VOLUME - 12, ISSUE - 05, MAY - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

General Medicine

THE MORFOFUNCTIONAL STATE OF PERIPHERAL ERYTHROCYTES AT CHRONIC HEART FAILURE AND VITAMIN D DEFICIENCY IN

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ABSTRACT

The aim of the work To study the effect of vitamin D deficiency in blood plasma on the morphofunctional state of peripheral blood erythrocytes after exercise in patients with chronic heart failure. **Methods:** scanning electron microscopy of peripheral blood erythrocytes, enzyme immunoassay to determine the level of vitamin D. **Results.** A morphological study revealed heterogeneity of erythrocyte subpopulations in 90% of patients with CHF and vitamin D deficiency. In peripheral blood, in addition to normocytes with sufficient hemoglobinization, subpopulations of macro- and microcytes were recorded. In most patients with vitamin D deficiency, several types of morphological changes in the shape of erythrocytes were revealed, manifested in the form of echino-, acantho- and stomatocytosis, which indicates destabilization of the erythrocyte's membrane. After a 6-minute walk test in CHF patients with vitamin D deficiency, the number of discocytes decreases, the ability to deform decreases, lytic cells were recorded with a reduced number of normocytes. **Conclusion:** The identified morphobiochemical changes indicate the destructurization of the erythrocyte membrane components and have negative consequences, taking into account the likely impact on the somatic health of patients who, in addition to changes characteristic of CHF, receive significant stress during physical exertion against the background of hypovitaminosis. D.

KEYWORDS : Chronic heart failure, vitamin D deficiency, erythrocytes, physical activity.

INTRODUCTION.

Chronic heart failure (CHF). is the most common and unfavorable prognostic complication of most cardiovascular diseases (CVD). One of the additional risk factors for this pathology is 25-(OH)D deficiency.

The receptors of this essential substance are located in such target cells: smooth myocytes, endotheliocytes, cardiomyocytes and blood cells [1,2,3]. Scientific literature data indicate the important role of fat-soluble vitamins in the processes of building and stabilizing cell membranes and protein synthesis [4]. Thus, vitamin D normalizes Ca^{+2}-dependent synthetic processes in the body, including development, an increase in the number of peripheral blood erythrocytes (PBE), i.e. about the positive effect on hematopoiesis in general [5].

At the same time, data on the relationship between vitamin D deficiency and the shape of peripheral blood erythrocytes in patients with CHF are not enough. But the data of some authors [6] indicate the important role of PBE in the processes of vitamin D transport to various tissues of the body.

According to several researchers, the interaction between vitamin D and the cardiovascular system includes the influence of the former on myocardium contractile function, blood pressure regulation, remodeling of cardiac muscle cells, and decreasing of left ventricle hypertrophy [7].

According to V.V. Povoroznyuk et al. [8], in a significant percentage of patients with CHF, not only vitamin D deficiency is observed, but also a distorted state of peripheral blood erythrocytes. Patients with vitamin D deficiency are more likely to develop CHF, exceeding the rates of the disease almost twice as compared with patients with sufficient levels of this vitamin in the blood [8]. However, data on morphological changes in PBE depending on vitamin D deficiency in patients with CHF are limited in the scientific literature [9].

The aim of the work is to evaluate and study the effect of the level of vitamin D in blood plasma on the morphofunctional state of PBE in patients with chronic heart failure. Materials and Methods The study included 25 patients (10 men and 15 women), residents of Ivano-Frankivsk aged 63.7±7.8 years, who were included in the main group (MG). All were diagnosed with CHF IIA (according to Vasilenko-Strazhesko), functional class (FC) I-II, which developed against the background of coronary heart disease (in 62.3% of patients) and / or arterial hypertension (AH) (in 37.7% patients).). The control group (CG) included 25 practically healthy individuals (11 men and 14 women) with an average age of 62.5±6.2 years. Exclusion criteria: chronic obstructive pulmonary disease, bronchial asthma, taking hormonal drugs, diabetes mellitus, oncological diseases.

To confirm the FC of CHF in accordance with the NYHA [10] and the national recommendations of Ukraine [11], all patients were offered to perform physical exercises (PE) in the form of a 6-minute walk test [12]. During the test, the patient had to walk the maximum distance at a comfortable pace in 6 minutes along a corridor marked every meter.

