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Hemoglobin-Oxygen Affinity and Gaseous Transmitters (Nitric Oxide, Hydrogen Sulfide) in Patients with COVID-19

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Abstract

Background: Although the approaches to management of Coronavirus Disease-2019 (COVID-19) have been improved, there is no clear understanding of the essence of this disease characterized by pronounced signs of hypoxic respiratory failure.

Objectives: The purpose of this study was to investigate hemoglobin-oxygen affinity and the gaseous transmitters Nitric Oxide (NO) and Hydrogen Sulfide (H_2S) in patients with COVID-19.

Methods: The object of the study was patients with COVID-19 whose major parameters of blood oxygen transport (hemoglobin-oxygen affinity) and the gaseous transmitters Nitric Oxide (NO) and Hydrogen Sulfide (H_2S) were determined.

Results: As compared to healthy individuals, the patients with COVID-19 showed decreased SO_2 and pCO_2 and elevated pH of the blood. We found an increase in the values of $p50_{st}$ and $p50_{act}$ by 14% and 6.4%, respectively ($p<0.05$), and the corresponding shift in the oxyhemoglobin dissociation curve rightwards, which was apparently a response for tissue hypoxia developed due to pulmonary circulation insufficiency. The above patients demonstrated an elevation in the nitrate/nitrite content and a reduced concentration of blood plasma hydrogen sulfide.

Conclusion: The alterations found in blood oxygen-binding properties resulted from the changes in the levels of the gaseous transmitters NO and H_2S, which is essential in the pathogenesis of hypoxia developed under this pathology.

Keywords: Coronavirus disease-2019; Hemoglobin-oxygen; Nitric oxide; Hydrogen sulfide

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Introduction

A great interest in studies on the pathogenesis of the new Coronavirus Disease-2019 (COVID-19) has recently become apparent. Although approaches to management of COVID-19 change with accumulation of new information, there is yet no clear understanding of the essence of this disease [1]. It is characterized by a significant impairment in oxygen supply to the body. The clinical characteristics of COVID-19 include pronounced signs of hypoxic respiratory failure: Patients experience a reduction of arterial blood saturation (less than 93%) [2]. Disturbances in blood oxygen transport, observed in the course of the disease, can also be important. Changed Hemoglobin Oxygen Affinity (HOA), which is a pivotal factor in compensation for hypoxic respiratory failure under various pathologic states, underlies the processes of adaptation to hypoxia [3]. However, at present, there is no clear insight into the state of blood oxygen transport, particularly in relation to severity of COVID-19, and the few available works dealing with the problem are contradictory [4,5]. In this connection, this study was aimed to investigate HOA and the gaseous transmitters Nitric Oxide (NO) and Hydrogen Sulfide (H_2S) in patients with COVID-19.

Material and Methods

Subjects

The object of research was patients with COVID-19. The group included 15 subjects who were diagnosed with COVID-19 by verification of the virus using qualitative determination of RNA SARS-CoV-2 in scrapings of pharyngo-oral cells by means of the polymerase chain reaction as well as on the basis of the typical clinical status and the changes in the lungs detected by X-ray

Computed Tomography (CT) of thoracic organs. The severity of viral pneumonia was determined according to the regional classification of COVID-19 diagnostics and treatment where the absence of signs of viral pneumonia was designated as CT-0, whereas mild pneumonia with areas of ground-glass opacity and lung injury of less than 25% was designated as CT-1, moderate pneumonia with lung injury of 25% to 50% was designated as CT-2, mild to severe pneumonia with lung injury of 50% to 75% was designated as CT-3 and severe pneumonia with more that 75% lung injury was designated as CT-4. These changes corresponded to the classification of the Dutch Association for Radiology, CO-RADS 4-5. The group of patients included 6 men and 9 women of the mean age of 58.0 years. The group of healthy individuals comprised 15 apparently healthy subjects aged 54 years.

