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THE STATE OF THE HEMOSTASIS SYSTEM IN PATIENTS WITH SENSONEUROLOGICAL HEARING DAMAGE CAUSED BY ACOUSTIC INJURIES IN COMBAT ZONE

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The problem of early diagnosis and treatment of acute traumatic injury and its complications has been sharply actualized in today's circumstances of combat actions. Acubarotrauma (ABT) is a specific damage characterized by manifestations from the side of the auditory system and those not related directly to the structures of the auditory system, such as severe forms of sensorineural deafness (SND). The development of SND can be associated with vascular pathology, hydrodynamic impact on blood vessels, damage to the vascular endothelium and uncontrolled release of various clotting factors into the bloodstream. The purpose of this work was to estimate the state of the hemostasis system in patients who received ABT injuries in combat zone and have sensorineural hearing disorders. The following parameters in the patient's blood plasma were measured: fibrinogen concentration, activated partial thromboplastin time (APTT), prothrombin time (PT), functionally inactive forms of prothrombin (FIFP), ecamulin time, the levels of protein C (PC), soluble fibrin-monomer complexes (SFMCs), soluble fibrin (SF) and D-dimer. The control group was formed by 15 healthy persons with normal hearing. 94 patients with combat ABT injuries were divided into 4 groups according to the severity of hearing impairment and the period after getting ABT: 1, 2 – patients with a mild hearing impairment, who contacted a medical facility within 2 months or more than 2 months after the injury respectively; 3, 4 – patients with a more severe hearing impairment who contacted a medical facility within 2 months or more than 2 months after the injury, respectively. Patients of all groups had significantly prolonged APT and increased SF, SFMCs and prethrombin-1 levels in the blood plasma compared to the control parameters. In patients of the 4th group, a PT prolongation was noted. The protein C level in the 1st, 2nd, and 3rd groups showed a tendency to decrease and was statistically reduced compared to the control in the 4th group. Concentration of D-dimer decreased in patients of the 1st and 2nd groups, remained at the control level in the 3rd, and increased in the 4th group. The fibrinogen level and ecamulin time ET in all studied groups remained at the level of control values. The observed dysfunctions of the hemostasis system links in patients with combat ABT indicate a slow development of disseminated intravascular coagulation syndrome. That is why patients with a severe hearing impairment due to combat ABT, regardless of the period of seeking medical help, need examinations after the injury according to a special algorithm of laboratory signs.

K e y w o r d s: hemostasis system parameters, acubarotrauma, intravascular coagulation syndrome, sensoneurological hearing damage.

Today's circumstances have sharply actualized the problems of military medicine. Among them, the development of acute traumatic injury and its complications occupies a special place. Acubarotrauma (ABT) is a specific damage to the auditory system due to exposure to high-intensity sounds. At the same time, significant damage to both peripheral and central structures is possible [1]. Treatment of patients with significantly pronounced perceptual hearing disorders is quite difficult. Early diagnosis, timely treatment and preventive measures can prevent the development of

complications, in particular – severe forms of sensorineural deafness (SND). ABTs are characterized by multifaceted manifestations both from the side of the auditory system and those not related directly to the structures of the auditory system. For these reasons, the treatment in such cases should be complex [2]. The development of SND can also be associated with vascular pathology of the atherosclerotic brain. That is why vascular pathology is both an etiological and a pathogenetic factor. The shock wave and its mechanical effect in ABT cause a hydrodynamic impact on blood vessels. This causes damage to the vascular endothelium with the uncontrolled release of various clotting factors into the bloodstream. In addition, the rupture of microvessels leads to direct contact of blood with collagen of the vessels' outer layers, which is a powerful blood clotting factor. Uncontrolled activation of the descending link of the hemostasis system causes the development of thrombus formation and microvessel blockage [3]. All these determine the interest in determining the relationship between the state of the hemostasis system and the vascular factor in the development of sensorineural hearing disorders at ABT. The search for new approaches for diagnosing and treating sensorineural disorders at combat-caused ABT requires a comprehensive examination with the application of modern methodological approaches. The aim of this work was to study the features of the hemostasis system in patients with sensorineural hearing disorders who received ABT injuries in the combat zone.

