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ATP-DEPENDENT ION-TRANSPORT SYSTEMS FUNCTIONING IN IMMUNOCOMPETENT CELLS OF MEN WITH ERECTILE DYSFUNCTION DUE TO COMBAT TRAUMA

Roman Fafula ✉, ***Dmytro Vorobets***, ***Mykola Vorobets***, ***Oksana Melnyk***, ***Olena Onufrovych***, ***Anna Besedina***, ***Zoryana Fedorovych***, ***Zinoviy Vorobets***

Danylo Halytsky Lviv National Medical University, 69 Pekarska St., Lviv 79010, Ukraine

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Background. The study of the problem of physical and mental health of people who have survived combat trauma is a component of a wide field of research on the problem of stress, which manifests itself at all levels of the organization of the body. Combat injuries, in particular those of the areas of pelvis and genitourinary system, stressful events, lead to the development of erectile dysfunction (ED). In recent decades, a sufficient number of scientific facts have been accumulated, which confirm the significant influence of stress factors on the reduction of sexual desire and sexual activity. Therefore, determination of Ca^{2+},Mg^{2+}- and Na^{+},K^{+}-ATPase activity on a convenient model like peripheral blood lymphocytes add complexity to the understanding of the development of the pathophysiological and pathobiochemical mechanisms of the body, the result of which is the development of ED.

Materials and Methods. The research was conducted on peripheral blood lymphocytes of men injured as a result of combat operations (shrapnel and bullet wounds) in the Russian-Ukrainian war and treated at the Military Medical Clinical Center of the Western Region. The research group of men with combat injuries was divided into two subgroups: men aged 20–39 years (subgroup 1) and men aged 40–53 years (subgroup 2). The control group consisted of 48 practically healthy men without complaints of sexual dysfunction or cardiac, neurological or endocrinological pathology. Among the men of the control group were 30 men aged 20–39 years (subgroup 3) and 18 men aged 40–53 years (subgroup 4).

Results. It has been shown that in the peripheral blood lymphocytes of men injured as a result of hostilities, there is a decrease in Na^+,K^+-ATPase activity and Ca^{2+},Mg^{2+}-ATPase activity of the plasma membrane and endoplasmic reticulum, which leads to overloading of the cytosol with Na^+ and Ca^{2+} ions, respectively, which is characteristic of pathological processes.

Conclusion. Erectile dysfunction due to combat trauma is accompanied by a decrease in both Ca^{2+},Mg^{2+}-ATPase activity of the plasma membrane and Ca^{2+},Mg^{2+}-ATPase activity of endoplasmic reticulum of blood lymphocytes. As the age of patients with disorders of sexual function increases, the decrease in Ca^{2+},Mg^{2+}-ATPase activities becomes more expressed. In men with erectile dysfunction due to combat trauma, the activity of Na^+,K^+-ATPase is also inhibited. According to the ROC curve, Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum in blood lymphocytes is a potential biomarker of erectile dysfunction.

Keywords: erectile dysfunction, Ca^{2+},Mg^{2+}-ATPase, Na^+,K^+-ATPase, lymphocytes, combat trauma

INTRODUCTION

From the experience of wars and local conflicts, in particular, of recent decades, it is known that wars inflict not only physical but psychological injuries too, which can manifest both during the war and after it (Serkin *et al*., 2010; Al-Azzawi & Koraitim, 2014; Ritchie & Elspeth Cameron, 2017). The study of the problem of physical and mental health of people who have survived combat trauma is a component of a wide field of research on the problem of stress, which manifests itself at all levels of the organization of the body (Serkin *et al*., 2010; Ritchie & Elspeth Cameron, 2017). The theory of traumatic stress is largely based on biological studies of disturbances in metabolism and regulatory systems as a result of an expressed reaction to traumatic stress (Ritchie & Elspeth Cameron, 2017). Combat injuries, in particular those of the areas of pelvis and genitourinary system, stressful events, lead to the development of erectile dysfunction (Al-Azzawi & Koraitim, 2014; Ritchie & Elspeth Cameron, 2017).

The modern scientific understanding of erectile dysfunction (ED) indicates the predominant secondary nature of sexual disorders in relation to the diseases that cause them (Vorobets, 2010; Horpynchenko *et al*., 2016). This especially applies to military personnel – participants in hostilities. Overall, ED is highly correlated with overall men's health.

The study of biochemical mechanisms underlying the development of ED is often conducted on the endothelium of cavernous bodies. This endothelium functions as a receptor-effector organ, responding to every physical or chemical stimulus, modulating vascular smooth myocytes to contract and relax, etc. (Horpynchenko *et al*., 2016; Samuel *et al*., 2021). However, endothelial cells are difficult to access for research, in particular when it concerns not experimental animals, but humans and, moreover, with combat trauma. In this regard, peripheral blood lymphocytes, due to their ability to quickly respond to any changes in homeostasis in the body and the fact that the modulation of enzyme activity in lymphocytes occurs much earlier than other biochemical and morphological indicators change, are a convenient object for conducting research associated with various pathologies, in particular erectile dysfunction (Koval *et al*., 2011). Lymphocytes express unique antigen-specific receptors, the variety of which actually

corresponds to the variety of environmental antigens. In its complexity, the immune system approaches the nervous system. Lymphocytes respond to almost all mediators of neuronal origin. Lymphocytes are capable of synthesizing the same biologically active substances expressing the same receptors as nerve or endothelial cells, as well as actively participating in the induction and regulation of the body's stress response through the synthesis and secretion of various factors. Lymphocytes are involved in the pathological process not only in blood diseases, they also undergo significant changes in structure and function in diseases of various genesis (Koval *et al*., 2011). The phenomenon of structural disorganization and impaired function of plasma membranes is a universal reaction of cellular systems during pathological processes of various genesis (Fafula & Vorobets, 2019; Fafula *et al*., 2020; Krebs, 2022). There are general laws of the response of cells to various pathogenic influences, typical pathological processes unfold in them, which are implemented according to a single scenario regardless of the primary initiating factor. The impact of various damaging factors of both psychogenic and organic nature on cells causes the launch of a universal response due to the action of similar molecular mechanisms of damage, regardless of its cause. These include, first of all, the intensification of lipid perocsidation, a decrease in the activity of the antioxidant defense system, and a change in the activity of ion transporting systems (Horpynchenko *et al*., 2016; Fafula *et al*., 2020).

