prostatic carcinoma and its correlation with microvessel density. Journal of the Egyptian National Cancer Institute. 2017; 29(1): 25-31.

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Conflict of interest:
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Received: 08.11.2021
Accepted: 14.11.2022

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

SCIENTIFIC ACHIEVEMENTS OF UKRAINIAN SCIENTIST IN DIAGNOSTICS OF TEMPOROMANDIBULAR JOINT DISEASES (LITERATURE REVIEW)

DOI: 10.36740/WLek202302126

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ABSTRACT

The aim: A systematic analysis and generalization of scientific information of Ukrainian scientists about planning of the stages of diagnosis of patients with TMJ diseases, which will allow to optimize the commonly accepted examination algorithm.

Materials and methods: Scientific analysis and generalization of obtained data of literary sources of Ukrainian scientists articles with characteristics of planning stages of diagnosis of patients with TMJ diseases is based on such databases as Scopus, Web of Science, MedLine, PubMed, NCBI, the study of which does not exceed 6 years, including monographs and results clinical research.

Conclusions: The results of scientific research by Ukrainian scientists are the basis for increasing the effectiveness of diagnosis of TMJ diseases by improving complex examination methods and implementing clinical algorithms, which will allow to choose adequate treatment methods.

KEY WORDS: temporomandibular joint, diagnostic methods, efficiency improvement, Ukrainian scientists

Wiad Lek. 2023;76(2):427-432

INTRODUCTION

Modern integral medicine considers temporomandibular joint (TMJ) to be the center of body's postural balance, and providing dental rehabilitation is possible only if optimal aesthetics, function, and morphology of the dento-maxillofacial area of an individual are achieved in general, and balanced work of the TMJ in particular [1-4].

Unfortunately, with prevalence of dento-maxillofacial anomalies, a significant place in structure of dental diseases is occupied by TMJ dysfunction [2, 5-7]. TMJ pathology is one of the urgent problems of modern dentistry, which in terms of prevalence ranks third among diseases of temporomandibular system after caries and periodontal lesions [8, 9]. According to various authors, the prevalence of TMJ diseases ranges from 20 to 70% among all patients seeking dental care [10, 11]. Research by the American Dental Association shows that at least 35% of the world's population have TMJ pathology. Among the total number of patients, TMJ dysfunction occurs in up to 80% of women. It has a polyetiological feature and direct dependence on the hormonal state, especially gynecological status, and the leading causes of its occurrence are considered to be anatomical, occlusive, muscular, neurogenic, post-traumatic and general somatic [12-14].

Due to the presence of a large number of manifestations of clinical signs, absence of generally accepted classification and a single view at pathogenesis, the lack of systematization of TMJ pathology diagnostics methods leads to a fact that patients are treated by specialists of various profiles: dental surgeons, orthopedists, orthodontists, neurodentists, otolaryngologists, rheumatologists [15].

Diagnosis of TMJ diseases is important, because occlusal disorders of maxillofacial system can be the cause and aggravating factors at a whole complex of dental diseases [6]. However, the methods of diagnosis and forecasting of TMJ diseases development need to be improved. All of the above mentioned applies to the population of our country.

THE AIM

The aim of the study is a systematic analysis and generalization of scientific information of Ukrainian scientists about planning of the stages of diagnosis of patients with TMJ diseases, which will allow to optimize the commonly accepted examination algorithm.

MATERIALS AND METHODS

Scientific analysis and generalization of obtained data of literary sources of Ukrainian scientists articles with characteristics of planning stages of diagnosis of patients with TMJ diseases is based on such databases as Scopus, Web of Science, MedLine, PubMed, NCBI, the study of which does not exceed 6 years, including monographs and results clinical research.

REVIEW AND DISCUSSION

Clinical examination of patients is based on generally accepted methodology and includes assessment of general somatic and dental status: clarification of complaints, life and medical history , examination, palpation. It is carried out according to the principle of a screening study, which allows to reveal the main aspect of pathology in a short period of time [3].

Patients with TMJ muscle-articular dysfunctions are recommended to undergo diagnostic psycho-screening and psychotherapist consultation with the aim of mandatory subsequent differentiated correction of behavior in accordance with existing leading psychopathological manifestations [13, 14].

When examining a patient, attention should be paid to the patient's usual posture, head position , cervical and thoracic spine, and upper limbs, as well as to clarify how the intensity of pain syndrome changes according to the change in a body position [4, 13].

Clinical examination includes, in addition to general dental external and intraoral, the following examination methods: symmetry assessment of facial and masticatory muscles functioning, position of the lower jaw, displacement of chin relative to nose at rest and when mouth is fully opened (deviation from the median axis), the size and symmetry of mouth opening (deviation and deflection), determination of joint noises, palpation of masticatory muscles, neck and shoulder muscles, determination of their tenderness, detection of occlusal sound and its asymmetry, analysis of eccentric occlusion of teeth and its trauma [3, 4, 13]. Advanced clinical analysis of the TMJ condition is carried out by lateral and dorsal palpation of the articular heads, applying a dynamic joint compression test, studying acoustic phenomena in the joint and their nature (friction, crunching, clicking, etc.), depending on a phase of mandible movements.

At clinical examination stage, special attention is paid to manual functional analysis, which indirectly determines the condition of individual TMJ anatomical structures, such as bilaminar zone, joint capsule, and the muscular system of masticatory apparatus. When evaluating movements of the lower jaw, capacity of active mouth opening, passive and loading movements of the lower jaw, dynamic compression (to determine the state of the ligamentous apparatus and joint capsule), articular surfaces, as well as passive compression(to characterize the adaptation of bilaminar zone) must be determined [15-17].

Objectively, palpation of the lateral pterygoid muscle is carried out behind the tubercle of the upper jaw, followed, if necessary, by its infiltration with an anesthetic without a vasoconstrictor [4].

An effective method of non-injection diagnosis of lateral pterygoid muscle condition can be considered an isometric test, available in clinical conditions, which does not require special equipment. Also, among the methods of express diagnostics, there is a clinical test for a presence of a certain pathology of joint, which is carried out by opening a mouth from position of maximum protrusion [3, 4, 13].

Palpation should be carried out not only in the area of the joints, but also in all parts of face: parotid salivary glands, exit points of trigeminal nerve branches, lymph nodes of face and neck, chewing muscles both at rest and when they are tense.

When examining the oral cavity, it is especially important to assess the character of occlusion, a ratio of the jaws and occlusal planes, presence of dental defects, state of fillings in previously treated teeth, and a state of chewing surfaces of the teeth. Partial loss of teeth, especially complicated by secondary deformations of dentitions, leads to TMJ and masticatory muscle pathology [3, 4, 8].

TMJ and masticatory muscle dysfunctions are also observed in patients with complete dentition, which is caused by anomalies, deformations of the dentition, which lead to a violation of occlusal-articulation equilibrium and muscle balance, spatial orientation of the lower jaw relative to the upper [4-6].

Electromyography is one of the leading methods of functional research of neuromuscular apparatus, which allows to optimize diagnostic algorithm, as well as differential diagnosis and dynamic monitoring of patients with diseases of temporo-mandibular joint. Electromyographic research can be used not only for the purpose of primary diagnosis, but also for qualitative differential diagnosis of various forms of pathology of temporo-mandibular joint, which will contribute to the development of the most rational treatment plan and increase the quality of medical care [18].

It has been shown that the method of functional diagnostics proved to be quite informative when assessing the state of masticatory muscles in patients with temporomandibular joint diseases, as it allows to detect indicators changes at different levels of disorders [19, 20].

According to the results of EMG masticatory muscles, in patients with TMJ dysfunction, the masticatory cycle, which is determined by the activity of central neural generator of mastication in brain stem, undergoes a significant transformation. The duration of this cycle in the presence of TMJ pathology is significantly shorter on average, and the average frequency of chewing movements is higher than in controls, mainly due to reduction of the silent phase in the generated cyclic activity. This is a confirmation of the existing significant modulation of mechanisms activity of chewing action central control by nociceptive influences from the periphery [21-25].

According to the results of T.M. Kostyuk research [26, 27] of EMG and MRI, a decrease in a distance from the back edge of a joint head to the back edge of a joint cavity is responsible for the presence of pain symptoms (8–10 points) and on EMG corresponds to an increase in ratio of bioelectric activity and bioelectric rest of muscles and an increase in degree of severity muscle damage. The number, thickness, and localization of linear compressions of muscle fibers of masticatory muscles (according to MRI data) and the intensity of changes in EMG activity of these muscles in TMJ dysfunction are directly correlated with the value of dysfunction index ($P < 0,05$). The degree of compaction in the mentioned muscles directly depends on the bioelectrical activity indicator and the amplitude of contractions of superficial masticatory muscles of the opposite side. Almost all patients with TMJ muscle and joint dysfunction (up to 95,3%) have noticeable disorders of the vascular bed of the head and neck (deficiency of blood flow in the system of external carotid artery and compression of internal carotid artery); this allows pathogenetically substantiate and include ischemic concept of the development of TMJ dysfunction to the formation of diagnostic and treatment model. An application program «PR» was developed for the systematization of diagnostic indicators and analysis of depth of changes in TMJ structures. This makes it possible to assess objectively dynamics of clinical course and organize a sequence of specific treatment and diagnostic and rehabilitation measures in patients with TMJ muscle and joint dysfunction. A device and, in accordance with it, a method of registering occlusal ratios in patients with TMJ dysfunction, has been developed, which allows to carry out instrumental registration when articulation is limited in patients with this pathology, to improve the quality of orthopedic care for patients and to shorten the duration of diagnostic and treatment period.

Ultrasonography may be a useful alternative in cases of internal TMJ disease and in cases where the patient has contraindications to MRI, such as claustrophobia or the presence of a pacemaker. This research method demonstrates high sensitivity and accuracy in diagnostics of an articular disc location in patient's rest state to identify the anatomical position or to identify a displacement of the disc. On the other hand, this method does not provide enough information to analyze displacements of articular discs in patients with an open mouth and to analyze morphological changes of the disc or articular head [26].

Another non-invasive and easy-to-use method of TMJ auscultation with the help of an electronic stethoscope is used to detect the presence of noise phenomena (crepitations, clicks, friction sounds of articular surfaces). The purpose of the study was to evaluate TMJ auscultation method using an electronic stethoscope as a step in comprehensive diagnosis of TMJ internal disorders in comparison with other methods, such as clinical examination methods (interview, examination, palpation), analysis of diagnostic models in the articulator, radiological examination, electroneuromyography. Audio diagnostics is carried out using a Littmann electronic stethoscope (3M ESPE), after it phonograms are analyzed using a computer program according to the main characteristics of sounds [3, 4, 23].

Axiography can be used as a method that allows to obtain accurate graphic data about trajectory of the articular head during movements of the lower jaw. This research method is used for the purpose of functional diagnosis of TMJ, as well as for adjusting articulators according to the individual parameters of a patient. This individual setting of the articulator eliminates the need to use additional methods of registration of the lower jaw position and allows to achieve maximum occlusal accuracy [4].

When conducting a comparative analysis of an application of software-hardware complex for digital orthopantomography and analog orthopantomograph for TMJ tomography, it was found that the use of digital orthopantomographs is more convenient than analog ones, because they give smaller projection distortions, a patient receives a picture immediately after tomography, there is a possibility of specific processing, analysis , copying and storing the received information [21].

The main diagnostic method for detecting arthropathies today is radiography, which is fairly simple and inexpensive method. With the help of radiography, pathological changes in bone structures of the joint can be fully and reliably detected, but, as a rule, at the late stages of the disease [3, 4]. TMJ is studied on plain X-rays films of the skull, on spot X-ray films of the joint in the lateral projection and on spot X-ray films of the temporal bone in the side projection.

The correctness of the ratios in TMJ is determined by comparing the spot-film radiography in the lateral projection with a patient's mouth closed [21].

Based on known X-ray morphometric indices of the lower jaw, V.M.Novikov [11, 14] proposed a protocol for X-ray morphometric measurements of TMJ based on paired radiographs of the joint according to Parma technique. After visual evaluation and preliminary diagnosis, all radiographs were digitized using a scanner. Evaluation of X-ray density was performed on X-ray visiograph using the densitometric function.

Arthrography or arthrotomography is used to visualize the joint disc, which is a combination of linear tomography and arthrography. It becomes possible to assess visually the articular disc, its shape, position and establish its intra-articular relationship with the bony structures of the joint, and when opening or closing mouth, also determine its biomechanics in motion [3]. But this method has a number of disadvantages: invasiveness of the procedure, emergence of the risk of inflammatory reactions due to joint puncture and development of allergic reactions in response to administration of a contrast agent. In addition, the technique is contraindicated in patients with tumor lesions of the joint and arthritis due to the high risk of developing inflammatory complications. Tomography and contrast arthrotomography, associated with a sufficiently large radiation dose, are quite voluminous methods of examination [4]. Such complications significantly limit the use of arthroscopy and arthrography.

Advent of zonography made it possible to reduce radiation exposure compared to tomography [2, 4], but the problem of clear visualization of TMJ structures remained, because when using orthopantomography, the joint is depicted in oblique projections, which disturb the picture of bony elements, the joint space. It does not eliminate the problem of obtaining a clearer image of TMJ structures and the use of special orthopantomograph programs.

Cone-beam computed tomography and other three-dimensional tomographic imaging tools are considered to be more accurate than panoramic radiographs for evaluating of bony components of TMJ and morphologic changes with high resolution. With the help of spiral computed tomography, it is possible to obtain images of TMJ and surrounding tissues in three mutually perpendicular planes: sagittal, coronal, and axial, to carry out measurements, to evaluate volumetric symmetry of TMJ elements, but the analysis of the condition of joint soft tissue structures remains unavailable [5, 6]. The use of computer densitometric analysis allows to determine the quality of a bone in different segments of the jaws in the form of digital indicators [4].

One of the safest, non-invasive diagnostic methods is magnetic resonance imaging (MRI) of TMJ as the gold standard for joint research, which allows to obtain images of periarticular tissues of TMJ without radiation exposure. Other advantages of MRI are sensitivity, specificity, and diagnostic accuracy [3, 4, 17].

V.M. Novikov et al. [11] proposed a set of diagnostic measures for patients with unilateral and bilateral TMJ disc dislocations associated with the presence of deficit or excess of interdental space with intact dentitions, which includes an assessment of the length of occlusal surface of dentitions, research topographical and anatomical changes of TMJ articular discs by MRI method and assessment of quality of life using the questionnaire «WHOQOL - 100».

T.M.Kostyuk in the research [31-32] developed and scientifically based multidisciplinary algorithm for diagnosis of TMJ muscle-articular dysfunctions. The author characterized for the first time the relationship between the state of vascular bed of head and neck in accordance with the development of dysfunctional disorders of TMJ according to the data of duplex scanning of the vessels and it is scientifically confirmed that 95,3% of all patients with muscle and joint dysfunction of TMJ have disorders of the state of vascular bed of head and neck .

In each clinical situation, it is important to prescribe such additional examination methods that would have adequate diagnostic value with the least contraindications, taking into account its availability [4] for a patient. With the development of modern technologies, Ukrainian scientists have an opportunity to decode received diagnostic information, but the development of new and further improvement of existing research methods remains topical, which is important for determining both the dynamics of TMJ diseases and for the preliminary diagnosis of disorders of structural and functional state of bone tissue.

CONCLUSIONS

The results of scientific research by Ukrainian scientists are the basis for increasing the effectiveness of diagnosis of TMJ diseases by improving complex examination methods and implementing clinical algorithms, which will allow to choose adequate treatment methods.

PROSPECTS FOR FURTHER RESEARCH

To develop an applied computer program for the diagnosis of TMJ diseases with the aim of synchronizing data, simplifying work of dentist and establishing more correct diagnosis.

