ISSN 0030-5987. Orthopaedics, traumatology and prosthetics. 2024. № 2

УДК 616.74-001.4:616.15-005.4](045)

DOI: http://dx.doi.org/10.15674/0030-59872024211-16

Biochemical indicators of military blood of the ischemic and denervation process in muscles after a mine-explosive injurys

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The injuries received are accompanied by massive damage to the soft tissues of the limbs, main vessels and nerves, and the development of compartment syndrome. Violation of muscle function in the case of a limb injury can be both a consequence of denervation — as a result of damage to peripheral nerves, and ischemia with subsequent necrosis and fibrosis — as a result of direct or indirect damage to muscles, «tenotomy syndrome» — in case of traumatic damage to tendons and muscles yams, with the simultaneous combination of denervation and ischemia. Markers for assessing the state of muscle tissue — levels of Na^+, K^+ and Ca^{++} electrolytes, indicators of kidney function (urea, creatinine); glucose and the products of its transformations — lactate (lactic acid) and pyruvate (pyruvic acid) and, finally, muscle enzymes, namely creatine kinase (CK) and lactate dehydrogenase (LDH). Objective. To study the biochemical indicators of blood (activity of energy metabolism enzymes and electrolyte content) of military personnel with ischemic contracture and muscle denervation after a mine-explosive injury. Methods. The blood serum of 45 people, who were divided into IV groups, was analyzed. The average age of patients of the II group was 33.75 ± 3.46; III — 37 ± 3.66; IV — 35.86 ± 2.35. The results. The activity of LDH and CK and the content of electrolytes Na^+, K^+, Ca^{++} were determined in blood serum. Conclusions. LDH plays a key role in lactate metabolism and maintenance of energy balance in skeletal muscle. Its increased activity indicates the presence of hypoxia due to ischemic contracture of the limb and damage to peripheral nerves. KK enzyme protects cell membranes from damage. The tendency to decrease the activity of CC is a negative sign that reflects the initial process of destruction of the cell membranes of muscles damaged by a mine-explosive injury. The obtained results can be used to determine the severity of pathological processes in patients after traumatic damage to peripheral nerves and ischemic contracture, as well as when assessing the expediency of surgical intervention.

Отримані поранення супроводжуються масивними ушкодженнями м'яких тканин кінцівок, магістральних судин і нервів, розвитком компартмент-синдрому. Також через денервацію — ушкодження периферичних нервів, ішемію з подальшим некрозом і фіброзом — у результаті прямого або опосередкованого ураження м'язів, «синдрому тенотомії» — за травматичного ушкодження сухожилків і м'язів, за одночасного поєднання денервації й ішемії. Маркери для оцінювання стану м'язової тканини — рівні електролітів Na^+, K^+ і Ca^{++}, показники функції нирок (сечовина, креатинін); глюкоза та продукти її перетворень — лактат (молочна кислота) й піруват (піровиноградна кислота) і, нарешті, м'язових ферментів, а саме: креатинкінази (КК) і лактатдегідрогенази (ЛДГ). Мета. Дослідити біохімічні показники крові (активність ферментів енергетичного обміну та вмісту електролітів) військових з ішемічною контрактурою та денервацією м'язів після мінно-вибухового ураження. Методи. Проаналізовано сироватку крові 45 осіб, яких розподілили на IV групи. Середній вік пацієнтів II групи становив 33,75 ± 3,46; III — 37 ± 3,66; IV — 35,86 ± 2,35. Результати. У сироватці крові визначали активність ЛДГ і КК, і вміст електролітів Na^+, K^+, Ca^{++}. Висновки. ЛДГ відіграє ключову роль у метаболізмі лактату й підтриманні енергетичного балансу в скелетних м'язах. Підвищена її активність вказує на наявність гіпоксії за ішемічної контрактури кінцівки й ушкодження периферичних нервів. Фермент КК захищає клітинні мембрани від ушкодження. Тенденція до зниження активності КК — негативна ознака, яка відображає початковий процес руйнування клітинних мембран м'язів, ушкоджених мінно-вибуховою травмою. Отримані результати можуть бути застосовані для визначення важкості патологічних процесів у пацієнтів після травматичного ушкодження периферичних нервів і ішемічної контрактури, а також під час оцінювання доцільності проведення оперативного втручання. Ключові слова. Ішемія, скелетні м'язи, лактатдегідрогеназа, креатинкіназа.

