

9. Montaruli A, Castelli L, Mulè A, Scurati R, Esposito F, Galasso L, et al. Biological Rhythm and Chronotype: New Perspectives in Health. *Biomolecules*. 2021;11(4):487. Published 2021 Mar 24. doi:10.3390/biom11040487.
10. Obi EE. Surgical considerations in skin of colour: minimizing pathological scars. *Clin Exp Dermatol*. 2022;47(8):1429–1437. doi:10.1111/ced.15147.
11. Schulz A, Shoham Y, Rosenberg L, Rothermund I, Perbix W, Christian Fuchs P, et al. Enzymatic Versus Traditional Surgical Debridement of Severely Burned Hands: A Comparison of Selectivity, Efficacy, Healing Time, and Three-Month Scar Quality. *J Burn Care Res*. 2017;38(4):e745–e755. doi:10.1097/BCR.0000000000000478.
12. Seago M, Shumaker PR, Spring LK, Alam M, Al-Niaimi F, Rox Anderson R, et al. Laser Treatment of Traumatic Scars and Contractures: 2020 International Consensus Recommendations. *Lasers Surg Med*. 2020;52(2):96–116. doi:10.1002/lsm.23201.
13. Tarasenko LM, Neporada KS, Klusha V. Stress-protective effect of glutapyrone belonging to a new type of amino acid-containing 1,4-dihydropyridines on periodontal tissues and stomach in rats with different resistance to stress. *Bull Exp Biol Med*. 2002;133(4):369–371. doi:10.1023/a:1016250121896.

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## MORPHOLOGICAL AND FUNCTIONAL CHANGES OF ERYTHROCYTES AND ENZYMES OF THE ANTIOXIDANT SYSTEM IN MULTIPLE SCLEROSIS

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In the pathochemical complex formed in multiple sclerosis, the central role is given to the disintegration of metabolism, activation of lipid peroxidation processes, and suppression of the antioxidant defense system. The results of the conducted research indicate that in the pathogenesis of multiple sclerosis, significant importance is attributed to the activation of enzymatic and free radical lipid oxidation of erythrocyte cell membranes in conditions of complete or partial inability of antioxidant defense mechanisms. The consequence is significant changes in the structure of erythrocyte membranes and their functions. The detected destructive changes in erythrocytes correlate with the severity of clinical manifestations of multiple sclerosis, thus, they can be used not only to determine the severity of the disease but also for predicting its course, as well as monitoring the quality and effectiveness of therapeutic interventions in such patients.

**Key words:** multiple sclerosis, antioxidant defense mechanisms, erythrocyte membranes.

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## МОРФО-ФУНКЦІОНАЛЬНІ ЗМІНИ ЕРИТРОЦИТІВ ТА ФЕРМЕНТИВ АНТИОКСИДАНТНОЇ СИСТЕМИ ПРИ РОЗСІЯНОМУ СКЛЕРОЗІ

У патохімічному комплексі, що формується при розсіяному склерозі, центральне місце відводиться дезінтеграції метаболізму, активації процесів перекисного окислення ліпідів і пригніченню системи антиоксидантного захисту. Результати проведеного дослідження свідчать, що в патогенезі розсіяного склерозу значне місце належить активації ферментативного та вільнорадикального окиснення ліпідів клітинних мембран еритроцитів в умовах повної або часткової неспроможності механізмів антиоксидантного захисту. Наслідком є значні зміни структури клітинних мембран еритроцитів та їх функції. Виявлені деструктивні зміни еритроцитів корелюють з важкістю клінічних проявів розсіяного склерозу, а значить, багато в чому їх визначають, що можна використовувати для визначення не тільки важкості захворювання, але й для прогнозування його перебігу, а також контролю за якістю та ефективністю терапевтичних заходів у таких пацієнтів.