Blood oxygen transport (hemoglobin-oxygen affinity)

Blood was drawn from the cubital vein after blood flow recovery on admission of patients to hospital. The blood was assayed for partial Oxygen Pressure (pO_2) and Carbon Dioxide (pCO_2), pH and blood Oxygen Saturation (SO_2) with a Star Profile pHOx plus L gas analyzer (Nova Biomedical, USA). The Siggaard-Andersen nomograms were used to calculate the acid-base parameters: Standard Bicarbonate (SBC), Actual Base Excess/Standard Base Excess (ABE/SBE), Hydrocarbonate (HCO_3-) and the total blood plasma Carbonic Acid (TCO_2). HOA was calculated by p50 (the blood PO_2 corresponding to its 50% oxygen saturation) which was determined spectrophotometrically (the $p50_{act}$). The Severinghaus formulas [6] were applied to calculate p50 at standard values of pH, pCO_2 and temperature ($p50_{st}$) as well as position of the oxyhemoglobin dissociation curve.

Gaseous transmitters

Production of Nitric Oxide (NO) was measured by the content of blood plasma Nitrates/Nitrites (NO_3–/NO_2–) using the Griess reagent and a Solar PV1251C spectrophotometer at a wavelength of 540 nm [7]. The content of Hydrogen Sulfide (H_2S) was measured by a spectrophotometric method based on the reaction between the sulfide anion and an acidic solution of N,N-Dimethyl-p-phenylenediamine dihydrochloride in the presence of ferric chloride at a wavelength of 670 nm [8].

Statistical analysis

Distribution of the body of data obtained was evaluated in compliance with the law of normal distribution using the Shapiro-Wilk test. The methods of nonparametric statistics and the StatSoft Statistica-10.0 software were applied. With allowance for the small sample and multiple comparisons, the statistical significance of the differences was evaluated by the Mann-Whitley U-test. The results were presented as a Median (Me) [25th percentile; 75th percentile]. The p value of <0.05 was considered significant.

Results

The clinical characteristics of COVID-19 patients from the group examined summarizes in Table 1. The patients of this group had histories of Stage 2 arterial hypertension and coronary heart disease (40% of the cases), as well as Functional Class II stable angina pectoris (46. 67%). Some of the examined patients (13.3%) had histories of cancer. The analysis of the laboratory parameters (Table 2) revealed elevated activities of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase and well as an increased content of C-Reactive Protein (CRP) which reflected an acute inflammatory process. Raised levels of ferritin and D-dimer were also observed, indicating activation of thrombogenesis. The characteristics of COVID-19 severity in the above patients are listed in Table 3. The changes in pulmonary tissue under COVID-19 (ground-glass opacity ± pulmonary consolidation) corresponding to CT-1, CT-2 and CT-3 lung injuries according to the Regional classification of COVID-19 diagnostics and treatment were detected in 6.67, 13.33 and 13.33% of the patients, respectively. The patients developed such disturbances in respiratory function as cough, dyspnea and anosmia. The values for blood oxygenation (SpO_2) were less than 93% in 46.66% of the cases.

Table 1: Clinical characteristics of examined patients.

Parameter	Value
Men/women	6/9 (40%/60%)
Age	58
Obesity (BMI>30.0 kg/м^2)	12 (80%)
Arterial hypertension	9 (60%)
Coronary heart disease: Functional Class II, stable angina pectoris (in past history)	8 (53.3%)
Cancer diseases	13 (/86.7%/)

Table 2: Laboratory parameters of examined patients.

Parameter	Value
Leucocytes, abs., 10^9/l	5.6 (3.9/8.0)
Lymphocytes, abs., 10^9/l	26.0 (18.0/31.0)
Thrombocytes, abs.	175.0 (157.0/260.0)
Total protein, g/l<65	67.0 (64.0/68.0)
Lactate dehydrogenase, u/l >220	406.0 (327.0/466.0)
Aspartate aminotransferase, >35u/l	51.0 (32.0/57.0)
Alanine aminotransferase >40 u/l	74.0 (32.0/97.0)
C-reactive protein >6 mg/l	44.0 (12.0/54.0)
Ferritin >400 µg/l	567.0 (314.0/654.0)
D-dimers >240 µ/l	570.0 (510.0/832.0)

Table 3: Characteristics of disease severity in patients with COVID-19.

