ORIGINAL ARTICLE

CONTENTS

Influence of oxidative stress caused by a blast wave in acoustic barotrauma on the auditory analyzer

Olga Rzhevska[1], Vasyl Troian[2], Irina Sinaiko[2], Olena Lobova[3], Olena Solomko[3], Kseniia Zolotarova[4], Kateryna Shtrakh[1]

[1] V. N. KARAZIN KHARKIV NATIONAL UNIVERSITY, KHARKIV, UKRAINE
[2] ZAPORIZHZHIA STATE MEDICAL-PHARMACEUTICAL UNIVERSITY, ZAPORIZHZHIA, UKRAINE
[3] PRIVATE INSTITUTION OF HIGHER EDUCATION «KYIV MEDICAL UNIVERSITY», KYIV, UKRAINE
[4] ODESA NATIONAL MEDICAL UNIVERSITY, ODESA, UKRAINE

ABSTRACT

Aim: To determine the intensity of posttraumatic oxidative stress (OS) in patients with mild explosive traumatic brain injury (METBI) complicated by acoustic barotrauma and its association with sensorineural hearing loss (SHL) occurrence.

Materials and Methods: This study included 43 patients with METBI form October 2023 to May 2024.

Results: METBI initiated an increase in OS markers in blood: aldehyde phenylhydrazone (APH) by 53.54% ($p<0.05$), carboxy phenylhydrazone (CPH) - by 50.51% ($p<0.05$) and with a deficiency of the antioxidant system of reduced glutathione by 47.4% ($p<0.05$), total thiols by 61.7% ($p<0.05$), decrease in the level of nitric oxide in blood by 54.22% ($p<0.05$), which led to microcirculatory disorder. A direct correlation was established between the frequency of SHL and an increase of APH ($r=+0.72$, $p<0.05$), CPH ($r=+0.66$, $p<0.05$) and an inverse correlation with a reduced glutathione deficiency ($r=-0.85$, $p<0.05$).

Conclusions: An imbalance of antioxidant protection led to significant microcirculatory disorders. The data obtained are important for the neutralization of the effect of damaging factors of OS on the inner ear microcirculation.

KEY WORDS: barotrauma, carboxyphenylhydrazone, aldehydphenylhydrazone audiometry, histomorphometry

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INTRODUCTION

During the period of full-scale aggression of Russia against Ukraine, the number of cases of traumatic brain injury from the exposure of a blast wave has increased significantly. In approximately 90% of cases, such traumatic brain injuries are complicated by acoustic barotrauma with sensorineural hearing loss [1-3]. Therefore, sensorineural damage to the organ of hearing in traumatic brain injury as a result of a blast wave is currently an important military-civil problem in Ukraine.

Sensorineural hearing loss due to exposure to a blast wave can be caused by several factors, such as the occurrence of microbleeds in the perilymphatic region of the cochlear labyrinth, as well as the occurrence of edema and death of hair cells of the organ of Corti, which form neuroelectric impulses[4]. However, it should be remembered that the exposure of a blast wave is not a strictly isolated organ of hearing. A blast wave has a very powerful short-term general mechanical effect on the entire surface of the victim's body. As a result, free radical reactions are activated in the injured patient's damaged tissues, causing oxidative damage to the endothelium of the microcirculatory circle. Thus, oxidative stress is initiated in such patients, which leads to microcirculation disorders in the inner ear structures [5-11].

Taking into account the above data, there is an objective need to study the parameters of pro- and antioxidant systems and determine their role in the pathogenesis of sensorineural hearing loss in patients injured by a blast wave in the early period after traumatic brain injury. These studies will contribute to a deeper understanding of the processes occurring in the early period after acoustic barotrauma and can become the basis for developing pathogenetically substantiated therapy aimed at mitigating the consequences of traumatic brain injury.

