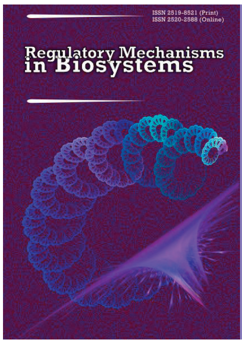

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The mechanism of adaptation of the organism of patients with chronic heart failure combined with vitamin D deficiency and the morphofunctional state of peripheral blood erythrocytes

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The problem of structural changes in peripheral blood erythrocytes in patients with chronic heart failure in combination with vitamin D deficiency during exercise stress remains insufficiently studied. Vitamin receptors are located on smooth myocytes, endothelial cells, cardiomyocytes and blood cells. It affects the state of the cell membrane, the contractile function of the myocardium, the regulation of blood pressure, cardiac remodeling and reduction of left ventricular hypertrophy. Therefore, it is important to assess the level of vitamin D in blood plasma in individuals with chronic heart failure and to identify the effect of its deficiency on the state of peripheral red blood cells when performing a 6-minute walk test. A total of 75 patients of the main group with chronic heart failure stage II A, I–II functional class with different levels of vitamin D deficiency were examined. The control group included 25 patients with chronic heart failure stage II A, functional class I–II without signs of vitamin D deficiency. The average age of patients was 57.5 ± 7.5 years. All patients were asked to undergo the 6 minutes walking test. The level of total vitamin D in plasma was determined by enzyme immunoassay. Morphological studies of erythrocytes were performed on the light-optical and electron-microscopic level. The obtained results showed that patients of the main group with chronic heart failure had a decrease in vitamin D by 2.2 times compared with the control group. Correlation analysis showed a directly proportional relationship between vitamin D deficiency and the number of red blood cells of a modified form and red blood cells with low osmotic resistance. Dosed exercise stress in patients with chronic heart failure against a background of vitamin D deficiency leads to an increase in the number of reversibly and irreversibly deformed erythrocytes and a decrease in their osmotic stability. This indicates a disorder in the structural integrity of their membrane and can have negative consequences for the somatic health of such patients.

Keywords: chronic heart failure; vitamin D; erythrocytes; physical activity; morpho-biochemical changes.

Introduction

The most widespread and prognostically adverse complication of most cardiovascular diseases (CVD) is chronic heart failure (CHF) (Vinogradova, 2019). The topicality of the study of CHF comes from the high prevalence and frequency of rehospitalizations, a significant level of disability and mortality. According to Sinha et al. (2013) and Zhang & Wang (2018), limited motor activity and decreased physical working capacity are the main causes of such a decreased quality of life (QOL) of patients. In such patients decreased physical working capacity is caused by skeletal muscle structural changes due to the decreased vasodilating ability of intramuscular blood vessels during CHF (Imaizumi et al., 2012). On the other hand, low working capacity causes deterioration in the patient's condition even during everyday physical activity, which is one of the factors decreasing the quality of life. This also manifests in decreased time spent in the fresh air and areas open to sunlight. The importance of insolation for these patients becomes clear from the results obtained by Rai & Agrawal (2017), who revealed that one of the additional development factors of this pathology is deficiency of 25-hydroxycholecalciferol vitamin D, production of which depends in direct proportion on degree of insolation and quality of diet. It was determined that patients with CHF and vitamin D deficiency consume little food rich in this biologically active product (Zittermann et al., 2013). The receptors to this essential substance are present in many target cells including smooth myocytes, endothelial cells, cardiomyocytes and blood cells (Meems et al., 2012). According to some researchers, the interaction of vitamin D with the tissues that are part of the cardiovascular organs includes its influence on myocardial contraction function, blood pressure regulation, cardiac remodeling and decrease in left ventricular hypertrophy (Dalbeni et al., 2014).

The scientific data point to the significant role of fat-soluble vitamins in the processes of cellular metabolism and protein synthesis (Lim, 2016). So, vitamin D normalizes synthetic processes in the body, induces biosynthesis of Ca^{+2}-dependent proteins, influences the differentiation and proliferation of hematopoietic stem cells, increases the number of circulating of peripheral blood erythrocytes (EPC), namely it has a positive influence on hematopoiesis in general (Holick, 2017). So vitamin D deficiency can lead to the impairment of the separate units of hematopoiesis significantly, the most important of which is erythropoiesis (Witham, 2011; Wang & Wells, 2015). The impairment of the latter leads to the development of different types of anemia (Katz, 2004).