The distance covered by the patient in 6 minutes is > 551 m with CHF FC 0, 426-550 m with CHF FC I, 301-425 m with CHF FC II, 151-300 m with CHF FC III and <150 m with CHF IV FC. At the time of inclusion in the study, all patients received complex pathogenetic therapy for coronary artery disease according to E.S. Atroshchenko et al. recommendations, [13] and national recommendations and the standard of the Ministry of Health of Ukraine on the secondary prevention of vitamin D deficiency [14].

We conducted a survey on the exogenous intake of vitamin D with dietary products rich in vitamin D (cod liver oil, omega-3 fatty acids, fish oil), as well as with vitamin D preparations. The level of total vitamin D in blood plasma was assessed by enzyme immunoassay with determination of the volume of total 25-hydroxycholecalciferol ($25(OH)D_{tot} = 25(OH)D_3 + 25(OH)D_2$). The volume of 25(OH)D in blood plasma > 30 ng/mL was regarded as optimal, 20–29 ng/mL as deficiency, less than 20 ng/mL as deficiency, and less than 10 ng/mL as severe deficiency [15]. ,16]. Blood sampling for $25(OH)D_2$ in the blood plasma was carried out in all examined patients in the period from October to December.

The hemoglobin level and hematocrit were determined by conventional methods [42]. To calculate the total number of PBE, blood smears were analyzed using a Leica CME microscope with subsequent digitalization of the image (SonyExwaveHad SSC-DC58AP camera). PBE were stained according to the original method for clearer display and better measurement quality (Karle, 2009). Within 10 minutes, blood smears were fixed in formalin vapor, treated with 1-butanol for 2 minutes, then immersed in a 0.01% solution of bromothymol blue mixed with ethyl acetate (5:4:1) for 10 minutes, and after a second wash 1 -butanol, dried for 2 minutes for examination under a microscope. Counting was performed on 500 cells.

Photomicrography was done with the help of OlympusCamediaC-480 ZOOM digital camera (Olympuscorp., Japan). The images were analyzed using NIHimage (Macintosh) and ScionImage (PC) packs. Morphological studies of PBE microrelief were performed on the JEOL-25M-T220A scanning electronic microscope (Japan) with generally accepted methods with determining of trace element composition of PBE [17,18]. We measured the maximal and minimal diameter of PBE, the length of their circumference, determining the percentage of their morphotypes before and after the 6 Minute Walk Test or physical activity.

Statistical processing of the results was performed using the STATISTICA 10.0 package (SN AXAR207F394425FA-Q). Data are presented as mean and standard deviation (M±SD), with a non-normal distribution, as median (Me) and interquartile range [LQ-UQ]. Correlation analysis with Spearman's rank correlation coefficient (R) was used to assess the correlation between variables. Differences were considered significant at $p < 0.05$.

Research results. The results of the survey showed that not a single person from the MG, as well as from the CG, consumes foods rich in vitamin D, and also does not take vitamin D supplements regularly.The average level of 25(OH)D in blood plasma in patients with MG was 17.2 (10.8; 23.6) ng/ml and was 2.2 times lower ($p = 0.01$) than in CG patients (38.4 (27.9)). ; 48.8)) ng/ml, which was regarded as vitamin D deficiency.

The results of the examination showed that before the 6-minute walk test, in all patients with OH, the indicators of erythrocytes, hemoglobin and hematocrit were within the limits of physiological values with a moderately increased number of reticulocytes in the blood compared to CG. (Table 1).

Table 1 Changes in values of red blood cells in the patients of the study and control groups before and after the 6 Minute Walk Test (M±m)

Values	Study group (n=25)		Control group (n=25)	
	Before PE	After PE	Before PE	After PE
Erythrocytes, 1012/L	3.9±0.11	4.2±0.16 *#	4.2±0.22	4.9±0.23
Hemoglobin, g/L	144.8±2.64	128.2±2.52#	146.0±4.14	146.2±2.93
Hematocrit, %	44.1±2.83	46.5±1.87#	44.89±3.95	46.76±2.42#
Mean corpuscular volume, fL	88.7±5.01	87.1±4.53#	88.7±2.43	92.9±2.93*#
Red blood cell distribution width-CV, %	11.2±1.36	12.7±1.19#	7.4±1.02*	8.8±1.32 *#
Reticulocytes, %	1.3±0.39	1.8±0.39 #	1.0±0.32*	1.0±0.33 *

Note: CV is the coefficient of variation; FE is the physical exercise; * – P<0.05 compared to the control group; # – P<0.05 compared to observation data before the PE.