Keywords. Ischemia, skeletal muscle, lactate dehydrogenase, creatine kinase

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Introduction

The war in Ukraine resulted in a significant increase in the number of victims of blast injuries both among the military and civil citizens. The resulting injuries are accompanied by massive damage to the soft tissues of the limbs, main vessels and nerves, and the development of compartment syndrome [1, 2]. Peripheral nerve injuries, which in peacetime amounted to 4 % of all injuries, were characterized by a significant and long-term decrease in the function of the limb, a high level of disability of patients and constituted a significant medical and social problem [3]. Currently, there are no clear statistics of such injuries in Ukraine, but due to military operations, it has probably increased dramatically.

Violation of muscle function in the event of a limb injury can be both a consequence of denervation in the case of peripheral nerve damage, and ischemia, with subsequent necrosis and fibrosis due to direct or indirect damage to the muscles, "tenotomy syndrome" in the case of traumatic damage to tendons and muscles also in the simultaneous combination of several of these processes (denervation + ischemia, etc.). In this regard, the role of not only adequate assessment of the severity of nerve damage, but also the nature of muscle damage is increasing in polystructural limb injuries [3].

As a result of ischemic and denervation damage of muscle tissue, a significant amount of protein factors are released into the blood, which also mediate inflammatory processes. Their release causes the beginning of a complex cascade of biochemical reactions and affects the course of ischemia [4]. The release of intracellular enzymes into the serum is an indicator of tissue injury and physiological cell metabolism [5]. Thus, the measurement of intracellular enzymes in blood serum is important for diagnosing damaged muscles, monitoring the course and severity of the disease, and evaluating the effect of therapy.

Markers that are determined in blood serum and used to assess the condition of muscle tissue include the levels of sodium (Na^+), potassium (K^+) and calcium (Ca^{++}) electrolytes, indicators of kidney function (urea and creatinine levels); glucose and the products of its transformations — lactate (lactic acid) and pyruvate (pyruvic acid) and, finally, muscle enzymes, namely creatine kinase (CK) and lactate dehydrogenase (LDH) [6, 7]. Creatine kinase is a key enzyme in the metabolism of skeletal muscle contraction and relaxation. It is present in tissues and cells that require energy (skeletal and cardiac muscle) and is considered the best marker for detecting and monitoring skeletal muscle diseases. Being in the myocyte cytoplasm, CK is a catalyst for the reaction of creatine and adenosine triphosphate, which leads to the formation of phosphocreatine and adenosine diphosphate. Phosphocreatine formed as a result of this reaction is the main depot of muscle energy [8]. The activity of total CK is a recognized marker of skeletal muscle destruction, which mostly occurs as a result of trauma. At the same time, myocytes disintegrate and CK enters the blood from the cytoplasm, which allows it to be used as a marker of muscle damage [8, 9]. An increase in the activity of CK in the blood serum may indicate the injury of skeletal muscles and can be observed in a number of pathological conditions. Therefore, monitoring changes in serum CK levels is important for patients with muscle pain or weakness [10].

Lactate dehydrogenase is a glycolytic enzyme that participates in the final stages of glucose conversion (catalysis of the interconversion of lactate and pyruvate). It is a component of all body cells, more often cytoplasm. Its highest activity was found in the liver, cardiac and skeletal muscles, and kidneys. The content of the enzyme in most tissues is much higher than in blood plasma. The increase in the activity of LDH or its individual isoforms in blood plasma occurs as a result of the release of enzymes from damaged or destroyed cells [6]. An increase in the activity of the LDH enzyme is registered in patients with progressive muscular dystrophy, especially in the early and intermediate stages of the disease, as well as during such pathological processes in muscles as: dystrophy, myopathy, traumatic injury [6, 11]. However, information on the activity of LDH and CK in case of traumatic damage to peripheral nerves is contradictory. Both a decrease and an increase or preservation of its activity at the control level were detected [12].

To date, numerous publications have proven the regulatory role of calcium in ensuring energy processes in muscles, their reduced activity. Therefore, further investigation of the intracellular content of calcium and other electrolytes is important for a more complete understanding of the mechanism of metabolic processes in connective and nervous tissues under normal and pathological conditions. As you know, catabolic processes prevail in the muscles in case of nerve innervation disorders.

They are manifested both by changes in the chemical composition of muscle tissue, and by sharp shifts in the speed of enzymatic reactions that provide energy for muscle activity. Disruption of catabolic processes, first of all, affects the regeneration of ATP, and

also decreases the supply of substrates necessary for biosynthetic processes (anabolism). In turn, damage to anabolic processes triggers a violation of the reproduction of functionally important compounds-enzymes, hormones necessary for catabolism [13].