According to Swedberg et al. (2013), anemia is the most widespread comorbid condition in several chronic diseases. It can be a cause, as well a threatening complication of CHF which worsens prognosis, complicates its course and increases morbidity indices. However, the insufficiently clear picture of anemia pathogenesis in CHF and ambiguity of results of clinical studies generate many unsolved problems related to the problems of laboratory data interpretation of EPC morphology in patients with CHF (Kleijn et al., 2013).

At the same time, there is a lack of data on the correlation between vitamin D deficiency and EPC condition in patients with CHF. In this context, the significant role of EPC in transporting processes of vitamin D to different body tissues is known. This is indicated by the results of the studies by some authors (Demir et al., 2013; Lim, 2016). According to data of Katz (2015) and Kleijn (2013), a significant percentage of people with CHF have not only vitamin D deficiency, but also damaged cellular membrane in EPC. In patients with vitamin D deficiency, CHF occurrence exceeds prevalence indices of the disease twice compared to the patients with sufficient level of this vitamin in the blood (Witham, 2011). However, the data on morphological changes of EPC depending on vitamin D deficiency in patients with CHF are limited in the scientific literature (Swedberg et al., 2013).

Also, the problem of EPC response to physical exercise (PE) in these patients requires further study. Chronic cardiorespiratory diseases can lead to impaired tolerance to physical exercise and limited physical activity which is revealed in research on the quality of life (Komoltri et al., 2011). Physical efficiency can be determined only with the help of physical exercise tests. The gold standard for determining the functional condition of the cardiorespiratory system is ergospirometry. However, this examination method requires complicated and expensive equipment not always available in practice.

An alternative is an examination with the use of 6-minute step test (6MST) that can be performed out of a department for functional diagnostics because conducting this test does not require expensive equipment (Leslie et al., 2013; Singh et al., 2015).

Patients with CHF need continuous rehabilitation and the determination of physical capacity can be one of the criteria of effectiveness of rehabilitation programmes (Sinha et al., 2013). At the same time, the EPC can be quite a sensitive marker of tolerance to physical load. However, scientific literature has few data on EPC changes during the 6MST in patients with CHF. Therefore, it is of certain interest to ascertain the pattern of EPC structural rebuild as an informative marker for determining factors contributing to decreased exercise tolerance in patients with CHF and its correlation with vitamin D level indices.

The study objective was finding characteristics of EPC structural rebuild in peripheral blood after physical exercise in patients with chronic heart failure against the background of vitamin D deficiency.

Materials and methods

A total of 75 patients (40 males and 35 females), citizens of Ivano-Frankivsk at the age of 57.5 ± 7.5 years were examined and included in the main group. All were diagnosed with CHF II A according to Vasylenko-Strazhesko, functional class (FC) I–II which developed against the background of ischemic heart disease (in 62.3% patients) and/or arterial hypertension (AH) (37.7% patients). The control group comprised 25 patients with chronic heart failure II A, functional class I–II without signs of vitamin D deficiency (11 males and 14 females). Exclusion criteria were: chronic obstructive pulmonary disease, asthma, taking hormonal medications, diabetes mellitus, oncological diseases.

At the time of inclusion into the study groups and during the whole observational period the patients received complex pathogenetic therapy according to the clinical guideline of diagnostics and treatment of CHF proposed by the American Heart Association (Marín-García, 2010): angiotensin-converting-enzyme inhibitors (ramipril 5 mg twice a day) or angiotensin receptor antagonists (losartan 50–100 mg a day), and diuretics (indapamide 2.5 mg a day or hydrochlorothiazide 12.2–25.0 mg a day) including treatment of comorbid arterial hypertension or ischemic heart disease.

