ORIGINAL ARTICLE

PECULIARITIES OF DYNAMICS OF INDICATORS OF PROTEINS OXIDATIVE MODIFICATION AND MATRIX METALLOPROTEINASE-9 ACTIVITY IN PATIENTS WITH PARANOID SCHIZOPHRENIA DEPENDING ON THE DISEASE DURATION

DOI: 10.36740/WLek202312111

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ABSTRACT

The aim: The objective of the research was to study the indicators of oxidative modification of proteins (OMP) and the activity of matrix metalloproteinase-9 (MMP-9) in patients with paranoid schizophrenia depending on the disease duration.

Materials and methods: 320 patients were included in the examination. 20 patients were with "Primary psychotic episode" (Comparison Group) and 300 patients were diagnosed with "Paranoid schizophrenia" (Experimental Group): 60 of them have suffered from this disease for a duration from 3 to 5 years (Subgroup I); 60 patients have suffered for a period from 6 to 10 years (Subgroup II); 60 individuals – from 11 to 15 years (Subgroup III); 60 patients have suffered for a duration from 16 to 20 years (Subgroup IV); 60 patients – from 21 years and longer (Subgroup V).

Results: The presented data showed that the levels of OMP indicators in Subgroup I constituted 0.826±0.046 conventional units at a wavelength of 356 nm; 0.864±0.051 conventional units at a wavelength of 370 nm; 0.444±0.019 conventional units at a wavelength of 430 nm; 0.176±0.007 conventional units at a wavelength of 530 nm, which is 1.99; 1.6; 1.13 and 1.43 times higher than in the Comparison Group. The content of OMP products was higher by 2.24; 1.74; 1.17, and 1.43 times in Subgroup II, respectively, by 2.4; 1.80; 1.36 and 1.46 times in Subgroup III, respectively; by 2.5; 1.9; 1.4; 1.6 times in Subgroup IV, respectively; by 2.5; 2.02; 1.54; 1.7 times in Subgroup V, respectively. The conducted correlation analysis indicated a direct correlation between OMP indicators and the disease duration. The concentration of MMP-9 in the patients of the Comparison Group was equal to 892.84±87.80 pg/ml, which was 11.2% less compared to the Experimental Subgroup I, where this indicator was 992.84±67.50 pg/ml. MMP-9 constituted 1092.53±47.20 pg/ml on average in the patients of Subgroup II, which was 22.36% higher than in the Comparison Group. This indicator was 1702.84±37.60 pg/ml in Subgroup III, which was 90.7% higher than in the Comparison Group. It constituted 1492.84±47.29 pg/ml in Subgroup IV, which was 67.2% higher than in the Comparison Group; and 2037.21±57.80 pg/ml in Subgroup V, which was more than two times higher than in the Comparison Group ($p<0.05$). The conducted correlation analysis showed a direct relation between MMP-9 expression and the increase in OMP indicators. This relation was more significant between MMP-9 and OMP products of a neutral nature. The correlation strength between MMP-9 and OMP products of a basic nature was somewhat less significant.

Conclusions: According to the results of the conducted analysis, the examined patients had the signs of decompensation of reactive-adaptive biomolecular mechanisms which activated radical reactions with the subsequent accumulation of oxidation products.

KEY WORDS: oxidative modification of proteins, reactive oxygen species, matrix metalloproteinase-9, paranoid schizophrenia, disease duration, oxidative stress

Wiad Lek. 2023;76(12):2625-2631

INTRODUCTION

According to scientific data, proteins are the main components of most biological systems and make up about 70% of the dry mass of cells and tissues. As a result, it is natural that the proteins sustain the most damage. Certainly, the degree of these damages and especially their biological significance can be very diverse and depend on many factors affecting the body [1, 2]. Therefore, it is not surprising that it is proteins of cell membranes that are primarily exposed to reactive oxygen species (ROS) under the conditions of oxidative stress (OS) causing cell lysis and depolymerization. It has been scientifically proven that some processes of protein oxidative modification contribute to the loss of protein function, cleavage or aggregation, and some lead to proteotoxicity and disruption of cellular homeostasis [3]. It is important to note that similar modification processes are the initial reaction of the cell to a change in its functioning conditions. It should be noted that products of oxidative modification of proteins (OMP) are quite stable by their nature. As a target for specific neutral

and alkaline proteases action, they are practically not recoverable and, therefore, become a trigger for many pathological processes and OS early marker. In addition, proteins are a natural source of free radicals. In the process of their modification, primary amino acid radicals are formed that can interact with neighboring amino acid residues. Almost all amino acid residues of proteins are capable of oxidation leading to changes in their functions. As a result, proteins begin to lose their natural structure and biological properties, such as metabolic or regenerative ones.