REFERENCES

1. Zhachko N.I., Nespriadko-Monborgne T.S., Skrypnyk I.L. et al. Improving dental health - is improving quality of life. Wiad Lek. 2021;74 (3):722-725.
2. Dmytrenko M.I., Nesterenko O.N. Results of follow-up study of occlusion state in Ukrainian schoolchildren. J. of Stomatology. 2016;69(6):725-728.
3. Rybalov O.V. et al. Kompresiino-dyslokatsiina miazovo-suhlobuva dysfunktsiia skronevo-nyzhnoshchelepnykh suhlobiv [Compression-dislocation musculo-articular dysfunction of the temporomandibular joints. Poltava: "ACMI ". 2019, 19p. (In Ukrainian).
4. Makeev V.F. et al. Skronevo-nyzhnoshchelepni rozlady [Temporomandibular disorders]. Monograph. Lviv National Medical University named after Danylo Halytsky. Lviv: Kvart. 2018; 43 p. (in Ukrainian).
5. Kuroedova V.D., Stasiuk A.A., Makarova A.N. et al. Symmetry of elements of temporomandibular joint (TMJ). Wiad Lek. 2017; 70(6):1079 – 1082.
6. Smagliuk L.V., Lyakhovska A.V. Osoblyvosti likuvannia patsiientiv iz zuboshchelepnymy anomaliiamy, uskladnenymy dysfunktsiieiu skronevo-nyzhnoshchelepnoho suhloba. [Peculiarities of treatment of patients with dento-mandibular anomalies complicated by temporomandibular joint dysfunction]. Ukrainian dental almanac. 2019; 1: 26-30.(In Ukrainian)
7. Dmytrenko M.I., Rybalov O.V., Lunkova Yu.C. Likuvannia miazovoi dysfunktsii skronevo-nyzhnoshchelepnoho suhloba u patsiientiv iz ortodontychnoiu patolohiieiu. [Treatment of temporomandibular joint dysfunction in patients with orthodontic pathology]. Bulletin of problems of biology and medicine. 2019, 2 (1): 304-307. (In Ukrainian)
8. Volovar O., Malanchuk V., Lytovchenko N. et al. Clinical manifestations of somatic pathology in patients with temporomandibular joint disorders. Journal of Research in Medical and Dental Science. 2017; 2 (5): 26–32.
9. Makeev V.F., Telishevska U.D., Telishevska O.D. et al. Rol i znachennia syndromu Kostena v dysfunktsionalnykh stanakh skronevo-nyzhnoshchelepnykh suhlobiv [Role and significance of Kosten's syndrome in dysfunctional conditions of temporomandibular joints]. Ukrainian Dental Almanac.2020;3:34-39. (In Ukrainian).
10. Novikov V.M., Lunkova Yu.S., Berezii M.V. Rezultaty otsinky yakosti zhyttia patsiientiv v dynamitsi ortopedychnoho likuvannia pry vyvykhakh meniskiv skronevo-nyzhnoshchelepnoho suhloba [Results of assessment of the quality of life of patients in dynamics of orthopedic treatment for dislocations of menisci of temporomandibular joint]. Visnyk Ukrainskoi medychnoi stomatolohichnoi akademii "Aktualni problemy suchasnoi medytsyny". 2017;172 (58): 244-250. (In Ukrainian).
11. Novikov V.M., Rezvina K.Y., Shvets A.I. et al. Vzaiemozviazok dysfunktsii skronevo-nyzhnoshchelepnoho suhloba v patsiientok iz hinekolohichnymy zminamy v anamnezi za danymy anketuvannia. [Interrelationship of temporomandibular joint dysfunction in patients with gynecological changes in the anamnesis according to questionnaire data]. Ukrainian Dental Almanac. 2020; 4: 53–56. (In Ukrainian).
12. Kostiuk T.M. Fizykalne obstezhennia patsiientiv iz dysfunktsiieiu skronevo-nyzhnoshchelepnoho suhlobu. Physical examination of patients with temporomandibular joint dysfunction. Ukrainian Journal of Medicine, Biology and Sport. 2018; 4 (13): 149–153. (In Ukrainian)
13. Kostiuk T.M. Osoblyvosti psykholohichnoho suprovodu khvorykh na miazovo-suhlobovu dysfunktsiiu skronevo-nyzhnoshchelepnoho suhlobu.[Peculiarities of psychological support for patients with musculo-articular dysfunction of the temporomandibular joint]. Visnyk Vinnytskoho natsionalnoho medychnoho universytetu. 2019; 4 (23): 594–598..(In Ukrainian).
14. Tumakova O.B., Novikov V.M., Lunkova Y.S. Koreliatsiinyi zviazok mizh dovzhynoiu zubnykh riadiv i topohrafoanatomichnymy pokaznykamy suhlobovoho dyska (meniska) v patsientiv iz dysfunktsiinymy stanamy SNSchS za danymy MRT [Correlation between length of dentions and topographic-anatomical indicators of articular disc (meniscus) in patients with TMJ dysfunctional conditions according to MRI data]. Ukrainian dental almanac. 2017; 3: 22-25. (In Ukrainian).
15. Smagliuk L.V., Lyakhovska A.V. Electromyography in Dentistry. Poltava: Copy Center A. Tkalich. 2021, 69 p.
16. Smaglyuk L., Liakhovska A., Smaglyuk V. et al. EMH-kharakterystyka zhuvalnykh miaziv u patsiientiv iz bruksyzmom [emg-characteristics of masticatory muscles in subjects with bruxism]. Ukrainian Dental Almanac. 2020;(1): 43-49. (In Ukrainian).
17. Smagliuk L.V., Lyakhovska A.V. Masticatory muscle activity in individuals with temporomandibular disorder. Acta Balneologica. 2021; 3(164): 179-182.
18. Smaglyuk L., Liakhovska A., Kulish N. Features related to EMG-activity of masticatory muscles in patients with unilateral posterior crossbite: a cross-sectional study. Orthodontic Forum. 2021; 17(2): 144-121. doi:10.5114/for.2021.107531.
19. Smaglyuk L.V., Liakhovska A.V. EMG-characteristic of masticatory muscles in patients with class II malocclusion and temporomandibular disorders. Wiad Lek. 2019;72(5):1043-1047.
20. Smaglyuk L., Solovei K., Liakhovska A. Characteristics of emg-activity of masticatory muscles in functional treatment of patients at retention stage. Georgian Med News. 2018;(274):42-47.
21. Smaglyuk L., Liakhovska A., Smaglyuk V. et al. EMG-activity of muscles of the cranio-mandibular system during functions of the dento-faci al region. World of Medicine and Biology. 2020; 1 (71): 128-132.
22. Yatsenko P.I., Rybalov O.V., Yatsenko O.I. et al. Electromyographic characteristics of temporal and masticatory muscles in patients with compression-dislocation dysfunction of temporomandibular joints of different severity. Wiad Lek. 2018; 71(3): 663-670.

23. Novikov V.M., Yasenko P.I., Rybalov O.V. Miofastsialnaya simptomatika v klinike kompressorno-dislokatsionnoy disfunktsii v visochno-nizhnechelyustnogo sustava [Myofascial symptoms in clinic compression of dislocation of the temporomandibular joint dysfanction]. Ukrainian dental almanac. 2017; 1: 32-36. (In Russian).
24. Rybalov O.V., Yatsenko P.I., Yatsenko O.I. et al. Hypermobility of the articular heads of the temporomandibular joint: pathology or variant of the norm? Wiad Lek. 2019;72 (10): 1883-1889.
25. Yatsenko P.I., Yatsenko O.I., Rybalov O.V. et al. Differential and diagnostic criteria for hypermobility of the articular heads of the mandible, muscle and joint contracture and compression-dislocation dysfunction of temporomandibular joint (according to the data of tmj zonography). World of medicine and biology. 2019; 2(64): 112-114.
26. Kostiuk T., Koval I., Tyshko D., Koval M. Analisis diagnostics and newest pathogenesis aspects of temporomandibular dysfunction (review). Georg Med News. 2018; 7–8 (280–281): 44–48.
27. Kostiuk T.M. Elektromiohrafichne doslidzhennia roboty zhuvalnykh miaziv pry dysfunktsiinykh rozladakh skronevo-nyzhnoshchelepnoho suhloba [Electromyographic study of masticatory muscles in dysfunctional disorders of temporomandibular joint]. Aktualni problemy suchasnoi medytsyny. 2018; 16 (3): 212–8 (In Ukrainian).
28. Kostiuk T.M. Mahnitno-rezonansna tomohrafiia v diahnostytsi miazovo-suhlobovoi dysfunktsii skronevo-nyzhnoshchelepnoho suhloba. [Magnetic resonance imaging in the diagnosis of musculoskeletal dysfunction of the temporomandibular joint]. Suchasna stomatolohiia. 2020; 1 (112): 108–111.(in Ukrainian).
29. Novikov V., Korostashova M., Dodatko V. et al. The role of magnetic resonance research in the treatment of dysfunction of the temporomandibular joint. Ukrainian Dental Almanac. 2021; 2: 59-63. (In Ukrainian).
30. Smagliuk L.V., Lyakhovska A.V., Kulish N.V. Stan skronevo-nyzhnoshchelepnoho suhloba u patsiientiv z perekhresnym prykusom zi zmishchenniam nyzhnoi shchelepy za danymy renthenolohichnykh metodiv doslidzhennia [The state of the temporomandibular joint in patients with a cross bite and mandible displacement based on x-ray methods]. World of medicine and biology. 2021; 1(75): 152-156.
31. Kostiuk T.M., Kaniura O.A. Rannia diahnostyka miazovo-suhlobovoi dysfunktsii skronevo-nyzhnoshchelepnoho suhlobu za dopomohoiu kompiuternoi systemy-dodatku [Early diagnosis of musculo-articular dysfunction of the temporomandibular joint using a computer system-application]. Ukrainskyi naukovo-medychnyi molodizhnyi zhurnal. 2019; 4 (112): 6–11. (In Ukrainian).
32. Kostiuk T.M., Kaniura A., Shinchukovskiy I. et al. Reseach of the chewing muscles in dysfunction disorders of TMG. Neurophisiology. 2020; 1 (52): 50–53.

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

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Received: 08.07.2022
Accepted: 12.12.2022

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

PROCEDURAL STATUS OF PERSONS SUFFERING FROM MENTAL DISORDERS: INTERNATIONAL STANDARDS

DOI: 10.36740/WLek202302127

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ABSTRACT

The aim: To identify, group and analyze international standards in terms of regulating the criminal procedural status of persons suffering from mental disorders.
Materials and methods: In preparing the article, the following issues were worked out: the provisions of international legal acts; legal positions of the European Court of Human Rights regarding the observance of the right to a fair trial of persons suffering from mental disorders; scientific research to ensure the rights of persons suffering from mental disorders in criminal proceedings. The methodological basis of the research is dialectical, comparative-legal, systemic-structural, analytical, synthetic, complex research methods.
Conclusions: Universal international standards of human rights retain their validity for persons suffering from mental disorders; today, a clear synchronization of universal (global) and European standards for determining the procedural status of persons suffering from mental disorders is being followed; the most justified is a differentiated approach to solving the issue of personal participation of a person suffering from a mental disorder in a court hearing.

KEY WORDS: mental health, international standards, mental disorder, ECHR practice

Wiad Lek. 2023;76(2):433-438

INTRODUCTION

In order to ensure the effectiveness of criminal proceedings, the legislation of European states provides for the possibility of limiting the rights and freedoms of persons participating in the criminal process, which requires the existence of a system of procedural guarantees that ensure the legality and justification of such restrictions and the proportionality of interference with human rights and freedoms. At the same time, the set of rights and responsibilities, embodied in the procedural status of a participant in criminal proceedings, reflects the community's vision of a reasonable balance between the public reaction to socially dangerous encroachments and the observance of the rights and freedoms of a person in the process of implementing such a reaction. The question of finding a balance, formalized in the procedural status, is extremely acute in relation to persons who, due to objective reasons, are unable to fully realize the appropriate range of procedural opportunities or fulfill the obligations provided for by law, in particular, persons suffering from mental disorders. In this perspective, international standards that establish the procedural status of such persons, in particular, in criminal procedural relations, have significant research potential.

THE AIM

The aim of this work is to identify, group and analyze international standards in terms of regulating the criminal procedural status of persons suffering from mental disorders.

MATERIALS AND METHODS

During the preparation of the article, the following issues were worked out: the provisions of international legal acts of the universal and regional level regarding the regulation of the procedural status of persons suffering from mental disorders; the legal positions of the European Court of Human Rights (hereinafter referred to as the ECHR) regarding the observance of the right to a fair trial of persons suffering from mental disorders (we analyzed 9 decisions in which the ECHR highlighted this issue in the context of the requirements of Article 6 of The European Convention on Human Rights (hereinafter referred to as the Convention)); scientific research on ensuring the rights of persons suffering from mental disorders in criminal proceedings.

A complex of general scientific and special methods of cognition (dialectical, comparative-legal, system-

ic-structural, analytical, synthetic, complex research methods) was used in the research process.

REVIEW AND DISCUSSION

Depending on a number of essential criteria, international norms on one or another issue can be universal or occupy a regional level. An indicator of the level of universality of the provisions is, on the one hand, the subject of creation (for example, the United Nations Organization (hereinafter referred to as the UN) or the Council of Europe), on the other hand, the territorial scope (for example, Europe, the states of North and South America, or Africa). This criterion will be used to structure the research material.

UNIVERSAL LEVEL

The expediency of considering international standards of a universal level within the framework of a narrow direction – the procedural status of persons suffering from mental disorders is due to the fact that universal standards retain their validity in relation to the specified persons as well, as evidenced by the following provisions of:

a) general international legal acts of a universal level. For example, Art. 1 of the Universal Declaration of Human Rights, adopted by resolution 217 A (III) of the UN General Assembly of December 10, 1948, declares everyone equal in their dignity and rights from birth, and according to Art. 2 of the same act, a full set of rights and freedoms is granted to every person, regardless of race, color, sex, language, religion, political or other beliefs, national or social origin, property or *other status* (italics added) [1];

b) normative legal acts of a universal level, specially devoted to the regulation of proper guarantees of persons suffering from mental disorders. For example, the Declaration on the Rights of Mentally Retarded Persons, adopted by resolution 2856 (XXVI) of the UN General Assembly of December 20, 1971, defines that a mentally retarded person has, as far as possible, the same rights as other people (clause 1) [2].A similar rule is reflected in the Declaration on the Rights of the Disabled, adopted by resolution 3447 (XXX) of the UN General Assembly of December 9, 1975 (clause 2) (hereinafter referred to as the Declaration on the Rights of the Disabled) [3]. A more specific rule is contained in the Principles for the Protection of Persons with Mental Illness and Improvement of Psychiatric Care, which is an appendix to Resolution 46/119 "Protection of Persons with Mental Illness and the Improvement of Mental Health Care", adopted by the UN General Assembly on February 18, 1992 (hereinafter referred to as the Principles for the Protection): every person with a mental illness shall have the right to exercise all civil, political, economic, social and cultural rights as recognized in the Universal Declaration of Human Rights, the International Covenant on Economic, Social and Cultural Rights, the International Covenant on Civil and Political Rights, and in other relevant instruments (the Declaration on the Rights of Disabled, the Code of Principles for the Protection of All Persons Subject to Detention or Imprisonment in any Way, adopted by resolution 43/173 of the UN General Assembly of December 9, 1988 (hereinafter referred to the Code of Principles for the Protection of Prisoners (clause 5 of the principle 1) [4]).

The original provisions that laid the foundation for the international understanding of procedural guarantees of persons suffering from mental disorders are contained in the Declaration on the Rights of Mentally Retarded Persons. In our opinion, such guarantees can be conditionally divided into several groups:

1) *representative direction:* provides for the right to use the qualified services of a guardian in cases where it is necessary to protect the well-being and interests of a mentally retarded person (clause 5) [2]. Despite the fact that this provision does not directly indicate the legal nature of guardianship, the terms "qualified services", "to protect the well-being and interests" used in it give reasons to believe that it was in this way that the foundations were laid at the international level for legal, as well as professional legal representation of a person suffering from a mental disorder;

2) *judicial direction:* in the case of prosecution for committing any act, the right to the proper implementation of legality is assumed, which would fully take into account the degree of mental development (clause 6) [2]. In the given guarantee, the intention to differentiate the generally accepted procedures of judicial proceedings by adapting them to the peculiarities of the mental health of the person in respect of whom it is carried out is traced;

3) *limiting direction:* provided that, as a result of a significant disability, a mentally retarded person is not able to properly exercise all his rights or there is a need to limit or cancel some or all rights, the procedure must provide for proper procedural guarantees against any abuses (justified by a qualified assessment by specialists of the person's socially useful capabilities, periodic review, possibility of appeal to higher authorities) (clause 7) [2]. This provision has the most pronounced procedural nature and lays the foundations for international standards regarding expert assessment of a person's mental state, as well as periodic and appellate reviews of the reasonableness of limiting a person's rights.