To confirm CHF FC according to NYHA classification and national recommendations of Ukraine (Tavazzi, 2001), all patients were proposed a physical exercise (PE) in the form of 6MST (Leslie et al., 2013). During the test, each patient had to walk as long a distance as possible along a corridor at a comfortable tempo in 6 min with each 1 m marked. Factors limiting the physical exercise or any other complaints were evaluated according to the modified Borg Scale of Perceived Exertion. The distance covered by a patient in 6 minutes was by CHF FC "0" at > 551 m, by CHF FC I at 426–550 m, by CHF FC II at 301–425 m, by CHF FC III at 151–300 m, and by CHF FC IV at < 150 m. The difference between distances was considered clinically reliable if it had been > 54 m (5) between tests. If the patient could not continue the test due to asphyxia, he could rest, but the time was not stopped. The 6MST result was measured in absolute, reference values (Singh et al., 2015) as well as the amount of activity during performance of the test which was determined as a product of distance covered (m) over body mass (kg).

To evaluate the quality of life, SF-36 questionnaires were used (Stewart, 2007). There were questions on exogenic vitamin D consumption with food products high in vitamin D (cod liver oil, omega-3 fatty acids, fish oil), as well with medications containing vitamin D. The SF-36 questionnaire consists of 8 components: physical functioning; role limitations due to physical problems; bodily pain; general health perceptions; vitality; social functioning; role-limitations due to emotional problems; mental health. The evaluation is done using a 100-point scale: the higher the score, the higher the quality of life (Komoltri et al., 2011; Zhang & Wang, 2018).

Total level of vitamin D in blood plasma was evaluated with the enzyme-linked immunosorbent assay determining an amount of total 25-hydroxycholecalciferol. The blood plasma was obtained through centrifuging of 10 ml blood at 1,500 rpm. Vitamin D amount in blood plasma > 30 ng/mL was evaluated as optimal, 20–29 ng/mL as insufficient, less than 20 ng/mL as a deficiency, less than 10 ng/mL as a severe deficiency (Ekwaru et al., 2016). To determine vitamin D level in blood plasma, the blood was collected from all the examined patients in the time from October to December.

Hemoglobin and hematocrit levels were determined using standard methods (Kenny et al., 2016; Warren, 2017). To count the total number of EPC, blood smear analysis was performed using a Leica CME Microscope with further conversion of the image to a digital file (Video Camera Sony ExwaveHad SSC-DC58AP).

The blood smears were fixed in formalin pairs during 10 minutes, processed with n-butanol during 2 minutes, then were immersed into bromophenol blue 0.01% solution in ethanol dioxane-acetic acid mixture (5 : 4 : 1) for 10 minutes, and after rewashing in y n-butanol, were dried during 2 minutes to observe under the microscope (Löffler et al., 2018). The counting was done per 500 cells. Micrography was performed using a digital camera Olympus Camedia C-480 ZOOM (Olympus corp., Japan). The images were analyzed in software packages NIHimage (Macintosh) and ScionImage (PC).

Osmotic fragility of EPC was determined according to the concentration of hypotonic sodium chloride solution using standard methods (Gorshkova et al., 2017). Morphological tests of EPC microrelief were done in a scanning electron microscope JEOL-25M-T220A (Japan) according to the generally accepted common method (Popel' et al., 2017). We measured the maximal and minimal diameter of EPC, their circumference, determined the percentage ratio of their morphotypes before and after the 6-minute walk test.

Statistical processing of the study results was done using software package Statistica 10.0 (StatSoft Inc., USA). The data were presented as an average value and standard deviation (x ± SD), at distribution distinct from the normal one as a median (Me) and interquartile range (LQ-UQ). To evaluate relationship between differences, we used correlation analysis according to Spearman correlation coefficient (r). The differences were considered significant at the level P < 0.05.

Results

Average vitamin D level in blood plasma in the patients of the main group was 17.5 ± 0.04 ng/mL and was 2.2 times lower (P < 0.05) than in the patients of the control group – 38.4 ± 1.22 ng/mL, which was evaluated as vitamin D deficiency.

The indices of the SF-36 questionnaire in the patients of the main group were: physical functioning 67.8 ± 1.8%; role limitations due to physical problems 56.1 ± 2.7%; bodily pain 67.6 ± 2.4%; general health perception 31.2 ± 1.2%; vitality 40.3 ± 1.8%; social functioning 37.9 ± 2.1%; role-limitations due to emotional problems 64.9 ± 3.1%; mental health 62.3 ± 1.6%. The patients of the control group had slightly different results: physical functioning 77.9 ± 2.6% (Δ = 10.1); role limitations due to physical problems 38.5 ± 1.5% (Δ = –17.6); bodily pain 49.2 ± 1,8 (Δ = –18.4); general health 50.3 ± 21.2% (Δ = 19.1); vitality 59.6 ± 1.9% (Δ = 19.3); social functioning 66.5 ± 1.8% (Δ = 28.6); role-limitations due to emotional problems 33.7 ± 1.1% (Δ = –31.2); mental health 64.1 ± 2.5% (Δ = 1.8). According to questionnaire results, it was revealed that no patients of either the main group or the control group consumed products high in vitamin D, nor did they regularly take vitamin D medications.