Morphological examination revealed an increased content of PBE with altered surface topography in almost all examined patients with MG.

Under physiological conditions, the allowable volume of irreversibly altered cells should not exceed 8.0%, and the volume of irreversibly altered erythrocytes should not exceed 1.0% of their total number [49].

In most patients with MG, in addition to normocytes in the peripheral blood (Fig. 1), subpopulations of macrocytes and microcytes clitin were recorded with a reduced level of hemoglobin (see Table 1).

In 87% of patients with OH, there were reversibly and irreversibly altered forms of PBE, the number of which exceeded the limit of the physiological norm.

These forms of PBE are individually characteristic only for each patient and do not have statistically significant group characteristics (P>0.05). Thus, we observed echinocytes with a tiny erosion of the outer membrane (Fig. 2), and in some patients, peripheral blood erythrocytes were presented as acanthocytes, stomatocytes (Fig. 3) and echinocytes (Fig. 4).

Mixed structural forms of peripheral blood erythrocytes indicate a high risk of intravascular hemolysis, especially in patients with MG with reversibly altered forms, and the development of a pathological condition, which in medicine is called anemia during PE.

After the 6-minute walk test, 43.0% of patients with PE had an increase in the number of erythrocytes, and in 57.0% of patients this number decreased.

At the same time, we did not observe statistically significant changes in the level of hemoglobin and hematocrit parameters, when microscopy of blood smears in patients with a reduced number of PBE revealed their lysis (Fig. 5).

Under physiological conditions, age and sex characteristics [18] of peripheral blood erythrocytes affect their differentiation in size, structure, metabolism, and other physicochemical properties determined by them.

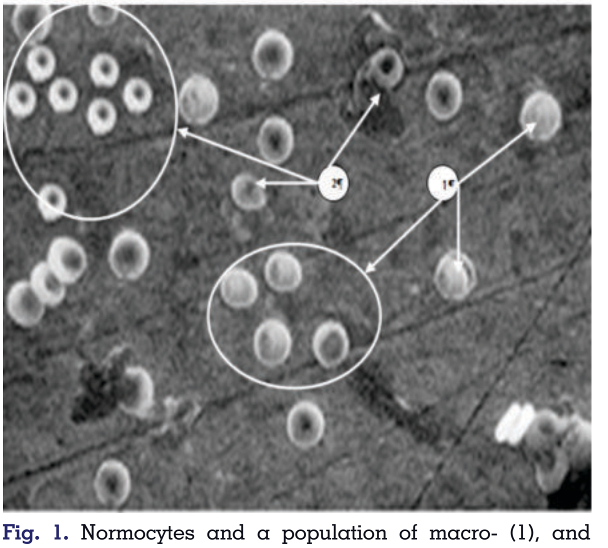

Fig. 1. Normocytes and a population of macro- (1), and microcytes (2) in the blood of the patients with chronic heart failure and vitamin D deficiency. Method: scanning electronic microscopy. Bar. (magnification) : x1350.

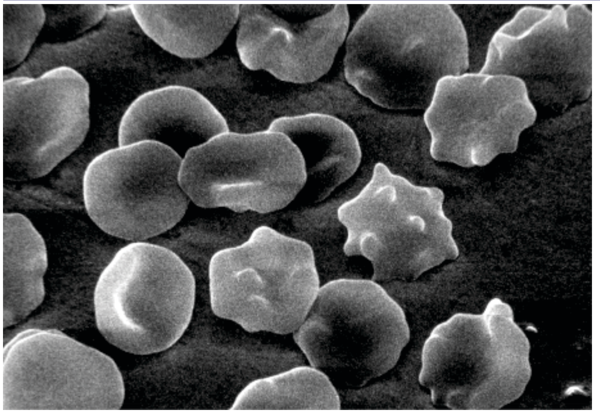

Fig. 2. Echinocytes (pointed by arrows) in the blood of patients with chronic heart failure and vitamin D deficiency. Method: scanning electronic microscopy, b) coloring according to Romanovsky. Mag.: a) x5000, b) x1350.