In addition, international standards for the procedural status of persons suffering from mental disorders are detailed in the Principles of Protection. In order to facilitate their perception, we distinguish them according to the objective criterion (the key issue for the solution of which

they are fixed).

DETERMINING THE VALIDITY OF PLACEMENT IN A PSYCHIATRIC INSTITUTION

Placement in a psychiatric institution is allowed by decision of a court or other body authorized by national law on the basis of competent and independent medical consultations (clause 3 of principle 20 of the Principles of Protection). The adoption of such a decision is preceded by the determination of a mental illness in accordance with international standards (clause 1 of principle 4 of the Principles of Protection), carried out during a medical examination, which can be compulsory only if it is carried out in accordance with domestic legislation (principle 5 of the Principles of Protection). The resolution of the issue of forced hospitalization is accompanied by the right of a person during any proceedings to provide an independent psychiatric report or other oral or written evidence, appropriate and admissible for the resolution of the issue by the competent authority (clause 3 of principle 18 of the Principles of Protection). The detailing of the requirements for the subject composition is also interesting: a) assessment of the legality of forced hospitalization must be carried out by a judicial or other independent and impartial body formed and acting on the basis of domestic legislation; b) assistance from one or more qualified and independent psychiatric workers must be provided during the rendering of the decision (clause 1 of principle 17 of the Principles of Protection).

DUE PROCESS OF LAW FOR A PERSON SUFFERING FROM A MENTAL DISORDER

A person suffering from a mental disorder is guaranteed the free choice of a lawyer for representation, in particular, during the appeal of decisions (clause 1 of principle 18 of the Principles of Protection). It is significant that the international understanding of the institution of representation has undergone development in the direction of distinguishing professional legal (lawyer) and personal representation (personal representative). In addition, the procedural guarantees can also include those enshrined in principle 18 of the Principles of Protection, in particular: a) the right to the assistance of an interpreter (clause 2); b) the right to receive documents that are submitted for review by a competent authority, except for cases when familiarization with them may cause significant harm to a person's health or pose a danger to other persons (clause 4); c) the right to personal participation during any proceedings, in particular, to express arguments (clause 5); d) factors that must be taken into account when determining the mode (open or closed) of conducting the review: the person's opinion, respect for his personal sphere, as well as the sphere of other persons, the possibility of causing significant damage to the health of the person or other persons (clause 7); e) the right to review and receive a final decision, reasoned in writing (clause 8) [4]. The Principles of Protection have also established procedural guarantees that accompany a person after a decision on the use of forced treatment: the possibility to appeal the decision on hospitalization to a court of higher instance (clause 7 of principle 17), as well as to initiate a review of the question of expediency before an authorized body after reasonable intervals of time continuation of forced treatment (clause 4 of principle 17) [4].

EUROPEAN LEVEL

The scientific understanding of international standards at the European level in the context of the researched topic was repeatedly addressed as individual authors of this article [5,6], as well as other domestic scientists [7,8]. Certain aspects of this topic were the subject of scientific interest of foreign researchers, in particular, of Peter Verbeke, Gert Vermeulen, Tom Vander Beken, Michaël Meysman [9], Stephen J. Morse [10], Peter Bartlett, Oliver Lewis, Oliver Thorold [11] etc. However, the issue of finding ways to introduce European standards into domestic legislation does not lose its relevance. The normative basis of the standards of the procedural status of persons suffering from mental disorders at the European level are the provisions of the ECHR and the decision of the ECHR regarding the interpretation of Articles 3, 5, 6, 8 of the ECHR, the Convention on Human Rights and Biomedicine in the Field of Biomedical Research dated April 4, 1997, Recommendations of the Parliamentary Assembly of the Council of Europe No. 818 (1977) on the situation with mental illnesses of October 8, 1977, as well as Recommendations of the Committee of Ministers of the Council of Europe to the participating states: 1) No. R (83)2 regarding the legal protection of persons suffering from mental disorders who are forcibly kept as patients; 2) No. R (99)4 on the principles relating to the legal protection of incapacitated adults; 3) No. Rec (2004)10 on the protection of human rights and dignity of persons with mental disorders (hereinafter referred as the Recommendation No. Rec (2004)10), etc.

A unified statement of European standards can be found in the norms of Recommendation No. Rec (2004)10), among which the European interpretation of universal standards can be traced. Thus, a significant array of rules actually duplicates the global understanding of proper legal procedures for persons suffering from mental disorders. For example, the following norms differ in full compliance with universal standards: establishment of a mental disorder based on international medical criteria (clause 1, article 2),

differentiation of a representative and a personal defender (clause 3, article 2), prohibition of substantial limitation of the convention rights of persons suffering are distinguished by full compliance with universal standards for mental disorders (clause 2, article 4), requirements for the subject of consideration of the issue of compulsory treatment (article 21, clause 1 of article 34), procedural features of the appeal and review of the feasibility of continuing compulsory treatment (article 25, clause 1 of article 34) and others.

In addition, a significant volume of standards was developed by the ECHR within the framework of the interpretation and application of the norms of the Criminal Procedure Code, which was already partially considered by the authors of this article together with Vasyl Y. Tatsiy [7], Olena A. Leiba [5]. Developing the results of previous studies, we note that it seems most interesting to consider the issue of personal participation of a person suffering from a mental disorder in court proceedings through the prism of European standards. Therefore, in favor of the mandatory participation of a person suffering from a mental disorder, the following arguments supported by the positions of the ECHR can be given:

1) direct participation in the trial is one of the key components of the right to a fair trial (Article 6 of the Convention). The European Court of Human Rights has repeatedly emphasized that the essence of the right to a fair trial derives from the general principle regarding the right of a person accused of committing a criminal offense to be present and effectively participate in the court proceedings (§ 100 of the ECHR decision in the case of "Proshkin v. Russia" [12], § 106 of the decision of the ECHR in the case of "Romanov v. Russia" [13]). With regard to persons suffering from mental disorders, the positions of the ECHR follow the priority of ensuring their personal participation in court proceedings [7], which the ECHR emphasized particularly clearly in § 102 of the decision in the case of "Proshkin v. Russia" [14];

2) although one of the forms of exercising the right to personal participation is the involvement of a defender in the proceedings, under certain circumstances representation does not allow effective compensation for the absence of a person and the inability to express one's arguments (§ 104 of the ECHR decision in the case of "Proshkin v. Russia" [14]), which, in turn, prevents direct evaluation of evidence by the court (§ 112 of the ECHR decision in the case of "Romanov v. Russia" [15]);

3) a person suffering from a mental disorder plays a dual role in the proceedings: he is an interested party and at the same time the main subject of the judicial investigation, and therefore: a) the direct or indirect participation of such a person is inseparable from a "fair and lawful procedure" (§ 62 of the ECHR decision in the case of "Zagidulina v. Russia") [14]; b) the direct participation of a person allows the judge to form his own opinion about his mental state based on a short visual contact and, if possible, an interrogation (§ 72, 73 of the ECHR decision in the case of "Shtukaturov v. Russia") [15].

At the same time, the expediency of limiting the right of a person suffering from a mental disorder to directly participate in a court hearing follows from:

1) the possibility of exercising such a right not only personally, but also through a representative office. According to the established practice of the ECHR, the person in respect of whom the question of treatment is decided must have access to the court in person or through a certain form of representation (§ 65 of the decision of the ECHR in the case of "Vaudelle v. France" [16], § 39 of decision of the ECHR in the case of "Gorshkov v. Ukraine" [17], § 60, 61 of the decision of the ECHR in the case of "Winterwerp v. the Netherlands" [18]);

2) recognition by the ECHR as legitimate of the restriction of such a right to ensure the proper administration of justice, protection of the health of the relevant person, etc. (§ 68 of the decision of the ECHR in the case of "Shtukaturov v. Russia") [17], as well as the range of circumstances that are taken into account when assessing the legality of such a restriction. Thus, in the case of "Romanov v. Russia", the failure to involve the applicant in the trial was recognized as a violation of the right to a fair trial, taking into account the fact that the person did not demonstrate unbalanced behavior and there were no such manifestations of his physical or mental state that would prevent him from appearing to court (§ 109) [15]. A similar approach was used in the case of "Anatoly Rudenko v. Ukraine", where the ECHR drew attention to the absence of "no proper reason for preventing the applicant from participating in the proceedings", as well as the lack of evidence to consider the mental state of the applicant "such that it does not allow him to participate effectively in court sessions" (§ 114) [19].

Therefore, the analysis of the given positions of the ECHR makes it possible to come to the conclusion that the ECHR recognizes as forms of realization of the conventional right to a fair trial both the personal participation in the trial of a person suffering from mental disorders and the alternative participation of his or her representatives [20]. In our opinion, both of the extreme positions (which can be conventionally called the "rule of personal participation" and the "rule of proceedings in the absence of a person") do not adequately reflect the actual level of legal personality of a person, which is determined, first of all, by the nature and degree of mental disorder. In view of the above, a differentiated approach has the greatest potential effectiveness. Its essence consists in solving the question of the possibility of a person's participation in court proceedings depending on the actual ability to exercise procedural powers and correct behavior in court.

Scientific interest in ensuring the rights of persons suffering from mental disorders in criminal proceedings

is due to their special vulnerability, which has repeatedly been the subject of scientific discussions. Thus, the issue of normative regulation of the procedural status of persons suffering from mental disorders in the context of international standards and precedent practice of the ECHR has already been investigated by individual authors of this article [6] together with Vasyl Y. Tatsiy [7], Olena A. Leiba [5]. Other domestic scientists also turned to the scientific understanding of international standards at the European level in the context of the researched topic [8-10, 21]. Certain aspects of this topic were the subject of scientific interest of foreign researchers, in particular, Peter Verbeke, Gert Vermeulen, Tom Vander Beken, Michaël Meysman [11], Stephen J. Morse [12], Peter Bartlett, Oliver Lewis, Oliver Thorold [13], etc. David Latham's report "Mental health and fair trial" provided recommendations for protecting the rights of people suffering from mental disorders [22]. Despite the considerable amount of work, a number of issues remain debatable and require further scientific research, in particular, in terms of improving the normative regulation of the procedural status of persons suffering from mental disorders.

CONCLUSIONS

1. The expediency of considering international standards of the universal level within the framework of a narrow direction – the procedural status of persons suffering from mental disorders is due to the fact that the universal standards retain their validity in relation to the specified persons as well, as evidenced by the provisions of both general international legal acts of the universal level and regulatory legal acts of a universal level, specially devoted to the regulation of adequate guarantees for persons suffering from mental disorders.
2. The initial provisions that laid the foundation for the international understanding of procedural guarantees for persons suffering from mental disorders can be grouped into separate directions, in particular: (a) representative direction; (b) judicial direction; (c) limiting direction; (d) the direction of determining the grounds for placement in a psychiatric institution; the direction of determining the proper legal procedure for a person suffering from a mental disorder.
3. Synchronization of universal (global) and European standards for determining the procedural status of persons suffering from mental disorders is being monitored today. Thus, the provisions of European international acts regarding: establishment of a mental disorder on the basis of international medical criteria, differentiation of a representative and a personal defender, prohibition of substantial limitation of the convention rights of persons suffering from mental disorders, requirements for the subject of consideration of the issue of forced treatment, procedural features of reviewing the expediency of continuing forced treatment.
4. The most ambiguous issue today remains the issue of the personal participation of a person suffering from a mental disorder in court proceedings through the prism of European standards. Given that the actual level of legal personality of a person is determined, first of all, by the nature and degree of mental disorder, a differentiated approach is the most justified. Its essence consists in solving the question of the possibility of a person's participation in court proceedings depending on the actual ability to exercise procedural powers and correct behavior in court.

REFERENCES

1. The Universal Declaration of Human Rights, adopted and proclaimed in resolution 217 A (III) of the UN General Assembly of December 10, 1948. https://zakon.rada.gov.ua/laws/show/995_015#Text [date access 26.09.2022]
2. Declaration on the Rights of Mentally Retarded Persons, adopted by General Assembly resolution 2856 (XXVI), December 20, 1971. https://zakon.rada.gov.ua/laws/show/995_119#Text [date access 26.09.2022]
3. Declaration on the Rights of Disabled Persons, adopted by General Assembly resolution 3447 (XXX), December 9, 1975. https://zakon.rada.gov.ua/laws/show/995_117#Text [date access 26.09.2022]
4. Resolution 46/119 "Protection of persons with mental illness and improvement of mental health care", adopted by the UN General Assembly on February 18, 1992 on the report of the Third Committee (A/46/721). https://zakon.rada.gov.ua/laws/show/995_905#Text [date access 26.09.2022]
5. Tyshchenko O., Leiba O., Titko A. European standards of respect for human rights in the application of compulsory medical measures in criminal proceedings. Wiadomości Lekarskie. 2019:72(12):2445-2450.
6. Tatsiy V., Tyshchenko O., Titko I. Mental health of a person as a criterion of personal participation in the trial during criminal proceedings. Wiadomości Lekarskie. 2020;72(12):2737-2742.
7. Teteriatnyk H. Protection of the rights and legitimate interests of unconvicted and limitedly convicted persons at the stage of pre-trial investigation in the criminal process of Ukraine: thesis for obtaining the scientific degree of Candidate of Legal Sciences. Classic private University. Zaporizhzhia. 2012, 226p.

8. Turenko D. The issue of legal regulation of the use of coercive measures of a medical nature in international legal acts and the legislation of certain foreign countries. Law and security. 2021;1(80):173-179.
9. Verbeke P., Vermeulen G., Beken T. et al. Protecting the fair trial rights of mentally disordered defendants in criminal proceedings: exploring the need for further EU action. International journal of law and psychiatry. 2015;41:67–75.
10. Morse S.J. Mental disorder and Criminal law. The journal of Criminal law & criminology. 2011;101(3):885–968.
11. Bartlett P., Lewis O., Thorold O. Mental Disability and the European Convention on Human Rights. 2007, 90p.
12. Case of Proshkin v. Russia, application no. 28869/03, judgment of 7 February 2012. http://hudoc.echr.coe.int/eng?i=001-108961. [date access 26.09.2022]
13. Case of Romanov v. Russia, application no. 63993/00, judgment of 20 October 2005. http://hudoc.echr.coe.int/eng?i=001-70685. [date access 26.09.2022]
14. Case of Zagidulina v. Russia, application no. 11737/06, judgment of 2 May 2013. http://hudoc.echr.coe.int/rus?i=001-119043. [date access 26.09.2022]
15. Case of Shtukaturov v. Russia, application no. 4400905, judgment of 27 March 2008. http://hudoc.echr.coe.int/eng?i=001-85611. [date access 26.09.2022]
16. Case of Vaudelle v. France, application no. 35683/97, judgment of 30 January 2001. https://www.legal-tools.org/doc/8a5ca1/pdf . [date access 26.09.2022]
17. Case of Gorshkov v. Ukraine, application no. 67531/01, judgment of 8 November 2005. http://hudoc.echr.coe.int/eng?i=001-70855. [date access 26.09.2022]
18. Case of Winterwerp v. the Netherlands, application no. 6301/73, judgment of 24 October 1979. http://hudoc.echr.coe.int/eng?i=001-57597. [date access 26.09.2022]
19. Case of Anatoliy Rudenko v. Ukraine, application no. 50264/08, judgment of 17 April 2014. http://hudoc.echr.coe.int/eng?i=001-17185. 3. [date access 26.09.2022]
20. Tyshchenko O. The right of a person suffering from mental disorders to personal participation in court proceedings: key positions of the ECHR. Criminal justice of modern Ukraine: challenges and prospects. Odesa. 2021, 194p.
21. Lapkin A., Yevtieieva D., Karelin V. European standards of respect for human rights in the application of compulsory medical measures in criminal proceedings. Wiadomości Lekarskie. 2019;72(12):2479-2484.
22. Mental health and fair trial. A Report by JUSTICE Chair of the Committee Sir David Latham. 2017, 114p. https://files.justice.org.uk/wp-content/uploads/2017/11/06170615/JUSTICE-Mental-Health-and-Fair-Trial-Report-2.pdf [date access 26.09.2022]

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

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Received: 19.09.2022
Accepted: 23.01.2023

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

REVIEW ARTICLE

JUNGLES OF UKRAINIAN LEGISLATION ON EGG DONATION

DOI: 10.36740/WLek202302128

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ABSTRACT

The aim: To study the current regulation on egg donation in Ukraine as one of the most attractive destinations for reproductive tourism, establish the current loopholes in the legal framework to be addressed when amending Ukrainian legal rules.