The 6MST results analysis showed that 60.0% of the patients had 6MST result equaling 250.5 ± 15.96 m on average, which corresponded to CHF FC III. A total of 13.3% of patients had a result at the level of FC IV. In 20.0% this result corresponded to FC II and only in 6.7% was the test result at the level of FC I. Among 3 6MST parameters, the distance covered in meters had the strongest correlation with the values of the quality of life. We found no exceeses of the absolute values of the amount of work performed in comparison with the reference values. The test result had a stronger correlation with the values of the quality of life which characterized physical functioning.

The strongest correlation was shown by the test result with values of the quality of life that characterized physical functioning and role limitations due to physical problems (Table 1). Approximately similar correlation, average in value, was observed between quantitative values of completed activity derived from testing of physical capacity and values of the quality of life such as physical functioning, role limitations due to physical problems, general health perceptions, vitality and mental health. The correlation between the 6MST results and the quantitative values of the quality of life is presented in Figure 1 which shows that the higher the score of the quality of life is, the longer the distance the patient with CHF can cover.

Table 1
Correlation between the 6 minute walk test data and the quality of life in patients with chronic heart failure and vitamin D deficiency (n = 75)

Value	6MST, m	6MST, %	Amount of completed activity 6MST, kg × m
Physical Functioning	0.672**	0.493**	0.521**
Role limitations due to physical problems	0.645**	0.294*	0.483*
Bodily Pain	0.284*	0.147	0.077
General health perceptions	0.544**	0.372*	0.441*
Vitality	0.572**	0.348*	0.489**
Social Functioning	0.361*	0.252*	0.223
Role-limitations due to emotional problems	0.322*	0.115	0.264*
Mental Health	0.431*	0.255	0.428*

Note: * – P < 0.05; ** – P < 0.01.

The average amount of hemoglobin in the blood in the patients of the main group was at the level 99.8 ± 4.16 g/L (those of the control group 119.1 ± 2.15 g/L). Mild anemia was diagnosed in 71.4%, moderate anemia in 28.6% patients of the main group. The control group had 92.1% and 7.9% of such patients respectively.

The duration of anamnesis of patients with CHF against the background of ischemic heart disease was 7.8 ± 2.4 years, among which 20.9% of the patients had acute myocardial infarction, 59.7% of the patients were diagnosed with arterial hypertension, 20.3% had diabetes mellitus. Systolic myocardial dysfunction with left ventricular ejection fraction <45% was recorded in 34.9% patients; diastolic myocardial dysfunction by the relaxation type was diagnosed in 76.1% of the patients. In 41.7% of the cases, we recorded stage 1 of chronic kidney disease with prevalent chronic pyelonephritis, urolithiasis, and renal cysts. However, chronic kidney failure was not observed to accompany these conditions.

During morphological examination practically all the examined patients presented an increased percentage of EPC with changed surface relief. This correlates closely (r = 0.816; P < 0.01) with decrease of their osmotic fragility (Table 2).

At the concentration of 0.54% sodium chloride solutions, hemolysis occurred in 36.7% of the patients of the main group and in 23.9% of the patients of the control group; at the concentration of 0.45% it was observed in 66.3% of the patients of the main group and 44.7% of the patients of the control group; at 0.40% it occurred in all the patients independently from examination group. In our study, practically all patients of the main group were observed to have decreased EPC osmotic resistance, the severity of which depended on cell shape. Increase in the number of low-resistant cells has an average correlation with spherical EPC (r = 0.582; P < 0.05) and correlates closely with vitamin D deficiency (r = 0.877; P < 0.01).