**Ключові слова:** розсіяний склероз, антиоксидантний захист, мембрани еритроцитів.

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The steady increase in prevalence and severe socio-economic consequences of multiple sclerosis (MS) make it necessary to further analyze this pathology, especially the study of its pathogenesis, as a key to finding promising methods of pathogenetic therapy [6]. In the pathochemical complex formed in MS, the central place is given to the disintegration of metabolism, the activation of lipid peroxidation processes (LPP) and the inhibition of the antioxidant defense system (ADS). The relationship between disorders in the immune system and the accumulation of LPP-lipoperoxide products in the body has been established. Excessive retention of LPP products in the body serves as one of the factors in the formation of autoimmune responses, while the state of the health system can determine the degree of recovery of neurological deficits. To reveal the pathogenetic mechanisms of tissue and cellular damage formation in MS, it is of great interest

to study the role of the erythron system as an important link in ensuring compensatory and adaptive reactions of the whole organism. Peripheral blood erythrocytes (PBER), in close contact with all tissues and entering into morpho-functional relationships with them, reflect the physiological and pathological changes occurring in the body through their own qualitative and quantitative restructuring, thereby conditioning the so-called expository (reflected) reactions of knowledge about which can serve as effective prognostic markers for a number of chronic diseases.

The multifunctional role of PBER in the mechanisms of adaptation and compensation in conditions of hypoxia, in the implementation of gas transport processes and other vital functions explains the high informativeness of the results of the study of structural and functional changes in these cells. At the same time, an insufficiently researched aspect of hypoxia is the enzymatic regulation of the processes of formation and destruction of hydrogen peroxide in PBER. Taking into account the data on the direct participation of reactive oxygen species (ROS) ( $O_2$ ,  $H_2O_2$ ) and antioxidant protection enzymes superoxide dismutase (ESD) and catalase (CT) in the processes of hemoglobin oxygenation [7], it is interesting to study the nature of changes in the activities of these enzymes aimed at increasing structural and functional adequacy of PBER, which is required for adequate oxygen transport in MS.

**The purpose** of the study was to establish the structural and functional features of peripheral blood erythrocytes and antioxidant defense enzymes in patients with multiple sclerosis, depending on their clinical forms and the stage of development of the pathological process.

**Materials and methods.** The research was conducted at the clinical base of the Department of Neurology and Neurosurgery of the Ivano-Frankivsk National Medical University in the Regional Clinical Hospital of the Ivano-Frankivsk Regional Council.

62 patients who were being treated in the neurological department with a diagnosis of multiple sclerosis according to MacDonald criteria were examined, the mean age of the patients was  $40.4 \pm 0.87$  years, PBER researches were carried out during hospitalization (within 14 days) and during an exacerbation disease. According to modern recommendations, all patients were divided into three groups: patients of the 1st group – with relapsing multiple sclerosis (RMS), II group – with secondary-progressive multiple sclerosis (SPMS) and grade III group – with primary progressive multiple sclerosis (PPMS). The control group (CG) consisted of 20 practically healthy people who were comparable in age.

Special research methods are presented:

1. By studying the content of products of free radical oxidation of lipids in PBER membranes: diene conjugates (DK) of polyunsaturated fatty acids of phospholipids and Schiff bases (SHO) [7].

2. Assessment of the level of antioxidant protection (activity of superoxide dismutase (SOD) – an enzyme of antiradical protection, catalase (CT) – an enzyme of the ADS, glucose-6-phosphate dehydrogenase (G-6-FDGD) [8, 11].

3. Determination of phospholipase activity. The surface architecture of PBER was studied with the help of scanning electron microscopy (SEM) (electron microscope “JEOL-25A-T3225”; Japan) with sample preparation according to the method of G. I. Kozynets and co-authors. Various morphological forms of PBER were counted according to the classification of G. I. Kozynets and co-authors.

4. Determination of the reserve index of erythrocyte deformability (IED) according to our own method [1] and blood oxygen saturation using pulse oximetry with the Jziki-Fingertip Oximeter device.

Part of the research was carried out with a hemoanalyzer (LabAnalyt30000Plus (Finland). Determination of the microelement composition of PBER was carried out using energy-dispersive X-ray structural analysis on the EDAR microanalysis attachment to the REMMA-202E SEM (Sumy, Ukraine).

Statistical analysis was performed using the standard SAS 8.0 software package (SAS Inc., USA). The  $t$  and  $\chi^2$  criteria were used. The results were considered reliable at  $p < 0.05$ .

