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The Role of Na^+/K^+-ATPase in the Development of Hyponatrenemia under Conditions of Hypoxic Stress in Patients with SARS-CoV-2 Infection

S. H. Jafarova[1], S. A. Adnaev[2], R. T. Guliyeva[1], and N. H. Jafar[2]

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We studied laboratory parameters of patients with COVID-19 against the background of chronic pathologies (cardiovascular pathologies, obesity, type 2 diabetes melitus, and cardiovascular pathologies with allergy to statins). A decrease in pH and a shift in the electrolyte balance of blood plasma were revealed in all studied groups and were most pronounced in patients with cardiovascular pathologies with allergy to statin. It was found that low pH promotes destruction of lipid components of the erythrocyte membranes in patients with chronic pathologies, which was seen from a decrease in Na^+/K^+-ATPase activity and significant hyponatrenemia. In patients with cardiovascular pathologies and allergy to statins, erythrocyte membranes were most sensitive to a decrease in pH, while erythrocyte membranes of obese patients showed the greatest resistance to low pH and oxidative stress.

Key Words: *erythrocyte membranes; lipid peroxidation; Na^+/K^+-ATPase; COVID-19; hypoxia*

For successful treatment and timely intervention, great importance is attached to the results of laboratory tests that allow predicting the course of the disease. For assessing the severity of the condition in patients with COVID-19, lactate dehydrogenase (LDH), D-dimer, C-reactive protein (CRP), *etc*. in blood plasma are used as the main criteria [10,12]. However, these indicators, which are nonspecific for infectious (including viral) diseases and just demonstrate the intensity of oxidative-destructive processes developing due to the presence of serious chronic pathologies such as diabetes, obesity, cardiovascular diseases with allergic complications [4,7-9]. It is known that CRP is involved in the development of oxidative stress in the body in various pathologies, leading to LPO activation in tissues [3,9]. One of the main LPO products, diene conjugates accumulating in blood vessels in cardiovascular pathologies stimulate the formation and accumulation of D-dimer [8]. Increased blood level of LDH attests to intensification of destructive processes in tissues under hypoxic conditions; it is not specific for SARS-CoV-2 infection, and occurs in advanced diabetes, vascular pathologies, and allergic conditions [10,12]. The main symptom of COVID-19, reduced blood oxygen level, is present in all patients with SARS-CoV-2 infection [12]. Hypoxic stress accompanying viral infections impairs oxygen-transport function of erythrocytes, which can contribute to a decrease in oxygen saturation and tissue malnutrition [1].

Our aim was to identify laboratory parameters reflecting the development of hypoxia in patients with COVID-19 against the background of cardiovascular pathologies, diabetes, obesity, and allergic conditions

[1]Laboratory of Ecological Biophysics, Institute of Biophysics of Azerbaijan National Academy of Sciences, Baku, Azerbaijan; MediClub Hospital, Baku, Azerbaijan. ***Address for correspondence:*** jafsa68@gmail.com. S. H. Jafarova

and to evaluate the influence of these pathologies on the resistance of erythrocytes to acidification of the environment.

MATERIALS AND METHODS

Two consecutive series of studies involving patients of the MediClub medical service company clinic were carried out. All patients (mean age 61±7 years) signed informed consent form for participation in the studies. For series I, 43 patients with confirmed PCR analysis for COVID-19 and with severe and moderate course of the disease were divided into 5 groups: control (no chronic pathologies; *n*=8; group 1), patients with cardiovascular pathologies (CVP; *n*=12; group 2), patients with obesity (*n*=8; group 3), patients with type 2 diabetes mlitus (DM2; *n*=10, group 4), and patients with CVP+allergy to statins (*n*=5; group 5). Laboratory tests were performed using a Cobas 411 biochemical analyzer (Roche) and a Sysmex XT-2000i hematological analyzer (Sysmex). All patients received treatment according to WHO protocols. In series II, 38 COVID-19 patients with chronic diseases outpatiently examined in the MediClub clinic were divided according to the same principle into 5 groups: control (*n*=7), CVP (*n*=9), obesity (*n*=8), DM2 (*n*=9), CVP+allergy (*n*=5).