Table 2
Correlation of vitamin D level in blood plasma with morphological reconstruction of erythrocytes in peripheral blood and their osmotic fragility (n = 75)

Value	Normocytes	Spherocytes	Echinocytes	Ovalocytes	Stomatocytes	Decreased osmotic resistance
Optimal vitamin D level (> 30 ng/mL)	0.981**	0.123	0.145	0.122	0.161	0.117
Vitamin D deficiency (<20 ng/mL)	0.252	0.775*	0.896**	0.861**	0.873**	0.877**
Decreased osmotic resistance	0.112	0.582**	0.815**	0.816**	0.823**	–

Note: * – P < 0.05; ** – P < 0.01.

At the same time, it was found that, unlike the patients of the control group, most patients of the main group were observed to have subpopulations of microcytic cells in peripheral blood along normocytes (Fig. 1*a*).

Some patients of the main group had reversibly (this potential reflects not only a number of morpho-functional adaptations of the human body but also is a direct reflection of the influence of external, social environmental factors) and irreversibly changed EPC forms, the amount of which exceeded the limit of the physiological norm, which creates a rather diverse morphological picture.

It is necessary to note that the presence of such EPC forms has purely individual characteristics and has no statistically reliable group characteristics (P > 0.05). So, we observed echinocytes with mild erosion of the outer membrane (Fig. 1*b*), and in some patients echinocytes, acanthocytes and stomatocytes were found (Fig. 1*c*, *d*).

After the 6MST test, all patients of the main group were observed to have a decrease of EPC level. At the same time, statistically reliable decrease in hemoglobin level and increase in hematocrit indices were observed. Electronic microscopy of EPC in the patients of the main group with decreased EPC level indicates their hemolysis (Fig. 1*e*). At the same time, unlike the patients of the control group, the main group had increased coefficient of their deformation (in 17.9%) and stronger aggregate capacity of EPC (aggregate level increased in 15.6%), which is presented well in Figure 1*f*.

Discussion

Recent scientific work has revealed that vitamin D deficiency could be a factor influencing the course of such diseases as arterial hypertension, aortic aneurysm, several angiopathies and CHF, which are causes of decreased adaptational potential of the body. This potential reflects not only a number of morpho-functional adaptations of the human body but also is a direct reflection of the influence of external, social environmental factors. One of such leading external factors is an index of the quality of life (Sinha et al., 2013). Some authors (Motsinger, 2012; Ekwaru et al., 2016) consider that the aim of medical and preventive measures in patients with CHF is prolongation of life, which depends on the quality of life according to many parameters.

During the study of the parameters of quality of life, we have shown that the patients of the main group spent little time engaged in physical activity and were less active socially compared those of the control group. This, against the background of the higher role of physical problems in limiting vitality and the significant influence of the emotional sphere on the general condition, and perception of problems with their health, has a significant effect on mental health. Because of this many people often lose the motivation to take an active role in life, start to break their daily regimen, follow a sedentary daily routine, lead irrational lifestyle, acquire negative habits, eat irrationally, etc. (Stewart, 2007; Zhang, W., & Wang, 2018). The complex influence of negative psychosomatic factors can be not only the cause of cardiovascular diseases (Komoltri et al., 2011; Marín-García, 2010), but also cause hypo- and avitaminoses, and in particular vitamin D deficiency (Demir et al., 2013; Mohammed et al., 2015). The studies conducted earlier considering the adaptation potential of patients with different CVD showed that they had a lower level of functional body reserves during vitamin D deficiency (Meems et al., 2012; Akin et al., 2014).