Materials and methods: The article is based on studying international and regional legal acts, jurisprudence of European Court of Human rights, pieces of national Ukrainian legislation, law drafts submitted to Ukrainian parliament and legal doctrine. The methodology of the article includes dialectical, comparative method and the method of systematic and structural analysis.

Conclusions: Existing legal framework in Ukraine has some serious lacunas that can result in violation of rights and interests of donors and of the children. Firstly, the state does not keep the unique state register of donors. Secondly, there are no rules on compensation for egg donor. Lastly, the current Ukrainian legislation does not contain provisions ensuring protection of the child`s right to know about one`s genetic origin, and thus to obtain the identifying information about the donor. All these issues should be addressed in order to establish a fair balance between the rights and the interests of donors, recipients, the child and the society.

KEY WORDS: reproductive rights; egg donation; assisted human reproduction; right to the information on one`s genetic origin

Wiad Lek. 2023;76(2):439-444

INTRODUCTION

Egg donation is a method of assisted human reproductive technologies, under which a healthy woman donates her egg to another woman, who does not have healthy eggs. In accordance with the latest available official statistics in Ukraine there were 2799 cycles of egg donation in Ukraine in 2020 [1] and the size of the egg donation market was valued in 2020 as over 1 billion with potential almost 20% growth by 2027 [2]. For some couples egg donation can be the only means to procreate, that is why this method of infertility treatment is very popular. In the meantime the method itself can be harmful for the donor and thus, if it is allowed by the legislation, it is very important to provide a legal regulation, ensuring that the rights and interests of donors and recipients are balanced and protected in a due way.

Legislation on egg donation vary in different countries and in a lot of jurisdictions egg donation is forbidden. For example, in Switzerland egg donation was long time prohibited under Article 4 of Federal Act on Medically Assisted Reproduction. The reasoning behind this prohibition was to follow the Roman principle *"Mater Semper Certa Est"* (the mother is always certain), meaning that the motherhood is established by the fact of birth and the motherhood of the woman that gave birth to the child can not be challenged [3]. Egg donation can result in situation, when the child is genetically connected with one woman, gestated by another woman (the surrogate) and brought up by the third woman, and the Swiss legislator has specifically opted for avoidance of such situation. Just recently in September 2022, the Swiss Senate voted in favour of allowing the egg donation and now the government has the obligation to create the legal mechanism for egg donation [4]. It is interesting that Swiss legislation contained a discriminative provision towards women, allowing only sperm donation before recent developments. Egg donation can be not allowed also due to religious reasons. For example, the Catholic Instruction Dignitas Personae on Certain Bioethical Questions [5], does not specifically ban the egg donation, but it provides for three fundamental goods for the treatment of infertility, that exclude the egg donation. Egg donation is also prohibited by Al-Azhar Fatwa (being one of the sources for creation of legislation in Islamic countries), because "it involves the fertilization of a woman's egg by the semen of a stranger" and "bears resemblance with adultery and it also leads to the confusion of lineages" [6].

In a number of jurisdictions egg donation is allowed: this is the case for Ukraine, the Kingdom of Spain, the United Kingdom of Great Britain and Northern Ireland,

the United States of America, the Czech Republic etc. The legal rules provide for different types of egg donation, e.g. it can either be anonymous (when donor and recipient do not know each other) and non-anonymous (when the donor gives the egg to the recipient she knows. This can be donation of eggs to close relatives or friends etc.). The egg donor can undergo a cycle of donation with the aim to donate the eggs or can give the "spare" eggs that were left after the donor`s own cycle of in vitro fertilisation treatment. In the latter case it is called the "egg sharing". The donor can donate the eggs for free (altruistic donation) or can do it on financial basis (commercial egg donation).

Before the brutal war, started by the Russian Federation against Ukraine, Ukraine was a popular touristic destination for infertility treatment, as the country has very good geographical location, excellent medical services with affordable prices. Ukraine allows egg donation, but its legal rules on egg donation have some loopholes which will be addressed in the article. Ukrainian legislation on egg donation is a fertile ground for certain improvement.

THE AIM

This research aims to study the current regulation on egg donation in Ukraine as one of the most attractive destinations for reproductive tourism, establish the current loopholes in the legal framework to be addressed when amending Ukrainian legal rules.

MATERIALS AND METHODS

The article is based on studying international and regional legal acts, jurisprudence of European Court of Human Rights, pieces of national Ukrainian legislation, law drafts submitted to Ukrainian parliament and legal doctrine. The author has chosen the methods taking into account the aim of this research. Dialectical method was used to study the gaps of legal regulation of egg donation in connection with the need to balance private and public interests. Comparative method was used to analyse the rules on egg donation in the other jurisdictions. Method of systematic and structural analysis was used to formulate the loopholes of the current Ukrainian legal framework on egg donation.

REVIEW AND DISCUSSION

Regulation of egg donation is conducted at different levels. For instance, at the *international level* the right to life and right to health are protected by such universal international agreements and covenants as International Covenant on Civil and Political Rights [7], International Covenant on Economic, Social and Cultural Rights [8], Universal Declaration of Human Rights [9]. At *regional (European) level* the regulation of egg donation is conducted by the Convention for the Protection of Human Rights and Fundamental Freedoms (in particular – by Article 8 of this Convention, protecting the right to respect for private and family life) [10].

Given that the practice of European Court of Human Rights (hereinafter – ECtHR) is a source of law in Ukraine under Article 17 of the Law of Ukraine "On Execution of Judgements and Application of Practice of European Court of Human Rights" [12], Ukrainian regulation on egg donation should also comply with the practice of ECtHR. The ECtHR has issued a decision on egg donation regulation in the case "S.H. and others v. Austria" [13]. In this case two married couples alleged the violation of their rights under Article 8 and Article 14 read in conjunction of Article 8 of the Convention for the Protection of Human Rights and Fundamental Freedoms, because the provision of the Austrian Artificial Procreation Act prohibited ova donation and sperm donation for in vitro fertilisation, which was the only medical technique that could be used by the applicants to conceive children. In particular, the third applicant was completely infertile; she suffered from agonadism, meaning that she could not produce her own ova. Her husband, on the contrary, was fertile and the only way for this couple to conceive would be to implant into her uterus the embryo conceived by the sperm of her husband (the fourth applicant) and the donor`s egg. Such procedure in Austria is prohibited by the Artificial Procreation Act with the main aim to prevent extended parental relations, when the child, conceived with the egg of the donor, will have two mothers (genetic one and the one who carried the child) and also to protect the women from being exploited. In its decision, the ECtHR has noted that there is no European consensus on whether to allow the egg donation for in vitro fertilisation or not; there is a big margin of appreciation granted to the European legislators in the matter. Austria has not overstepped the said margin of appreciation in respect of prohibition of ovum donation for in vitro fertilisation and the ECtHR has not found a violation of Article 8 of the Convention for the Protection of Human Rights and Fundamental Freedoms and ruled that it is not necessary to examine the application also under Article 14 of the Convention read in conjunction with Article 8 [13].

The national legal rules on egg donation in Ukraine can be found in different laws and sub-laws: the Civil Code of Ukraine [14], Family Code of Ukraine [15], the Law of Ukraine "Fundamentals of the Legislation of

Ukraine on Healthcare" [16], the order of the Ministry of Healthcare of Ukraine "On Adoption of the Order of Application of Assisted Reproductive Technologies in Ukraine" (hereinafter – the ART Order) [17] and other acts. Part 3 of Art. 123 of the Family Code of Ukraine foresees that the married couple shall be recognized as the parents of the child, born by the wife after the transfer of embryo, conceived by her husband and other woman as a result of application of assisted human reproduction technologies. The Civil Code of Ukraine provides for a right of woman and man who reached the age of majority to conduct medical programs on assisted human reproduction if they have medical condition in accordance with the order and conditions established by the legislation (Part 7 of Art. 281). Article 290 of the Civil Code of Ukraine stipulates that person who has reached the age of majority and has full civil capacity can donate gametes. Donation of gametes shall be made in accordance with the law. Following Art. 48 of the Law of Ukraine 'Fundamentals of the Legislation of Ukraine on Healthcare', application of artificial conceiving and implantation of the embryo is conducted in accordance with the conditions and the order, established by the central body of executive power, that is in charge of forming of the state policy in the sphere of healthcare, upon the medical indications of the woman that reached the age of majority, upon condition of written approval of the married couple, donor anonymity and medical secret non-disclosure.

The ART Order regulates mostly medical rules on egg donation. The ART Order allows either for egg donation (anonymous and donation by close relatives) and for egg sharing, and it lists the requirements for the egg donor. The egg donor should: be of certain age (from 18 to 36 years old); have her own healthy child; not have negative phenotypic manifestations; have good state of somatic health; not have contraindications, hereditary diseases, drug and/or alcohol addiction. Egg donorship, as a method of treatment, is allowed only upon medical indications, such as: the absence of eggs, risk of transfer of hereditary diseases, unsuccessful attempts of in vitro fertilization with a low quality of embryos and unsatisfactory response of the ovaries to controlled stimulation and repeated receipt of low-quality oocytes and embryos.

The ART Order has norms that tend to protect the egg donor, e.g. it limits the number of egg donation cycles to eight per donor. There is a provision that the clinics should provide donor with medical treatment during thirty days after aspiration of oocytes in case of complication. Further, the text of donor`s informed consent shows that the egg donor should be provided with all the information on potential complications after donation of eggs (including bleeding, inflammation, injury to adjacent organs, ovarian hyperstimulation syndrome, formation of ovarian retention cysts, allergic reactions and other side effects of medicines etc.). The donor is also provided with the information that in some cases hospitalization and surgery may be needed.

In the end of 2021 and at the beginning of 2022 three draft laws on assisted human reproduction were submitted to Ukrainian Parliament and all of them regulate egg donation. *The first draft of Law of Ukraine "On Assisted Human Reproduction"* No. 6475 dated December 12, 2021 was submitted by the Cabinet of Ministers of Ukraine [18] and it foresees the definitions of the terms "egg donation" and "donor of gametes" and lays down mandatory genetic screening of gametes` donors. Article 19 of this draft stipulates that the list of medical contraindications for the gametes` donors and the order of donor`s medical check should be approved by the Ministry of Health Care of Ukraine; the Ministry of Health Care of Ukraine should also adopt the requirements and the order of donation of gametes, order of their storage and recording of the donors. Article 19 also lays down the anonymity of egg`s donor and forbids the donors to have parental rights with regard to the intended child as well as to try to find information about the child and the personal data of recipients. In the meantime, it is allowed for the donor to give the clinic informed consent to disclose her personal data when it is necessary for the interests or for the health of the future child (e.g. the child`s treatment of hereditary diseases). Article 20 of the same draft ensures protection of the egg donor by the state, it obliges the clinic to inform the donor on her rights and duties and the order of conduction of assisted human reproduction treatment. Article 21 states the age limits for egg donor – from 18 to 36. Article 22 of the said draft foresees that donation of eggs can be conducted on commercial and non-commercial basis. The compensation to the donor should be conducted in account of the recipient. This draft law also entitles the Cabinet of Ministers of Ukraine to adopt the order of eggs transfer within Ukraine as well as in and out of Ukraine.

Alternative law draft was filed by the Member of Ukrainian Parliament, Oleksandr Danutsa. *This draft of Law of Ukraine "On Application of Assisted Reproductive Technologies" No. 6475-1 dated January 11, 2022* [19] also addresses the issues on egg donation. This law draft foresees the definition of "egg donation", "donor of gametes", "oocytes". Chapter IV of the law draft specifically deals with the donation of gametes and lays down the following rules. Egg donation can be done only on the basis of donor`s informed consent. Any medical contraindications for donors and the order of

their medical check should be adopted by the Ministry of Health Care of Ukraine. The draft excludes the donor`s parental rights to the future child and even forbids the donors to look for the child and the recipients. The state protects the donor and the donor should be informed by the clinic about her rights and duties and the procedure of donation. Egg donor should be 18 – 36 years old, should have her own healthy child and should not have any medical contraindications for donation. The draft law also provides with a minimum requirements for donor medical check (not less than listed in Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells). Rules on coding of donors should be adopted by the Ministry of Health Care of Ukraine, following the principle of traceability. Egg donorship could be anonymous or non-anonymous (in case of donation to relatives or if the donor and recipients have filed common application). Financial compensation to donors shall be provided by the clinics, altruistic donation is allowed. The Cabinet of Ministers, as in the case with the first law draft, is entitled to adopt the order of eggs transfer within Ukraine as well as in and out of Ukraine.

Another alternative *law draft "On Application of Assisted Reproductive Technologies and Surrogacy" No. 6475-2 dated January 13, 2022* [20] was filed by a group of Members of Ukrainian Parliament. Like the previous drafts, this draft also foresees the definitions of the terms "gametes` donation", "gametes` donor". Chapter III of the law draft is dedicated to egg donation. Egg donor should be 20 – 36 years old and have her own health child. Medical contraindications for donors and the order of their medical check should be adopted by the Ministry of Health Care of Ukraine. The draft foresees the obligation of the clinic to inform the egg donor on her rights and duties and the procedure of donation; the information should be given by competent person in appropriate way and using the language appropriate for the donor. The egg donor does not acquire any parental rights towards the future child and also is not allowed to look for the child and the recipients. Article 13 of the law draft foresees the rights and duties of the donor. Both anonymous and non-anonymous egg donation are allowed, the latter being legal in cases of donation to relatives or if the donor and recipient filed common application. Information about the donor can be disclosed to the recipients or other clinics upon voluntary informed consent of the donor when donor is the relative of recipients; upon mutual consent of recipients and donor and also in cases when disclosure of the information is necessary for treatment of the child`s hereditary disease. A very positive offer is that donor`s compensation shall be conducted in the order, adopted by the Cabinet of Ministers of Ukraine. Altruistic egg donation is also allowed. This law draft does not allow transfer and selling of gametes out of Ukrainian territory. The way of transfer of gametes within the borders of Ukraine, into the territory of Ukraine and conditions of their storage during the transfer should be regulated by the Cabinet of Ministers of Ukraine.

Despite the recent legislative initiatives, that are currently considered by Ukrainian parliament, the current rules of Ukrainian legislation on egg donorship have some loopholes that should be addressed.

First of all, there is *no state donor registry of egg donors*. The lack of such register can cause several problems – e.g. the egg donor can overgo through more than eight cycles of egg donation and it will be impossible to trace it. Secondly, the information about the donors is kept only by separate clinics and in case if the legislation will allow for the rights to information about the child`s genetic origin – it will be difficult to find the information about egg donors. The legal doctrine discusses also the necessity of limiting the number of eggs retrieved from one donor in order to avoid the possibility of future marriage between the children of the same donor [21]. Although, there is lack of studies on statistics of such cases in Ukraine, the mere "threat" of such situation should be avoided.

