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Changes in biochemical indicators of blood of rats of different sex in posttraumatic stress disorders

O.V. Denefil, S.V. Chorniy, V.A. Miroshnyk

I. Ya. Horbachevsky Ternopil National Medical University

Denefil Olha Volodymyrivna – Doctor of Science in Medicine, Professor, head of the Department of Pathological Physiology at I. Ya. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine; https://orcid.org/0000-0002-3606-5215

Chorniy Sofiya Volodymyrivna – 4th year student of Medical Faculty at I. Ya. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine; https://orcid.org/0000-0003-2718-5191

Miroshnyk Viktoria Andriivna – 5th year student of Medical Faculty at I. Ya. Horbachevsky Ternopil National Medical University Ministry of Health of Ukraine; https://orcid.org/0009-0001-9195-9537

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Abstract

Introduction. The war in Ukraine, the difficult ecological situation, socio-economic hardships, the acceleration of the rhythm of life and the increase in information load contribute to the emergence and development of constant tension in the body of modern man, the manifestation of which is the stress reaction. This provokes exhaustion and disruption of the adaptive capabilities of the body and the development of diseases. One of the types of such pathology is post-traumatic stress disorder (PTSD). The relevance of this problem currently determines the need for in-depth study of individual links of their pathogenesis.

The purpose of the study is to determine the effect of PTSD on biochemical blood indexes of different sexes rats.

Materials and methods. The experiment was performed on 40 different sexes Wistar rats, weighing 180-200 g, aged 4 months. They were kept on a standard vivarium diet with free access to drinking water throughout the entire experimental period. The rats were divided into 4 groups, according to sex: males and females in equal numbers – control males (I), control females (II), post-traumatic stress disorder, males (III), post-traumatic stress disorder, females (IV). Modeling of PTSD was carried out by 4 stressors using one by one twice. This preclinical model reflects molecular and physiological changes that repeat the same changes observed in post-traumatic stress disorder in humans. Biochemical methods of the study included determination in blood serum of the concentration of diene conjugates (DC), superoxide dismutase activity (SOD).

Research results and their discussion. When analyzing lipid peroxidation indicators in animals of group III, compared to animals of the control group (I), was detected an increase in DC by 49.5% ($p<0.001$) while in animals of group IV, compared to animals of the control group (II), was detected an increase in DC by 1.9 times ($p<0.001$). When comparing the impact of PTSD on LPO processes, it was found that in group IV animals, compared to group III, DCs were 1.1 times higher ($p<0.001$).

As for the antioxidant system, in the blood serum of rats of group III, compared to animals of I group, a decrease in SOD by 23.50% ($p<0.001$) was detected, and in animals of IV group, compared to animals of the control group, was detected a decrease in SOD by 10% ($p<0.001$). When comparing the degree of influence of PTSD on the enzymatic system of antioxidant defense in rats, it was found that in group III animals, compared to group IV, SOD was lower by 38.3% ($p<0.001$).

Conclusion. Posttraumatic stress disorder causes activation of lipid peroxidation in the body of rats, but its magnitude depends on gender and is more pronounced in females. In response to the activation of free radical

oxidation processes, the activity of the antioxidant system is suppressed, which is more pronounced in males.

KEY WORDS: lipid peroxidation, antioxidant activity, posttraumatic stress disorders, blood, rats, gender.

Introduction. The war in Ukraine, the difficult ecological situation, socio-economic hardships, the acceleration of the rhythm of life and the increase in information load contribute to the emergence and development of constant tension in the body of modern man, the manifestation of which is the stress reaction [2, 7,19]. This provokes exhaustion and disruption of the adaptive capabilities of the body and the development of diseases and disorders of a psychogenic nature.

One of the types of such pathology is post-traumatic stress disorder (PTSD). According to the ICD-10, PTSD occurs as a delayed or prolonged reaction to a stressful event or situation (short-term or long-term) of an exceptionally threatening or catastrophic nature, which in principle can cause general distress in almost any person (for example, natural or man-made disasters, combat operations, accidents, accidental presence at the scene of the violent death of others, victims of torture, terrorism, rape or other crime) [5, 8,

15]. PTSD develops in 20-25% of people who have been exposed to stress, but still have preserved their physical health; among the wounded, the prevalence of these disorders is about 40% [11, 14].

Such disorders of adaptation, behavior, the development of anxiety and amnesia were first described in soldiers of the First World War under the name mild contusion [9, 16]. Later, these disorders were defined as fatigue syndrome [5], and today – as PTSD [5, 10, 14]. The relevance of this problem currently determines the need for in-depth study of individual links of their pathogenesis.

The purpose of the study is to determine the effect of PTSD on biochemical blood indexes of different sexes rats.

Materials and methods. Experiments were performed on 56 white male Wistar rats aged 3.5-4 months.