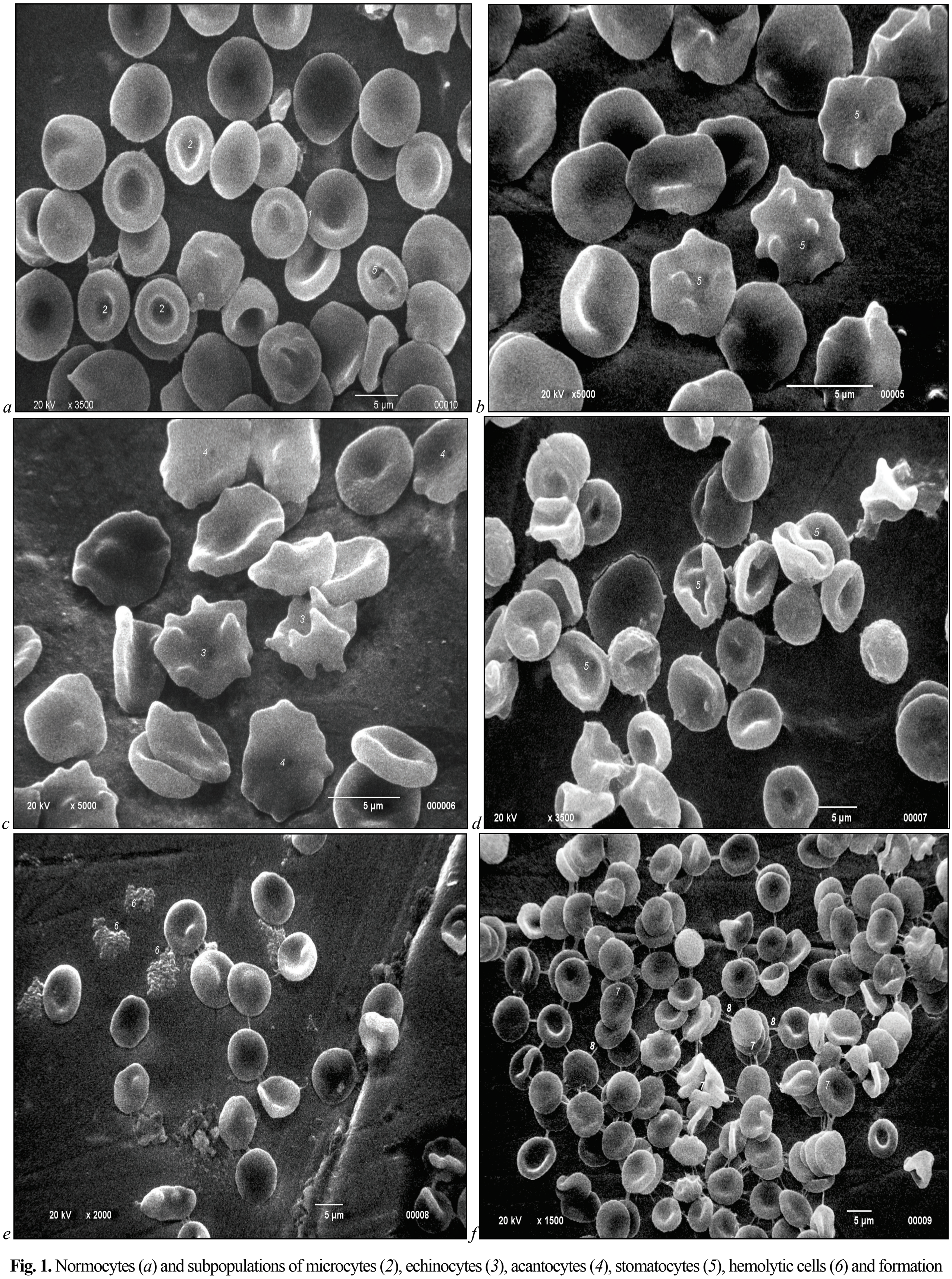

Fig. 1. Normocytes (*a*) and subpopulations of microcytes (*2*), echinocytes (*3*), acantocytes (*4*), stomatocytes (*5*), hemolytic cells (*6*) and formation of erythrocyte aggregates (*7*) and intercellular fibrin threads (*8*) in blood of the patients with chronic heart failure and vitamin D deficiency, after physical exercise (*b*–*e*)

At the same time, it is known that adaptation potential of the human and animal body depends strongly on the condition of the oxygen transport system (Popel' et al., 2017), to which EPC belong, and intracellular energy reserves (Chang & Bo, 2015). This requires closer attention of scientists of different specialties to the morpho-functional condition of EPC. At the same time, the clear interrelation which we found between the severity of vitamin D deficiency in patients with different tolerance level and PE during the 6MST indicates that the proposed grading of the morpho-functional changes of EPC can be used as a criterion to determine the volume of PE. This corresponds to similar criteria determined by other authors (Chang & Bo, 2015).

The PE of different intensity level plays a major part in the formation of general endurance of the organism, which manifests in different types of human life activities (Lambert, 2016; Popel' et al., 2017). It reflects the general level of the physical capacity of the human body. While being a multifunctional feature of the human body, endurance integrates into itself a large number of processes occurring at different levels: from the cellular and to the whole body (Chang & Bo, 2015; Lambert, 2016).