In recent decades, a sufficient number of scientific facts have been accumulated, which confirm the significant influence of stressful factors on the reduction of sexual desire and sexual activity (Ritchie & Elspeth Cameron, 2017; Fafula *et al*., 2020). Therefore, studies such as the determination of Ca^{2+},Mg^{2+}- and Na^{+},K^{+}-ATPase activity on a convenient model like a peripheral blood lymphocyte add complexity to the understanding of the development of the pathophysiological and pathobiochemical mechanisms of the body, the result of which is the development of ED. It is believed that the patterns of changes in the structure and function of lymphocytes with the corresponding fate of correction, due mostly to the species specificity of cells, can be extrapolated to other cells (Koval *et al*., 2011).

The aim of the present work is to study the activity of Ca^{2+},Mg^{2+}- and Na^{+},K^{+}-ATPase of peripheral blood lymphocytes in men with erectile dysfunction due to combat trauma.

MATERIALS AND METHODS

Study design. The research was conducted on peripheral blood lymphocytes of men injured as a result of combat operations (shrapnel and bullet wounds) in the Russian-Ukrainian war, and treated at the Military Medical Clinical Center of the Western Region (Lviv, Ukraine). The research was conducted in September–December 2023 and January 2024. The research group of men with combat injuries was divided into two age subgroups: men aged 20–39 years (subgroup 1, n = 42) and men aged 40–53 years (subgroup 2, n = 26). The control group consisted of 48 practically healthy men without complaints of sexual dysfunction or cardiac, neurological or endocrinological pathology. Among the men of the control group were 30 men aged 20–39 years (subgroup 3) and 18 men aged 40–53 years (subgroup 4). The collection of peripheral blood was carried out after the preliminary completion of their clinical examination, before assigning them a course of treatment.

Lymphocytes isolation. Peripheral blood lymphocytes were isolated according to the method of A. Boyum (Boyum, 1968). Blood, diluted in a ratio of 1:1 with physio-

logical solution, was layered in a density gradient of ficol-triumbrast (r = 1.08 g/cm^3) and centrifuged for 20 min at 500 g. The removed interphase rings of mononuclear cells were washed twice for 10 min with physiological solution. After the last centrifugation, a small amount of physiological solution was added to the sediment; it was resuspended and, with the help of trypan blue, the number of live and dead cells was counted in the Goryaev chamber. The integrity and viability of blood lymphocytes in all experiments was at least 95%. Saponin was added to the suspension to permeabilize blood lymphocyte membranes and reveal latent enzymatic activities. Blood lymphocytes were incubated for 10 min with moderate shaking in a solution containing saponin at a concentration of 0.2% (optimal concentration).

Enzyme assay. Na^+,K^+-ATP-ase activity of blood lymphocytes was determined by recording the process of ATP hydrolysis by the accumulation of P_i (Veklich, 2007). Determination of the total ATPase enzymatic activity of cells was carried out at 37 °C in an incubation medium (volume 1 mL) of the following composition (мМ): 120 NaCl, 30 KCl, 5 $MgCl_2$, 1.5 ATP, 1 EGTA, 1 NaN_3 (mitochondrial ATPase inhibitor), 20 Hepes-Tris buffer (pH = 7.4), 0.1 μM thapsigargin (selective inhibitor Ca^{2+},Mg^{2+}-ATP-ase of endoplasmic reticulum) (Veklich, 2016). The presence of the Ca^{2+} chelator EGTA in the incubation medium ensured the binding of endogenous Ca^{2+} ions in it.

The ATP-hydrolase reaction was initiated by adding an aliquot of cell suspension (100 μL) to the incubation medium; the amount of protein in the sample did not exceed 50–100 μg. The duration of incubation was 5 min. The enzymatic reaction was stopped by adding 1 mL of a cooled “stop solution” of the following composition: 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% trichloroacetic acid (pH = 4.3). The “basal” Mg^{2+}-ATPase activity of cells was tested in a similar incubation environment, but in the presence of 1 mM ouabain, a selective inhibitor of Na^+,K^+-ATPase (Veklich, 2007). The ouabain-sensitive Na^+,K^+-ATPase activity of blood lymphocytes was calculated by the difference between the value of the total ATPase and “basal” Mg^{2+}-ATPase activity and was expressed in μmol P_i per minute per 1 mg of protein. Controls for non-enzymatic ATP hydrolysis and the content of endogenous P_i were samples whose composition corresponded to the standard incubation medium but contained cells with previously inactivated ATPase by treating them with a “stop solution”.

Ca^{2+},Mg^{2+}-ATPase activity of blood lymphocytes in patients was determined by recording the process of ATP hydrolysis according to the accumulation of P_i (Veklich, 2016). Determination of total Ca^{2+},Mg^{2+}-ATPase activity was carried out at 37 °C in an incubation medium (volume 1 mL) of the following composition (mM): 150 KCl; 0.05 $CaCl_2$; 5 $MgC1_2$; 5 ATP; 1 NaN_3 (mitochondrial ATPase inhibitor); 1 ouabain (inhibitor of Na^+,K^+-ATPase); 20 Hepes-Tris buffer (pH = 7.4).