Materials and methods of the study. The experiment was performed on 40 different sexes Wistar rats, weighing 180-200 g, aged 4 months. They were kept on a standard vivarium diet with free access to drinking water throughout the entire experimental period. The rats were divided into 4 groups, according to sex: males and females in equal numbers – control males (I), control females (II), post-traumatic stress disorder, males (III), post-traumatic stress disorder, females (IV).

Modeling of PTSD was carried out by 4 stressors using one by one twice [16]. This preclinical model reflects molecular and physiological changes that repeat the same changes observed in post-traumatic stress disorder in humans [16].

Biochemical methods of the study included determination in blood serum of the concentration of diene conjugates (DC) [17], superoxide dismutase activity (SOD, K.F.1.15.1.1) [17].

All experiments were carried out in the first half of the day at a temperature of 18-22 o C, relative humidity of 40-60% and illumination of 250 lux. The experiments were performed in compliance with the norms of the

Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, March 18, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine No. 690 dated September 23 .2009.

Euthanasia of rats was performed by total bleeding from the heart after previous thiopental-sodium anesthesia (60 mg/kg of the animal's body weight intraperitoneally).

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.).

Research results and their discussion. When analyzing lipid peroxidation indicators (Table 1), in animals of group III, compared to animals of the control group (I), was detected an increase in DC by 49.5% ($p<0.001$) while in animals of group IV, compared to animals of the control group (II), was detected an increase in DC by 1.9 times ($p<0.001$).

Table 1 – Changes in indicators of lipid peroxidation in blood serum of rats with posttraumatic stress disorders ($M \pm \sigma$, n=10)

Group	Experimental conditions	Index
		DC, unit/mL
I	Control, males	1,13 ± 0,05
II	Control, females	1,01 ± 0,06 **
III	Posttraumatic stress disorders, males	1,69 ± 0,07 *
IV	Posttraumatic stress disorders, females	1,89 ± 0,08 *,**
Notes: 1. * – probable differences with the control; 2. ** – probable differences with with males.		

When comparing the impact of PTSD on LPO processes, it was found that in group IV animals, compared to group III, DCs were 1.1 times higher ($p<0.001$) (Fig. 1).

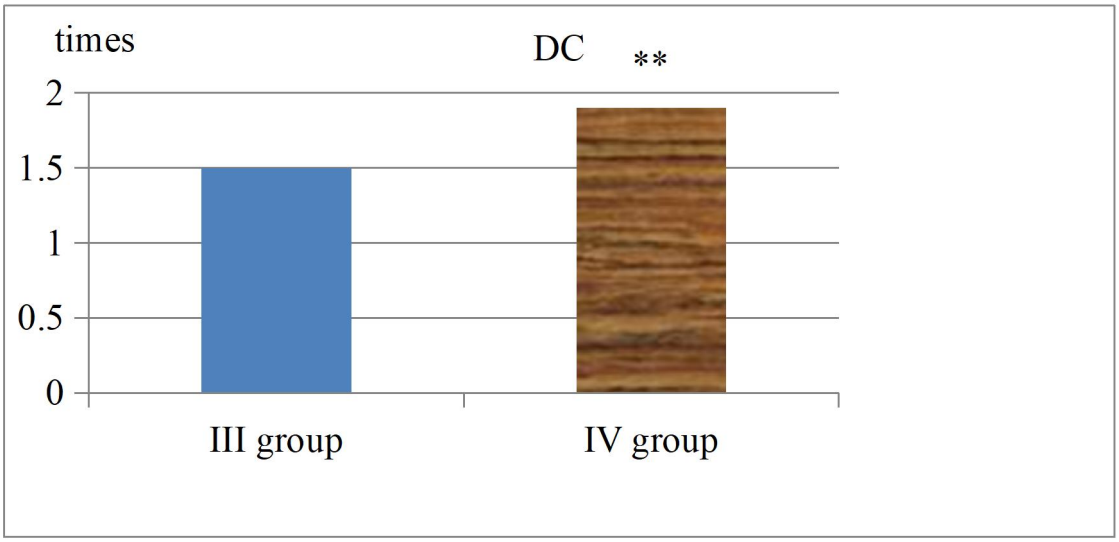

Figure 1. Gender difference of DC increase under the influence of PTSD

Notes. ** – probable differences with with males.

As for the antioxidant system, in the blood serum of rats of group III, compared to animals of I group, a decrease in SOD by 23.50% ($p<0.001$) was detected, and in animals of IV group, compared to animals of the control group, was detected a decrease in SOD by 10% ($p<0.001$) (Table 2).

Table 2 – Changes in indicators of antioxidant system in blood serum of rats with posttraumatic stress disorders (M ± σ, n=10)

Group	Experimental conditions	Index
		SOD, unit/mL
I	Control, males	0,95 ± 0,03
II	Control, females	1,12 ± 0,02 **
III	Posttraumatic stress disorders, males	0,73 ± 0,04 *
IV	Posttraumatic stress disorders, females	1,01 ± 0,06 *,**
Notes: 1. * – probable differences with the control; 2. ** – probable differences with with males.		