**Results of the study and their discussion.** The state of membrane-destabilizing processes is characterized by various criteria. This is, first of all, the level of functional activity of endogenous phospholipases and the dynamics of accumulation of lipoperoxides. As it follows from the results we obtained, the severity of membrane-destabilizing processes increases from the minimum values in patients of the 1st group. with the most favourable remitting form of MS (RMS) to the maximum level in Group I with secondary-progressive MS (SPMS) and Group III in primary-progressive MS (PPMS), which is manifested by an increase in the width of the hemogram and its change from a normal, unimodal type of hemogram to a bipolar type of PBER distribution according to their corpuscular size, and a significant shift of the distribution peak to the left.

In patients, Group II and Group III revealed changes not only in the form of PBER, but also in their chemical composition, which was most pronounced in patients of Group III. As MS progressed,

erythrocytes from doubly curved discs turned into spherocytes and pronounced microrelief irregularities appeared on their surface. The ratio of spherocytes to discocytes at the stage of MS compensation was 1:7, at the stage of subcompensation 1:4, and at the stage of decompensation 1:9 (in practically healthy individuals of CG and the same age, this ratio is 1:60). Under such conditions, in patients of Group III with an increase in the number of spherocytes compared to CG people, the amount of nitrogen – a well-known main chemical element of living proteins – decreases statistically significantly (by 3 times;  $p < 0.05$ ), which makes it possible to detect it with the “EDAR” microanalyzer attachment to a scanning electron microscope.

It is known that the proteins in the PBER membrane are located mainly on its inner side and form a network of filaments that support the biconcave (toroidal) shape of the PBER.

In the PBER of patients of Group II and Group III compared to the PBER of practically healthy CG of the same age, a probable ( $p < 0.05$ ) increase in the concentration of calcium (1.5–2 times), magnesium

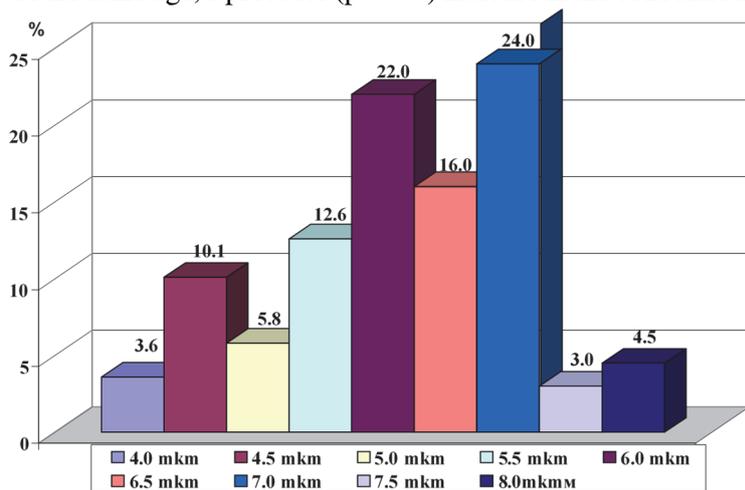


Fig. 1. The distribution width of peripheral blood erythrocytes of patients of Group III by corpuscular size.

(2 times) and aluminium (3 times) was found, which should be interpreted as a sign the development of the “calcium paradox” with a decrease in the number of free forms of nitrogen and an increase in nitric oxide during the activation of LPP, a violation of the permeability of cell membranes and the approach of PBER to hemolysis.

The hemogram of the distribution of PBER by size is asymmetric and multipolar due to an increase in the number of varying classes of the small (left) wing of cellular elements (Fig. 1).

At the same time, the biochemical indices of PBER change, which is especially evident when comparing the indices obtained during the exacerbation period and during the remission period of MS (Table 1).