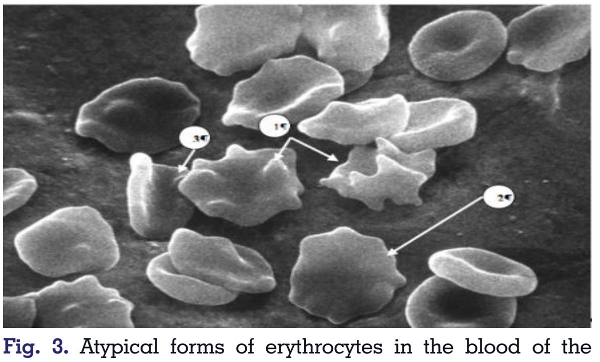

Fig. 3. Atypical forms of erythrocytes in the blood of the patients with chronic heart failure and vitamin D deficiency: echinocytes (1), acanthocytes (2), and stomatocytes (3). Method: a) scanning electronic microscopy,Bar.: x 5000.

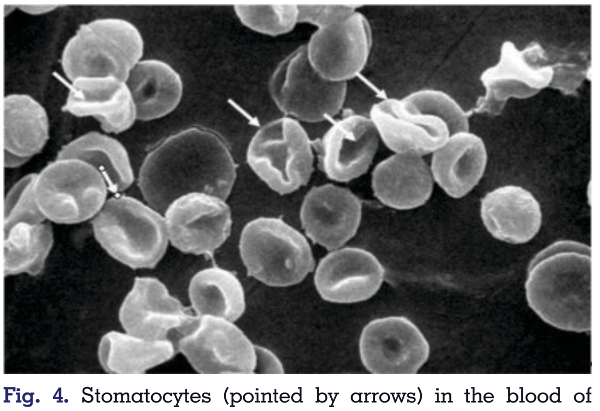

Fig. 4. Stomatocytes (pointed by arrows) in the blood of patients with chronic heart failure and vitamin D deficiency. Method: scanning electronic microscopy, Bar.: x 3000.

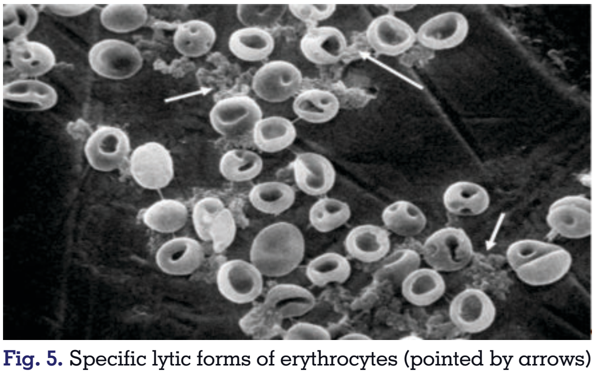

Fig. 5. Specific lytic forms of erythrocytes (pointed by arrows) in a blood smear in the blood of the patients with chronic heart failure and vitamin D deficiency. Method: scanning electronic microscopy, Bar.: x 1500.

DISCUSSION

Recent scientific studies have found that vitamin D deficiency can be a factor influencing the course of a number of diseases [19,20,21,22]. Previous studies of the adaptive potential of patients with various cardiovascular diseases (CVD) showed that they have a lower level of functional resources of the body with vitamin D deficiency [23]. At the same time, the fact is known that the adaptive potential of the human and animal organism strongly depends on the state of oxygen transport [18]. This requires closer attention of scientists from a number of specialties to the morphofunctional state of PBE. At the same time, a clear relationship was found between the severity of vitamin D deficiency in patients with different levels of tolerance to PE during the 6-minute walk test, which indicates that the proposed gradation of morphofunctional changes in PBE can be used as a criterion for determining the volume of PE . This is consistent with the same criteria established by other authors [24].