When comparing the degree of influence of PTSD on the enzymatic system of antioxidant defense in rats, it was found that in group III animals, compared to group IV, SOD was lower by 38.3% ($p<0.001$) (Fig. 2).

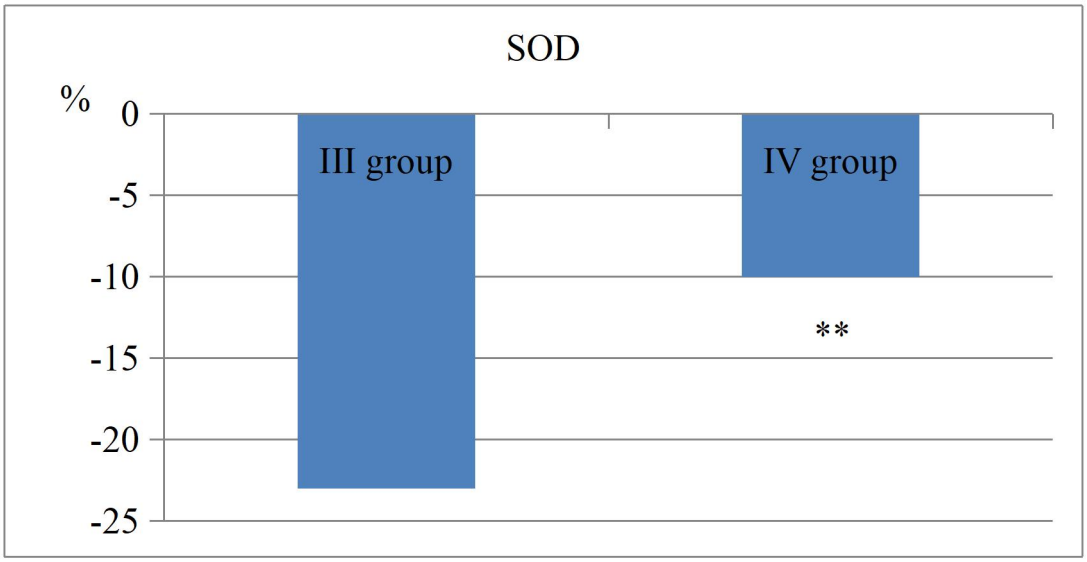

Figure 2. Gender difference of SOD decrease under the influence of PTSD

Notes. ** – probable differences with with males.

With any excessive effects on the human and animal body, the processes of free radical oxidation of lipids are activated, which is a nonspecific reaction of the body [12, 16, 18]. Due to chain reactions, the processes of peroxidation of lipids and proteins are activated. In our experiments, we see an increase in lipid peroxidation products. Such changes were sex-dependent, and a significantly greater increase in DC was noted in females compared to males. The results obtained indicate damage to the lipid layer of cell membranes.

With any excessive effects on the human and animal body, the processes of free radical oxidation of lipids are activated, which is a non-specific reaction of the body. Due to chain reactions, the processes of peroxidation of lipids and proteins are activated [25, 34]. In our experiments, we see an increase in lipid peroxidation products. Such changes were sex-dependent, and a significantly greater increase in DC was noted in females compared to males. The results obtained indicate damage to the lipid layer of cell membranes. At the same time, the level of SOD decreased in animals of both sexes. One might think that the processes of lipid peroxidation are activated, however, protection against free radical damage to lipids is slowed down, which indicates damage.

Conclusion. Posttraumatic stress disorder causes activation of lipid peroxidation in the body of rats, but its magnitude depends on gender and is more pronounced in females. In response to the activation of free radical oxidation processes, the activity of the antioxidant system is suppressed, which is more pronounced in males.

Perspectives of further research consist in studying further the protein modification processes in different organs under different duration of stress.

Supplementary Materials

Table S1 – Changes in indicators of lipid peroxidation in blood serum of rats with posttraumatic stress disorders (M ± σ, n=10)

Table S2 – Changes in indicators of antioxidant system in blood serum of rats with posttraumatic stress disorders (M ± σ, n=10)

Figure 1. Gender difference of DC increase under the influence of PTSD

Figure 2. Gender difference of SOD decrease under the influence of PTSD

Author Contributions

Conceptualization, O.V. Denefil.; writing – original draft preparation S. V. Chorniy and V. A. Miroshnyk; writing – review and editing – O.V. Denefil; project administration O.V. Denefil, S. V. Chorniy and V. A. Miroshnyk.

All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The experiments were performed in compliance with the norms of the Council of Europe Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, March 18, 1986), the resolution of the First National Congress on Bioethics (Kyiv, 2001) and the order of the Ministry of Health of Ukraine No. 690 dated September 23 .2009.

Informed Consent Statement

Not applicable.

Data Availability Statement

Publicly available datasets were analyzed in this study. This data can be found here: That work did in University Laboratory of I. Ya. Horbachevsky Ternopil National Medical University

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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