Intensification of the processes of proteins peroxidation depletes antioxidants reserves in the body. In particular, sulfo- and aminohydroxyl groups of amino acids are subject to peroxidation. In relation to the products of protein destruction, they accumulate in the research material in the form of aldehyde- and keto-derivatives of dinitrophenylhydrazones. According to the researchers, oxygen-dependent protein oxidation is an early indicator of damage to organs and tissues, so OMP indicators should be under continuous laboratory control.

More and more available data on schizophrenia pathogenesis focus on neuroinflammatory processes. Lu Y and co-authors report that the expression of matrix metalloproteinases has a close correlation with OS, namely the degree of its manifestation determining the relevance of studying the interaction of the state of the prooxidative-antioxidant system and the expression of matrix metalloproteinases in patients with schizophrenia [4]. In particular, matrix metalloproteinase-9 (MMP-9) cleaves various proteins of the extracellular matrix, significantly contributing to numerous physiological and pathological processes [5]. This extracellular protease is actively involved in the regulation of synaptic plasticity [6]. Its participation in the processes of neurons morphology and their transmission of signals has been scientifically confirmed [7]. Recent studies have confirmed that ROS produced during OS affect the expression of MMP-9, the increase of which increases the risk of cognitive impairment in case of schizophrenia [8]. In addition, according to Dickerson, F et al., people with higher levels of MMP-9 have significantly higher chances of developing schizophrenia [9, 10].

THE AIM

The objective of the research was to study the indicators of oxidative modification of proteins (OMP) and the activity of matrix metalloproteinase-9 in patients with paranoid schizophrenia tracing the peculiarities of t

heir dynamics depending on the disease duration.

MATERIALS AND METHODS

The study was conducted at the premises of Municipal Non-profit Enterprise "Prykarpattia Regional Clinical Center for Mental Health of Ivano-Frankivsk Regional Council" (MNE PRCCMHIFRC) and "Pohonia Psychoneurological Care Home". The study included 320 patients: 20 patients with "Primary psychotic episode" (Comparison Group) and 300 patients with a diagnosis of "Paranoid schizophrenia" (Experimental Group): 60 of them have suffered from this disease for a duration from 3 to 5 years (Subgroup I); 60 patients have suffered for a period from 6 to 10 years (Subgroup II); 60 individuals – from 11 to 15 years (Subgroup III); 60 patients have suffered for a duration from 16 to 20 years (Subgroup IV); 60 patients – from 21 years and longer (Subgroup V). The main criteria for inclusion in the Study Group were as follows: the presence of a "Paranoid schizophrenia" diagnosis and individual consent of the patient. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Cases of schizophrenia with the comorbidities of substance-related disorders or mental retardation were excluded. Spectrophotometric analysis of carbonyl groups formed during the interaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with oxidized amino acid residues of proteins was used to study the intensity of OMP. Determination of the optical density of aldehyde- and ketone-containing dinitrophenylhydrazines was conducted on a spectrophotometer SPECORD M 40 (Germany) of a neutral nature at the wavelength of 356 and 370 nm, and the basic nature at the wavelength of 430 and 530 nm. The content of MMP-9 in the blood plasma of all examined patients was determined by the enzyme immunoassay method by means of "Immuno Chem-2100, Microplate Reader" device using the laboratory kit "The RayBiotech Human MMP-9 Enzyme Immunoassay Kit" (USA) and was expressed in pg/ml. All examinations were conducted after the patients signed the informed consent, the terms of which were approved by the Bioethics Commission of the Ivano-Frankivsk National Medical University. The research was conducted according to the "Rules of Ethical Principles of Conducting Scientific Research with Human Participation" approved by the Declaration of Helsinki (1964-2013) and the ethical and moral and legal norms of the order of the Ministry of Health of Ukraine № 281, November 1, 2000.