Materials and Methods

The study was conducted by the requirements of bioethics in compliance with the provisions of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the SI "Prof. O.S. Kolomiichenko Institute of Otolaryngology of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine (protocol number 16 of 05.08.2025). All patients gave written informed consent to participate in the study. 94 patients with combat ABT were examined. Perceptual disorders of auditory function were found in all of them according to the results of subjective audiometry with a downsloping tonal curve. The control group consisted of 15 healthy persons with normal hearing who had no exposure to high-intensity sounds or industrial noise. All patients were informed and gave written consent to participate in the study.

Patients were divided into groups according to two criteria:

1. Severity of hearing impairment.
2. The period after receiving akubarotrauma.

In this way, 4 groups were formed:

Group 1 – patients with a milder hearing impairment who applied to a medical facility within 2 months of ABT.

Group 2 – patients with a less pronounced hearing impairment and who applied to a medical institution more than 2 months from the moment of ABT.

Group 3 – patients with a more pronounced hearing impairment who applied to a medical institution within 2 months of ABT.

Group 4 – patients with a more pronounced hearing impairment and who applied to a medical facility more than 2 months of ABT.

The research involved blood plasma obtained from the cubital vein on an empty stomach, collected in a plastic tube containing 3.8% sodium citrate at a 9:1 ratio. Plasma samples were obtained by centrifugation at 1000 g for 20 min at 20°C [4]. Fibrinogen concentration in the blood plasma was determined using a modified spectrophotometric method. Blood plasma (0.2 ml) and phosphate-buffered saline (1.7 ml) were mixed in a glass tube. Coagulation was initiated by adding 0.1 ml of thrombin-like enzyme from *Agkistrodon halys halys* snake venom (1 NIH/ml) to prevent fibrin cross-linking. The mixture was incubated for 30 min at 37°C. The fibrin clot was then removed and dissolved in 5 ml of 1.5% acetic acid. The protein concentration was measured using the Spectrophotometer OPTIZEN POP (Daejeon, South Korea) at 280 nm ($\varepsilon = 1.5$) [5]. Activated partial thromboplastin time (APTT) was studied by measuring the coagulation time of blood plasma in the presence of APTT-reagent and calcium ions (Renam-U, Ukraine). Under these conditions, the rate of formation of a fibrin clot is determined by the activity of factors of the internal and general coagulation pathways [6]. Prothrombin time (PT) was determined by initiating the polymerization of a blood plasma sample at an excess of tissue thromboplastin and calcium ions (Siemenskit, USA). The total prothrombin level was evaluated by the ecamulin time (ET) [5]. Ecamulin is a prothrombin-activating enzyme from the Echis multisquamatus venom that activates both prothrombin and its functionally inactive forms.

Comparison of PT and ET test results allowed evaluation of the prothrombin-1 (Pre-1) level.

This functionally inactive form of prothrombin is a marker of prothrombin autolysis by thrombin and indicates activation of the blood clotting system. The calculation of Pre-1 concentration was performed using the formula obtained from the calibration curve with the use of prethrombin-1 (1).

$$C = \frac{EI/PI - 0.9919}{0.141} - 0.1, \quad (1)$$

where C – Pre-1 concentration, μg/ml; EI – ecamulin index (the ratio of clotting time of blood plasma donors and patients, which corresponds to the total level of prothrombin in blood plasma); PI – prothrombin index (the ratio of clotting time of blood plasma donors and patients, which corresponds to the level of intact prothrombin in blood plasma).