AIM

The purpose of this study is to determine the intensity of posttraumatic oxidative stress in patients in early period

after mild explosive traumatic brain injury complicated by acoustic barotrauma and to find out its association with occurrence of sensorineural hearing loss.

MATERIALS AND METHODS

PARTICIPANTS

Patients were enrolled in the study between October 2023 and April 2024.

The study group included 43 patients with mild traumatic brain injury caused by the exposure of a blast wave complicated by acoustic barotrauma. The duration of the disease at the time of treatment was from 1 to 3 days after receiving the acoustic barotrauma. The age of the patients was from 25 to 54 years; average age – 35,2 ± 5.7 years old. Among the examined, men essentially predominated than women (96.3 % *vs* 3.7 %).

The comparison group included 15 clinically healthy persons with comparable anthropometric indicators, who expressed consent to participate in the study. Average age was 38.3±3.2 years old. The main criteria for selecting patients for the study: absence of disorders of higher mental functions and vestibular disorders.

PROCEDURES

In all patients general clinical examination and ENT investigation methods were carried out according to current protocols, which consisted of rhinoscopy, pharyngoscopy, otoscopy, establishing the function of auditory analyzers (subjective tonal threshold audiometry and tympanometry) and neuroimaging method (MRI).

Subjective tinnitus was assessed using the Tinnitus Handicap Inventori scale, which consists of 25 questions and is rated as never - 0 points, sometimes - 2 points, always - 4 points.

Neuroimaging studies of the brain were carried out in the patients by using MRI, on the «Hitachi Echelon» tomograph with 1.5 T magnetic field induction in T1 and T2 modes.

The following biochemical methods were used to assess the intensity of oxidative stress induced by the impact of the blast wave:

1. Determination of markers of oxidative modification of proteins - aldehyde phenylhydrazones (APH), carboxy phenylhydrazones (CPH).
2. The state of the antioxidant system was assessed by the level of total thiols and reduced glutathione in the blood plasma by the fluorometric method.
3. Determination of the number of stable metabolites of nitric oxide in the blood plasma was carried out according to the Griess method.
4. The expression of morphological changes in the vascular network of the head was studied using computer histomorphometry at the light-optical level (Axioplan microscope - 2, «Carl Zeiss», Germany) with a video camera DXS - 151A («Sony», Japan).

STATISTICAL ANALYSIS

Statistical processing of the obtained data was performed using computer programs of the «STATISTICA® for Windows 6.0» package (StatSoft Inc., No AXXR712D833214FAN5). The statistical significance of the compared indicators and the interdependence of their changes were established using the Spearman rank correlation coefficient, to compare the difference in two independent groups - the Mann-Whitney U test.

ETHICS

All examined patients gave informed consent in accordance with the Declaration of Helsinki of the World Medical Association «Ethical Principles of Medical Research Involving Human Subjects» Finland, June 1964, and with most amendments adopted at the 64th World Medical Assembly, Fontaleza, Brazil, October 2013.

RESULTS

According to our data, the most frequent and most pronounced complaints in patients in the early period of acoustic barotrauma received in the combat zone were decreased hearing function - 43 (100.0 %) patients, subjective tinnitus - 35 (81.39 %), headache - 33 (76.74%). Hearing loss was unilateral in 12 (27,9 %) of cases, bilateral in 31 (72.09 %), and unilateral deafness in one case. Among all the processed audiograms, a descending, often steep, type of audiometric curve was detected in 25 (58.13 %) of patients. Perforations of the tympanic membrane were diagnosed in 7 (16.27 %) of cases. Analysis the subjective audiometry data in military personnel with combat acoustic barotrauma showed that in 19 (44.18%) of patients a descending, often steep type of audiometric curve was observed with a peculiar peak of threshold increase at a frequency of 3 or 5 kHz (Cover figure, Fig. 1).

According to the results of neuroimaging studies of the brain (MRI), conducted 2 - 3 days after acoustic barotrauma, only 8 (18.6%) of patients had signs of initial cerebral microangiopathy (dyscirculatory leukoencephalopathy according to the Fazekas - 1 scale) (Fig. 2).