Diseases that lead to neuromuscular disorders cause the release of organ-specific enzymes from the muscles into the blood plasma. Their appearance in the bloodstream as a result of leaching from cells with increased membrane permeability or structural damage makes it possible to assess the metabolic state of muscle tissue [5, 8, 9].

Purpose: to investigate the biochemical indicators of blood (activity of energy metabolism enzymes and electrolyte content) of military personnel with ischemic contracture and muscle denervation after a blast injury.

Material and methods

The study was conducted after the approval of the ethics committee of the Institute of Traumatology and Orthopedics (Protocol No. 4 dated 11.11.2023) in accordance with the Helsinki Declaration of 2000, European Society Directive 86/609 on human participation in biomedical research and orders of the Ministry of Health of Ukraine No. 690 of 23.09.2009, No. 944 dated 14.12.2009, No. 616 dated 03.08.2012. All patients signed an informed consent form regarding participation in the study.

The blood serum of 45 people, who were divided into IV groups, was analyzed. The average age of patients of Group II was 33.75 ± 3.46; III — 37 ± 3.66; IV — 35.86 ± 2.35.

Of them, 10 conditionally healthy patients were included in Group I (control), 8 patients in Group II with a diagnosis of ischemic contracture of a limb based on history and clinical data — local hypertensive syndrome, signs: atrophy of muscle fibers characteristic of the reactive-restorative period of ischemic contracture; 10 in Group III — damage to the peripheral nerves of the limb (based on clinical and instrumental indicators: using ultrasound and electroneuromyography, peripheral nerve injury was determined); 7 in Group IV — ischemic contracture of a limb with damage to peripheral nerves based on the combination of the clinical picture of ischemic contracture with the data of instrumental studies that indicated peripheral nerve injury.

In blood serum, the activity of LDH and CK was determined using standard Roche Diagnostics test systems on a Cobas-311 biochemical analyzer. The content of electrolytes Na^+, K^+, Ca^{++} was determined on an automatic EasyLyte Calcium analyzer (USA) using a package of reagents from MEDICA (Netherlands).

Statistical processing of the obtained results was carried out using the Origin Pro 8.5 software package. Average values of the obtained indicators (M) with standard deviations (SD) were calculated. The significance of the difference between groups with a normal distribution of the comparison was assessed by the t-Student test. At $p < 0.05$ changes were considered significant. In order to determine the statistical significance of differences between groups for quantitative (with a distribution other than normal) and ordinal variables, the Kruskal–Wallace test was used. Comparison of quantitative and ordinal variables in dependent samples was performed using the Wilcoxon test. Indicators on the graphs are presented in the form of median and 5–95 percentiles.

Results and their discussion

According to the data we received, the average indicator of LDH activity in the blood serum of patients in the control group was (82.5 ± 2.1) units/l (Table). In the rest of the patients, its probable growth was noted. Thus, LDH activity increased by 142.4 % in Group II, by 122.8 % in Group III, and by 153.3 % in Group IV ($p < 0.05$, Fig. 1).

LDH plays a key role in lactate metabolism and maintenance of skeletal muscle energy balance. Its increased activity may indicate hypoxia of skeletal muscles due to ischemic contracture of the limb and damage to peripheral nerves. Insufficient supply of oxygen to tissues causes a decrease in the share of aerobic, i. e. oxidative, phosphorylation. As a result, under conditions of hypoxia, muscles accumulate lactic acid, instead of using it as an energy source. This leads to acidosis, which inhibits phosphofructokinase. As a result, glycolytic formation of ATP also stops.

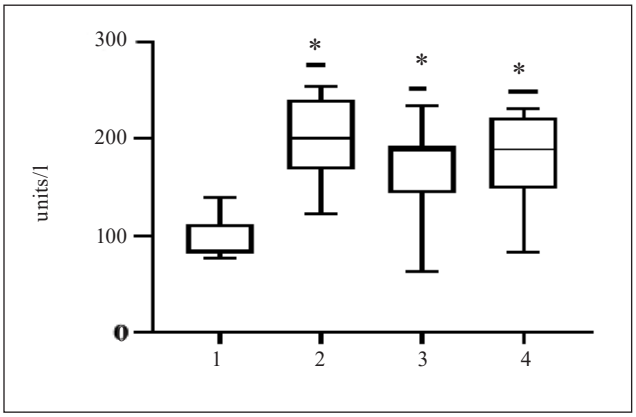

Fig. 1. LDH activity in blood serum of patients of the control group (1), with ischemic contracture (2), peripheral nerve damage (3), ischemic contracture and peripheral nerve damage (4). * $p < 0.05$ relative to the control group

Therefore, determination of LDH activity during diseases accompanied by traumatic damage to skeletal muscles and destruction of their cells is an important marker of tissue destruction.