Table 1

**Indices of PLA2 activity and the content of LPP products in the PBER of patients with MS depending on the form of the disease, M±m**

Indices	RF patients n=60			
	Healthy n=20	Group I	Group II	Group III
Phospholipase α 2 % hemolysis	4.44±0.22	15.21±1.1 *	27.05±1.3 */ **	25.93±1.33 */ **
Diene conjugate Ml.	48.43±3.5	109.11±3.7 *	180.3±5.1 */ **	170.1±7.9 */ **
Schiff bases	18.3±1.02	21.73±5.1 *	30.1±2.3 */ **	33.1±2.5 */ **
Index of deformation capacity of erythrocytes	2.03±0.05	1.44±0.02	1.39±0.03	1.08±0.02
Indices	RF patients n=60			
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Schiff bases	18.3±1.02	21.73±5.1 *	30.1±2.3 */ **	33.1±2.5 */ **
Index of deformation capacity of erythrocytes	2.03±0.05	1.44±0.02	1.39±0.03	1.08±002

Notes: IDRE is the reserve index of deformation resistance of erythrocytes; \* – probability of differences between indices of healthy and sick people,  $p < 0.05$ ; \*\* – probability of differences between indicators of patients with RMS and indices in two other groups of patients with MS,  $p < 0.05$ ; \*\*\* – probability of differences between indicators of patients with SPMS and PPMS,  $p < 0.05$ .

In patients with MS, regardless of the form of the disease, a multiple increase in the content of lipid products (diene conjugates (DC) and Schiff bases (SHB), as well as phospholipase-α2 (PL-α2) activity was found compared to controls ( $p < 0.05$ ). At the same time, as the disease worsens, the content of LPP products (DC, SHB) naturally increases ( $p < 0.05$ ) and is closely correlated ( $r = 0.93$ ) with an increase in the amount of PBER in an irreversibly altered form (Fig. 2).

These data can serve as an unfavorable prognostic index in patients with MS, since the formation of acanthocytes and echinocytes leads to a sharp decrease in the area of effective metabolism with the PBER membrane, due to the very small area of their contact with endotheliocytes of the MCR of working organs, only a small surface on the tops of spinous growths and blood disorders. These outgrowths often injure the surface of the endotheliocytes of the microcirculatory bed (there is an abrasion of the surface glycocalyx, when the rough microrelief and structure of the echinocytes acquires the properties of a peculiar pipeline brush relative to the very weak surface of the endotheliocytes), which is the cause of microthrombotization of hemocapillaries with a corresponding violation of blood supply and metabolism in the microenvironment of hemocapillaries damaged in this way.

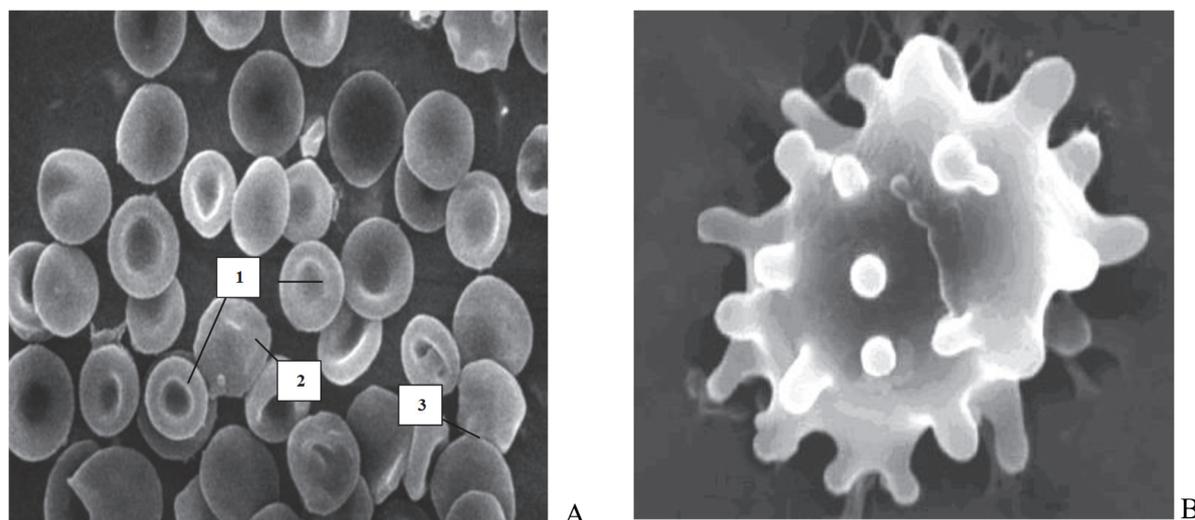


Fig. 2. Different forms of PBER: A discocyte (1), an erythrocyte with one outgrowth (2), an erythrocyte with a crest (3) and an irreversibly altered form of PBER – echinocyte (B) in a patient with primary progressive multiple sclerosis. Collection:  $\times 5000$  (A),  $\times 8000$  (B). The accelerating voltage is 20 kV 1