Secondly, *the scope of compensation for egg donors* is not regulated and should be addressed. For example, in the United States of America, the Ethics Committee of the American Society for Reproductive Medicine came to conclusion that "financial compensation of women donating oocytes for reproductive or research purposes is justified on ethical grounds and should acknowledge the time, inconvenience, and discomfort associated with screening, ovarian stimulation, oocyte retrieval, and postretrieval recovery and not vary according to the planned use of the oocytes or the number or quality of oocytes retrieved" [22]. In the United Kingdom of Great Britain and Norther Ireland, egg donor gets a compensation for egg donation in amount of up to £750 per donation [23]. The law draft *"On Application of Assisted Reproductive Technologies and Surrogacy" No. 6475-2 dated January 13, 2022* tries to address this issues by referring to adoption of the rules on donor`s compensation by the Cabinet of Ministers of Ukraine.

Thirdly, the current Ukrainian legislation *does not foresee the right to know about genetic origin and does not contain any rules on disclosure of egg donor`s anonymity if it is in the interests of the child*. The law drafts contain some rules on disclosure of donor`s anonym-

ity, however in our opinion, the legislation should be amended to follow the examples of the states when the child has full access to the information about the donor (e.g. following the example of the United Kingdom of Great Britain and Northern Ireland).

CONCLUSIONS

Ukraine is a very liberal jurisdiction, allowing egg donation. Before the war, started by the Russian Federation against Ukraine on February 24, 2022, Ukraine was a very popular touristic destination for infertility treatment. The rules on egg donation can be found in different laws and sub-laws of Ukraine and this fragmentary regulation is not sufficient. Currently Ukrainian Parliament considers three law drafts, dedicated to regulation of assisted human reproduction and all of the law drafts contain special norms of egg donation. Existing legal framework in Ukraine has some serious lacunas that can result in violation of rights and interests of donors and of the children. For example, the state does not keep the unique state register of donors and the donors potentially can donate more eggs than is allowed by the ART order. Secondly, there are no rules on compensation for egg donor and the simple check of the web-sites of the clinics shows that the scope of compensation may vary. Lastly, the current Ukrainian legislation does not contain provisions ensuring protection of the child`s right to know about one`s genetic origin, and thus to obtain the identifying information about the donor. All these issues should be addressed in order to establish a fair balance between the rights and the interests of donors, recipients, the child and the society.

REFERENCES

1. Informatsiyno-statystychnyi Dovidnyk pro Dopomizhni Reproduktyvni Tehnologii v Ukrayiny (Informational and Statistic Handbook on Assisted Reproductive Technologies in Ukraine). Ministerstvo Ohorony Zdorovya Ukrainy, Derzhavnyi Zaklad„Tsentr Medychnoyi Statystyky MOZ Ukrayiny". Kyiv. 2022 https://www.uarm.org.ua/index.php?option=com_attachments&task=download&id=500 (in Ukrainian). [date access 21.09.2022].
2. Egg Donation Market Size By Type (Fresh, Frozen), By Service Provider (Hospitals, Fertility Clinics), COVID-19 Impact Analysis, Regional Outlook, Application Potential, Competitive Market Share & Forecast, 2021 – 2027. https://www.gminsights.com/industry-analysis/egg-donation-market. [date access 21.09.2022].
3. Bühler N. Social Science Report. Egg Donation and IVF with Donated Eggs. Lessons to be Learned from Other Countries. University of Zurih. 2014, 98p.
4. Egg Cell Donation Approved by Swiss Parliament. Swissinfo.ch. 2022. https://www.swissinfo.ch/eng/egg-cell-donation-approved-by-swiss-parliament/47897310. [date access 21.09.2022].
5. Instruction Dignitas Personae on Certain Bioethical Questions by the Congregation for the Doctrine of Faith. https://www.vatican.va/roman_curia/congregations/cfaith/documents/rc_con_cfaith_doc_20081208_dignitas-personae_en.html. [date access 21.09.2022].
6. Shabana A. Islamic Normative Principles Underlying Fatwas on Assisted Reproductive Technologies: Al-Azhar Fatwa on Artificial Insemination. The Muslim World. 2021; 111: 511 – 533. doi: 10.1111/muwo.12406.
7. International Covenant on Civil and Political Rights. 1966. [http://www.un.org.ua/images/International_Covenant_on_Civil_and_Political_Rights_CCPR_eng1.pdf. [date access 21.09.2022].
8. International Covenant on Economic, Social and Cultural Rights. 1966. https://www.ohchr.org/en/instruments-mechanisms/instruments/international-covenant-economic-social-and-cultural-rights. [date access 21.09.2022].
9. Universal Declaration of Human Rights. 1948. https://www.ohchr.org/sites/default/files/UDHR/Documents/UDHR_Translations/eng.pdf. [date access 21.09.2022].
10. Convention for the Protection of Human Rights and Fundamental Freedoms. 1950. https://www.echr.coe.int/documents/convention_eng.pdf. [date access 21.09.2022].
11. Maydanyk R., Moskalenko K. Certain Limitations in Reproductive Rights`s Exercising (Cases of China and Austria). Wiad. Lek. 2021;74(11):3016-3020. doi: 10.36740/WLek202111226.
12. Zakon Ukrayiny "Pro Vykonannya Rishen ta Zastosuvannya Praktyky Evropeiskogo Sudu z Prav Lyudyny" (The Law of Ukraine "On Execution of Judgements and Application of Practice of European Court of Human Rights") No. 3477-IV. 2006. https://zakon.rada.gov.ua/laws/show/3477-15#Text [date access 21.09.2022]. (in Ukrainian).
13. Judgment of the European Court of Human Rights in the case of S.H. and others v. Austria (application No. 57813/00). 2011. https://www.globalhealthrights.org/wp-content/uploads/2016/05/CASE-OF-S.H.-AND-OTHERS-v.-AUSTRIA.pdf. [date access 21.09.2022].
14. Tsyvilnyi Kodeks Ukrayiny (Civil Code of Ukraine) No. 435-IV. 2003. https://zakon.rada.gov.ua/laws/show/435-15#Text [date access 21.09.2022]. (in Ukrainian).
15. Simeynyi Kodeks Ukrayiny (Family Code of Ukraine) No. 2947-III. 2002. https://zakon.rada.gov.ua/laws/show/2947-14#Text. [date access 21.09.2022] (in Ukrainian).

16. Zakon Ukrayuny "Osnovy Zakonodavstva Ukrayiny pro Ohoronu Zdorovya" (The Law of Ukraine 'Fundamentals of the Legislation of Ukraine on Healthcare") No. 2801-XII. 1992. https://zakon.rada.gov.ua/laws/show/2801-12#Text [date access 21.09.2022] (in Ukrainian).
17. Nakaz Ministerstva Ohorony Zdorovya Ukrayuny "Pro Zatverdzhennya Poryadku Zastosuvannya Dopomizhnyh Reproduktyvnykh Tehnologiy v Ukrayini" (The order of the Ministry of Healthcare of Ukraine 'On Adoption of the Order of Application of Assisted Reproductive Technologies in Ukraine") No. 787. 2013. https://zakon.rada.gov.ua/laws/show/z1697-13#Text [date access 21.09.2022] (in Ukrainian).
18. Proekt Zakonu Ukrayiny „Pro Dopomizhni Reproduktyvni Tehnologii" (Law Draft „On Assisted Human Reproduction ") No. 6475. 2021. https://itd.rada.gov.ua/billInfo/Bills/pubFile/1135240 [date access 21.09.2022] (in Ukrainian).
19. Proekt Zakonu Ukrayiny „Pro Zastosuvannya Dopomizhnyh Reproduktyvnyh Tehnologii" (Law Draft "On Application of Assisted Reproductive Technologies") No. 6475-1. 2022. https://itd.rada.gov.ua/billInfo/Bills/pubFile/1158609 [date access 21.09.2022] (in Ukrainian).
20. Proekt Zakonu Ukrayiny „Pro Zastosuvannya Dopomizhnyh Reproduktyvnyh Tehnologii ta Zaminne Materynstvo" (Law Draft "On Application of Assisted Reproductive Technologies and Surrogacy") No. 6475-2. 2022. https://itd.rada.gov.ua/billInfo/Bills/pubFile/1163097 [date access 21.09.2022] (in Ukrainian).
21. Golombok S., Scott R., Appleby J. et al. Limiting offspring numbers: can we justify regulation? In: eds. Regulating Reproductive Donation. Cambridge: Cambridge University Press. 2016, 207 p.
22. Financial Compensation of Oocyte Donors: an Ethics Committee Opinion. 2021. https://www.asrm.org/globalassets/asrm/asrm-content/news-and-publications/ethics-committee-opinions/financial_compensation_of_oocyte_donors.pdf . [date access 21.09.2022]
23. Donating Your Eggs. Human Fertilisation & Embryology Authority. 2020. https://www.hfea.gov.uk/donation/donors/donating-your-eggs/. [date access 21.09.2022]
24. Moskalenko K. Pravo Osib, Narodzhenyh iz Zastosuvannyam Dopomizhnyh Reproducktyvnyh Tehnologiy, Na Informtsiyu pro Svoye Genetychne Pohodzehnnya (Right of Persons, Conceived with the Help of Assisted Human Reproduction Technologies, to the Information on their Genetic Origin). Entrepreneurship, Economy and Law. 2018; 1: 27 – 30. (in Ukrainian).

This work was supported by the University Research Priority Program "Human Reproduction Reloaded" of the University of Zurich.

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Conflict of interest:

The Author declare no conflict of interest.

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Received: 22.09.2022.
Accepted: 18.01.2023

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

REVIEW ARTICLE

FEATURES OF EXERCISING THE RIGHTS OF PATIENTS TO MEDICAL TREATMENT IN WARTIME CONDITIONS

DOI: 10.36740/WLek202302129

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ABSTRACT

The aim: Study of Ukraine's international obligations in the field of access to medical treatment and analysis of Ukrainian legislation in respect of exercising the rights of patients to medical treatment in the conditions of Russia's war against Ukraine.

Materials and methods: The analysis of regulatory legal acts of Ukraine and international standards was carried out using the comparative method.

Conclusions: The health care system of Ukraine has shown its effectiveness and focus on the protection of human rights and freedoms, as well as a vector for the harmonization of Ukrainian legislation with the EU in the field of health care.

KEY WORDS: Human immunodeficiency virus (HIV), rights of patients, medical treatment, war in Ukraine, intellectual property rights

Wiad Lek. 2023;76(2):445-451

INTRODUCTION

On 24 February 2022, Russia launched a military offensive on the territory of the independent European state of Ukraine. In connection with Russia's military aggression against Ukraine, the President of Ukraine introduced martial law in Ukraine by his Decree No. 64/2022 dated 24 February 2022. From the very first days, Russia has been shelling and bombing not only military targets, but also industrial sites, critical infrastructure, medical facilities, defiantly shelling and destroying civilian homes, committing war crimes and crimes against humanity.

The conditions of martial law add the problems of logistics and access of patients to continuous treatment and, as a result, create an urgent need for measures to be taken by the state. In connection with military operations, a large part of the south and east of Ukraine became temporarily occupied. In the territories temporarily occupied by Russia, patients remain who need continuous access to pharmaceuticals and medicinal products. A significant part of Ukrainians was forced to leave for EU and other countries in search of a safe place. According to the UN, Europe is experiencing the biggest migration crisis since World War II. The Office of the United Nations High Commissioner for Refugees has estimated that more than 8.7 million people have left Ukraine and more than 8 million Ukrainians became internally displaced persons. This is the fastest forced population displacement since World War II [1]. For example, Poland is one of the countries that accepted the largest number of Ukrainians who became forced migrants. Most countries grant the right to social benefits, access to housing and medical care to Ukrainians who are forced to stay outside of Ukraine.

Since the first days of the war in Ukraine, the state, represented by the Public Health Center of the Ministry of Health of Ukraine, has begun developing mechanisms for obtaining access to medical treatment for persons living with HIV who were forced to leave Ukraine due to the war or remain in the temporarily occupied territory of Ukraine. Half a year after the war has begun, the Public Health Center of the Ministry of Health of Ukraine is proceeding with continuous accumulation and improvement of the mechanisms of access to medical treatment for patients who temporarily stay outside Ukraine and in the temporarily occupied territory of Ukraine to exercise their inalienable right to treatment.

THE AIM

The aim of our study is to highlight two aspects of exercising the rights of patients to medical treatment in wartime conditions: patient's physical access to treatment (including access to the patient's medical record) and access in the context of removing the patent monopoly on medicinal and medical products. Another objective is to find out an effective state approach to exercising the rights of patients to treatment in wartime conditions.

MATERIALS AND METHODS

In order to achieve the results of our research, we analyzed international documents and standards in the field of human rights protection, in particular the right to exercise access to medical treatment, including persons living with HIV.

Research methods were used to achieve the set goal and address the work task, taking into account the specifics of the study. In the course of the research, general scientific methods and the logical method were used in the development of recommendations for clarifying an effective state approach to exercising the rights of patients to medical treatment in wartime conditions.

REVIEW AND DISCUSSION

Important legal steps for Ukraine's integration into the European Union were taken as far back as 2014, including the health care sector of Ukraine, when the Association Agreement was signed between Ukraine, on the one part, and the European Union, the European Atomic Energy Community and their member states, on the other part (hereinafter "the Association Agreement"), where it was agreed that the parties shall develop cooperation in the field of health care in order to increase the level of its safety and protection of human health as a prerequisite for sustainable development and economic growth. Article 427 of the Association Agreement defines the prevention and control of communicable diseases, such as HIV/AIDS and tuberculosis, as one of the areas for cooperation [3].

In the first block of our study, it is proposed to consider the patient's physical access to treatment taking into account the patient's consent to access his medical documents.

According to Ukrainian legislation, access to medical documentation is closed to unauthorized parties, as it contains confidential information. Thus, according to Article 39-1 of the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Healthcare", a patient has the right to secrecy about his/her state of health, the fact of seeking medical help, diagnosis, as well as information obtained during the medical examination. It is prohibited to request and provide information about the patient's diagnosis and treatment methods at the place of work or study. Moreover, in accordance with Part 3 of Art. 13 of the Law of Ukraine "On Countering the Spread of Diseases Caused by the Human Immunodeficiency Virus (HIV) and Legal and Social Protection of People Living with HIV", information about the results of testing a person for the purpose of detecting HIV, about the presence or absence of HIV infection in a person is confidential and constitutes a medical secret. We must also take into account the provisions of Article 16 of the Law of Ukraine "On the Protection of Personal Data" and Article 10 of the Law of Ukraine "On Access to Public Information".

Let us return to Article 39-1 of the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Healthcare", which contains the definition of medical secret. A medical secret means information about the disease, medical inspection, examination and their results, intimate and family aspects of a citizen's life, which became known to a health care provider or another person in connection with the performance of professional or official duties [4].

In accordance with Article 40 of the Law of Ukraine "Fundamentals of the Legislation of Ukraine on Healthcare", health care providers and other persons who, in connection with the performance of professional or official duties, became aware of an illness, medical examination, inspection and their results, intimate and family aspects of a citizen's life, do not have the right to disclose this information, except for cases provided by legislative acts when the medical secret can be disclosed without the consent of the person or his/her legal representatives [4].

Part 1 of Article 286 of the Civil Code of Ukraine stipulates that a natural person has the right to secrecy about his/her state of health, the fact of seeking medical help, diagnosis, as well as information obtained during medical examination [5].

Given the specified legal norms, the patient's rights regarding disclosure of information about his/her health are reliably protected by Ukrainian legislation. However, during active warfare within Ukraine, both patients and physicians were forced to leave the territory of Ukraine going to other countries, or to become internally displaced persons in search of safer places to stay. In connection with such events, there was a disruption of communication and physical links between the patient and the physician / hospital where the patient received treatment. At the same time, the effective provision of medical care to people living with HIV, especially in wartime conditions, depends on the ability to access the patient's medical record in order to find out the treatment regimen, possible adverse reactions to medication and concomitant conditions. In the first days of the war, the Ukrainian healthcare sector was challenged to provide treatment for patients who had lost contact with their physician and the hospital where they received antiretroviral therapy. The Public Health Center of the Ministry of Health of Ukraine started working to develop mechanisms for obtaining information from the patient's medical record and arrange cooperation between medical institutions in

the event of the need to provide medical assistance to people living with HIV, including cooperation with medical institutions of other countries.