To separate the total Ca^{2+},Mg^{2+}-ATPase activity into components – thapsigargin-insensitive Ca^{2+},Mg^{2+}-ATPase of the plasma membrane and thapsigargin-sensitive Ca^{2+},Mg^{2+}-ATPase of endoplasmic reticulum membranes – a Ca^{2+},Mg^{2+}-ATPase inhibitor thapsigargin (0.1 μM) was added to the standard Ca^{2+}- and Mg^{2+}-containing incubation medium. The activity of “basal” Ca^{2+}-independent, Mg^{2+}-dependent ATPase of blood lymphocytes was determined under the same conditions, but in the absence of $CaCl_2$ and with the addition of 1 mM EGTA and 0.1 μM thapsigargin. Ca^{2+},Mg^{2+}-ATPase of the plasma membrane was calculated as the difference between Ca^{2+},Mg^{2+}-ATPase activity in the presence of thapsigargin and “basal” Ca^{2+}-independent, Mg^{2+}-dependent ATPase activity. Ca^{2+},Mg^{2+}-ATPase of endoplasmic reticulum membranes was estimated as the

difference between total Ca^{2+},Mg^{2+}-ATPase activity and Ca^{2+},Mg^{2+}-ATPase activity in the presence of thapsigargin. After stopping the enzymatic reaction with a “stop-solution”, the suspension was centrifuged (10 min, 1500 g) and the content of inorganic phosphorus P_i was determined in the obtained supernatant.

Statistical analysis. Experimental data were processed by methods of variation statistics using software MS Office and BioStat LE. Inter-group differences were determined using non-parametric Kruskal–Wallis test. P value of <0.05 or lower were interpreted as statisticslly significant. A study of prognostic efficiency tests of biomarker indicators was carried out using the ROC analysis method. The calculated indicators of ROC analysis were as follows: sensitivity is a relative indicator of correctly classified positive cases and specificity is the proportion of incorrectly classified negative cases, based on which the ROC curve is built – a graph that allows evaluating the effectiveness of diagnostic tests. The ROC curve is built in the sensitivity coordinates (part of true-positive test results) and 1 – specificity of the test (in part false-positive test results). Data analysis was performed using the commercially available statistical software packages (MedCalc Statistical Software trial version 22.014. (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2023). Diagnostic accuracy was assessed based on the area under the ROC curve.

RESULTS AND DISCUSSION

Currently, it is known that changes in the concentration of Na^+ ions in cells can reflect their physiological or pathological state (Babsky, 2014; Yan & Shapiro, 2016; Clausen *et al*., 2017; Pirahanchi & Jessu, 2023). The main enzyme that regulates Na^+ concentration in cells is Na^+,K^+-ATPase (Yan & Shapiro, 2016; Clausen *et al*., 2017; Pirahanchi & Jessu, 2023).

When studying different age groups of men with shrapnel and bullet wounds, it was found that in the blood lymphocytes of men of subgroup 1 (age 20–39 years), the Na^+,K^+-ATPase activity is 3.15 (2.1; 3.85) μmol P_i/min·mg of protein, and in men of subgroup 2 (age 40–53 years) enzyme activity is somewhat lower – 2.92 (2; 3.625) μmol P_i/min·mg of protein (**Fig. 1**). In healthy men of subgroup 3 (age 20–39 years), the Na^+,K^+-ATPase activity in peripheral blood lymphocytes is 4.4 (2.775; 8.125) μmol P_i/min·mg of protein, and in control subgroup 4 (age 40–53 years) – 4.1 (2.875; 7.15) μmol P_i/min·mg of protein. When comparing groups using the Kruskal–Wallis method, we observed a significant difference in Na^+,K^+-ATPase activity in blood lymphocytes between men with ED due to combat trauma and healthy men, specifically between subgroups 1 and 3 ($P < 0.05$) and subgroups 2 and 4 ($P < 0.005$). There is no significant difference in Na^+,K^+-ATPase activity between age subgroups both in men with ED due to combat trauma and healthy men.

Ca^{2+}, Mg^{2+}-ATPase activity of cells is also one of the indicators characterizing their functional state (Stafford *et al*., 2017; Boczek *et al*., 2021; Meskalo *et al*., 2020; Fafula *et al*., 2020; Krebs, 2022). It is known that there are two Ca^{2+}-dependent ATPases in cells: Ca^{2+},Mg^{2+}-ATPase of the plasma membrane and Ca^{2+},Mg^{2+}-ATPase of the endoplasmic reticulum.

Using specific and non-specific blockers of various ATPases (plasma membrane and endoplasmic reticulum), we identified the contribution of each of the ATPases to maintaining Ca^{2+} homeostasis in the cell. Thus, the component of the total ATPase activity, which was inhibited by the specific Ca^{2+},Mg^{2+}-ATPase blocker of the endoplas-

mic reticulum thapsigargin (0.1 μM), the Na^+,K^+-ATPase blocker ouabain (1 mM) and the mitochondrial H^+-ATPase blocker sodium azide (1 mM), was 2.95 (2; 4.125) μmol P_i/min·mg of protein (subgroup 3) and 2.7 (2.15; 3.6) μmol P_i/min·mg of protein (subgroup 4) (**Fig. 2**). This activity corresponds to the Ca^{2+},Mg^{2+}-ATPase activity of the lymphocyte plasma membrane. In peripheral blood lymphocytes of men with ED due to combat trauma, the Ca^{2+}, Mg^{2+}-ATPase activity of the plasma membrane is 1.7 (0.9; 3.63) μmol P_i/min·mg of protein in subgroup 1, and 2 (1; 2.45) μmol P_i/min·mg of protein in subgroup 2. A comparison of the groups using the Kruskal–Wallis method reveals a significant difference in Ca^{2+}, Mg^{2+}-ATPase activity of the plasma membrane in blood lymphocytes between men with ED due to combat trauma and healthy men, specifically between subgroups 1 and 3 (P <0.005) and subgroups 2 and 4 (P <0.005). There is no significant difference in Ca^{2+}, Mg^{2+}-ATPase activity of the plasma membrane between age subgroups both in men with ED due to combat trauma and healthy men.