We studied the intensity of oxidative stress and the parameters of pro- and antioxidant systems in patients with acoustic barotrauma, and we found a significant

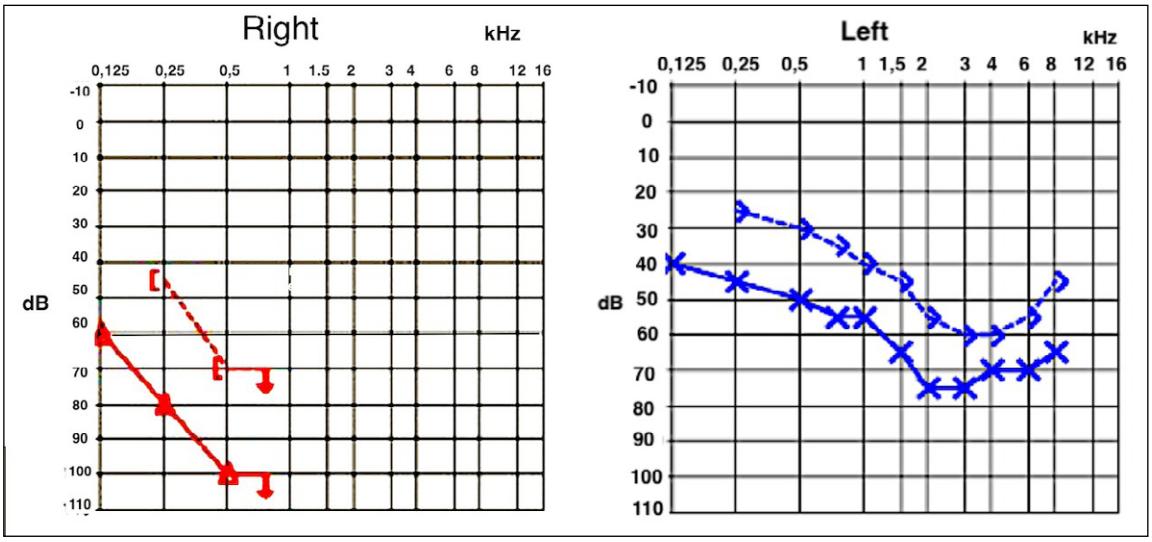

Fig. 1. An example of audiometry with a characteristic «acoustic traumatic tooth»