Protein C levels were determined using a test by the cleavage of the chromogenic substrate S2366 using the protein C activator (The Berichrom® Protein C, Siemens, Germany). Determination of soluble fibrin-monomer complexes (SFMCs) was carried out using the semi-quantitative method [7]. The absence of visible turbidity was evaluated as a negative result, clear turbidity as positive (+), which corresponds to a content of 0.03 g/l, lamellar sediment (++) – 0.06 g/l, formation of lamellar and thread-like structures – as (+++) – 0.09 g/l, gel-like sediment – (++++) – 0.12 g/l. Soluble fibrin (SF) was detected using a sandwich ELISA with monoclonal antibodies produced at the Palladin Institute of Biochemistry of NAS of Ukraine. A fibrin-specific monoclonal antibody I-3C was used as a capture antibody. A biotinylated monoclonal antibody II-4, which has an epitope at the NH_2-terminal fragment of the γ-chain of the D-region of the fibrinogen molecule, was used as a tag antibody. Optical density was measured at 450 nm using the Multiplate Reader RT-2100C (Rayto, China) [5, 8]. D-dimer was detected using a sandwich ELISA as described above for SF with modification. DD-specific monoclonal antibody III-3B that has an epitope at the NH2-terminal fragment of the Bβ-chain of the D-region of fibrin(ogen) was produced at the Palladin Institute of Biochemistry of NAS of Ukraine and used as the capture antibody [5, 9]. Statistical analysis of the results was carried out using WinPEPI, a package of programs for statistical processing of biometric data. To evaluate the difference between patients and the control group, the Student's *t*-test was used [10].

Results and Discussion

Thrombosis of various localizations is a leading cause of disability or death resulting from different pathological conditions. At the same time, diagnosing thrombosis solely based on clinical manifestations is not sufficiently informative [11, 12]. However, as with many pathological conditions, clinical signs of thrombosis are often preceded by a complex of metabolic disturbances at the molecular and cellular levels. Therefore, the most informative laboratory methods for thrombophilia diagnosis are based on the quantitative determination of specific activation markers of various links of the coagulation cascade. This contributes not only to the overall assessment of the patient's condition but also to the identification of a broken link in hemostasis [13]. The results of the study of patients with hearing impairment and different periods of seeking medical help after ABT are shown in Table 1.

According to Table 1, fibrinogen levels in patients of all studied groups did not change signifi-

Table 1. The fibrinogen level and the duration of APTT and PT in the blood plasma of patients with SND and different periods of seeking medical help after combat ABT, M ± m

Groups	Indices		
	Fibrinogen, g/l	APTT, sec	PT, sec
Practically healthy persons (*n* = 15)	2.5 ± 0.6	45.0 ± 3.0	18.0 ± 3.0
Patients with ABT:			
Group 1 (*n* = 21)	2.7 ± 0.3	73.0 ± 6.0*	21.3 ± 1.2
Group 2 (*n* = 25)	2.8 ± 0.5	66.0 ± 6.0*	25.0 ± 1.5
Group 3 (*n* = 26)	2.9 ± 0.2	65.0 ± 5.0*	21.8 ± 1.4
Group 4 (*n* = 20)	3.0 ± 0.4	66.0 ± 6.0*	28.3 ± 2.1*

Note: *Statistically significant difference between the group of practically healthy persons and the corresponding indicators of the group of patients ($P < 0.02–0.01$)

cantly and remained practically at the control level. To characterize the activation of the factors of the intrinsic pathway of blood coagulation and identify imbalances between procoagulant and anticoagulant links of the hemostasis system, APTT coagulation time was measured. Group 1 patients with sensorineural deafness (SND) had a significantly prolonged APTT (1.6 times) compared to control values. In the blood plasma of patients of the 2nd, 3rd, and 4th groups, APTT was significantly prolonged significantly, on average 1.5 times the control.

To evaluate the activation of factors of the external blood coagulation pathway and identify disturbances in the balance between procoagulants and anticoagulants, the PT was determined. According to the data in Table 1, the PT in patients with SND of the 1st, 2nd, and 3rd groups was within the control range. At the same time, in patients of the 4th group, a statistically significant prolongation of the PT by 1.6 times was observed compared to the control. This may suggest a disturbance in the balance between the links of the blood clotting system. For a more complete characterization of the state of this system and prediction of the development of thrombotic complications, it is advisable to use additional methods.