Thus, a regularity can be observed, which is manifested by an increase in the activity of phospholipase- $\alpha 2$  in comparison with similar indicators in healthy people with CG, and it can be argued that it is the high activity of phospholipase- $\alpha 2$  that ensures a less favorable course of MS in patients with the 1st group. This is evidenced by the quantitative characteristics of the activity of endogenous phospholipases and membrane lipid peroxidation processes, depending on the stage of the disease. It was found that the content of lipoperoxides and the activity of phospholipase- $\alpha 2$  in PBER membranes are statistically significantly ( $p < 0.05$ ) increased in patients with MS, regardless of the stage of the disease, and in the period of clinical remission, when these indices do not correspond to the normative parameters. At the same time, we can claim that the indicated changes are pathogenetically significant in case of exacerbation of MS (the difference between the analyzed indices during exacerbation and remission of the disease is statistically significant,  $p < 0.05$ ). Significant differences in PL- $\alpha 2$  activity depending on the stage of MS allow us to claim that this index has pathogenetic significance, increasing during exacerbation and decreasing during remission of the disease. The state of the antioxidant defense system in the body is also one of the most important mechanisms that regulate the activity of LPP and, indirectly, the functional significance of endogenous phospholipases. Presented in the table. 3 results may indicate that an unfavorable variant of MS (SPMS and PPMS) in patients with II and III gr. there is a disruption of the compensatory capabilities of the investigated links of the ADS, while in patients with the 1st gr. no significant differences from controls were found in RMS.

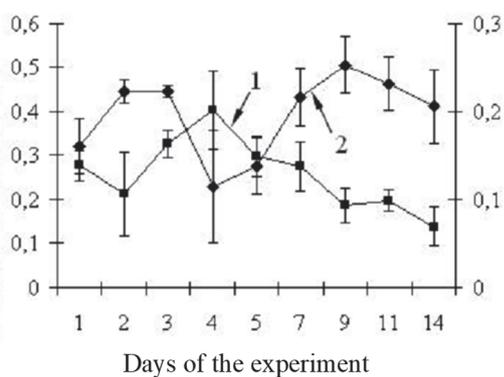
The change in the activities of catalase and ESD in PBER depending on the group of patients in the dynamics of MS has an oscillatory character (Fig. 2).

An increase in catalase activity always occurs against the background of a probable decrease in ESD activity relative to catalase (CAT). For many enzymes, in particular ESD and catalase, the phenomenon of cross-regulation of their activity is characteristic. For CT, the superoxide anion radical is a negative effector, and  $H_2O_2$  is a positive effector, while ESD is the opposite.

The spherization of PBER and the accumulation of chemical elements in them appear to precede hemolysis, that is, the destruction of erythrocyte membranes with the release of hemoglobin into the blood. Thus, according to a number of authors, the hemolysis of PBER passes through a number of stages: 1) before the hemolytic stage, which is characterized by spherization of erythrocytes; 2) the stage of osmotic

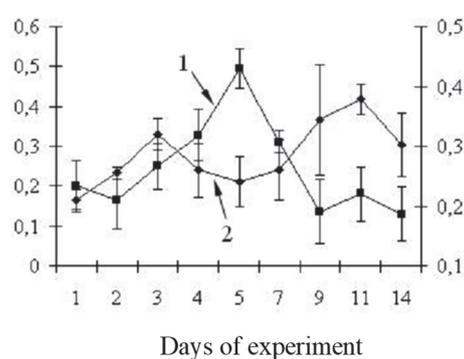
hemoglobinolysis, which is accompanied by the swelling of the erythrocyte beyond the volumetric critical size (which in our case determines the expansion of the hemogram of the distribution of PBER by volume) with a change or damage to the cell surface and the release of most of the hemoglobin into the plasma; 3) the stage of chemical hemoglobinolysis, which is characterized by a change in the chemical composition of the cell and, accordingly, a change in the electrochemical and colloid-osmotic properties of PBER with complete cleavage of hemoglobin; 4) stage of complete destruction of cellular structures. It would be logical to expect the development of anemia, mainly hemolytic character, also in patients with MS. Indeed, the syndrome of anemia in the patients examined by us occurred in 6.7–11.5 % of cases with exacerbation of MS and in 33.3 % of cases with a decrease in blood oxygenation (determined by pulse oximetry). According to the scientific literature, cardiovascular mortality in patients with MS increases proportionally with the deviation of the hematocrit index both in the direction of anemia and in the direction of polycythemia. Thus, a 1.6 % increase in mortality is associated with each percent deviation from the norm in the hematocrit level in either direction.