Our results are consistent with a number of other publications [6, 14, 15], which report changes in biochemical markers, in particular, LDH activity, under conditions of ischemia and damage to peripheral nerves.

During the measurement of CK activity, no probable changes were detected in the blood serum of patients of Groups II, III and IV compared to control values. However, a tendency to a decrease of this enzyme in the blood serum of patients of Groups II and III was noted, respectively, by 11.5 and 19.7 %, and in the IV group, on the contrary, an increase by 4.5 % ($p > 0.05$, Table, Fig. 2). These results are

Table

The activity of creatine kinase and lactate dehydrogenase, the content of K^+, Na^+, Ca^{++} ions in the blood serum of patients with ischemic and denervation processes in muscles (M ± m)

Indicator	Control, n = 10	Ischemic contracture, n = 8	Damage to peripheral nerves, n = 10	Ischemic contracture with damage to peripheral nerves, n = 7
Lactate dehydrogenase, units/l	82.50 ± 2.10	200.00 ± 16.13*	183.80 ± 8.21*	209.00 ± 11.85*
Creatine kinase, units/l	137.00 ± 2.00	121.25 ± 28.7	110.20 ± 18.94	143.23 ± 11.85
Na^+, mmol/l	142.00 ± 1.90	152.25 ± 5.74*	143.94 ± 9.40	141.26 ± 2.79
K^+, mmol/l	4.50 ± 0.10	4.81 ± 0.26	5.32 ± 0.18	4.81 ± 0.12
Ca^{++}, mmol/l	1.23 ± 0.06	1.12 ± 0.04	1.16 ± 0.03	1.17 ± 0.03

Note. * $p < 0.05$ relative to the control group.

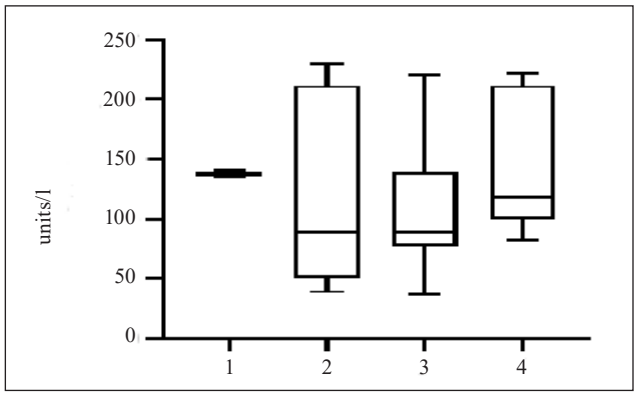

Fig. 2. Creatine kinase activity in blood serum of patients of the control group (1), with ischemic contracture (2), peripheral nerve injury (3), ischemic contracture and peripheral nerve injury (4)

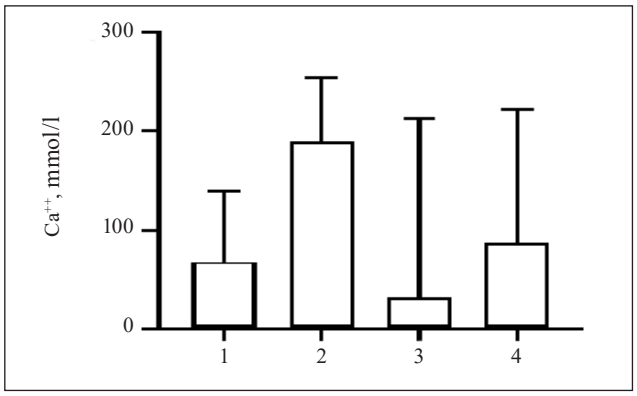

Fig. 4. Concentration of Ca^{++} in blood serum of patients of the control group (1), with ischemic contracture (2), peripheral nerve injury (3), ischemic contracture and peripheral nerve injury (4)

