The Role of Na⁺/K⁺-ATPase in the Development of Hyponatremia under Conditions of Hypoxic Stress in Patients with SARS-CoV-2 Infection
S. H. Jafarova¹, S. A. Adnaev², R. T. Guliyeva¹, and N. H. Jafar²

Translated from Byulleten’ Eksperimental’noi Biologii i Meditsiny, Vol. 172, No. 9, pp. 268-272, September, 2021
Original article submitted May 11, 2021

We studied laboratory parameters of patients with COVID-19 against the background of chronic pathologies (cardiovascular pathologies, obesity, type 2 diabetes mellitus, 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 hyponatremia. 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
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 miltitus (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 from hemolysate of isolated erythrocytes to precipitate membranes to a final concentration of 0.8-1.2 mM. LnCl₃ 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 erythrocyte ghosts as described previously [6]. 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 analog of leukotriene, and 8-isoprostane, a product of peroxidation of arachidonic acid, a constituent of phosphatidylserine, an annular analog of leukotriene.

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       | LDH, U/liter     | CRB, mg/liter     | Na+, mmol/liter | K+, mmol/liter | D-dimer, mg/liter | SO2, % | 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) | 7.45 (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.

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.
REFERENCES

1. Bakanov MI, Vasilieva EM, Elagina IA, Zubkova IV, Lozovskaya LS, Matkovskaya TA. Biochemical Modifications in the Erythrocytes due to Interaction with Virus Koksaki A18. The Influence of the L-arginin and the Antiviral Preparation – Phosphonphormiate. Vestn. Nov. Med. Tekhnol. 2005;12(2):10-12. Russian.

2. Boldyrev AA. Function of Na/K-pump in Excitable Tissues (Review). Zh. Sib. Fed. Univer. Ser. Biol. 2008;1(3):206-225. Russian.

3. Vel’kov VV. C-reactive protein — structure, function, methods of detection, clinical value. Lab. Med. 2006;(8):1-7. Russian.

4. Konoshenko SV, Yolkina NM, Kazakova VV, Zagnoenko NE, Kucharik ON, Martojan MM. Indexes of destructive processes in erythrocytes under allergy. Uchenye Zapiski Krym. Fed. Univer. Biol. Khim. 2019;5(1):67-73. Russian.

5. Sheremet’ev IA, Uspenskij AN, Sheremet’eva AV, Smirnov VN, Shevchenko EA, Smirnova DV. Patent RU No. 2309754. Method for production of erythrocyte membranes. Bull. No. 31. Published November 10, 2007.

6. Petrova PA. The comparative analysis of the activity assay methods for Mg²⁺-dependent Na’/K’-activated ATPase in erythrocyte membranes. V Mire Nauch. Otkryt. 2017;9(4-2):150-166. doi: 10.12731/wsd-2017-4-2-150-166. Russian.

7. Abe H, Semba H, Takeda N. The roles of hypoxia signaling in the pathogenesis of cardiovascular diseases. J. Atheroscler. Thromb. 2017;24(9):884-894. doi: 10.5551/jat.RV17009

8. Bhatia S, Jenner AM, Li H, Ruberu K, Spiro AS, Shepherd CE, Kriel JJ, Kain N, Don A, Garner B. Increased apolipoprotein D dimer formation in Alzheimer’s disease hippocampus is associated with lipid conjugated diene levels. J. Alzheimers Dis. 2013;35(3):475-486. doi: 10.3233/JAD-122278

9. Cottone S, Mulè G, Nardi E, Vadalà A, Guarnieri M, Brilotta C, Arseno R, Palermo A, Riccobene R, Cerasola G. Relation of C-reactive protein to oxidative stress and to endothelial activation in essential hypertension. Am. J. Hypertens. 2006;19(3):313-318. doi: 10.1016/j.amjhyper.2005.09.005

10. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Pleban M, Lippi G. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: a pooled analysis. Am. J. Emerg. Med. 2020;38(9):1722-1726. doi: 10.1016/j.ajem.2020.05.073

11. Huseynov TM, Guliyeva RT. Oxidative resistance and peroxidase activity of hemoglobin of G-6-PhD deficient erythrocytes under the action of high tension electric fields. J. Kafqaz University. Physics. 2015;3(1):23-28.

12. Lagadinou M, Salomou EE, Zareifopoulos N, Marangos M, Gogos C, Velissaris D. Prognosis of COVID-19: Changes in laboratory parameters. Infez. Med. 2020;28(Suppl. 1):89-95.

13. Sitprija V. Altered fluid, electrolyte and mineral status in tropical disease, with an emphasis on malaria and leptospirosis. Nat. Clin. Pract. Nephrol. 2008;4(2):91-101. doi: 10.1038/ncpneph0695