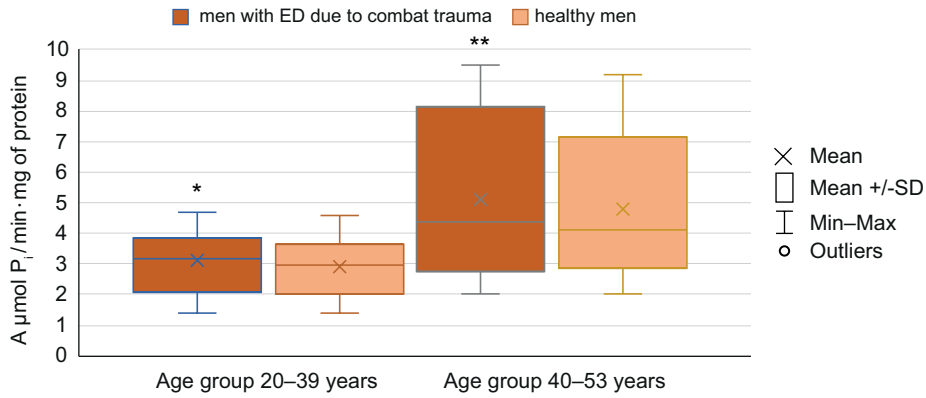

Fig. 1. Na^+,K^+-ATPase activity of peripheral blood lymocytes in men with erectile dysfunction due to combat trauma and healthy men, graphical interpretation of the Kruskal–Wallis test, n = 48–68, * P <0.05; ** P <0.005

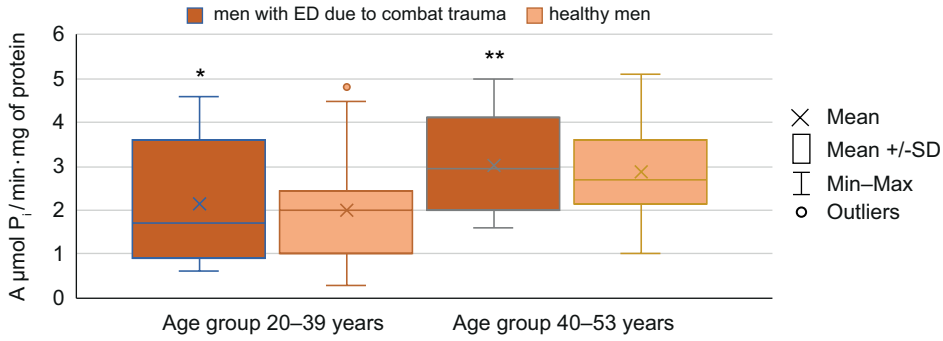

Fig. 2. Ca^{2+},Mg^{2+}-ATPase activity of plasma membrane of peripheral blood lymphocytes in men with erectile dysfunction due to combat trauma and healthy men, graphical interpretation of the Kruskal–Wallis test, n = 48–68; * P <0.005; ** P <0.005

The component of the total Ca^{2+},Mg^{2+}-ATPase activity that was inhibited by 0.1 μM thapsigargin corresponded to the Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticu-

lum. In men of subgroup 1, the activity of this enzyme was 1.65 (0.9; 2.125) μmol P_i/min·mg of protein, and in subgroup 2 – 1.1 (0.8; 2.05) μmol P_i/min·mg of protein (**Fig. 3**). In control subgroup 3, Ca^{2+},Mg^{2+}-ATPase activity of endoplasmic reticulum was 2.32 (1.175; 3.1) μmol P_i/min·mg of protein and in subgroup 4 – 2.6 (2.05; 3) μmol P_i/min·mg of protein. A comparison of the groups using the Kruskal–Wallis method shows a significant difference in Ca^{2+}, Mg^{2+}-ATPase activity of the endoplasmic reticulum in blood lymphocytes between men with ED due to combat trauma and healthy men, specifically between subgroups 1 and 3 ($P < 0.05$) and subgroups 2 and 4 ($P < 0.001$). There is no significant difference in Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum between age subgroups both in men with ED due to combat trauma and healthy men. It can be seen that activities of Ca^{2+},Mg^{2+}-ATPases, both PMCA and ERCA, decrease in men with ED due to combat trauma compared to heathy men. This leads to an overload of the cytosol with ions. However, a more pronounced decrease occurs with the increasing age of men.

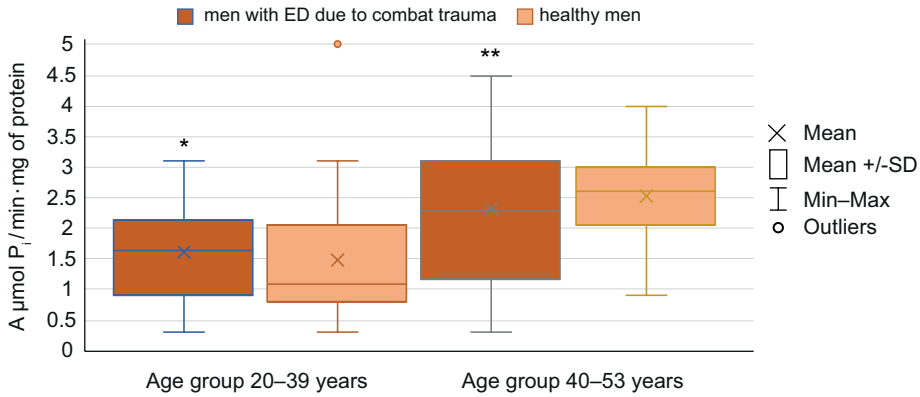

Fig. 3. Ca^{2+},Mg^{2+}-ATPase activity of endoplasmic reticulum plasma membrane of peripheral blood lymphocytes in men with erectile dysfunction due to combat trauma and healthy men, graphical interpretation of the Kruskal–Wallis test, n = 48–68; * $P < 0.05$; ** $P < 0.001$

The receiver operating curve (ROC) analysis for Na^+,K^+-ATPase activity, Ca^{2+},Mg^{2+}-ATPase of the plasma membrane and Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum in various groups of individuals are shown in **Figs 4–6.** The sensitivity and specificity of indicators (activity of Na^+,K^+-ATPase, PM Ca^{2+},Mg^{2+}-ATPase, and ER Ca^{2+},Mg^{2+}-ATPase) were calculated and are summarized in **Table**.