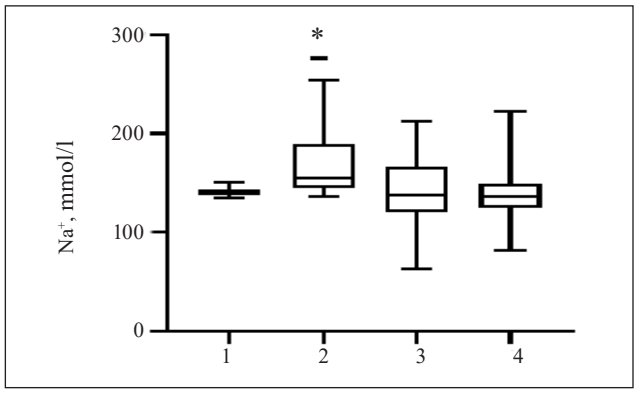

Fig. 3. Concentration of Na^+ in blood serum of patients of the control group (1), with ischemic contracture (2), peripheral nerve injury (3), ischemic contracture and peripheral nerve injury (4). * $p < 0.05$ relative to the control group

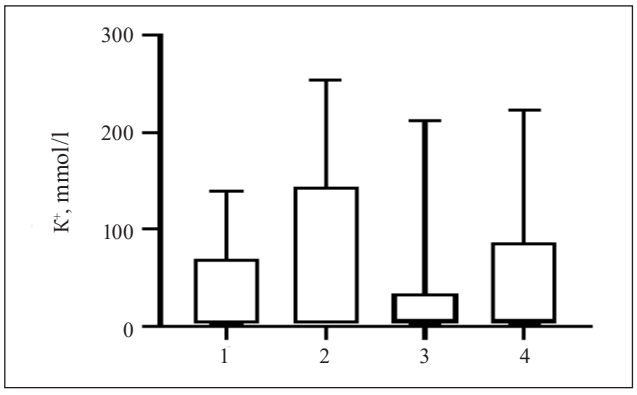

Fig. 5. The content of K^+ ions in the blood serum of patients of the control group (1), with ischemic contracture (2), peripheral nerve injury (3), ischemic contracture and peripheral nerve injury (4)

consistent with other researchers [12]: CK activity can decrease/increase or remain at the control level. However, the CK enzyme protects cell membranes from damage. Its formation and accumulation is one of the factors of membrane protection. Therefore, the tendency to decrease the activity of CK is rather a negative sign, which reflects the initial process of destruction of cell membranes of muscles injured by a blast injury [6].

The content of Na^+ ions in the blood serum of patients of Group II probably increased by 7 % ($p < 0.05$, Table, Fig. 3) from the control values.

Whereas the concentration of K^+ and Ca^{++} ions in blood serum of patients of all studied groups did not differ from the control values. Although a tendency to decrease Ca^{++} was noted in all groups of patients. It was the largest in the blood serum of individuals of Group II — 8.9 %, and III and IV — by 5.7 and 4.9 %, respectively ($p > 0.05$, Table 1, Fig. 4).

The content of K+ ions in the blood serum of patients of Groups II and IV tended to increase by 6.9 %, and in III by 18.2 % ($P > 0.05$, Table 1, Fig. 5).

The lack of ATP and the shift in pH to the acidic side change the polarization of the membrane. Na^+ and Ca^{++} ions enter, and K+ leaves skeletal muscle cells. As a result of acidosis, the influx of extracellular Ca^{++} decreases. It is released from complexes with troponin, ATP activity of myosin is inhibited. This leads to a decrease in contractility of skeletal muscles. During prolonged ischemia, both the sarcoplasmic reticulum and plasma membranes are damaged. The concentration of Na^+, Ca^{++}, lactate, pyruvate, ATP, etc. increases in the cell. These highly hydrophilic components lead to the swelling of cells and their organelles, therefore the intensity of peroxidation of membranes, entry of enzymes into the blood and damage to skeletal muscles increases [14, 15].

Conclusions

Lactate dehydrogenase plays a key role in lactate metabolism and maintenance of energy balance in skeletal muscle. Increased activity of the enzyme indicates the presence of hypoxia due to ischemic contracture of the limb and damage to peripheral nerves. CK enzyme protects cell membranes from injury. The tendency to decrease the activity of CK is a negative sign that reflects the initial process of destruction of the cell membranes of muscles damaged by a blast injury.

The obtained results can be used to identify the severity of pathological processes in patients after traumatic damage to peripheral nerves and ischemic contracture, as well as when determining the expediency of surgical intervention.

Conflict of interest. The authors declare no conflict of interest.

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The article has been sent to the editors 17.01.2024

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