Activity of superoxide dismutase,  $\mu\text{mol}/\text{min mg Hb}$



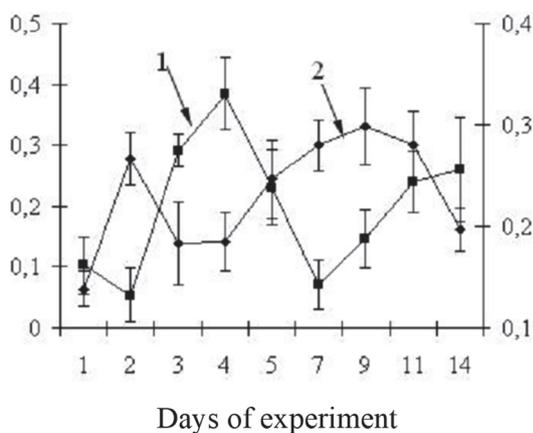
A

Activity of superoxide dismutase,  $\mu\text{mol}/\text{min mg Hb}$



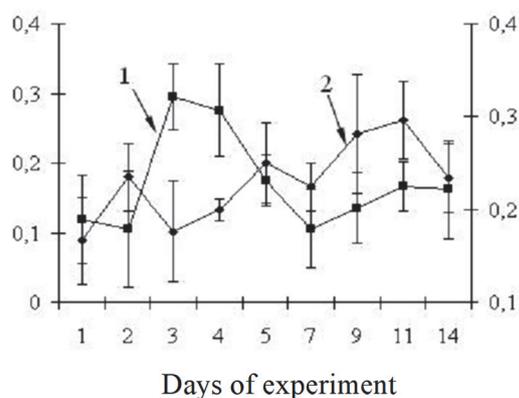
B

Catalase activity,  $\text{kmol}/\text{min mg Hb}$



C

Catalase activity,  $\text{kmol}/\text{min mg Hb}$



D

Fig. 3. Changes in the activity of ESD (1), catalase (2) in the dynamics of peripheral blood erythrocytes of patients of Group I (A), Group II (B), Group III (C) and in the period of exacerbation (D).

Therefore, with the chronic course of MS and against the background of membrane-destabilizing processes in the PBER, a more unfavorable prognosis for life is expected than with the usual chronic form of MS with minor neurological deficits that developed against the background of an acute stage of the disease combined with destructive changes in the PBER. Indeed, firstly, the compensatory capabilities of the PBER are significantly less during the development of LPP processes due to the smaller thickness of the PBER membrane, and, secondly, the degree of hypoxia in the presence of metabolic pathology increases significantly. Among the patients with MS examined by us, the mortality rate in the case of exacerbations in the neurologically deficient type was 0.93 %, and in the autonomically deficient type – 28.6 %, which confirms our thesis about a pronounced extrinsic reaction of PBER in MS. Changes in the morpho-functional state of PBER in the form of their spherulation and the development of a chemical imbalance (loss of nitrogen by cells and accumulation of calcium inside them, which is described by the term “calcium paradox” and other chemical elements) can be considered signs of an unfavorable prognosis for the life of

patients with chronic neurological insufficiency in MS, and the SEM method of blood samples is diagnostically significant in such patients.