The material for the study was plasma and erythrocyte membranes.Washed erythrocytes isolated from venous blood were incubated for 30 min at 37°C in 0.15 M sodium phosphate buffer with a pH range from 6.9 to 7.5. Erythrocyte ghosts were obtained as described elsewhere [5]. $LnCl_3$ was added to the hemolysate of isolated erythrocytes to precipitate membranes to a final concentration of 0.8-1.2 mM. Activity of Na^+/K^+-ATPase was measured in a suspension of erythrocyte ghosts as described previously [6]. Changes in ATPase activity were assessed by accumulation of inorganic phosphorus (Pi) in the incubation medium. Na^+/K^+-ATPase activity was expressed in μmol/Pi/mg protein. LPO intensity was assessed by changes in malondıaldehyde (MDA) content [11]. Protein content was measured spectrophotometrically at 260-280 nm on an SF-46 spectrophotometer (LOMO).

Statistical processing of the results was carried out using the Student's *t* test (Microsoft Excel 2017). The results of laboratory tests were presented as Me (Q1; Q3), Na^+/K^+-ATPase activity and content of LPO products were presented as *M*±*m*. The differences were significant at $p<0.05$.

RESULTS

The data of clinical laboratory tests showed a 1.5-3.0-fold increase in plasma LDH activity during the first week in patients with CVP and DM2 in comparison with the control (COVID-19 patients without chronic diseases) (Table 1). After 15 days of treatment, LDH values in all groups, except group 5, significantly decreased and were below the levels observed at admission.

Plasma D-dimer concentration increased during the first week in all groups, except the group of obese patients, and 10-fold surpassed the control by the end of this period. By the end of the second week, D-dimer values in groups 2 and 5 were still high. Lactate level by the end of the first week was increased by 2-7 times in all groups; this increase was most pronounced in patients of CVP and allergy to statins (by 8 times). Plasma level of CRP increased during the first 2 weeks of the disease; significant fluctuations of this parameter were found in patients with CVP and allergy to statins (by 23 times). The increase in CRP levels was associated with an increase in plasma 8-isoprostane, a product of peroxidation of arachidonic acid, a constituent of phosphatidylserine, an annular lipid for Na^+/K^+-ATPase in erythrocyte membranes, which affects the activity of the enzyme and its transport function [2,3,9]. Over the 2 weeks of observation, the content of potassium and sodium ions in the plasma of patients of all test groups deviated significantly from the normal (3-7%) (Table 1). Changes of sodium and potassium inflow into the cell in various diseases, including viral ones, can be either as a result of damage to the cell membrane or inhibition of Na^+/K^+-ATPase [1,2,13]. Our findings attested to a direct relationship between the increase in hypoxia (decrease in saturation) and significant fluctuations in the content of electrolytes in the blood plasma (hyponatrenia and hyperkalemia) in patients with COVID-19. At the same time, plasma pH in all groups was shifted to the acidic values and reached by the end of the second week values close to 7.0 in groups 2 and 5. Analysis of the above data indicates a possible dependence of the resistance of erythrocyte membranes and Na^+/K^+-ATPase on LPO intensity and changes in plasma pH during hypoxic stress in COVID-19 patients and various pathologies.

In model experiments, we studied the resistance of Na^+/K^+-ATPase of erythrocytes isolated from COVID-19 patients with various pathologies (series I) to changes in medium pH and oxidative stress. Changes in the activity of Na^+/K^+-ATPase induced by medium acidification were most pronounced in erythrocytes of group 5 patients (CVP+allergy to statins): in comparison with the control group at pH 7.4, enzyme activity at pH 6.9 in this group decreased by 35% (Fig. 1). In other experimental groups, enzyme activity was also changed upon pH shift to acidic values (in groups 2 and 4 it decreased by 31 and 28%,

TABLE 1. Laboratory Findings in COVID-19 Patients (Me (Q1-Q3))