Attention also should be drawn to the fact that access to medical information of a patient living with HIV is closed even between different providers of these medical services in order to eliminate the stigmatization of a person regarding his/her diagnosis. Therefore, an important step was to develop a reliable and effective mechanism to get access to medical records of patients without violating their rights, including the right to privacy about health status. In order to continue the antiretroviral therapy cycle according to the scheme by which the patient was treated, it is important for the physician to know this scheme, since people living with HIV can change the treatment scheme only once in their life. It is no longer possible to return to the previous treatment scheme, and it will become necessary to provide the patient with other medicinal products. Antiretroviral therapy is a mandatory component of comprehensive medical care for HIV-infected persons, since continuous lifelong antiretroviral therapy ensures maximum suppression of HIV replication, restoration of immune system function, prolongation and improvement of the quality of life of PLHIV, prevention of the development of AIDS-related diseases and HIV transmission [6].

According to this protocol, when a person living with HIV applies to clinics in Europe, the following is provided for:

- filling out of the "Application for providing information on health status and treatment" by the patient;
- submission of this application to the department of HIV infection management and prevention of the Public Health Center to the e-mail _ in order to facilitate provision of information on HIV infection treatment from the attending physician or health care institution where the patient had been under medical supervision;
- filling out of information about the patient;
- patient applications are subject to registration.

An interesting initiative was launched within the framework of the pilot project of Poland and Ukraine with the aim of effective treatment of people living with HIV who were forced to stay in Poland. WHO has started funding two of four Polish NGOs, the project activities of which are aimed at facilitating access to the health care system and HIV prevention, care and treatment for people living with HIV/AIDS and at-risk population groups, focusing on refugees arriving in Poland from Ukraine. Pilot initiatives will continue until December 31, 2022. [2]

We should note the positive dynamics regarding the implementation of access to patient treatment with the adoption of the new Law of Ukraine "On Medicinal Products" (hereinafter "the Law"), which is designed to expand access of patients to exercising their natural right to health. On 28 July 2022, the Verkhovna Rada of Ukraine adopted in the second reading the Law of Ukraine "On Medicinal Products", the purpose of the Law being to ensure health care for the population of Ukraine by providing access to effective, high-quality, modern and safe medicinal products, as well as to implement certain provisions of the EU legislation regarding medicinal products for human use [8]. The adoption of the Law is a long-awaited step to bring its provisions on the regulation of the medicinal products circulation in line with the EU standards and expand access to treatment. The Association Agreement imposed an obligation on Ukraine to develop cooperation in the field of health care. Thanks to the Association Agreement signed in 2014, which laid the foundation for harmonization of the Ukrainian legislation with the EU legislation, the Law has become a logical continuation on the way to the European space, and Ukraine's obtaining the status of a candidate for the EU membership is a confirmation of that. The Law entered into force on 18 August 2022 and will be carried into effect 30 months after the end of martial law in Ukraine, with the exception of certain regulations. However, the Law of Ukraine "On Medicinal Products" No. 123/96-VR dated 04.04.1996 continues to be in effect until the entry into force of the Law No. 2469-IX dated 28.07.2022. Among the main provisions of the Law, the following innovations are worthy of attention:

- The mechanisms were established for conducting programs of extended access of patients to unregistered medicinal products and programs of access of research subjects (patients) to the researched medicinal product after the completion of a clinical study (trial), which fully complies with the norms of the EU legislation and expands human rights to access to medical treatment (Art. 7).
- An improved approach to clinical studies (trials) of medicinal products was also established (Art. 10).

With the adoption of the Law, we see these innovations as a positive transformation of patients' access to medical treatment in post-war times. Therefore, let us pay attention to the practice of the European Court of Human Rights regarding health care, since most of its resolutions are a source of national law, including Ukraine. An interesting case that we draw attention to in the context of our research is the case No. 47039/11 and 358/12 *Hristozov and Others v. Bulgaria* on access to experimental treatment or medicinal product [9]. We can highlight the following from the case materials: the applicants in the case are terminal cancer patients who tried to obtain from the Bulgarian government

the possibility to legally take experimental anti-cancer pharmaceuticals. The pharmaceuticals have not been approved for treatment in any of the European countries. However, in a number of countries including Germany they were allowed for "compassionate use", that is, for such patients whom the conventional treatment methods were no longer able to help. The applicants appealed to the authorities to allow the use of these medicinal products, which have not passed all phases of clinical study in other countries, in Bulgaria. However, they were refused on the grounds that Bulgarian legislation does not provide for such a mechanism. In its resolution, the ECtHR noted that the development of new medicinal products is a field that is constantly influenced by scientific research and technological progress. By denying the applicants (terminal patients) any access to these products, the Bulgarian authorities actually have completely disregarded their strong interest in being able to try a treatment which, although involving the acceptance of additional risk uncertainty, could turn out to be their only chance to try to save one's own life. The ECtHR recognized that in this case there was a violation of Article 8 "Right to respect for private and family life" of the Convention for the Protection of Human Rights and Fundamental Freedoms (European Convention on Human Rights).

Therefore, we believe that Articles 8 and 10 of the new Law of Ukraine "On Medicinal Products" will significantly expand access to treatment, including experimental medicinal product, which for some patients are the last chance and hope for recovery.

We also believe that these regulations will have positive dynamics in post-war times as well, for example, during Russia's military aggression against Ukraine, according to UN data, more than 100 cases of war crimes of sexual violence were recorded as of the beginning of summer 2022 [10], such war crimes entailing injury and mutilation of the victim, as well as high risks of infection with HIV and other venereal diseases. At the same time, the regulations of the new Law of Ukraine "On Medicinal Products" will allow patients to participate in clinical trials of the latest and innovative medicinal products.

The next block which we will cover concerns the exercise of patient rights by removing the patent monopoly on medicinal and medical products.

The problem of ensuring access to treatment gained worldwide importance at the beginning of the 21st century already, when the patent monopoly came into conflict with fundamental human rights [7].

For many years, the scientific and human rights communities in Ukraine have been raising painful issues of human rights to health and access to the latest medicinal products.

For a long time, the Ukrainian legislature needed to address the issues of issuing patents for surgical or therapeutic treatment methods and methods of diagnosing the human body, which violated the interests of a person in one of the most important benefits such as access to innovative treatment methods. Another aspect was the possibility of providing legal protection to a utility model patented as a substance, which in turn allowed the pharmaceutical to be patented again and again, and thus Ukrainian patients did not have the opportunity to buy medicines at affordable prices due to the "patent monopoly".

On 21 July 2020, the Verkhovna Rada of Ukraine adopted the Law of Ukraine "On Amendments to Certain Legislative Acts of Ukraine (Regarding the Reform of Patent Legislation)", which from now on has the status of the Law of Ukraine No. 816-IX and changes the patent system, preventing the abuse of patent rights by patent owners, and therefore, expands the possibilities of patients to access affordable, innovative, high-quality medicines and methods of diagnosis and treatment. After all, ensuring access to basic medicinal products is a fundamental duty of the state in respect of human rights.

Ukraine has reached a historical moment of shifting emphasis in the field of legal protection of intellectual property items towards the implementation of the relevant provisions of the Agreement on Trade-Related Aspects of Intellectual Property Rights (hereinafter "the TRIPS Agreement") and international obligations in terms of legal protection of inventions and utility models.

The patent reform which has finally taken place in Ukraine will not extend legal protection to items declared as inventions (utility models), namely: diagnostic, therapeutic and surgical methods of treating humans or animals, from now on, they are excluded from the scope of patentable subjects, such a provision is stipulated by Art. 27.3(a) of the TRIPS Agreement. Ukrainian physicians will have legal rights to use the best methods of patient treatment and diagnostics without the need to obtain permission from the patent owner. In this case, the inventor of the treatment procedure or method will have a personal non-property right to be the author of such treatment procedure or method.

Let us find out how the patent reform will affect the field of medicine and pharmacy and ensure access for Ukrainian patients to high-quality and affordable medicinal products. Ukraine has a sufficient inventory of both original (branded) and generic medicinal products, as well as, until recently, the opportunity to patent procedures and methods of human treatment and diagnostics. Physicians faced the difficult question

of choosing a medicinal product and treatment method for the rational pharmacotherapy of the patient, the most important aspect being the price of the treatment cycle. Because of the high cost of medicinal products, 48.8% of Ukrainian patients refused treatment, and 56.5% reduced the number of prescribed pharmaceuticals or used only a part of them for financial reasons. Such statistical data were presented in the results of the study "Free Medicine".

The question arises why medicines are so expensive for patients in Ukraine? The main reason is the imperfect patent system that made it possible to patent the original medicinal product repeatedly, despite the fact that the legal protection term is 20 years for the invention and 10 years for the utility model, unscrupulous patent owners patented the same active substance that was no longer innovative through treatment procedures and methods, which is absurd for European countries. Most often, it was patented as a useful model, the intellectual property rights for which were previously acquired according to a simplified procedure. The legal protection was thus continued, preventing generic medicinal products from entering the market and providing affordable and high-quality pharmaceuticals to patients.

In Ukraine, in accordance with the previous version of the Law of Ukraine "On Legal Protection of Inventions and Utility Models", the concepts of "invention" and "utility model" were defined exactly the same, and in the second part of Article 6, legal protection was provided to the object of the invention (utility model), respectively for: the product (device, substance, microorganism strain, plant and animal cell culture, etc.); process (method), as well as a new application of a known product or process. Thus, manufacturers of medicinal products had legal grounds in Ukraine to obtain a patent for the same product after the expiration of the patent through a process, method, and new application of a known product or method.

Thus, in the EU, legal protection of procedures and methods of treatment and surgical techniques was abandoned. Such monopolization of intellectual property rights is considered a violation of human rights to life and health. This is the reason why we did not find any judicial resolution of the Practice of the European Court of Human Rights from EU patients.

Harmonization of the provisions of the Ukrainian legislation with the provisions of the EU in this direction provides patent protection to innovations in the field of medicine, and not to fake patents for the procedure of surgical intervention or the method of treatment, etc. Intellectual property is designed to protect the true new pharmaceutical findings of inventors and plays a significant role in scientific progress and the development of innovation, allowing inventors to protect the results of their creative activity without abusing them.

But, unfortunately, our search in the database of "inventions (utility models) in Ukraine" revealed a valid patent No. 104476 *Annular Bundle and Method of its Manufacture* [11], therefore, in Ukraine the patent still protects previously patented absurd inventions the effective term of which is still valid: medical tourniquet bundles and other medicinal and medical products that received a patent for such an intellectual property item before the new standards of patent reform came into effect. This does not apply to medical products that are imported to Ukraine today as humanitarian aid for the needs of servicemen and civilians who were injured to stop bleeding. A problem may arise if tourniquet bundles that are subject to patent protection are brought to Ukraine as humanitarian aid.

It should be noted that in order to resolve the humanitarian problems faced by the Ukrainian health care system due to the Russian invasion, the Ukrainian Government shall address the issue of the supply of medicinal and medical products to patients applying all possible legal mechanisms, including those envisaged by intellectual property laws.

Ukraine is a party to a number of bilateral and multilateral international treaties that regulate the protection of intellectual property rights, including the TRIPS Agreement. The TRIPS Agreement establishes minimum standards for the protection of IP rights that all members of the World Trade Organization (WTO), including Ukraine, are obliged to ensure. As part of its assumed obligations to ensure effective protection and implementation of intellectual property rights, Ukraine must comply with the requirements of the TRIPS Agreement. However, the TRIPS Agreement also contains certain exceptions to the fulfillment of such obligations. Given the fact that Ukraine is currently a state at war, it is possible to apply the provision of Article 73 "Security Exceptions" of the TRIPS Agreement. The said provision goes as follows: "Nothing in this Agreement shall be construed: …b) to prevent a Member from taking any action which it considers necessary for the protection of its essential security interests: … iii) taken in time of war or other emergency in international relations…". In other words, paragraph (b) of Article 73 contains an introductory part that qualifies actions in taking of which by a WTO member there may not be any interference, namely the actions "which it considers necessary for the protection of its essential security interests". Such actions may be taken despite a member's obligations under TRIPS. That is, this provision guarantees that WTO members have the right to refer to the protection of their essential security interests in order to justify ac-

tions that are not actually corresponding to TRIPS. Thus, Article 73 of the TRIPS Agreement essentially acts as an exception or defense against allegations of violation of TRIPS obligations [13].

The Ukrainian state has every right to apply Article 73 of the TRIPS Agreement to protect the lives and health of people in Ukraine, which are fundamental especially during wartime, and the restriction of intellectual property rights to critically needed medicinal or medical products precisely means the protection of the state's security interests.

The Scientific Research Institute of Intellectual Property of the National Academy of Law Sciences of Ukraine has developed, for the purpose of implementation into the national legislation in the field of intellectual property rights of the legal grounds available in international law for the limitation of intellectual property rights in order to provide the population with critically important medicinal and medical products, the Draft Law of Ukraine "On Providing the Population with Critically Needed Medicinal and Medical Products During the Martial Law and for the Period of Overcoming the Consequences of the Military Aggression of the Russian Federation against Ukraine" which was submitted to the Committee of the Verkhovna Rada of Ukraine for Economic Development and to the Committee of the Verkhovna Rada of Ukraine for National Health, Medical Care and Medical Insurance on the basis of Article 73 of the TRIPS Agreement and of the Law of Ukraine "On the Legal Regime of Martial Law". The said Draft Law proposes to the Verkhovna Rada of Ukraine to establish restrictions on intellectual property rights regulated by the legislation of Ukraine, including patents, supplementary protection certificates, copyrights, industrial designs, trade secrets, exclusive data and other intellectual property rights (except for trademarks) related to medicinal and medical products applied to save life and health of people who suffered both directly as a result of the military actions of the aggressor and indirectly due to the impossibility of supplying a sufficient amount of critically needed medicinal and medical products to Ukraine.

It is important for Ukraine to introduce such a limitation of intellectual property rights precisely on the basis of Article 73 of the TRIPS Agreement, since this Article directly defines war as one of the grounds for the introduction of such exceptions, and also, unlike Article 31 of the TRIPS Agreement, does not require after the end of a war or other state of emergency the payment of compensation to the owner of intellectual property rights, which is especially important given the extremely limited financial resources of the state budget.

During the period of overcoming the consequences of the war, we will talk about other mechanisms for overcoming the patent monopoly, namely the compulsory licensing and use of patented medicinal and medical products for the public interest (Articles 30, 31 of the Law of Ukraine "On Inventions and Utility Models").

CONCLUSIONS

The analysis of the Ukrainian legislation in terms of bringing it into line with the EU standards in the conditions of war in Ukraine showed that the Ukrainian health care system has begun European integration processes, and amendments have been made to laws and subordinate legislation to provide accessible medical treatment to the population of Ukraine. The legislative proposals presented in the paper regarding the improvement of the regulatory framework in the area under consideration will contribute to the progress of our state on the way to unconditionally guaranteeing access to treatment for every person.

The Ukrainian authorities have implemented a number of positive measures to ensure exercising of human rights to access to treatment in such difficult times for Ukraine. The health care system of Ukraine has shown its effectiveness and focus on the protection of human rights and freedoms, as well as a vector for the harmonization of Ukrainian legislation with the EU laws exactly in the field of health care.