Parameter	Value
Lung injury	
CT1 (mild pneumonia with ground-glass opacity areas and less than 25% pathologic changes)	14 (93.3%)
CT2 (moderate pneumonia, 25%-50% lung injury)	10 (66.7%)
CT3 (moderate to severe pneumonia, 50-75% lung injury)	13 (86.7%)
CT4 (severe pneumonia, more than 75% lung injury)	15 (100%)
Respiratory function	
Cough	15 (100%)
Dyspnea	10 (66.7%)
Anosmia	13 (86.67%)
SpO_2<93	8 (53.4%)

Compared to healthy individuals, patients with COVID-19 had reduced SO_2 and pCO_2 and increased pH of the blood demonstrated in Table 4. An elevation of $p50_{st}$ by 14% (p<0.05) was found, which was evidently a typical response to tissue hypoxia that developed due to insufficiency of pulmonary circulation. The values for other parameters did not differ from those in healthy individuals. The $p50_{act}$ was elevated by 6.4% (p<0.05) in comparison with the control group. Attention should be given to the rightward shift in the oxyhemoglobin

Table 4: Changes in parameters of blood oxygen transport in patients with COVID-19.

Parameter	Healthy individuals	PatientswithCOVID-19
n	15	15
$p50_{act}$, mmHg	28.2 (27.9; 29.1)	30.0 (29.1; 31.45)*
$p50_{st}$, mmHg	27.9 (27.5; 28.9)	31.8 (29.5; 34.3)*
pH, units ед.	7.367 (7.352; 7.407)	7.456 (7.421; 7.497)*
pCO_2, mmHg	48.5 (46.7; 49.8)	44.2 (41.5; 47.7)*
pO_2, mmHg	40.0 (38.0; 47.0)	33.5 (25.2; 45.7)
HCO_3-, mmol/l	29.4 (27.3; 31.0)	30.8 (27.5; 33.3)
TCO_2, mmol/l	31.0 (28.8; 32.5)	32.4 (29.5; 34.8)
ABE, mmol/l	3.5 (2.5; 5.6)	7.5 (2.5; 9.2)*
SBE, mmol/l	3.6 (2.1; 6.0)	7.3 (3.0; 8.8)*
SBC, mmol/l	26.6 (25.3; 28.3)	30.1 (26.6; 31.9)*
Hemoglobin, g/l	141 (117; 144)	121 (92; 130)*
SO_2, %	65.4 (60.6; 67.8)	59.9 (44.7; 72.8)

* p<0.05 *vs*. healthy individuals

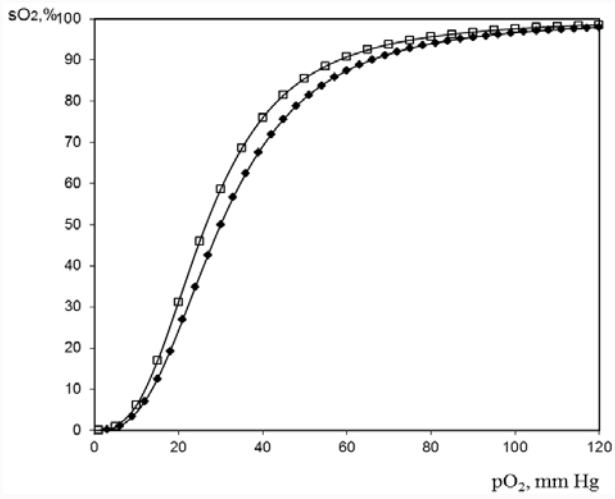

Figure 1: Oxyhemoglobin dissociation curve venous blood in healthy individuals (□) and patients with COVID -19(♦).

dissociation curve (Figure 1). This can probably be associated with a certain exhaustion of the compensatory response of the body to hypoxia, as evidenced by the decreased pO_2 and SO_2 contents. The elevated p50 contributes to enhancement of the oxygen flow to tissues under normoxia or mild hypoxia, whereas in impaired tissue oxygen utilization, its reduction can cause an adverse, pro-oxidant, effect [9].