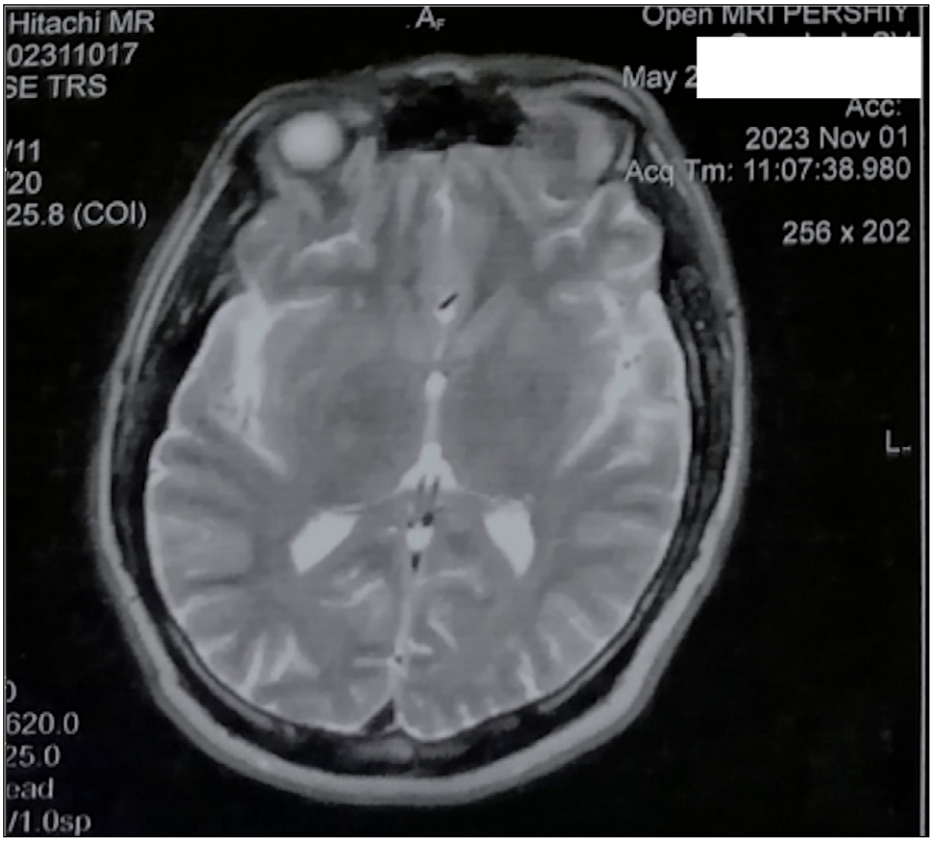

Fig. 2. Axial T2-weighted MRI in theT1. In the subcortical white matter of the left frontal lobe and in the projection of the basal nuclei on the right, single hyperintense in T2 and isointense in T1 foci of vascular gliosis 2-3 mm in diameter are noted

increase in the earliest indicators of oxidative damage to the endothelium of the microcirculatory channel. The elevated level of aldehyde phenylhydrazone by 53.54 % was discovered in patients with traumatic brain injury compared to control group (4.80 ± 0.37 vs 2.23 ± 0.07; $p < 0.05$) and carboxy phenylhydrazone by 50.51 % (2.89 ± 0.15 vs 1.43; $p < 0.05$) (Table 1).

At the same time, in patients with traumatic brain injury, there was a decrease in the level of stable nitrogen metabolites with a vasodilating effect by 54.22 % compared to the control group (4.99 ± 0.44 vs 10.90 ± 0.26; $p < 0.05$), which led to a spastic condition of the microcirculatory circle in the injured tissues.

We also found a significant deficiency of reduced glutathione in patients in the early post-traumatic period – by 47.4 % compared to the control group (16.91 ± 0.73 vs 32.15 ± 0.22; $p < 0.05$). Reduced glutathione, as an important component of the antioxidant system, interacted with nitric oxide and formed nitrosoglutathione, thus increasing its bioavailability and enhancing the endothelial protective effect. A decrease in the level of reduced glutathione in patients with traumatic brain

Table 1. The content of markers of oxidative stress and stable metabolites of nitric oxide in the blood plasma of subjects of the control group and patients after TBI

Patients	Markers of oxidative protein modification		Nitrogen metabolites
	aldehyde phenilhydrasones	carboxy phenylhydrasone	
Control group (n=15)	2,23±0,07	1,43±0,04	10,90±0,26
Patients after TBI* (n=15)	4,80±0,37	2,89±0,15	4,99±0,44
P	<0,05	<0,05	<0,05

TBI* - traumatic brain injury

Table 2. Indicators of the antioxidant system in the patients after TBI and the control group

Patients	Indicators of the thiol-disulfide system	
	Glutathione reduced	Common thiols
Control group (n=15)	32,15±0,22	27,60±0,49
Patients after TBI* (n=15)	16,91±0,73	10,57±0,53
P	<0,05	<0,05

TBI* - traumatic brain injury

injury occurred against the background of suppression of reduced equivalents of the thiol-disulfide system – by 61.7 % compared to the control group (10.57 ± 0.53 vs 27.60 ± 0.49; $p < 0.05$), which led to a deficiency of endogenous antioxidants and required their replenishment through exogenous administration (Table 2).