REFERENCES

1. The UN and the war in Ukraine: key information. https://unric.org/en/the-un-and-the-war-in-ukraine-key-information/ [date acces: 04.09.2022]
2. Emergency in Ukraine: external situation report #19, published 11 August 2022: reporting period: 28 July – 10 August 2022. https://www.who.int/europe/publications/i/item/WHO-EURO-2022-5152-44915-65715 [date access 04.09.2022]
3. Senyuta I.Ya., Harasymiv O.Yu. Compendium of Case Law Related to HIV/AIDS and Tuberculosis in Ukraine: Practitional Guide for Judges. United Nations Development Programme in Ukraine. English text editing:. K.. 2021, 124 p.
4. Fundamentals of the Legislation of Ukraine on Healthcare. The Law of Ukraine. https://zakon.rada.gov.ua/laws/show/2801-12#Text [date access 04.09.2022]
5. The Civil Code of Ukraine. https://zakon.rada.gov.ua/laws/show/435-15#Text [date access 06.09.2022]

6. Guide on the International Standards, ECHR Case Law and National Case Law on HIV / AIDS and TB: Lawyer's Legal Tools. https://www.undp.org/uk/ukraine/publications/posibnyk-iz-mizhnarodnykh-standartiv-praktyky-yespl-ta-natsionalnoyi-sudovoyi-praktyky-u-sferi-vil/snidu-ta-tb-pravovyy?fbclid=IwAR3E1TRIyByMSQdU5RzQqrq9ydSyVInVBSLWxUsvR0HQ3dPzLTtt3qFR63g [date access 06.09.2022]
7. Kashyntseva O. Y., Trofymenko M. M. Legal measures to expend the access to treatment during the pandemic in Ukraine and in the world. Medical Law. 2020. doi:10.25040/medicallaw2020.02.034.
8. On Medicinal Products. The Law of Ukraine 2469-IX. https://zakon.rada.gov.ua/laws/show/2469-20#Text [date access 06.09.2022]
9. Case Hristozov and Others v. Bulgaria: judgment of European Court of Human Rights 13 November 2012. https://hudoc.echr.coe.int/eng#%20 [date access 06.09.2022]
10. Sexual Violence "Most Hidden Crime" Being Committed against Ukrainians, Civil Society Representative Tells Security Council. 2022. https://press.un.org/en/2022/sc14926.doc.htm [date access 06.09.2022]
11. Patent No. 104476 for annular bundle and method of its manufacture. https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=196608 [date access 06.09.2022]
12. Standardized protocol for clinical management and medical data-sharing for people living with HIV among refugees from Ukraine. 2022. https://apps.who.int/iris/handle/10665/353083?locale-attribute=en [date access 06.09.2022]
13. Gurgula O. B. Limitation of intellectual property rights during wartime to ensure access to critically needed medicines in Ukraine based on Article 73 of the TRIPS Agreement. Legal issues of epidemic safety during martial law : materials of international round table. (16 June 2022) electronic scientific publication. National Academy of Law Sciences (NALS) of Ukraine. Kharkiv : Pravo Publ. 2022, 158 p. (in Ukrainian)

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

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Received: 24.09.2022
Accepted: 23.01.2023

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

CASE STUDY

BRUGADA-LIKE ECG PATTERN AND TUMORS INVOLVING RIGHT VENTRICULAR OUTFLOW TRACT – CASE SERIES AND LITERATURE REVIEW

DOI: 10.36740/WLek202302130

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ABSTRACT

Mechanical heart compression, including that from mediastinal tumor, may cause Brugada-like ECG pattern. Such ECG pattern might also be observed in intracardiac tumor obstructing the right ventricular outflow tract (RVOT). Eight cases with Brugada-like ECG and tumors involving RVOT have been described so far; 4 growing in the mediastinum (with one example of inflammatory mass), 3 with intracardiac location, and 1 being an organized pericardial hematoma.

The authors present other 3 cases of intracardiac metastatic tumors in RVOT and Brugada-like ECG pattern with coved ST-segment elevation in the right precordial leads. All patients had negative history of cardiovascular disease or familiar malignant arrhythmia occurrence. ECG were done routinely; none of the patients had chest pain or an increased level of cardiac troponins. In all patients, neoplastic disease was at advanced stage. A 76-year-old male, had a history of four neoplasms: bladder cancer was being treated with chemotherapy, while prostate, tongue, and lung cancers had been resected years ago and no signs of local relapse were found. A 78-year-old female, was diagnosed with colon cancer 1 month after an episode of venous thromboembolism. Six months after the resection of cancer, second focus of adenocarcinoma was found in the rectum. Third patient, a 65-year-old-male had undergone nephrectomy for renal cancer a year before cardiac metastasis diagnosis.

KEY WORDS: Brugada-like ECG, right ventricular outflow tract, mediastinal tumor, intracardiac tumor

Wiad Lek. 2023;76(2):452-457

INTRODUCTION

Mechanical heart compression may be a cause of Brugada-like ECG pattern with specific (coved or saddle-back) ST segment elevation (STE) in patients without true Brugada syndrome, i.e., Brugada phenocopy [1, 2]. Clinical conditions include: pectus excavatum [3], chest trauma [4], large pericardial effusion [5], acute pneumothorax [6], spontaneous pneumopericardium [7], retrosternal stomach reconstruction (in esophageal cancer patients) [8] and mediastinal tumors growing in the neighborhood of the right ventricular (RV) wall, especially RV outflow tract (RVOT) [2]. However, Brugada-like ECG pattern might also be observed in intracardiac tumor obstructing RVOT. Eight cases with Brugada-like ECG and tumors associated with RVOT have been described so far; 4 growing in the mediastinum (including one inflammatory mass) [9-12], 3 with intracardiac location [13-15], and one being an organized pericardial hematoma [16].

THE AIM

The purpose of this paper is to present 3 new Brugada-like ECG cases associated with intracardiac metastatic tumor and to analyze the clinical data of all published ones with such ECG pattern in the presence of tumor involving RVOT.

CASE SERIES REPORT

All of 3 our patients were at advanced cancer stage in the moment of intracardiac metastatic tumor diagnosis, all were examined due to the right ventricular heart failure exacerbation; two of them died during hospitalization, one soon after discharge. They had negative history of cardiovascular disease or familiar malignant arrhythmia occurrence. Their ECG, echocardiography, and computed tomography (CT) are collected in figure 1.

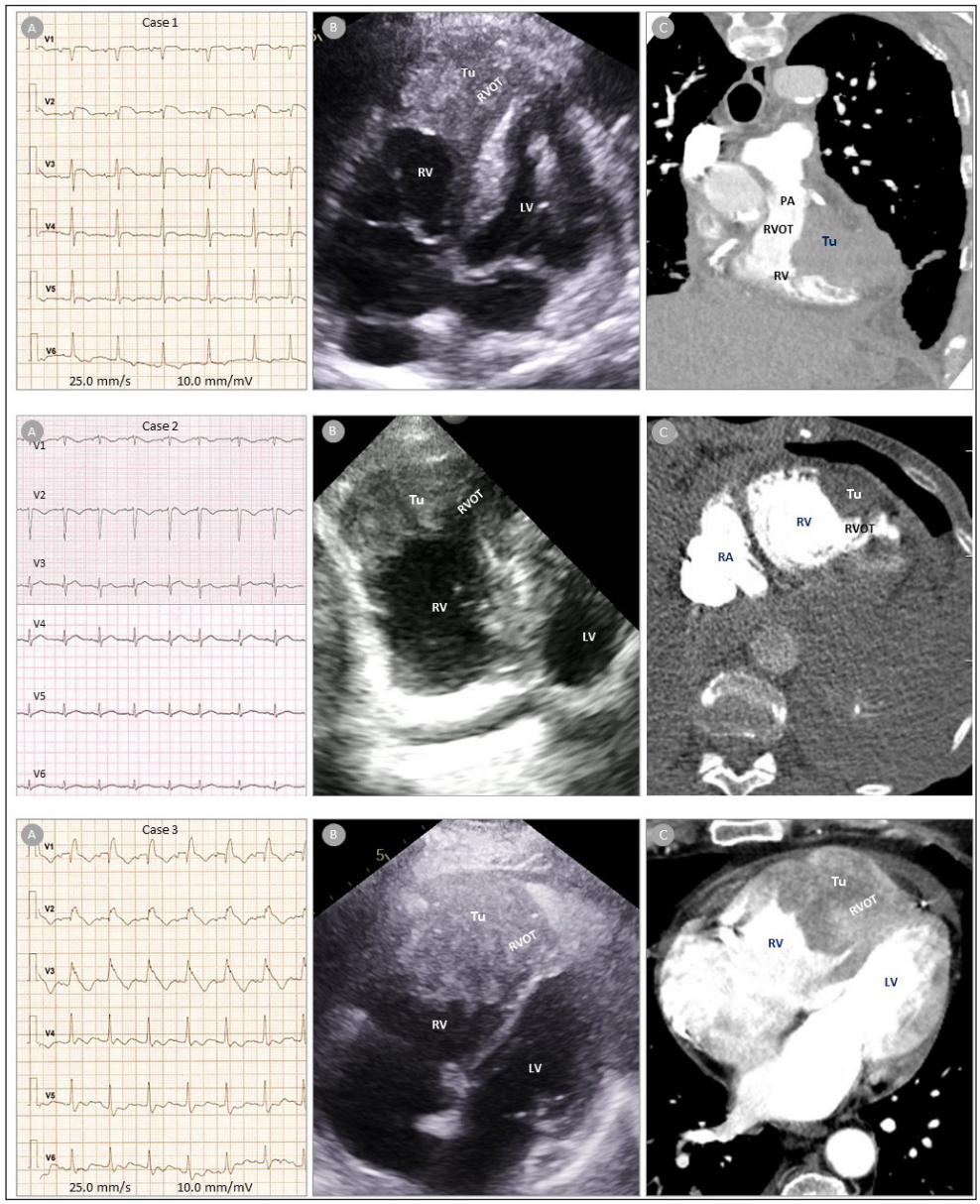

Fig. 1. Case 1-3. ECG - precordial leads (A), intracardiac metastatic tumor (Tu) in RVOT in TTE (B) and in CT (C). TTE - transthoracic echocardiography, CT - computed tomography, RVOT - right ventricular outflow tract, RV - right ventricle, LV - left ventricle, RA - right atrium, PA - pulmonary artery.

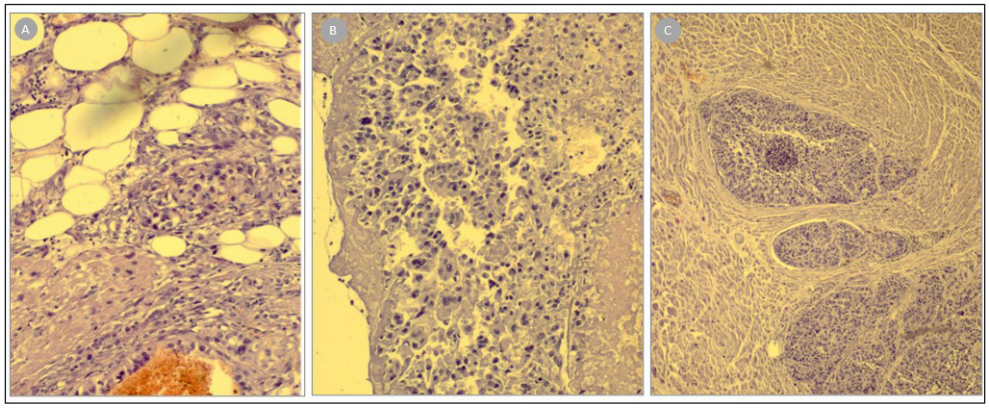

Fig. 2. Heart histopathology (case 3): the infiltration of renal carcinoma cells into the pericardial fat and RV myocardium (A), cancer mass in RV cavity covered with fibrin, deeper - necrotic mass (B), neoplastic involvement of interventricular septum vessels (C); magnification: 100x (A-B), 40 x (C); hematoxylin and eosin stain

ECG which showed Brugada-like STE in the right precordial leads (in case No 3 with coexistent right bundle branch block -RBBB) were done routinely; none of the patients had chest pain or an increased level of cardiac troponins. Previous ECG had been all normal. Echocardiography was the first examination which revealed tumor. First patient, a 76-year-old male, had a history of four neoplasms: bladder cancer was being treated with chemotherapy, while prostate, tongue, and lung cancers had been resected years ago and no signs of local relapse were found. Second patient, a 78-year-old female, was diagnosed with colon cancer 1 month after

Table I. Clinical data of published cases with Brugada-like ECG and mediastinal tumor compressing RVOT or intracardiac tumor obstructing RVOT

Case number Author (publication date)	Patient age/ gender/ symptoms/ other	Tumor description	Method of tumor imaging and underlying disease diagnosis	Brugada ECG pattern, other ECG findings	Therapy/ outcome
1. Tarín 1999 [9]	52/M/ palpitations, recurrent chest pain/ PAF	Large anterior mediastinal tumor displaced RVOT, metastatic breast carcinoma	echocardiography, MRI	type 1 (STE in V1-V3), coexistent incomplete RBBB	tumor removal, adjuvant radiotherapy, clinical improvement STE disappearance
2. Tomcsányi 2002 [16]	44/F/ dyspnea/history of rheumatoid arthritis	pericardial organized hematoma	echocardiography, MRI	type 1 (STE in V1-V3)	tumor removal, clinical improvement, STE disappearance
3. Nakazato 2003 [10]	52/F/ history of pulmonary stenosis and recurrent RVHF	mediastinal inflammatory mass compressing RV	echocardiography, MRI	type 1 (STE in V1-V4), coexistent incomplete RBBB	antibiotics, amelioration of RV failure symptoms, STE resolution
4. Nguyen 2011 [13]	17months/F	rhabdomyoma in the anterior interventricular septum, extending into RV and LV free walls	echocardiography	type 1	tumor reduction, ECG normalization at age 21 months, negative genetic testing
5. Sato 2011 [14]	65/F/ fatigue, palpitations, pitting edema	massive intracardiac tumor invading RA and RV wall, projecting to RVOT/ primary intracardiac lymphoma	CT, intracardiac echocardiography with biopsy	type 1 (STE in V1-V3)	chemotherapy and stem cell transplantation remission of lymphoma, ECG normalization, 2-year follow-up
6. Asteriou 2013 [11]	33/F/ dyspnea, palpitations	mediastinal giant lipoma (20 x 14 x 7 cm) extending from the right lateral thoracic wall to the pericardium	CT, histopathology of tumor excised	type 2 (STE in V1-V2), coexistent incomplete RBBB	tumor excision, ECG normalization, negative provocative test
7. Schnegg 2016 [15]	66/F/ negative history for chest pain, palpitations, syncope	intracardiac metastasis of rectal cancer in RVOT	PET, CT, histopathology (needle biopsy of perimyocardial region of RVOT	type 1 in V1, negative T waves in V2-V4	wide QRS tachycardia after biopsy, palliative treatment of cancer
8. Pérez-Riera 2018 [12]	79/F/ precordial pain with epigastric radiation, dyspnea, syncopal episode/ coexistent CAD	large mediastinal NHL compressing PA and RVOT	echocardiography (?), CT, histopathology (transthoracic needle aspiration)	type 1 (STE in V1-V2), coexistent ST depression in inferolateral leads	stent implantation (RCA), chemotherapy, immunotherapy, radiotherapy, reversal of STE, negative provocative test
9. Elikowski 2023, [present case No 1]	76/M/ dyspnea, RVHF	intracardiac metastatic tumor in RVOT, history of 4 neoplasms	echocardiography, CT, probably bladder cancer metastasis	type 1 (STE in V1-V3)	palliative treatment, terminal care, death soon after discharge
10. Elikowski 2023, [present case No2]	78/F/ dyspnea, RVHF/ PAF	intracardiac metastasis of colorectal cancer in RVOT	echocardiography, CT, histopathology of primary (colorectal) foci	type 1 (STE in V1-V2)	palliative treatment, death during hospitalization
11. Elikowski 2023, [present case No 3]	65/M/ dyspnea, RVHF	intracardiac metastasis of renal cancer in RVOT (nephrectomy 1 year earlier)	echocardiography, CT, heart histopathology (autopsy)	type 1 (STE in V1-V3), coexistent RBBB	palliative treatment, death during hospitalization

RVOT - right ventricular outflow tract, RV - right ventricle, RA - right atrium, LV - left ventricle, PA - pulmonary artery, RCA - right coronary artery, STE - ST- segment elevation, RBBB - right bundle branch block, MRI - magnetic resonance imaging, CT - computed tomography, PET - positron emission tomography, PAF - paroxysmal atrial fibrillation, RVHF - right ventricular heart failure, CAD - coronary artery disease, NHL - non-Hodgkin's lymphoma

an episode of venous thromboembolism. Six months after the resection of cancer, second focus of adenocarcinoma was found in the rectum. Cardiac involvement was diagnosed on echocardiography when her clinical status deteriorated after the operation. Third patient, a 65-year-old-male had undergone nephrectomy for renal cancer a year before cardiac metastasis diagnosis. In retrospective analysis of CT performed before nephrectomy (not shown), small cancer metastasis in RVOT could be identified. Heart histopathology in case 3 revealed the infiltration of renal carcinoma cells into the pericardial fat and RV myocardium, cancer mass covered with fibrin in RV cavity and neoplastic involvement of interventricular septum vessels (Fig. 2).