Thus, to determine the content of prothrombin and identify its functionally inactive forms, a method was developed using ecamulin – a prothrombin activator from the venom of Echis multisquamatus. Ecamulin can activate both prothrombin and its functionally inactive forms. In the absence of vitamin K, this leads to the formation of decarboxylated forms of prothrombin and prothrombin-1. The latter appears in circulation under the influence of thrombin and is one of the markers of intravascular blood clotting [14]. The results of the study of ET and prethrombin-1 levels in the blood plasma of patients with different degrees of hearing impairment and different periods of seeking medical help after combat acubarotrauma are shown in Table 2.

According to the data presented in Table 2, the ET in the blood plasma of patients of all studied groups did not undergo significant changes and remained at the level of control values.

The prethrombin-1 level in the blood plasma of Group 1 patients with SND had an increased level compared to control values, but this was not reliable due to significant differences in individual indicators. In the blood plasma of patients of the 2nd, 3rd, and 4th groups, the level of Pre-1 was significantly increased by 11.0, 5.6, and 12.5 times, respectively, compared to the control. This suggests an acute stage of blood coagulation system activation, thrombin generation, and an overall risk of intravascular thrombus formation.

Moreover, it was found that the level of protein C in the blood plasma of patients with SND in the 1st, 2nd, and 3rd groups tended to decrease compared to the control group. On the other hand, in group 4, this level was significantly reduced by 1.8 times. It is known that the anticoagulation system of protein C (protein C, protein S, thrombomodulin, thrombin, protein C inhibitor) plays an important role in the regulation of blood coagulation. The system primarily aims to inhibit factors VIIIa and Va and to inactivate the plasminogen activator inhibitor (PAI-1). Disruptions in this system cause thrombosis of various localization [15, 16]. The anticoagulant effect of protein C is associated with proteolysis and inactivation of activated factors VIIIa and Va, which is traditionally considered one of the main physiologi-

Table 2. ET, Pre-1concentration and the level of protein C in the blood plasma of patients with SND with hearing impairment and different periods of seeking medical help after combat ABT, M ± m

Groups	Indices		
	ET, sec	Pre-1, μg/ml	Protein C, %
Practically healthy persons (n = 15)	120.00 ± 15.00	0.20 ± 0.09	100.00 ± 15.00
Patients with ABT:			
Group 1 (n = 21)	132.20 ± 6.90	0.61 ± 0.32	91.00 ± 5.00
Group 2 (n = 25)	126.50 ± 16.00	2.30 ± 0.82*	81.00 ± 10.00
Group 3 (n = 26)	132.50 ± 9.30	1.11 ± 0.50*	93.00 ± 6.00
Group 4 (n = 20)	127.90 ± 11.10	2.50 ± 0.50*	55.00 ± 5.00*

Note: *Statistically significant difference between the group of practically healthy persons and the corresponding indicators of the group of patients (P< 0.02)

cal barriers to thrombosis [17]. According to the literature, an increase in the levels of prethrombin-1 as well as a decrease in the level of protein C have been observed in individuals with a prothrombotic state [18, 19]. Together with the prolongation of APTT and PT (Table 1), these findings may indicate a violation of the balance between pro- and anticoagulants.

It was established that the level of SFMCs, in the blood plasma of patients of the 1st group was 25.0 ± 10.0 μg/ml, and that of patients of the 2nd group was 18.0 ± 6.0 μg/ml. In the blood plasma of patients of the 3rd group, this indicator was 57.0 ± 11.0 μg/ml, and in the patients of the 4th group, it was 28.0 ± 14.0 μg/ml. Such changes in the SFMC level may indicate activation of the blood coagulation system and a disturbance of the dynamic balance between the blood coagulation and fibrinolysis systems.

Accumulation of prethrombin-1 and SFMCs clearly indicates activation of the blood clotting system, which, together with the decrease in PC levels, makes the control of hemostasis in patients with ABT the most vital problem to solve.