But as the results of modern scientific studies show, in most cases a leading role in studies of endurance belongs to determination of factors which contribute to the activation of energy exchange and vegetative systems of its provision, cardiovascular and respiratory ones, as well the central nervous system. At the same time, studies of cellular reactions at physical exercise, especially EPC in the process of adaptation of patients with CHF against the background of vitamin D deficiency remain out of sight (Mohammed et al., 2015; Montero & Lundby, 2019).

In physiological conditions, erythrocytes circulating in peripheral blood differ in size, structure, metabolism and other properties due to their age peculiarities. In this case, EPC serves as a convenient object for such studies since they take part in the processes related to maintaining homeostasis at the level of the whole body (Popel' et al., 2017). These cells, apart from the specific gas transport function intrinsic to them, have the ability to participate in regulation of acid-base and water-electrolyte balance, microrheological properties of blood, immune reactions, binding and transportation of amino acids and lipids, which is of immediate interest in the context of development of general body endurance in patients with CHF against the background of vitamin D deficiency (Akin et al., 2014).

Because of PE, statistically probable increase of hematocrit level by 5.0% and decrease hemoglobin level by 2.7% occur (Mairbäurl, 2013). However, PE following increase in number of EPC, due to the development of hemoconcentration, caused decrease in EPC level in some patients of the main group. This phenomenon can be caused by the destructive influence on structurally changed erythrocytes of the factors occurring during muscle activity: increased blood circulation, its temperature, acidosis or alkalosis, etc. (Zhao et al., 2013). One-time PE causes increase in EPC of a larger size by approximately 3.0% ($P < 0.05$). Amount and volume of EPC determine the rheological properties of the blood. Change in these parameters under physical exercise can significantly influence the oxygen transport function of the blood and can cause disorders in the microcirculation system, thus changing the level of functional body condition of patients with CHF (Akin et al., 2014). This mechanism of the development of adaptation processes during physical exercise in patients of the main group explains the increased aggregation capacity of EPC (content of aggregates increased by 15.6%) and increased coefficient of their deformity (by 17.9%), which is observed because of muscle activity and can contribute to increase in viscosity of circulating blood. Such changes in EPC indices are unfavourable for the realization of the oxygen transport function of the blood and indicate lack of adaptation to PE in patients of the main group (Lambert, 2016).

It is known that EPC have a phenomenal property to accumulate Ca^{+2} salts against the background of its decreased concentration in blood plasma, unlike other cells (including cardiomyocytes), which are deficient in it in such circumstances (Akin et al., 2014). The increased intracellular concentration of Ca^{+2} plays a major role itself in ATP deficiency and increased deformity of EPC (Chang & Bo, 2015).

In general, significant conformational change of EPC causes decrease of EPC membrane area when it does not contact its whole surface, but only contacts it over a highly limited area on the tops of thorny projections. This shows the extent of impairment of transmembrane transportation of essential substances at the level of hemomicrocirculatory metabolism in patients with vitamin D deficiency.

Therefore, under the influence of PE most (97.0%) patients with CHF were observed to have negative morphological changes in EPC caused by dysmetabolic disorders related to vitamin D deficiency. In such conditions, the structural integrity of EPC is impaired, which in its turn contributes to their intravascular hemolysis and can cause anemia (Chang & Bo, 2015; Popel' et al., 2017).

To characterize EPC of different quality, it was proposed to determine their resistance to biological, thermal agents, chemical substances, environmental hypotonia of different degree, etc. In our examinations, practically all the patients had decreased EPC osmotic resistance that was more expressed in the patients of the main group and depended on EPC shape. Decreased osmotic resistance, increased number of low-resistant cells on the erythrogram correlated with the prevalence of spherical EPC in blood. The results of our study coincide with other data of other authors (Tavazzi, 2010), whose results show that, compared to patients of the control group, patients of the main group were observed to have decreased EPC membrane resistance to EPC osmotic hemolysis. The EPC hemolysis is conditioned in many respects by impaired enzymatic processes in the cellular membrane, which often develop with vitamin D deficiency (Rai & Agrawal, 2017). Prevalence of content of EPC with changed superficial cytoarchitectonics, decreased resistance of the EPC membrane to osmotic hemolysis, as well as recording hemolyzing cells after the 6MST in blood of the patients of the main group could be caused by several factors resulting from the development of discoordination of metabolic processes during vitamin D deficiency (Akin et al., 2014; Dalbeni et al., 2014).