Clinical data of all 11 patients (3 males) with Brugada-like ECG and mediastinal or intracardiac tumor are collected in table I. Six patients were 65 or older, one female subject was a child. More important symptoms were dyspnea, RV heart failure and palpitations. All tumors were large in size. Mediastinal tumors were diagnosed as: lipoma, non-Hodgkin lymphoma, inflammatory mass. Two intracardiac masses were primary cardiac tumors (rhabdomyoma, lymphoma), remaining ones were metastases (two from colorectal cancer, one from renal cancer, one unspecified in a male with a history of 4 neoplasms). Benign mediastinal tumors were observed in younger adult patients (33-52 years). Advanced age (65-79) was associated with aggressive intracardiac metastatic cancer or intracardiac/mediastinal lymphoma. In 10 patients coved ST segment elevation (STE), meaning type 1 Brugada ECG pattern, was present; one female had saddle-back STE consistent with type 2 Brugada ECG pattern. Four patients had coexistent RBBB; in three cases block was incomplete. Ventricular arrhythmia was observed in only one patient, but it was a complication of myocardial biopsy. In six patients, proper therapy (surgery, radiotherapy, chemotherapy, stem cell transplantation, antibiotic therapy) caused clinical improvement as well as ECG normalization. In four older patients (including 3 ours) neoplastic disease was at advanced stage which led to death; therefore, ECG normalization was not possible.

DISCUSSION

Local pressure applied to the RV wall has been reported to induce Brugada-like ECG pattern [2]. The ability of local pressure to give rise to STE in the right precordial leads has been demonstrated experimentally in the arterially perfused RV wedge preparation; focal pressure was shown to cause loss of the action potential dome at some right epicardial sites [17]. Those observations explain why mechanical heart (especially RVOT) compression by mediastinal tumor or pericardial mass, may be responsible for generating specific ECG alterations of repolarization [9-12]. Considerable size of tumor [11] and location in the anterior mediastinum [10-12] particularly predispose to such situation. In the moment of diagnosis, reported tumors were large or even giant, e.g., lipoma described by Asteriou et al. [11], no matter if tumor is benign [11], malignant [9, 12], inflammatory [10] or is an organized pericardial hematoma [16].

Mechanism of Brugada-like STE in RV intracardiac tumor seems to be more complex than that resulting from mediastinal mass compression. The role of hemodynamic deterioration related to RVOT obstruction should not be excluded. However, in large intracardiac tumors (e.g., like in our case 1 and 3), changes of the heart position in the chest due to tumor proliferation may also be associated with compression (from the outside) of RV areas responsible for ECG alterations [14]. In patient reported by Schnegg et al., large neoplastic mass involved peri-myocardial region of RVOT [15]. Nguyen et al. observed Brugada phenocopy in female infant with large rhabdomyoma extending from interventricular septum into the RV and LV free wall and resulting in heart displacement [13]. Sato et al. described a patient with primary cardiac lymphoma invading a significant amount of the RV and causing marked cardiac distortion around the longitudinal axis [14]. On the other hand, myocardial infiltration in hematological disorder, without evident signs of intracardiac or extracardiac tumor, may also induce ECG pattern mimicking Brugada syndrome [18]. Neoplastic or inflammatory infiltration and local edema of RVOT myocardium may (unevenly in respective myocardial layers) disturb cells action potential which produce transmural gradient. Right ventricular metastases, including RVOT location, are observed in different cancers; e.g., colorectal [19], renal [20, 21], bladder [22], tongue [23, 24], and lung cancer [25]. By applying the approximate frequencies of cardiac tumors categorized by type and site, an intracavitary RVOT tumor is 70 to 140 times more likely to be malignant than benign [26]. Metastatic obstruction of RVOT should be considered in the absence of widespread malignancy because the heart was the sole site of metastasis in a half of autopsy patients [27].

Probably, Brugada-like STE in pulmonary embolism should be also considered in the aspect of RVOT mechanical compression, since it is observed in patients with severe RV dilatation [28]. Mechanical causes of Brugada-like ECG listed in the introduction, can be completed by some interventional procedures like pericardiocentesis [29] and radiofrequency catheter ablation of atrial fibrillation.

In all but one of the analyzed patients, type 1 Brugada pattern was present. It was also dominating type in other cases identified as examples of mechanical heart compression [1]. It should be remembered that mediastinal or intracardiac tumor sometimes manifests as arrhythmia and some patients complain with palpitation, which in the presence of STE simulating Brugada syndrome, favors misdiagnosis. Yet, only one female in the group, had ventricular tachycardia provoked by myocardial biopsy. Apart from Brugada-like ECG, other repolarization abnormalities, may be observed in patients with tumors involving RVOT. In some of them, STE mimicking myocardial infarction and extending to lateral precordial leads, can be seen [24]. Causes of STE associated with cardiac neoplasm include: direct compression of coronary arteries, tumor extension/embolization to coronary lumen, neoplastic pericardium invasion, direct myocardial injury or physiochemical action [30]. The most frequent ECG features in patients with RVOT tumor are right axis deviation and right bundle branch block [19].

As in the previously described by the authors case with Brugada-like ECG and fatal pulmonary embolism [28], provocative test was impossible to be done in our three cancer patients. It is hard to justify such testing in patients at advanced age who have never suffered from ventricular arrhythmia and are in end-stage of neoplastic disease.

CONCLUSIONS

Either neoplastic or non-neoplastic tumors located in the neighborhood of RVOT (in the anterior mediastinum, in the right ventricle cavity or in the pericardium) may cause Brugada-like (usually type 1) ECG pattern. Therefore, such ECG pattern may arise from RVOT compression as well as from RVOT obstruction. In the moment of diagnosis, all analyzed tumors had large size. Older patients (over 60 years) are more likely to have malignant intracardiac metastatic tumors or intracardiac/mediastinal lymphoma. Other mediastinal tumors, usually benign, can be found in younger adults and have better prognosis; after a proper treatment, ECG normalization can be observed.

REFERENCES

1. Baranchuk A, Nguyen T, Ryu MH, et al. Brugada phenocopy: new terminology and proposed classification. Ann Noninvasive Electrocardiol. 2012;17:299-314.
2. Francis J, Antzelevitch C. Brugada-like electrocardiographic pattern. Indian Pacing Electrophysiol J. 2003;3:91-92.
3. Awad SF, Barbosa-Barros R, Belem Lde S, et al. Brugada phenocopy in a patient with pectus excavatum: systematic review of the ECG manifestations associated with pectus excavatum. Ann Noninvasive Electrocardiol. 2013;18:415-420.
4. Kazmi F, Adil A, Miller S, et al. Trauma induced Brugada pattern. J Am Coll Cardiol. 2019;73(S1):2924.
5. Coppolino A, Valeri L. Pericardial Effusion and Brugada Type 1 Electrocardiogram Pattern. An Another Case of Brugada Phenocopy. Acta Scientific Clinical Case Reports. 2022;3(1): 59-64.
6. Barcos JC, Tello Santacruz IA, Monié CC, et al. Brugada phenocopy induced by severe pneumothorax. J Electrocardiol. 2018;51:343-345.
7. Khmao P, Long V, Ku N, et al. Brugada phenocopy or congenital Brugada syndrome in a patient with spontaneous pneumopericardium and pericarditis. J Arrhythm. 2020;37:246-248.
8. Sasaki A, Nakazato Y. Brugada-like electrocardiogram detected after reconstructive operation for oesophageal cancer. Europace. 2010;12:1542.
9. Tarín N, Farré J, Rubio JM, et al. Brugada-like electrocardiographic pattern in a patient with a mediastinal tumor. Pacing Clin Electrophysiol. 1999; 22: 1264-1266.
10. Nakazato Y, Ohmura T, Shimada I, et al. Brugada-like precordial ST elevation on ECG by anterior mediastinal infective mass lesion. Indian Pacing Electrophysiol J. 2003;3:184.
11. Asteriou C, Lazopoulos A, Giannoulis N, et al. Brugada-like ECG pattern due to giant mediastinal lipoma. Hippokratia. 2013;17:368-369.
12. Pérez-Riera AR, Barbosa Barros R, Daminello-Raimundo R, et al. Brugada phenocopy caused by a compressive mediastinal tumor. Ann Noninvasive Electrocardiol. 2018;23:e12509.
13. Nguyen T, Smythe J, Baranchuk A. Rhabdomyoma of the interventricular septum presenting as a Brugada phenocopy. Cardiol Young. 2011;21:591-594.
14. Sato N, Shimokawa M, Iwasaki H, et al. Brugada-Like ECG Associated with Primary Cardiac Lymphoma. J Clin Experiment Cardiol. 2011;2:1-2.
15. Schnegg B, Seiler J, Suter TM, et al. Brugada Type 1 ECG Caused by an RVOT Tumor. Scientific Pages Heart. 2016;1:002.
16. Tomcsányi J, Simor T, Papp L. Images in cardiology. Haemopericardium and Brugada-like ECG pattern in rheumatoid arthritis. Heart 2002;87:234.
17. Antzelevitch C, Dumaine R. Electrical heterogeneity in the heart: physiological, pharmacological and clinical implications. In: The Handbook of Physiology. New York: Oxford Univ. Press, 2002, pp. 654-692.

18. Kim YH, Lim HE, Kim SH, et al. Brugada-like ST-segment abnormalities associated with myocardial involvement of hematologic diseases. Pacing Clin Electrophysiol. 2008;31:761-764.
19. Tomiyama T, Shijimaya T, Sano Y, et al. Large Metastatic Cardiac Tumor from Ascending Colon Cancer with Autopsy. Case Rep Gastroenterol. 2021;15:703-708.
20. Briasoulis A, Siddiqui F, Siddiqui S, et al. Large right ventricular mass causing outflow tract obstruction secondary to metastatic renal cell carcinoma. Acta Cardiol. 2015;70:86-87.
21. Briosa E, Gala A, Dimarco A, et al. Subtotal Obstruction of the Right Ventricular Outflow Tract Caused by Isolated Intracardiac Renal Cell Carcinoma Metastasis. Circ Cardiovasc Imaging. 2019;12:e009714.
22. Doshi TV, Doshi JV, Makaryus JN, et al. A rare case of successfully treated cardiac metastasis from transitional cell bladder cancer. Am J Ther. 2013;20:307-310.
23. Chua S, Liu WH, Lee WC. Isolated huge right ventricular tumor: cardiac metastasis of tongue cancer. Korean J Intern Med. 2017;32:1119-1120.
24. Demir V, Turan Y, Ede H, et al. Electrocardiographic changes in right ventricular metastatic cardiac tumor mimicking acute ST elevation myocardial infarction: A case of misdiagnosis. Turk J Emerg Med. 2018; 19: 33-35.
25. Ya'qoub L, Payne K, Parikh S, et al. A Right Ventricular Mass in a Patient with Squamous Cell Lung Cancer: A Case Report and Review of Literature. Cureus. 2018;10:e2261.
26. Gopal AS, Stathopoulos JA, Arora N, et al. Differential diagnosis of intracavitary tumors obstructing the right ventricular outflow tract. J Am Soc Echocardiogr. 2001;14:937-940.
27. Labib SB, Schick EC Jr, Isner JM. Obstruction of right ventricular outflow tract caused by intracavitary metastatic disease: analysis of 14 cases. J Am Coll Cardiol. 1992;19:1664-1668.
28. Elikowski W, Łazowski S, Fertała N, et al. Brugada phenocopy in pulmonary embolism – clinicopathological case study and literature review. Pol Merkur Lekarski. 2022;50:378-383.
29. Lazaros G, Lazarou E, Tousoulis D. Brugada phenocopy in a patient underdoing pericardiocentesis for a large idiopathic pericardial effusion. J Electrocardiol. 2020;63:184-185.
30. Facin M, Pastore CA, Samesima N, et al. Ventricular repolarization abnormalities: the electrocardiographic track of cardiac tumoural involvement in an infant with tuberous sclerosis complex. A case report. Eur Heart J Case Rep. 2020; 4: 1-6.

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

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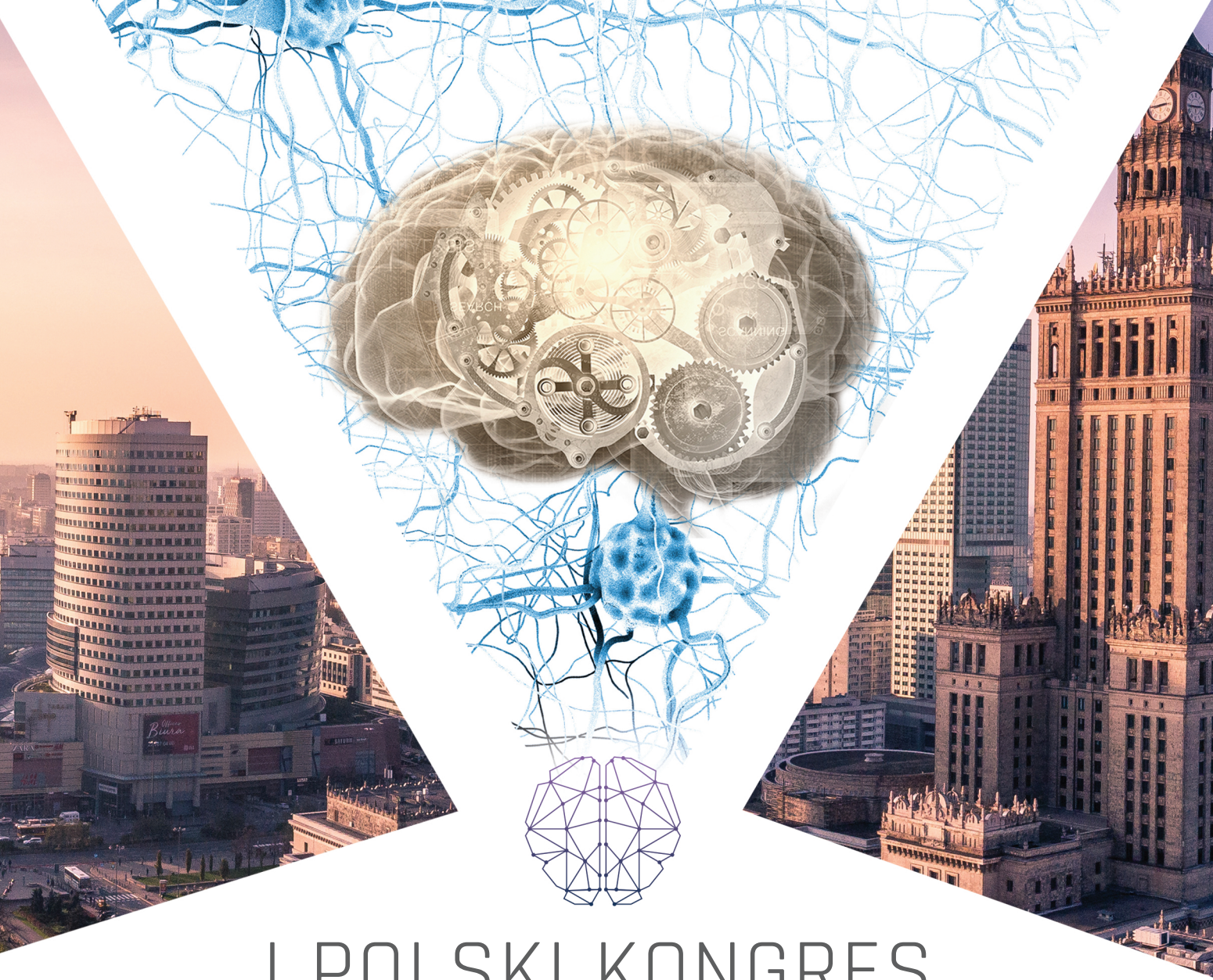
Received: 20.12.2022
Accepted: 05.02.2023

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



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