Statistical processing of the obtained results was conducted using "STATISTICA 7.0." program (StatSoft, Inc.) and the package of statistical functions of "Microsoft Excel, 2016" program. The reliabilty of the obtained results was confirmed based on the calculation of the Student's coefficient. Correlation analysis was per-

Table I. The content of the products of proteins oxidative modification in the blood serum of the examined patients

Groups		Products of a neutral nature (M±μ)		Products of a basic nature (M±μ)	
		356 nm., aldehyde derivatives	370 nm., keto derivatives	430 nm., aldehyde derivatives	530 nm., keto derivatives
Experimental Group, n=300 (conventional units)	Subgroup I, (n=60)	0.826±0.046*	0.864±0.051*	0.444±0.019*	0.176±0.007*
	Subgroup II, (n=60)	0.931±0.045*	0.946±0.067*	0.461±0,024*	0.177±0.005*
	Subgroup III, (n=60)	0.996±0.043*	0.981±0.068*	0.532±0,025*	0.180±0.006*
	Subgroup IV, (n=60)	1.034±0.042*	1.030±0.065*	0.551±0,023*	0.198±0.007*
	Subgroup V, (n=60)	1.048±0.041*	1.100±0.058*	0.606±0,023*	0.209±0.008*
Comparison Group, n=20 (conventional units)		0.414±0.032	0.543±0.031	0.391±0,087	0.123±0.043

Note: * – ($p<0.05$) the data are reliable between the Comparison subgroup and the studied one

formed using the Spearman method (rank method). Arithmetic mean (M), standard error (±m) was used to describe quantitative characteristics.

RESULTS

The results of the statistical analysis of the obtained data are presented in Table I.

The presented data showed that the levels of OMP indicators in Subgroup I constituted 0.826±0.046 conventional units at a wavelength of 356 nm; 0.864±0.051 conventional units at a wavelength of 370 nm; 0.444±0.019 conventional units at a wavelength of 430 nm; 0.176±0.007 conventional units at a wavelength of 530 nm, which was 1.99; 1.6; 1.13 and 1.43 times higher than in the Comparison Group. The content of OMP products was higher by 2.24; 1.74; 1.17 and 1.43 times in Subgroup II, by 2.4; 1.80; 1.36 and 1.46 times respectively in Subgroup III, by 2.5; 1.9; 1.4; 1.6 times in Subgroup IV, by 2.5; 2.02; 1.54; 1.7 times in Subgroup V.

The comparative characteristic of the optical density ratio of aldehyde- and ketone-containing dinitrophenylhydrazines in the examined patients is shown graphically in Figure 1. Here, the ratio of the values in all studied groups is clearly presented in the form of pie charts.

The dynamics of OMP indicators in the examined patients depending on the main disease duration is presented in Figure 2. According to the presented data, the OMP indicators determined by aldehyde- and keto-derivatives of a neutral and basic nature were significantly higher in the Experimental Group than in the Comparison Group and had an upward trend depending on the disease duration.

The conducted correlation analysis showed that there was a direct correlation between OMP indicators and the disease duration: OPM-356/ duration of the disease was strong ($r=+0.82$; $p<0.05$); OMP-370/ disease duration was strong ($r=+0.86$; $p<0.05$); OMP-430/duration of the disease was average ($r=+0.44$; $p<0.05$); OMP-530/ duration of the disease was weak ($r=+0.17$; $p<0.05$).

The results of the study of MMP-9 level in the serum of the examined patients are shown in Figure 3.

According to the presented data, the concentration of MMP-9 in the patients of the Comparison Group was equal to 892.84±87.80 pg/ml, which was 11.2% less compared to the Experimental Subgroup I, where this indicator constituted 992.84± 67.50 pg/ml. MMP-9 was 1092.53±47.20 pg/ml on average in the patients of Subgroup II, which was 22.36% higher than in the Comparison Group. It constituted 1702.84±37.60 pg/ml in Subgroup III, which was 90.7% higher than in the Comparison Group, and 1492.84±47.29 pg/ml in Subgroup IV, which was 67.2% higher than in the Comparison Group, and 2037.21±57.80 pg/ml in Subgroup IV, which was more than two times higher than in the Comparison Group ($p<0.05$).

The conducted correlation analysis showed a direct relation between the expression of MMP-9 and the growth of OMP indicators. This relation was more significant between MMP-9 and neutral OPM products: OMP-356/MMP-9 – strong ($r=+0.78$, $p<0.05$); OMP-370/ MMP-9 – strong ($r=+0.71$, $p<0.05$). The strength of the correlation between MMP-9 and OMP products of a basic nature was somewhat less pronounced: OMP-430/ MMP-9 – medium ($r=+0.57$, $p<0.05$); OMP-530/MMP-9 – medium ($r=+0.43$, $p<0.05$).