(PE of different intensity plays an important role in the formation of the overall endurance of the body, which is manifested in a number of human activities [25]. It determines the overall level of physical performance of the human body. Efficiency, as a multifunctional property of the human body, integrates a large number of processes occurring at several levels: from the cellular to the general organism [26]. But, the results of modern research have shown that in most cases the main role in endurance studies belongs to the determining factors that contribute to the activation of energy metabolism and autonomic systems for its provision, the cardiovascular, pulmonary and central nervous systems. At the same time, studies of the cellular response to physical activity, without special attention, to peripheral blood erythrocytes in the process of adaptation of patients with coronary artery disease and vitamin D deficiency were carried out [26,27].

PBE as a convenient object for such studies, since they are involved in the processes associated with homeostasis at the level of the whole organism [28,29,30]. In addition to their specific gas transport function, these cells can participate in the regulation of water-electrolyte and acid-base balance, blood microrheological status, immune reactions, binding and transport of amino acids and lipids, which is of direct interest. on the development of general body endurance in patients with coronary artery disease and vitamin D deficiency [31,32,33,34,35].

A statistically significant increase in hematocrit occurs in 5.0%, as well as hemoglobin in 2.7% as a result of PE. But PE with a general increased number of erythrocytes due to hemoconcentration causes a decrease in peripheral blood erythrocytes in some patients. This may be due to the fact that erythrocytes are destructively affected by factors that occur during muscle activity: increased blood circulation, elevated blood temperature, acidosis or alkalosis, etc. A single PE causes an increased number of larger PBE in approximately 93.0% of cases ($p<0.05$). The number and volume of PBE determine the rheological features of the blood. Changes in these indicators during exercise can significantly affect the oxygen transport function of the blood and cause microcirculation disorders, thereby changing the level of functional state in patients with CHF [36,37].

This adaptive mechanism during PE in patients with MG reveals an increased aggregation ability of erythrocytes (the volume of aggregates increased by 15.6%) and an increased coefficient of deformation (by 17.9%), which is manifested due to muscle activity and can contribute to an increase in the viscosity of circulating blood. . Such changes in the values of peripheral blood erythrocytes are unfavorable for the oxygen transport function of the blood and indicate insufficient adaptation of patients with MG to physical activity [38].

It is known that PBE have a phenomenal feature of accumulating Ca^{+2} salts with a decrease in its concentration in blood plasma (calcium paradox), unlike other cells (including cardiomyocytes), which experience their deficiency under such conditions [39]. An increased intracellular concentration of Ca^{+2} itself plays a major role in ATP deficiency, which reduces the deformability of PBE [39,40].

In general, significant conformational changes in PBE cause a decrease in the surface of their membrane upon contact not with its entire surface, but only with a small surface at the tops of the protrusions. Then we begin to understand the full depth of the distortion of transmembrane transport of vital substances at the level of metabolism in the hemo microcirculatory network with vitamin D deficiency. dysmetabolic disorders associated with vitamin D deficiency. Under such conditions, a violation of the structural integrity of PBE, in turn, contributes to their intravascular lysis and can cause anemia of PE [29].

CONCLUSIONS

1. A deeper study of the mechanisms of the influence of stressful physical exercises on the state of the blood oxygen transport system, the organization of hematological control at several stages of IHD therapy, as well as during corrective and restorative measures aimed at eliminating deficiencies. resulting from vitamin D deficiency is an urgent task and requires scientific justification of appropriate preventive measures to solve it.

2. Morphological examination reveals heterogeneity of erythrocyte cell subpopulations in almost all patients with coronary artery disease and vitamin D deficiency. In peripheral blood, in addition to normocytes with high hemoglobinization, subpopulations of macro- and microcytes were recorded. In most patients with vitamin D deficiency, several morphological changes in the shape of erythrocytes were revealed, which manifest themselves in the form of echino-, acantho- and stomatocytosis, indicating instability of the erythrocyte membrane. 3. Under the influence of the 6-minute walk test in IHD patients with vitamin D deficiency, the number of erythrocytes decreased, their deformability increased, lytic cells were recorded with a reduced number of normocytes. 4. The identified morphobiochemical changes indicate a violation of the structural integrity of the components of the erythrocyte membrane and have negative consequences, taking into account the likely impact on the somatic health of patients who, in addition to changes characteristic of CHF, receive significant stress during exercise under conditions of vitamin D deficiency.

Conflict of Interest: The authors have no conflict of interest to declare.

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