This is accompanied by damage to EPC membrane structures. Earlier studies researched the activity of enzymes which actively participate in EPC metabolism: glucose-6-phosphate dehydrogenase and glutathione reductase, which provide preservation of EPC cellular membrane at influence of different agents. These enzymes have a key role in intraerythrocytic enzyme processes, particularly take part in the reductive pentose phosphate cycle of glucose metabolism and glutathione cycle (Chang & Bo, 2015). About 90% of glucose assimilated by EPC transforms in the process of glycolysis into lactate and about 10% of it oxidizes through transformation of glucose-6-phosphate into 6-phosphogluconate, which is catalyzed by glucose-6-phosphate dehydrogenase. This reaction mostly provides EPC with the necessary amount of restored nicotinamide adenine dinucleotide phosphate, the most important hydrogen donor which is used for restoring glutathione. Restoration of glutathione occurs with direct participation of glutathione reductase. Glutathione is an intracellular restorative agent whose main function is to protect protein thiol groups while preserving them in restored condition. Rapid glutathione regeneration protects hemoglobin from oxidizing denaturation. In all this cascade of biochemical transformations, vitamin D participates directly, because all of them require the presence of intracellular Ca^{+2}, the amount of which is regulated precisely by this biologically active substance (Holick, 2017).

One examination (Limsuwan, 2010) showed that after 6MST the patients were observed to have lower activity of G6PD as well as GR. Deficiency of glucose-6-phosphate dehydrogenase and glutathione reductase in erythrocytes under the influence of oxidizing agents caused decreased glutathione level.

In exhausted glutathione regeneration processes, intracellular oxidoreduction processes slow down or stop, "chemical", and therefore, biological death of EPC occurs.

Such a development mechanism of increased deformability and hemolysis of EPC can be assumed in the case of the decreased amount of EPC and decreased tolerance to PE in patients with CHF against the background of vitamin D deficiency. An erythrocyte is either phagocytized by the cells' monocyte phagocyte system (during physiological ageing), or undergoes intravascular decay under the influence of oxidizing agents (Popel' et al., 2017).

Thus, under the influence of PE, patients have negative morphological changes of EPC, based on impaired oxygen transport system due to vitamin D deficiency. The basis of these changes is formed possibly by inadequate functional capacity of the body biosystems of the patients of the main group for the volume and intensity of physical exercise compared to the patients of the control group who have an adequate vitamin D level. In such conditions, structural rebuild of EPC occurs, which can contribute to intravascular hemolysis during PE and cause anemia (Swedberg, 2013). In our opinion, such type of anemia should be attributed to the anemia which accompanies chronic diseases (Katz, 2004; Kleijn et al., 2013). The presence of such changes usually has a negative influence on the results obtained from performing the 6MST, which requires appropriate correction of the therapeutic process, and also development and timely use of adequate measures to eliminate intravascular EPC hemolysis in patients with CHF against the background of vitamin D deficiency and elimination of possible negative consequences (Marín-García, 2010; Swedberg et al., 2013; Vinogradova, 2019).

Conclusions

In-depth study of mechanisms of the influence of physical exercise of stress level on the blood oxygen transport system, organization of hematological control at different stages of treatment of chronic heart failure, as well as during correctional restoration measures orientated towards diminishing disorders which occur as a result of vitamin D deficiency, is a relevant task and requires scientific justification of corresponding preventive measures.

During the morphological examination practically all patients with chronic heart failure and vitamin D deficiency were observed to have heterogeneity of cellular subpopulations erythrocytes in peripheral blood, when along with normocytes subpopulations of microcytes, echino-, acanto- and stomatocytes were recorded, which indicates instability of the membranes of erythrocytes in the peripheral blood.

After the 6-minute walking test, the patients with chronic heart failure and vitamin D deficiency were observed to have a decreased level of erythrocytes in the peripheral blood, although the deformability of the erythrocytes increased, lysis of cells was recorded against the background of decreased normocytes, which is confirmed by decreased osmotic resistance. This dictates the necessity to search for new ways of correcting the blood cellular components in this cohort of patients.

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