DISCUSSION

Today's attention focused on the OMP features and the expression of MMP-9 in patients with schizophrenia, particularly its paranoid form. Unfortunately, as it stands today, there is no single concept of this disease pathogenesis, the underlying complex genetic architecture remains elusive complicating significantly the development of pathogenetic treatment methods, and the mechanisms of its development and progression remain debatable [11].

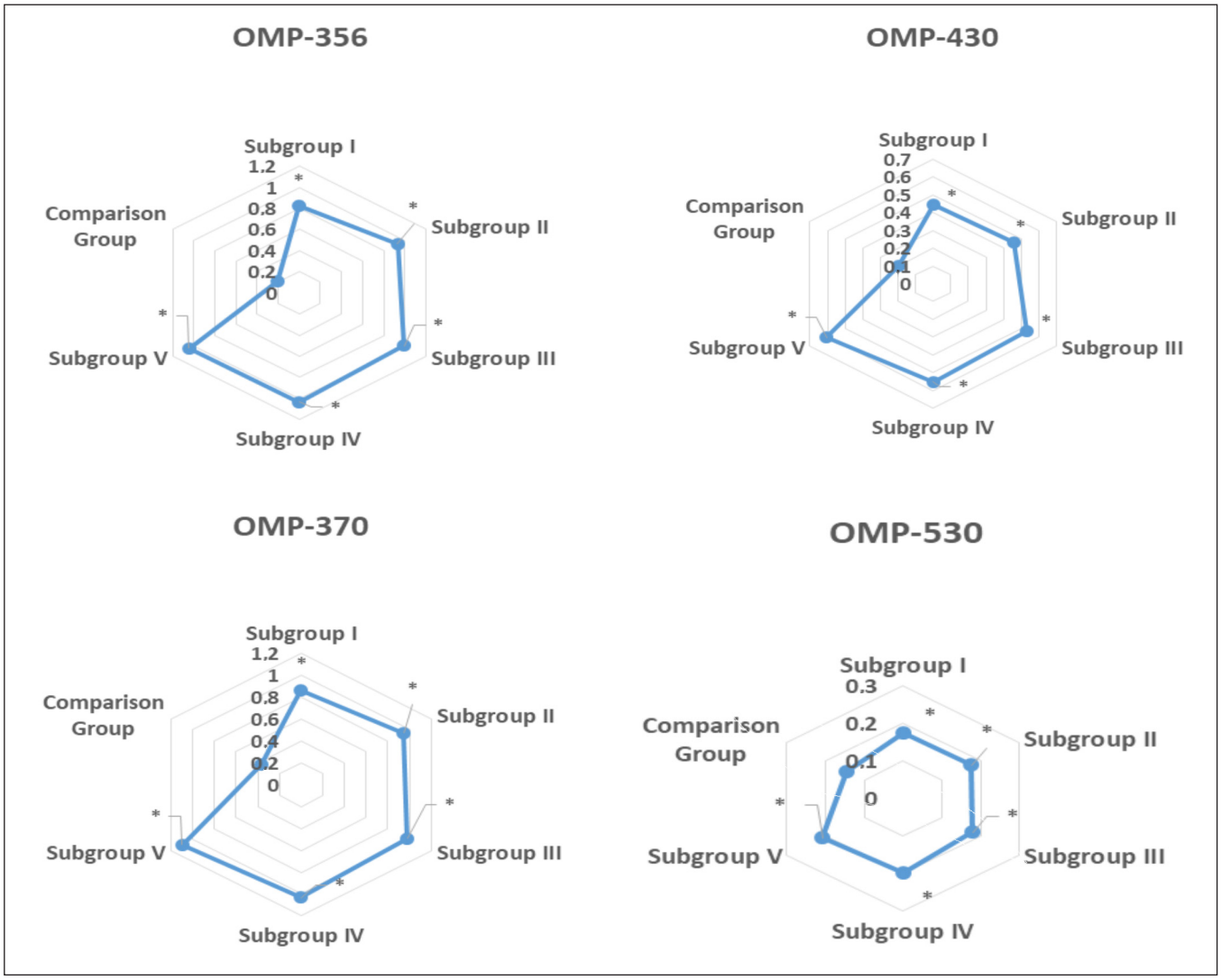

Fig. 1. Ratio of indicators of the optical density of aldehyde- and ketone-containing dinitrophenylhydrazines in the examined patients.
Note: * – ($p<0.05$) the data are reliable between the studied subgroups of Experimental Group and Comparison Group.