Results of analysis for Na^+,K^+-ATPase activity for young men patients showed that at a cut-off value of ≤4.7 μmol P_i/min·mg of protein, sensitivity was 100%, but specificity was reduced to 50.00%. In contrast, for value of <1.4 μmol P_i/min·mg of protein, specificity was 100% but sensitivity fell to 0.00%. The area under the ROC curve was equal 0.707 (95% CI 0.576 to 0.818, P = 0.0692) (see **Table**, **Fig. 4**). On the contrary, at a cut-off value of ≤4.6 μmol P_i/min·mg of protein sensitivity was 100%, but specificity was reduced to 46.67% for Na^+,K^+-ATPase activity for aged men patients. The specificity was 100%, but sensitivity fell to 0.00% at less than 1.4 μmol P_i/min·mg of protein. The area under the ROC curve was equal 0.737 (95% CI 0.608 to 0.842, P = 0.0644) (see **Table**, **Fig. 4**).

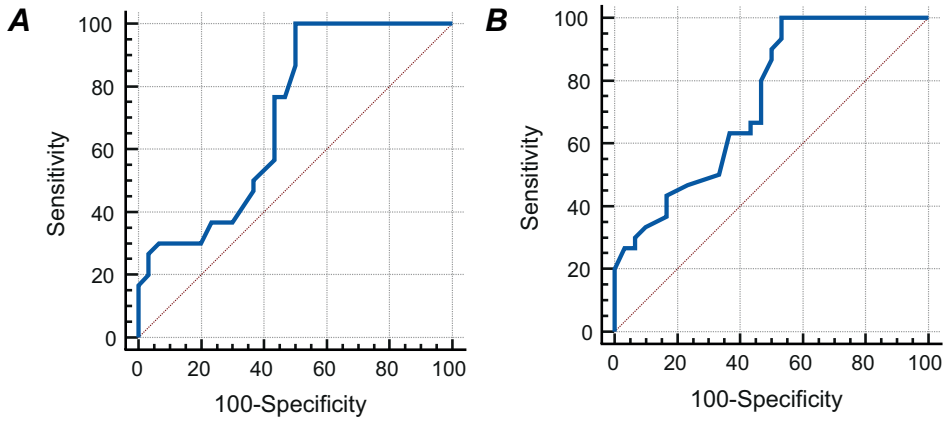

Fig. 4. Receiver operating characteristic (ROC) curve of Na^+,K^+-ATPase activity between (***A***) young men patients versus control group, and (***B***) aged men patients versus control group

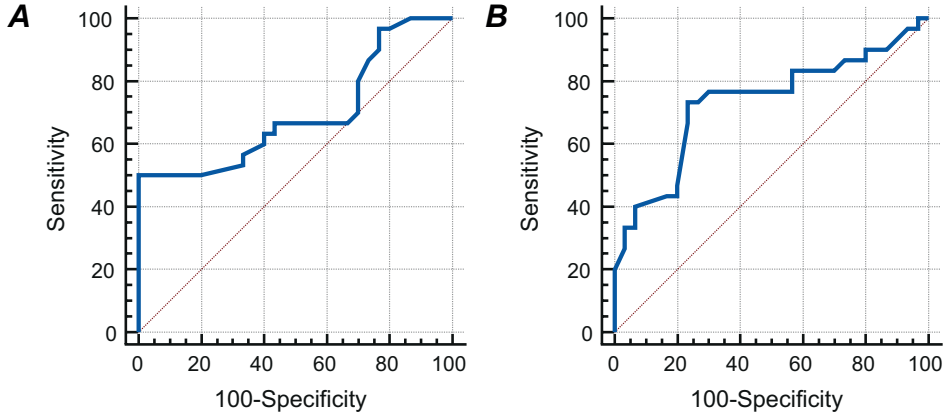

Fig. 5. Receiver operating characteristic (ROC) curve of Ca^{2+},Mg^{2+}-ATPase of the plasma membrane between (***A***) young men patients versus control group, and (***B***) aged men patients versus control group

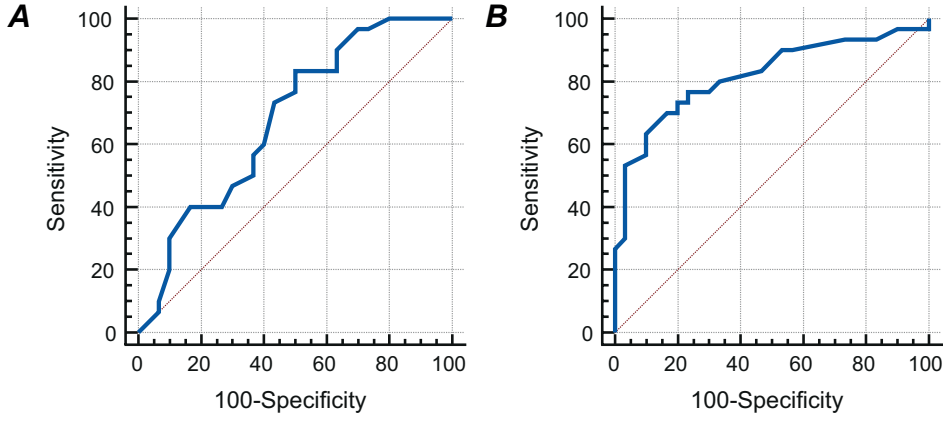

Fig. 6. Receiver operating characteristic (ROC) curve of Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum between (***A***) young men patients versus control group, and (***B***) aged men patients versus control group