To assess the balance between coagulation and fibrinolysis system in the blood plasma of patients with ABT, we measured the concentrations of D-dimer and soluble fibrin. Soluble fibrin was assumed to be the result of thrombin action on fibrinogen in the initial stages of clotting activation. On the other hand, D-dimer was a product of fibrinolysis of covalently stabilized fibrin, indicating the late onset of the thrombosis process [21, 22]. The results of the study of the content of D-dimer and soluble fibrin in the blood plasma of patients with different degrees of hearing impairment and different periods of seeking medical help after combat ABT are shown in Table 3.

According to the data in Table 3, the concentration of D-dimer in patients with SND of the 1st group was at the level of control values. On the other hand, in patients of the 2nd group, a 1.4-fold decrease in this indicator compared to the control was noted. Against a background of a slight decrease in D-dimer, the concentration of soluble fibrin in this group increased.

The rate of SF in the blood plasma of patients of the 1st group is slightly higher than that of the control. On the other hand, in patients of the 2nd group, a 3-fold increase in this indicator was found compared to the control. In patients of group 3, the concentration of D-dimer increased slightly, and in patients of group 4, a tendency to increase by almost 2 times was revealed. The concentration of soluble fibrin in patients of the 3rd group increased by 1.8 times, and in patients of the 4th group, it was 6.5 times higher than the norm. It is known that an increase in the SF level is an early prognostic indicator of the activation of the blood coagulation system. Simultaneous measurement of its content and the level of D-dimer in the blood plasma helps to establish the presence or absence of a balance and correlation between the accumulation of SF and its destruction [16, 20, 21]. It is known that in equilibrium between the blood coagulation and fibrinolysis systems, the amount of D-dimer is always proportional to the amount of split fibrin [20, 21]. An increased level of D-dimer can indirectly indicate the formation of fibrin in blood plasma, its stabilization by factor XIIIa, and lysis of stabilized fibrin by plasmin. The concentration of SF in the blood depends on the ratio of concentrations of three enzymes – thrombin, factor XIIIa and plasmin. This concentration ratio determines the rate of transformation of fibrinogen into fibrin, polymerization of fibrin with the formation of its soluble

Table 3. The content of D-dimer and SF in the blood plasma of patients with SND and different periods of seeking medical help after combat ABT, M ± m

Groups	Indices	
	D-dimer, ng/ml	Soluble fibrin, μg/ml
Practically healthy persons (*n* = 15)	70.00 ± 6.00	< 3.00
Patients with ABT:		
Group 1 (*n* = 21)	69.00 ± 7.00	3.50 ± 0.58
Group 2 (*n* = 25)	49.57 ± 6.00	8.55 ± 1.71
Group 3 (*n* = 26)	76.31 ± 15.70	5.45 ± 1.80
Group 4 (*n* = 20)	131.66 ± 58.00	19.37 ± 6.21

oligomers and their stabilization; the formation of a solid-phase fibrin framework of a thrombus and the degradation of fibrin by plasmin [21]. The content of SF is considered the main indicator of the degree of activation of the blood coagulation system and the risk of thrombus formation, and the content of D-dimer indicates the ability of the fibrinolytic system to cope with the degradation of both soluble fibrin and thrombi that have already formed [20, 21, 23].

Conclusions. Changes in the indicators of the coagulation and fibrinolytic links of hemostasis in patients with sensorineural hearing disorders caused by acutraumatic damage in the combat zone have a complex and multidirectional nature.

1. Patients with a significant degree of hearing impairment due to combat ABT, regardless of the period of seeking medical help, need examinations according to a special algorithm of laboratory signs after the injury to confirm or refute the presence of a pathological condition associated with a violation of the hemostasis system. The presence of such violations is a prognostically insusceptible sign and indicates more severe lesions due to combat acubarotrauma.

2. Prolongation of clotting time in APTT and PT can be a sign of accumulation of clotting inhibitors or the depletion of clotting factors during the activation of the blood clotting cascade. The latter was confirmed by decreased protein C levels and the accumulation of SFMCs.