	Group	LDH3, U/liter	CRB, mg/liter)	Na^+, mmol/liter	K^+, mmol/liter	D-dimer, mg/liter	SO_2, %	Lactate, mmol/liter	pH
				At admission to hospital					
1	Control	211 (110-305)	14,03 (4.26-27.47)	138 (136-145)	4.2 (3.4-5.1)	0.30 (0.18-0.45)	97 (94-100)	0.75 (0.54-1.50)	7.44 (7.34-7.46)
2	CVP	375 (277-412)	47.01 (36.69-58.46)	133 (130-138)	3.7 (3.2-4.9)	0.39 (0.20-0.57)	84 (69-88)	1.40 (0.57-1.74)	7.43 (7.33-7.44)
3	Obesity	215 (185-345)	8.33 (7.30-21.42)	134 (131-137)	3.5 (2.7-5.7)	0.22 (0.20-0.44)	75 (73-78)	1.10 (1.04-1.53)	7.15 (7.09-7.19)
4	DM2	265 (221-411)	41.86 (31.28-58.59)	135 (133-138)	4.3 (4.0-5.6)	0.45 (0.20-0.60)	85 (69-89)	1.50 (0.80-0.90)	7.39 (7.31-7.45)
5	CVP+allergy	543 (289-611)	51.77 (31.10-73.63)	133 (130-135)	3.4 (3.2-4.1)	0.70 (0.40-1.20)	76 (58-84)	1.20 (0.40-1.60)	7.54 (7.47-7.56)
				On days 5-7 of therapy					
1	Control	377 (248-510)	69.39 (48.05-106.62)	135.8 (136-144)	3.8 (3.5-5.1)	0.34 (0.28-0.60)	96 (83-97)	1.39 (1.10-2,03)	7.33 (7.26-7.42)
2	CVP	570 (418-801)	128.46 (67.14-139.54)	130 (129-134)	5.6 (4.4-6.2)	5.90 (1.67-6.90)	72 (59-89)	5.60 (1,07-6,50)	6.96 (6.86-7.09)
3	Obesity	724 (685-877)	79.00 (49.14-86.22)	133 (129-141)	3.2 (3.0-3.7)	0.59 (0.40-0.70)	92 (54-97)	1.47 (1,31-1,68)	7.34 (7.15-7.46)
4	DM2	401 (341-688)	34.24 (21.62-78.66)	131 (122-132)	5.4 (3.8-5.7)	4.73 (2.90-5.60)	85 (58-97)	3.80 (1,80-4,20)	7.14 (6.99-7.21)
5	CVP+allergy	738 (679-776)	148.10 (137.18-153.23)	130 (128-133)	4.9 (3.8-5.6)	5.10 (3.10-5.90)	87 (79-91)	4.20 (2,70-4,90)	7.10 (6.91-7.27)
				On days 10-15 of therapy					
1	Control	133 (128-185)	10,62 (9.20-48.30)	141 (136-145)	4.2 (3.4-4.7)	0.28 (0.11-0.38)	97 (96-100)	0.56 (0.45-1.05)	7.41 (7.31-7.42)
2	CVP	370 (301-479)	16.22 (8.11-18.74)	132 (129-138)	5.2 (4.2-5.8)	3.10 (2.80-3.90)	80 (64-93)	1.72 (0.59-2.80)	7.22 (7.14-7.30)
3	Obesity	275.2 (239-408)	20,80 (8,93-46,52)	136 (127-139)	3.1 (2.8-3.5)	1.20 (1.00-2.00)	91 (78-98)	1.07 (0.51-1.43)	7.41 (7.33-7.48)
4	DM2	307 (287-457)	11.60 (8.91-12.10)	136 (130-137)	3.7 (2.8-4.1)	0.90 (0.45-1.40)	94 (91-98)	1.20 (0,58-1.50)	7.33 (7.32-7.40)
5	CVP+allergy	702 (342-769)	35.20 (16.87-40.50)	129 (126-132)	5.1 (4.6-5.7)	3.70 (0.80-4.12)	67 (64-81)	8.00 (2.70-9.37)	7.01 (6.89-7.14)

Note. All differences from the control are significant (p<0.05).