Area under the ROC curve of Ca^{2+},Mg^{2+}-ATPase of the plasma membrane in young and aged men patients were AUC = 0.696 (95% CI 0.563 to 0.808, P = 0.0706) and AUC = 0.733 (95% CI 0.603 to 0.839, P = 0.0672), respectively. Cut-off values were ≤1.4 μmol P_i/min·mg of protein, and ≤2.1 μmol P_i/min·mg of protein, respectively. Ca^{2+},Mg^{2+}-ATPase of the plasma membrane in young men patients has the highest specificity (100.00%) and a relatively high sensitivity (50%) (see **Table**, **Fig. 5**).

AUC of Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum of young men patients was 0.680 (95% CI 0.547 to 0.795, P = 0.0699). Diagnostic specificity was 50.00% with the cut-off value of ≤2.2 μmol P_i/min·mg of protein, (see **Table**, **Fig. 6**).

The diagnosis value of individual indicators

Markers	AUC	Standard error (for AUC)	95% CI (for AUC)	Sensitivity (%)	Specificity (%)	P
Na^+,K^+-ATPase young men patients	0.707	0.0692	0.576 to 0.818	100.00	50.00	0.0027
Na^+,K^+-ATPase aged men patients	0.737	0.0644	0.608 to 0.842	100.00	46.67	0.0002
PM Ca^{2+},Mg^{2+}-ATPase young men patients	0.696	0.0706	0.563 to 0.808	50.00	100.00	0.0056
PM Ca^{2+},Mg^{2+}-ATPase aged men patients	0.733	0.0672	0.603 to 0.839	73.33	76.67	0.0005
EP Ca^{2+},Mg^{2+}-ATPase young men patients	0.680	0.0699	0.547 to 0.795	83.33	50.00	0.0100
EP Ca^{2+},Mg^{2+}-ATPase aged men patients	0.818	0.0568	0.698 to 0.906	76.67	76.67	<0.0001

The best value AUC = 0.818 (95% CI 0.698 to 0.906, P = 0.0568) was reached by a parameter Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum in aged men patients (see **Table**, **Fig. 6*B***), Cut-off value was ≤2 μmol P_i/min·mg of protein showing the sensitivity of 76.67% and the specificity of 76.67% (see **Table**, **Fig. 6**).

It is known that ion homeostasis is an important indicator of the body's functional activity. It is provided by the superposition of different ion transport systems of the cell, among which the leading role belongs to Na^+,K^+-, Ca^{2+},Mg^{2+}- and H^+-ATPases. Na^+,K^+-ATPase is the main enzyme of cytoplasmic membranes, which performs a highly specialized function in eukaryotic cells – energy-dependent transport of Na^+ and K^+ ions, and thereby ensures maintenance of the electrochemical gradient and osmotic potential of monovalent ions in the cell, which is a necessary precondition for their functioning (Babsky, 2014; Cao *et al*., 2020; Fafula *et al*., 2020; Krebs, 2022). In this regard, the enzyme plays a key role in the implementation of numerous cellular functions and processes that depend on the presence of ion gradients (Bartlett *et al*., 2018; Liu *et al*., 2018). The study of changes in Na^+,K^+-ATPase activity in pathological conditions is of

great interest to researchers in medical and biological practice. In particular, significant violations of the mechanisms of ouabain-sensitive and ouabain-resistant transport of monovalent ions have been shown in many mental illnesses (Clausen *et al*., 2017). Mutations in the Na^+,K^+-ATPase genes, in which the isoforms of the α-subunit of this enzyme are encoded, have severe physiological consequences, often causing neurological diseases (Clausen *et al*., 2017). Changes in kinetic properties of Na^+,K^+-ATPase were found in spermatozoa from fertile and infertile men (Fafula *et al*., 2019).

A significant decrease in the Na^+,K^+-ATPase activity of erythrocytes was revealed in patients with atrial fibrillation, ventricular and supraventricular extrasystole, hypertension; a violation of the working mechanisms of Na^+, K^+-ATPase was observed in combination with other cardiovascular diseases (Babsky, 2014; Clausen *et al*., 2017; Yan & Shapiro 2016). Cardiotonic steroids, such as digitalis, have been shown to mediate signal transduction through Na^+,K^+-ATPase in a process that has been reported to lead to the generation of reactive oxygen species (Yan & Shapiro, 2016). A direct relationship between the Na^+,K^+-ATPase activity and oxidative stress was also demonstrated by other authors (Bartlett *et al*., 2018). Over the past two decades, extensive research has been conducted to understand the signaling function of Na^+, K^+-ATPase and to define its role in physiological and pathophysiological conditions. It was shown that the Na^+,K^+-ATPase signaling cascade can function as an amplifier of reactive oxygen species, which can be initiated by cardiotonic steroids or by an increasing ROS concentration (Liu *et al*., 2018).