Our previous studies established that paranoid schizophrenia was manifested in an imbalance of BDNF and MMP-9 expression that can affect the processes of neurogenesis and synaptic plasticity. According to the PANNS scale, this affects emotions, thinking processes, cognition, causes a loss of interest to social and environmental phenomena, contributes to the deterioration of memory, so they can be considered as diagnostic markers of such pathology [12]. We found that BDNF expression significantly decreased and the expression of MMP-9 increased with an increase in the duration of the studied pathology, and the therapeutic approach of such patients should take these changes into account [13,14].

According to the presented data, the OMP indicators determined by aldehyde- and keto-derivatives of a neutral and basic nature were significantly higher in the Experimental Group than in the Comparison Group and had an upward trend depending on the disease duration. The conducted correlation analysis showed that there was a direct correlation between OMP indicators and the disease duration

The results of the study of the level of MMP-9 in the serum of the examined patients reflect its tendency to increase depending on the duration of the underlying disease. The conducted correlation analysis showed a direct relation between the expression of MMP-9 and the growth of OMP indicators. This relation was more significant between MMP-9 and neutral OPM products

The complex dynamics of redox regulation mechanisms and their modulation in case of schizophrenia are evidenced by separate scientific works [15]. Currently, there are scarce data that acquaint us with the features of OMP and MMP-9 expression in case of schizophrenia, and there are no data that would reflect the dynamics of this indicator among the specified category of patients, taking into account the duration of their disease. All of the above mentioned confirms the relevance and importance of further research in this direction.

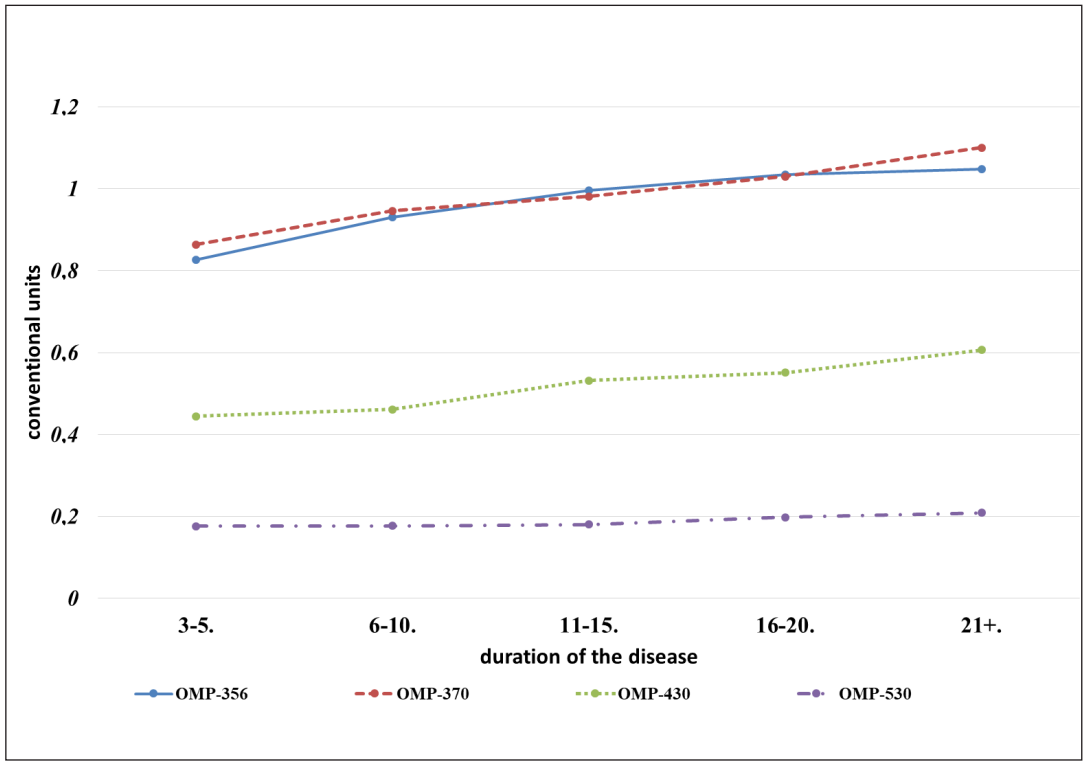

Fig. 2. Dynamics of indicators of oxidative modification of proteins in the examined patients