In order to determine the dependence of the identified disorders in the pro- and antioxidant systems with the frequency of sensorineural hearing loss, we conducted a correlation analysis. As a result of the analysis, a direct correlation between the frequency of sensorineural hearing loss and an increase in the content of markers of oxidative stress - aldehyde phenylhydrazones AFH ($r = + 0.72$; $p < 0.05$), carboxy phenylhydrasones CFH ($r = + 0.66$; $p < 0.05$) and inverse correlation with reduced glutathione deficiency ($r = - 0.85$; $p < 0.05$) were established.

Thus, we discovered an imbalance between the pro- and antioxidant protection system in patients after traumatic brain injury, leading to the appearance of significant damage to the microcirculatory circle. The identified data were confirmed using computer histomorphometry of the condition of the vessels of the superficial postauricular wound, which allows us to assess the state of the vascular system of the inner ear (Fig. 3, 4).

The changes in the microcirculatory channel, revealed by computer histomorphometry, were accompanied by pronounced vascular spasm, the appearance of microthrombi in the openings of the vessels, and plasmatic impregnation of the walls of the micro vessels. The Kernogan index was 0.54 ± 0.04 (N - 0.3), and the elongation coefficient was 0.69 ± 0.08 (with a norm of 1.0).

DISCUSSION

An analysis of publications on traumatical brain injury from exposure to a mild blast wave with acoubarotrauma complicated by sensorineural hearing loss indicates that they mainly cover the issue of diagnostics and treatment of this pathology in victims in the intermediate and late posttraumatic periods, which affects the possibilities of their effective rehabilitation [1, 4, 5].

Therefore, conducting research dedicated to studying the state of indicators of key links in the pathogenesis of sensorineural deafness in the early post-traumatic period will make it possible to carry out their etiopathogenetic correction in time in order to level the consequences of acoustic barotrauma.

Currently, there is a general opinion that the basis of the emergence of sensorineural pathology from the action of a blast wave is the appearance of microhemorrhages in the microvascular bed of the labyrinth of the inner ear, which cause swelling and death of the hair cells of the organ of Corti, which form neuroelectric impulses Shidl [1, 4-6, 8].

However, it is known that the effect of the blast wave is not isolated, but is a component of a short-term, general, very powerful mechanical impact on the entire surface of the victim`s body with the activation of free radical reactions in the damaged tissues and the appearance of pronounced changes in the pro-oxidant-antioxidant the balance of the victim's body [10, 11].

The resulting excess production of aldehyde phenylhydrazones, which are the earliest indicators of oxidative damage to the endothelium of the microcirculatory bed, which leads to significant disturbances of microcirculation in the inner ear [9-11].