A decrease in the activity of Na^+,K^+-ATPase and a corresponding increase in the concentration of Na^+ in cells in various pathological conditions is quite a common phenomenon. This is primarily evidenced by direct measurements of Na^+ concentration in cells using nuclear magnetic resonance (Babsky, 2014). Thus, a decrease in the activity of enzymes of the glycolytic cycle and Na^+,K^+-ATPase in diabetic cardiomyocytes led to an increase in the level of $[Na^+]_i$ (Babsky, 2014; Cao *et al*., 2019; Nwia *et al*., 2022). The authors suggest that the increase in $[Na^+]_i$ activates the Na^+/Ca^{2+}-exchanger in the mitochondrial membrane and leads to a decrease in the concentration of Ca^{2+} in the mitochondria, and thus to the inhibition of Ca^{2+}-dependent bioenergetic processes. It has been shown that Na^+ ions are involved in the restoration of heart functions through the normalization of intracellular concentrations of Ca^{2+} and H^+ (Yan & Shapiro, 2016). These processes are mediated mostly through Na^+/H^+- and Na^+/Ca^{2+}-ion transport mechanisms (Cao *et al*., 2020).

During a comparative analysis of changes in total tissue and intracellular Na^+ in RIF-1 fibrosarcoma tumors, it was found that against the background of an almost unchanged level of $[Na^+]_i$ in an untreated tumor, the level of $[Na^+]$ increases, obviously as a result of a decrease in the bioenergetic status of the cell and the activity of Na^+,K^+-ATPase. A decrease in the activity of Na^+,K^+-ATPase was established during malignant transformations of spleen cells. It was found that the increase in $[Na^+]_i$, due to the inhibition of Na^+,K^+-ATPase activity, is characteristic of various pathologies, in particular those associated with the development of hypoxic conditions in tissues (Babsky, 2014).

Direct measurement of Na^+ concentration in cells confirmed the possibility of using this cation as an integral indicator of metabolic processes (Babsky, 2014). Thus, $[Na^+]$ increases by 2.5 times after initiation of anoxia, primarily reflecting a decrease in the activity of Na^+,K^+-ATPase in acidified anoxic muscle tissue. As evidenced by the given

data, changes in the concentration of Na^+ ions in cells reflect physiological and metabolic transformations that occur in various pathological conditions, and can be used as an integral indicator of these transformations. The level of Na^+_i is much more sensitive to pathological changes than the level of Na^+_e (Babsky, 2014).

The ion-transporting activity of the Na^+,K^+-pump changes under the influence of hormones, growth factors, and stress factors. As a component of the cell's life support system, the sodium pump is under control of various types of regulatory mechanisms that ensure both rapid and long-term changes in the intensity of ion flows through the plasma membrane (Bartlett *et al*., 2018; Pirahanchi & Jessu, 2023). The change in sodium pump activity induced by the activation of hormone or growth factor receptors is associated with a change in the kinetic parameters of Na^+,K^+-ATPase subunits, as well as with the incorporation of new ATPase components from the inactive intracellular pool into the membrane (Bartlett *et al*., 2018; Pirahanchi & Jessu, 2023).

The activities of Ca^{2+},Mg^{2+}-ATPases of the plasma membrane and endoplasmic reticulum also characterize the functional state of cells and the whole organism (Fafula & Vorobets, 2019; Boczek *et al*., 2021). Micromolar concentrations of Ca^{2+} in cells are maintained due to Ca^{2+},Mg^{2+}-ATPases. It has been shown that the activity of Ca^{2+},Mg^{2+}-ATPase is inhibited in both type 1 and type 2 diabetes (El Haouari, 2008). At the same time, the content of saturated and polyunsaturated fatty acids increases, which is accompanied by a decrease in the activity of membrane-bound ATPases. Activation of lipid perocsidation processes in pathological conditions leads to changes in the activities of membrane-bound enzymes, in particular Na^+,K^+-ATPase (Bartlett *et al*., 2018; Liu *et al*., 2018). So, under the conditions of the development of ED, the activity of the enzyme decreases. This slows down the outflow of Ca^{2+} from the cytosol and may indicate that the concentration of Ca^{2+} in the cell is increasing. The accumulation of Ca^{2+} in cells and a decrease in the level of ATP leads to a decrease in the activity of ion pumps and overloading of the cytosol with calcium. On the other hand, an increase in Ca^{2+} concentration leads to the activation of Ca^{2+}-dependent phospholipase A2, as a result of which lysofractions of phospholipids and free fatty acids accumulate in membranes. The above is in agreement with the data which show that the increased accumulation of calcium ions in lymphocytes in diabetes and hypertension is due to a decrease in the activity of Ca^{2+},Mg^{2+}-ATPase of the plasma membrane and modulation of the Na^+/Ca^{2+} exchange (Nwia *et al*., 2022). An increase in the concentration of ionized calcium in the cytoplasm in various pathological conditions is a widespread phenomenon. These data fully agree with those obtained on platelets (El Haouari, 2008, 2009). In arterial hypertension, for example, Ca^{2+} channel blockers (diltiazem, nifedipine, nicardipine, etc.) are widely used to prevent excessive influx of Ca^{2+} into cells (Stafford *et al*., 2017). Disturbances in the functioning of the complex system of Ca^{2+}-binding and Ca^{2+}-transporting mechanisms naturally leads to a violation of calcium homeostasis, causes failures of the regulatory function of Ca^{2+} and multiple pathological changes and metabolic shifts that are harmful to the cell. With dysfunction of Ca^{2+},Mg^{2+}-ATPase and an increase in the concentration of Ca^{2+} in the cell, the activity of a whole complex of enzyme systems that are activated by calcium increases, including Ca^{2+}-dependent proteases, the intensification of which contributes to the degradation of proteins.

ROC analysis of markers for Na^+,K^+-ATPase activity for young men patients and aged men patients, Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum for young

men patients reported in Tables shows that they are more sensitive than specific. Ca^{2+}, Mg^{2+}-ATPase activity of the endoplasmic reticulum is a sensitive parameter for erectile dysfunction in aged men. This marker can be a predictive biomarker by ROC-AUC. ROC curve analysis showed good diagnostic accuracy for Na^{+},K^{+}-ATPase activity for both groups, Ca^{2+},Mg^{2+}-ATPase of the plasma membrane for aged men patients and Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum for aged men patients versus control group with the AUC of 0.707, 0.737, 0.733, 0.818, respectively, suggesting their potential use as biomarkers for diagnosis of erectile dysfunction.