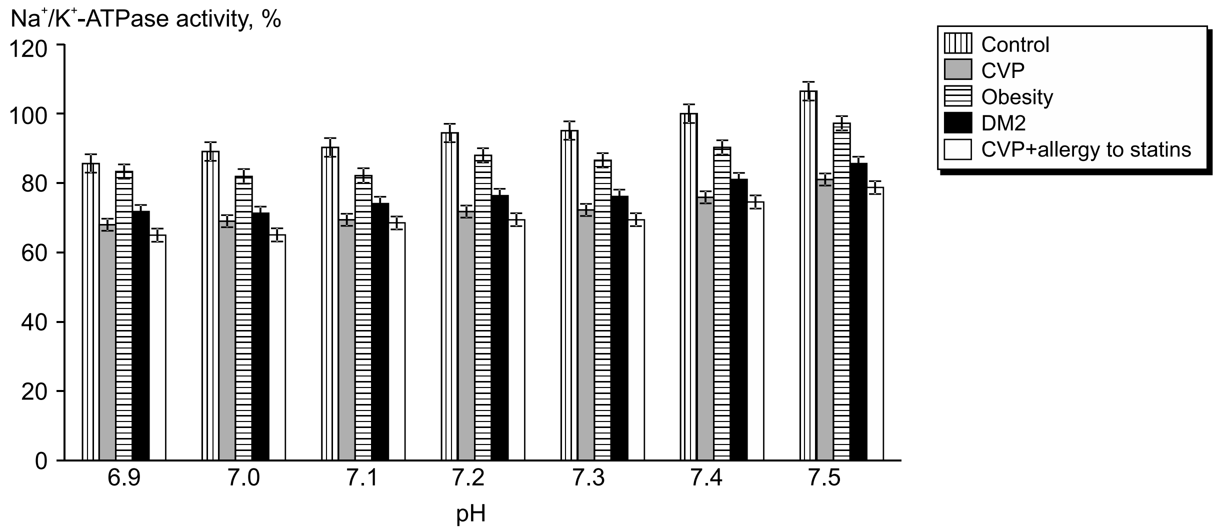

Fig. 1. Effect of different pH of the incubation medium on activity of Na^+/K^+-ATPase in erythrocytes from COVID-19 patients with chronic pathologies. Here and in Fig. 2: all differences from the control are significant at p<0.05.

respectively). Erythrocytes from obese patients (group 3) were most resistant to medium acidification: enzyme activity in this group decreased by 17% compared to the control at pH 7.4 (Fig. 2). At pH 6.9, the level of MDA in erythrocytes of this group increased by 1.6 times in comparison with the control group at pH 7.4. The destructive processes in erythrocyte membrane lipids caused by the decrease in medium pH were most intensive in groups 2 (CVP) and 4 (DM2), and especially in group 5 (CVP+statin allergy). In particular, MDA content at pH 6.9 in groups 2, 4, and 5 increased by 2.0, 1.9, and 2.3 times, respectively, in comparison with the control at pH 7.4.

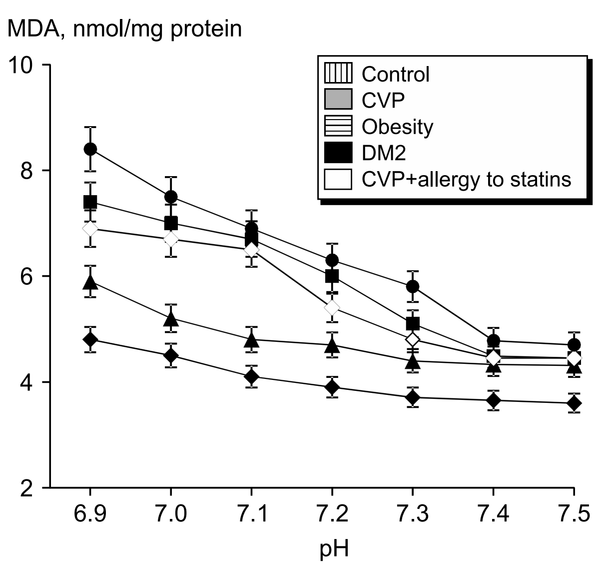

Fig. 2. Effect of different pH of the incubation medium on the content of LPO products in membranes of erythrocytes from COVID-19 patients with chronic pathologies.

Hypoxia leads to significant metabolic disorders in tissues, including accumulation of LPO products, lactate, and a decrease in medium pH. The substrate dependence of Na^+/K^+-ATPase changes under the influence of ROS and at different pH values, and leads to the loss of the ability of the enzyme protomers to interact with each other, affecting its activity. Hence, the observed decrease in Na^+/K^+-ATPase activity and intensification of LPO in erythrocytes of the test groups after 30-min incubation in phosphate buffer at low pH attested to a destructive effect of these factors on activity of the enzyme, and, as a result, transport of sodium ions. This was most pronounced in patients with vascular pathologies (groups 2 and 5). Despite taking measures to maintain electrolyte homeostasis in patients with moderate and severe complications of SARS-CoV-2 infection, a pronounced decrease in the plasma Na^+ level was observed in these patients. We believe that this is primarily due to impaired Na^+ transport across the erythrocyte membrane as a result of inhibition of Na^+/K^+-ATPase. The most significant impairments of the acid-base balance and electrolyte balance during coronavirus infection were observed in patients with allergies to statins against the background of CVP. In model experiments, analysis of the resistance of isolated erythrocytes to pH changes also revealed the lowest activity of Na^+/K^+-ATPase and higher levels of MDA in CVP patients with allergies. These observations showed that exacerbation of allergic reactions against the background of viral infections, intensification of LPO in erythrocytes leads to serious disturbances in Na^+/K^+-ATPase activity.

Our findings can be used to assess the severity of the condition of patients with COVID-19 and, if necessary, to change the treatment tactics.

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