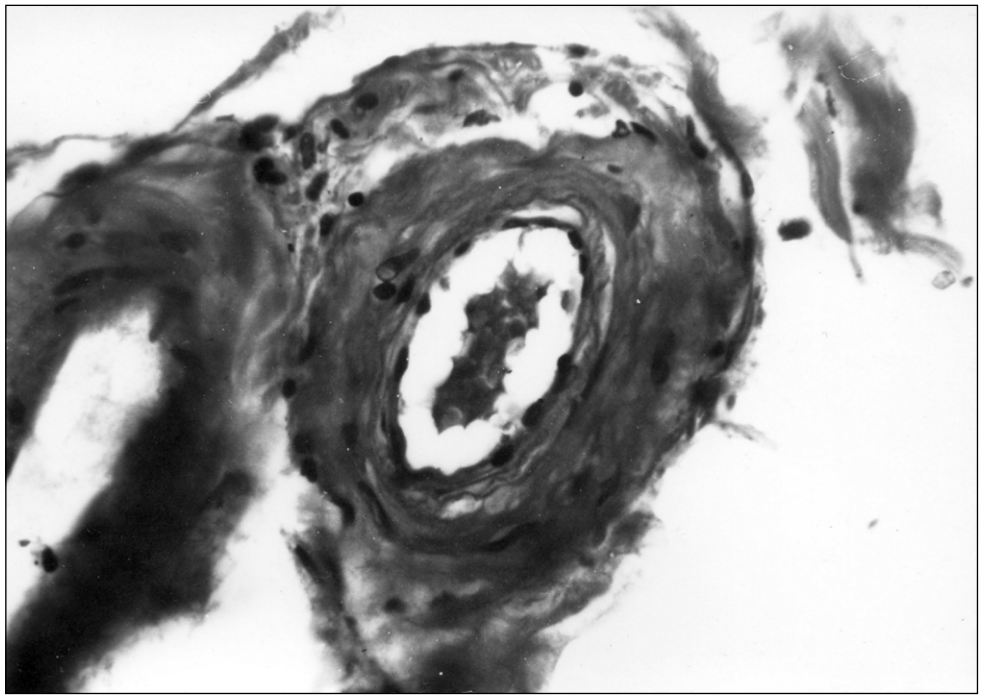

Fig. 3. Micro photo of a preparation of muscle tissue, taken during surgical treatment of a superficial post-auricular wound. Magnification 1:200. Staining with hematoxylin-eosin. There is a thrombus in the opening of a medium-sized arteriole, plasmatic impregnation of the vascular wall is observed

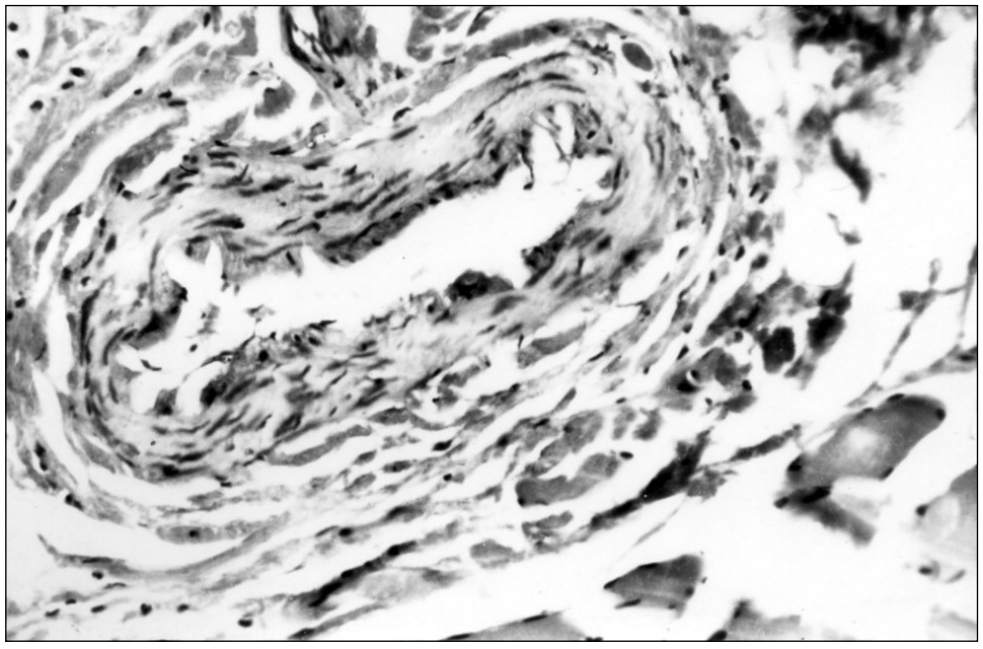

Fig. 4. Micro photo of the microvascular circle of a muscle tissue, taken during surgical treatment of a superficial post-auricular wound. Magnification 1:200. Staining with hematoxylin-eosin. Marked plasmatic infiltration of the arteriole wall and vascular spasm are observed

At the same time, we found a decrease in the content of stable nitrogen metabolites with a vasodilating effect in the victims by 54.22% ($p < 0.05$) (patients after TBI - 4.99 ± 0.44 μmol/l, control group - 10.90 ± 0.26 μmol/l), which led to a spastic state of the microcirculatory bed in injured tissues. The imbalance between the pro- and antioxidant protection system of the victim's body, which we discovered, led to the appearance of significant damage to the microcirculatory channel, which affected the microcirculation in the inner ear. The results obtained by us are confirmed by data published by scientists Fukuda S, Fradi A. and their co-authors [9-11].