CONCLUSION

Erectile dysfunction due to combat trauma is accompanied by a decrease in both Ca^{2+},Mg^{2+}-ATPase activity of the plasma membrane and Ca^{2+},Mg^{2+}-ATPase activity of endoplasmic reticulum of blood lymphocytes. As the age of patients with sexual function disorders increases, the decrease in Ca^{2+},Mg^{2+}-ATPase activities becomes more expressed. In men with erectile dysfunction due to combat trauma, the activity of Na^{+},K^{+}-ATPase is also inhibited. According to the ROC curve, Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum in blood lymphocytes is a potential biomarker of erectile dysfunction.

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COMPLIANCE WITH ETHICAL STANDARTS

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

Animal rights. This article does not include animal studies.

Human rights. All studies were conducted in accordance with the Declaration of Helsinki guidelines. Approval for the study was taken from the ethics committee of Danylo Halytsky Lviv National Medical University (protocol No 2 from 25 February 2019).

AUTHOR CONTRIBUTIONS

Conceptualization, [F.R.; O.O.; V.Z.]; methodology, [M.O.; O.O.; V.D.; V.Z.]; validation, [F.Z.; M.O.]; formal analysis, [M.O.; V.M.; F.Z.; B.A.].; investigation, [M.O.; V.D.; B.A.]; resources, [F.R., V.D.]; data curation, [F.R.; O.O.; F.Z.; B.A.]; writing – review and editing, [F.R.; M.O.; V.D.]; visualization, [M.O.; F.Z.] supervision, [F.R.; V.D.; V.Z.]; project administration, [F.R.]; funding acquisition, [F.R.; V.Z.].

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ФУНКЦІОНУВАННЯ АТФ-ЗАЛЕЖНИХ ІОН-ТРАНСПОРТУВАЛЬНИХ СИСТЕМ В ІМУНОКОМПЕТЕНТНИХ КЛІТИНАХ ЧОЛОВІКІВ ЗА ЕРЕКТИЛЬНОЇ ДИСФУНКЦІЇ, ОБУМОВЛЕНОЇ БОЙОВОЮ ТРАВМОЮ

Роман Фафула, Дмитро Воробець, Микола Воробець, Оксана Мельник, Олена Онуфрович, Анна Бесединa, Зоряна Федорович, Зіновій Воробець

Львівський національний медичний університет імені Данила Галицького
вул. Пекарaська, 69, Львів 79010, Україна

Вступ. Дослідження проблеми фізичного та психічного здоров'я людей, які пережили бойову травму, є складовою широкого поля досліджень проблеми стресу, що проявляється на всіх рівнях організації організму. Бойові травми, зокрема, ділянки тазу та сечостатевої системи, стресові явища призводять до розвитку еректильної дисфункції. За останнє десятиліття накопичилася достатня кількість наукових фактів, які підтверджують значний вплив стресорних чинників на зниження статевого потягу та сексуальної активності. Тому дослідження, такі як визначення Ca^{2+},Mg^{2+}- та Na^{+},K^{+}-АТФазної активності на зручній моделі – лімфоцитах периферичної крові, додають комплексності в розумінні розвитку патофізіологічних і патобіохімічних механізмів організму, результатом яких є розвиток ЕД.

Матеріали та методи. Дослідження проводили на лімфоцитах периферичної крові чоловіків, які постраждали унаслідок бойових дій (осколкові та кульові поранення) у російсько-українській війні та які проходили лікування у Військово-медичному клінічному центрі Західного регіону. Дослідну групу чоловіків із бойовими травмами розділили на дві: чоловіки віком 20–39 років (група 1) і чоловіки віком 40–53 роки (група 2). Контрольна група складалася зі 48 практично здорових чоловіків без скарг на сексуальну дисфункцію чи кардіологічну, неврологічну або ж ендокринологічну патологію. У контрольній групі було 30 чоловіків віком 20–39 років (група 3) і 18 чоловіків віком 40–53 роки (група 4).

Результати. З'ясовано, що в лімфоцитах периферичної крові чоловіків, які постраждали внаслідок бойових дій, відбувається зниження Na^{+},K^{+}-АТФазної активності та Ca^{2+},Mg^{2+}-АТФазної активності плазматичної мембрани й ендоплазматичного ретикулуму, що призводить до перевантаження цитозолю відповідно йонами Na^{+} і Ca^{2+}, а це характерне для патологічних процесів.

Висновки. Еректильна дисфункція, обумовлена бойовою травмою, супроводжується зниженням як загальної Ca^{2+},Mg^{2+}-АТФазної активності, так і Ca^{2+},Mg^{2+}-АТФазної активності плазматичної мембрани й ендоплазматичного ретикулуму

лімфоцитів, що свідчить про зростання концентрації йонізованого кальцію у клітинах і порушення регуляторних механізмів клітини. Зі зростанням віку пацієнтів із розладами сексуальної функції зниження Ca^{2+},Mg^{2+}-АТФазних активностей наростає. За еректильної дисфункції чоловіків інгібується також активність Na^{+},K^{+}-АТФази, що призводить до накопичення Na^{+} в цитоплазмі та до порушення клітинних функцій. Відповідно до кривої ROC аналізу Ca^{2+},Mg^{2+}-АТФазна активність ендоплазматичного ретикулуму лімфоцитів периферичної крові є потенційним біомаркером еректильної дисфункції.

Ключові слова: еректильна дисфункція, Ca^{2+},Mg^{2+}-АТФаза, Na^{+},K^{+}-АТФаза, лімфоцити, бойова травма

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