3. Comprehensive diagnostics of the blood coagulation system are strongly recommended for patients with sensorineural hearing disorders, particularly those who have sustained acubarotrauma (ABT) in a combat zone. Measuring fibrinogen concentration, together with the simultaneous assessment of D-dimer (a product of fibrinolysis of cross-linked fibrin clots) and soluble fibrin (a marker of active thrombin action), is considered the primary approach for accurately predicting thrombosis and preventing thrombotic complications.

4. Dysfunctions of the links of the hemostasis system, found in patients with combat ABT, which are characterized by their successive activation and exhaustion, may indicate the slow development of disseminated intravascular coagulation syndrome in these cases.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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СТАН СИСТЕМИ ГЕМОСТАЗУ ПАЦІЄНТІВ ІЗ СЕНСОНЕВРОЛОГІЧНИМИ ПОШКОДЖЕННЯМИ СЛУХУ, ЗУМОВЛЕНИМИ АКУСТИЧНИМИ ТРАВМАМИ В ЗОНІ БОЙОВИХ ДІЙ

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Проблема ранньої діагностики та лікування гострої травматичної патології та її ускладнень набула особливої актуальності в сучасних умовах бойових дій. Акубаротравма (АКБ) – це специфічне пошкодження, що характеризується проявами як з боку слухової системи, так і з ускладненнями, зокрема тяжкими формами нейросенсорної глухоти (НСП), які не пов’язані безпосередньо зі структурами слуху, але асоційовані з судинною патологією, гідродинамічним впливом на кровоносні судини, ушкодженням судинного ендотелію та неконтрольованим вивільненням у кровотік різних факторів згортання крові. Метою цієї роботи було оцінити стан системи гемостазу у пацієнтів, які отримали АБТ у зоні бойових дій та мають нейросенсорні порушення слуху. У плазмі крові пацієнтів визначали такі показники: концентрацію фібриногену, активований частковий тромбопластиновий час (АЧТЧ), протромбіновий час (ПЧ), функціонально неактивні форми протромбіну (ФНФП), екамуліновий час, протеїн С, розчинні фібрин-мономерні комплекси (РФМК), розчинний фібрин (РФ) та D-димер. Контрольну групу склали 15 здорових осіб із нормальним

слухом. 94 пацієнти з бойовими травмами були розділені на 4 групи залежно від ступеня порушення слуху та періоду після отримання АБТ: 1, 2 – пацієнти з легким порушенням слуху, які звернулися до медичного закладу протягом 2 місяців або більше ніж 2 місяці після травми, відповідно; 3, 4 – пацієнти з більш тяжким порушенням слуху, які звернулися до медичного закладу протягом 2 місяців або більше ніж 2 місяці після травми, відповідно. У плазмі крові пацієнтів усіх груп спостерігалося значно подовжене АЧТЧ та підвищені рівні РФ, РФМК та претромбіну-1 порівняно з показниками контрольної групи. У пацієнтів 4-ї групи відзначалося подовження ПЧ. Рівень протеїну С у 1-й, 2-й та 3-й групах мав тенденцію до зниження, а у 4-й групі був статистично зниженим порівняно з контролем. Концентрація D-димеру знизилася у пацієнтів 1-ї та 2-ї груп, залишалася на контрольному рівні в 3-й та збільшилася в 4-й групі. Рівень фібриногену та екамуліновий час у всіх досліджуваних групах залишалися на рівні контрольних значень. Відмічені дисфункції ланок системи гемостазу у пацієнтів із бойовою АБТ свідчать про повільний розвиток синдрому дисемінованого внутрішньосудинного згортання крові. Саме тому пацієнти з тяжким порушенням слуху внаслідок бойової АБТ, незалежно від терміну звернення за медичною допомогою, потребують обстеження після травми за спеціальним алгоритмом лабораторних показників.

Ключові слова: параметри системи гемостазу, акубаротравма, синдром внутрішньосудинного згортання крові, сенсоневрологічне пошкодження слуху.

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