We visualized and quantified most of the indicated manifestations of the state of the microvascular bed with the help of computer histomorphometry. The Kernogan index was 0.54 ± 0.04 (with a norm of 0.3), and the elongation coefficient was 0.69 ± 0.08 (with a norm of 1.0) and had a significant effect on microcirculation.

Blast traumatic brain injury of the explosive action of mild degree initiates oxidative stress in patients with a significant increase in the content of markers of oxidative stress in the blood plasma of aldehyde phenilhydrasones (AFH) by 53.54% ($p<0.05$), carboxy phenylhydrasone (CFH) - by 50.51% ($p<0.05$) and with a simultaneous deficiency in the antioxidant system of

reduced glutathione by 47.4% ($p<0.05$), common thiols by 61.7% ($p<0.05$), decrease in the level of stable nitric oxide metabolites in blood plasma by 54.22% ($p<0.05$), which leads to an angiospastic microcirculation disorder. The results obtained by us do not contradict the data of other scientists [10, 11].

In order to determine the dependence of the detected disorders in the pro- and antioxidant systems with the frequency of sensorineural deafness, we performed a correlation analysis that established a direct correlation between the frequency of sensorineural deafness and an increase in the content of markers of oxidative stress AFH ($r = +0.72$, $p < 0.05$), CFH ($r = +0.66$, $p < 0.05$) and inverse correlation with reduced glutathione deficiency ($r= -0.85$, $p < 0.05$). The obtained research results make it possible to correct and eliminate the consequences of oxidative damage to the endothelium of the microcirculatory channel already in the early post-traumatic period.

CONCLUSIONS

Conducting research showed that sensorineural hearing loss accompanied all cases of early period after explosive acoustic barotrauma. It was revealed that mild blast traumatic brain injury initiates oxidative stress, which is accompanied by an imbalance of antioxidant defense markers (a significant increase in the blood of aldehyde phenylhydrazone, carboxy phenylhydrazone, with a simultaneous decrease in reduced glutathione, total thiols, stable metabolites of nitric oxide), which leads to angio spastic disorder of microcirculation in inner ear structures. A direct correlation was established between the frequency of sensorineural hearing loss and an increase in the content of markers of oxidative stress AFH ($r = + 0.72$, $p < 0.05$), CFH ($r = + 0.66$, $p < 0.05$) and inverse correlation with reduced glutathione deficiency ($r = - 0.85$, $p < 0.05$). Analysis of the obtained data on the indices of pro- and antioxidant systems and related changes in microcirculation in the structures of the inner ear in the early period of acoustic barotrauma indicates the need to develop pathogenetically substantiated treatment. This therapy should have an antioxidant, endothelioprotective and vasodilator effect in order to neutralize the impact of damaging factors of oxidative stress on the microvascular bed of the inner ear.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Olga Rzhevska
V. N. Karazin Kharkiv National University
4 Freedom Square, 61022 Kharkiv, Ukraine
e-mail: Rzhevskaolga@ukr.net

ORCID AND CONTRIBUTIONSHIP

Olga Rzhevska: 0000-0002-6410-7769 C D
Vasyl Troian: 0000-0003-2918-4547 A D E F
Irina Sinaiko: 0000-0002-3714-0088 A B D
Olena Lobova: 0000-0003-4252-8690 B D F
Olena Solomko: 0000-0002-9956-8063 B D
Kseniia Zolotarova: 0000-0002-0853-2728 D E
Kateryna Shtrakh: 0000-0003-3563-0371 C D

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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