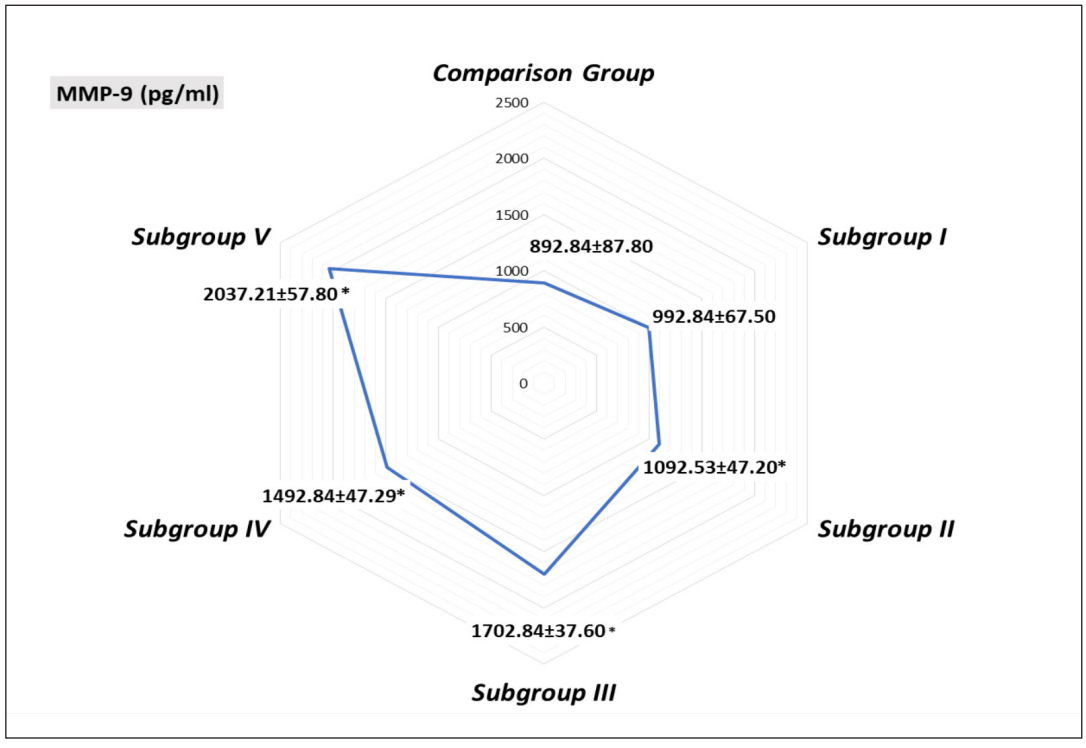

Fig. 3. The level of MMP-9 in the blood serum of the examined patients
Note: * – ($p<0.05$) the data are reliable between the study subgroups of Experimental Group and Comparison Group.

CONCLUSIONS

Thus, according to the results of the conducted analysis, the examined patients had the signs of decompensation of reactive-adaptive biomolecular mechanisms which activated radical reactions with the subsequent accumulation of oxidation products having a toxic effect. This was evidenced by the accumulation of aldehyde and ketone derivatives in blood serum, which were positively correlated with MMP-9 activity. Statistical analysis also confirmed a positive correlation between OMP indicators and the duration of the main disease course.

Prospects for further research are to monitor the dynamics of OMP indicators and MMP-9 activity and their mutual influence on the features of pathopsychological symptoms (according to the analysis of the PANSS scale) in the patients with paranoid schizophrenia depending on the duration of their disease.

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The work is a fragment of the research project "Mental and psychosomatic consequences of mental trauma as a result of hostilities in Ukraine among the military and civilian population. Issues of diagnosis, differential diagnosis and differential therapy" (№ state registration 0123U100350) on the Department of Psychiatry, Addiction and Medical Psychology, Ivano Frankivsk National Medical University.

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

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Received: 04.11.2022
Accepted: 24.09.2023

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