According to scientific literature, such a multidirectional change in the activities of these two enzymes is characteristic of hypoxia and energy deficiency of cellular metabolism, i.e. in conditions where the activation of membrane-destabilizing processes underlying the conformational transformation of PBER is observed. Obviously, this pattern is inherent in MS patients as well. Statistical analysis of the obtained experimental data showed the presence of a probable negative correlation between the activities of ESD and catalase of erythrocytes of patients of all studied groups. In CG patients, a high ( $r=+0.93$ ;  $p=0.0012$ ) positive correlation between the activities of these enzymes was recorded. There is an opinion that the reduction of hydrogen peroxide can be an additional source of molecular oxygen. Catalase, performing an antioxidant function, compensatory increases the coefficient of useful use of exogenous oxygen for energy purposes due to the partial return of molecular oxygen, which is restored in the body by a one-electron pathway, to the metabolic chains of oxidative phosphorylation. The destruction of myelin in MS leads to the release of neurolemmocytes proteolytic enzymes and other aggressive agents, which irreversibly changes the conformation of the protein molecules of damaged cells, which are already perceived by the immune system as "alien". For example, some authors [3] found morphological signs of destruction of erythrocytes in rats already on the 2nd day after exposure to stress factors. The authors note that a sharp decrease in the supply of oxygen to cardiomyocytes occurs both as a result of the development of swelling of the vessel wall and perivascular space, and as a result of direct destruction of the vessels of the hemomicrocirculatory bed, which is observed starting from the first days of the stress reaction. At the same time, it was shown that MS causes structural changes in the population of mitochondria in different types of neurons [4, 5]. The morpho-functional changes in the tissues of the thymus and kidneys at different times of action of stress factors during aging and hypoxia are described, which allows us to consider the reaction of the body of patients with MS to the pathological influence of the main myelin protein as specific and due to the discrepancy between the energy demand and the capabilities of the energy-generating systems that increase in MS compensation, in the heart, muscle, nervous and connective tissues. At the same time, inflammatory processes occur in their body primarily in the nervous tissue and find regular manifestations in the form of conformational changes of PBER, which we propose to use for diagnostic and prognostic purposes.

Therefore, the results of the research indicate that in the pathogenesis of MS, a significant place belongs to the activation of enzymatic and free radical oxidation of cell membrane lipids in conditions of complete or partial failure of antioxidant defense mechanisms. The consequence is significant changes in the structure of PBER cell membranes and their function. The changes detected in the PBER model are basic, correlated with the severity of the clinical manifestations of MS, which means that they are largely determined, which can be used to determine not only the severity of MS, but also to predict its course, as well as control the quality of therapeutic measures in such patients. Thus, the loss of the disk-like shape of PBER is explained by a deficiency of free nitrogen and an increase in the amount of nitric oxide, which is possible under nitrosolin stress conditions [9, 10] and the breakdown of protein structures of the cytoplasmic membrane of PBER. It is known that many diseases, including immuno-neurological ones, are characterized by the development of oxidative stress as a result of a violation of the pro-oxidant-antioxidant balance and an increased course of free-radical reactions involving active forms of oxygen. In recent years, a lot of data has been accumulated, indicating a close relationship between the production of oxygen free radicals and nitric oxide. One of the markers of nitrosative stress is the formation of low-molecular and high-molecular nitroso thiols [8], particularly protein nitrosylation products. Previously, it was shown that in ischemic heart disease (CHD) the reactions of LPP and oxidative modification of proteins in PBER are enhanced [1, 3]. There is evidence that nitric oxide (NO) is one of the universal regulators of the body's physiological functions with a fairly wide range of biological effects. Thus, it is known that in optimal concentrations, NO improves the endothelial function of peripheral vessels, has a positive effect on the activity of certain protein kinases, is capable of inhibiting caspases, and inhibiting the induction of apoptosis. However, the synthesis of nitric oxide in elevated concentrations exceeding the permissible stationary level can be the cause of the development of nitrosative stress caused by the formation of active forms of nitrogen (AFN), first of all, peroxy nitrite and the product of its degradation, nitrogen dioxide. Considering this and the fact that erythrocytes are involved in the pathological process in a number of diseases, we were interested in studying individual indices of the nitric oxide synthesis system and nitrosylation processes in erythrocytes [10, 11] of MS patients, which will be the goal of future research. It is known that the breakdown of proteins increases in the case of exacerbations of MS and in conditions

of ischemia [2]. However, this hypothesis requires a separate study and may become the task of further research.

### Conclusions

1. The different course of multiple sclerosis is accompanied by a change in the activity of the superoxide dismutase and catalase enzymes, which is associated with the activation of the processes of formation of reactive oxygen species, which occur against the background of a 2-fold decrease in the erythrocyte deformation index and nitrogen concentration in erythrocytes, which can be one of the pathogenetic factors progression of multiple sclerosis.

2. It is shown that in multiple sclerosis, the superoxide dismutase/catalase enzyme system of erythrocytes is characterized by the phenomenon of cross-regulation of activity, which manifests itself in a negative correlation between enzyme activities and is associated with the appearance of irreversibly altered forms of erythrocytes in the general bloodstream.

3. An increase in the ratio of superoxide dismutase/catalase above the level of the control group is observed in patients with secondary progressive multiple sclerosis. This fact is caused by a drop-in catalase activity and can be considered as a state of hypoxia.

4. Exacerbation of multiple sclerosis is accompanied by a decrease in the activity of both superoxide dismutase and catalase, as well as their ratio below unity. This is closely correlated ( $r=0.93$ ;  $p=0.0012$ ) with conformational changes of erythrocytes, which should be used for diagnostic and prognostic purposes in multiple sclerosis.

### References

1. Churpiy IK, Barila NI, Melnyk IV inventors; NI Barila, IV Melnyk, patent owners. A device for studying the deformation resistance of erythrocytes of human blood. Patent of Ukraine for a utility model UA 153105 U. 2023; 25(5) [in Ukrainian].
2. Chuprina G. Multiple sclerosis: etiology, pathogenesis, clinic, diagnosis, differential diagnosis, treatment (clinical lecture). in East European Journal of Neurology, 2017; 11 (20): 49–54. [https://doi.org/10.33444/2411-5797.2017.5\(17\).27-37](https://doi.org/10.33444/2411-5797.2017.5(17).27-37).
3. Demchenko AV. Glutathione system state in a hemolysate of erythrocytes among the patients with chronic cerebral ischemia. East European Journal of Neurology, 016;10(29): 221–234. [https://doi.org/10.33444/2411-5797.2016.4\(10\).37-43](https://doi.org/10.33444/2411-5797.2016.4(10).37-43).
4. Farogh A, Hassan A, Gull S, Khan M, Bashir G, Mahmood H, et al. The Influence of Preoperative Hematocrit Levels on Early Outcomes in Patients undergoing Cardiac Surgery. Pakistan Journal of Medical and Health Sciences. 2021; 10 (30): 115–128. <https://doi.org/10.53350/pjmhs2115102817>
5. Franco R, Navarro G, Martínez-Pinilla E. Antioxidant Defense Mechanisms in Erythrocytes and in the Central Nervous System. Antioxidants, 2019;18(2):56–63. <https://doi.org/10.3390/antiox8020046>.
6. Merzlyakov V, Drugova E, Lesnikova L. The use of an extract from brown seaweed saccharina japonica to restore physiological characteristics of rat erythrocytes during intoxication with tetrachloride. Chronos. 2016; 99–104.
7. Mir R, Alfardan S. Multiple Sclerosis: The Bystander Effect. Multiple Sclerosis and Related Disorders, 2020; 1(74): 69–71. <https://doi.org/10.1016/j.msard.2019.11.079>.
8. Muravyov AV. The mechanism of microrheological responses of erythrocytes to the action of gas transmitters – nitric oxide and hydrogen sulfide. Thrombosis, hemostasis and rheology. 2020; 2: 34–39.
9. Petrushanskaya KA, Kotov SV, Pismennaya EV, Lizhdvoy VJu, Dotsenko VI. Influence of the Exoskeleton training Course on the Energy parameters of Walking in patients with Multiple Sclerosis Bulletin of Rehabilitation Medicine. 2021; 20 (2): 29–41. <https://doi.org/10.38025/2078-1962-2021-20-2-29-41>
10. Romashchenko OV, Kamenev VF. Possibilities for predicting heart failure possibility of predicting heart failure using electron blood microscopy data. World medicine and biology. 2019; (4): 89–94.
11. Zavodnik IB. Mitochondrial dysfunction and compensatory mechanisms in liver cells during acute carbon tetrachloride-induced rat intoxication. Biomedical chemistry, 2015;106(11):45–52. <https://doi.org/10.18097/pbmc20156106731>.

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