Discussion

Some papers describing the pattern of changes in hemoglobin-oxygen affinity in patients with COVID-19 are available, but they are rather contradictory. According to Daniel et al. [4], the HOA tended to decrease in patients with this pathology. On the contrary, other authors such as Vogel et al. [5] noticed elevated HOA in patients with the above disease. Under hypoxia, the body supplies O_2 to the tissues by changing the mechanisms of HOA regulation, and even insignificant changes in HOA can maximize the arteriovenous oxygen difference and optimize O_2 transport to the tissues, maintaining a relatively low pressure on the hemodynamics [10]. The shift in the oxyhemoglobin dissociation curve rightwards in the examined patients with COVID-19 and the decreased blood pO_2 may be considered as an attempt of the body to compensate for hypoxia. However, under conditions of oxidative stress, when the tissue oxygen utilization is disturbed and a considerable amount of it is spent in oxygenase reactions to produce reactive oxygen species, this can activate free radical oxidation processes [11]. Nearly all patients with COVID-19 experience systemic cardiovascular system injury [12] which is accompanied by a pronounced damage of the endothelium. Sometimes this pathology is defined as endotheliitis [13]. The endothelial dysfunction developing in COVID-19 is a pathologic state under which the endothelium loses its ability to maintain the normal vascular tone. This brings about changes in production of different hemoglobin derivatives with released gaseous transmitters (NO, H_2S) [14].

We can suggest that the system of gaseous transmitters, predominantly that of nitric oxide and hydrogen sulfide, is responsible for the changes in blood oxygen-binding properties. As is known, the above gaseous transmitters contribute to modification of HOA. This is attained via various mechanisms such as production of sulfhemoglobin and modulation of the intraerythrocyte and L-arginine-NO pathway, as well as is mediated through systemic mechanisms of formation of hemoglobin functional properties [15]. In this connection, of interest is the work of Mortaz et al. [16] that shows an increased content of intraerythrocyte NO in patients with COVID-19. In this study, we found an 83.03% increase in the ratio of blood plasma NO_3-/NO_2- ($p<0.05$) and a 79.9% decrease of H2S ($p<0.05$) in the above category of patients (Figure 2). It is known that the gaseous transmitters (NO and H_2S) play a role of allosteric effectors of hemoglobin functional properties, changing hemoglobin-oxygen affinity and thus affecting O_2 transport [17]. The changed content of the gaseous transmitters (NO, H_2S) observed in our study contributes to alterations in blood oxygen transport realized through participation of the gaseous transmitters NO and H_2S (elevated concentrations of nitrates/nitrites and decreased hydrogen sulfide). The effects of both NO and the other gaseous transmitter, H_2S, on the formation of blood oxygen-binding properties can be significant for the processes of gaseous exchange and other physiological functions.

Many physiological effects of H_2S are due to its interaction with other gaseous intermediates (NO and CO) occurring both at the level of regulation of biosynthetic enzymes and targets for their effects. On this basis, we suggest to consider the gaseous mediators as a union of molecules regulating cell processes [15]. Hydrogen sulfide occupies a special position in regulation of physiological processes, particularly in regulation of contractile reactions of vascular smooth muscles, and its vasorelaxing effect is stipulated by not only dysfunction of endothelial smooth muscle interactions but also by a close interaction with NO-dependent mechanisms [18]. This is applicable to H_2S effects on red blood cells. It was shown that the hydrogen sulfide donor NaHS at a physiological concentration (6×10^{-5} mol) improved erythrocyte deformability, exerting a unidirectional effect that was similar in value to that of the nitric oxide donor sodium nitroprusside. However, at a higher concentration (10^{-3} mol) NaHS reduced erythrocyte deformability due to the increased limit of membrane fluidity [19]. Our study demonstrated an increase in nitric oxide and a decrease in hydrogen sulfide, indicating that the activities of the mechanisms of generation of these gaseous transmitters were not unidirectional. The mechanisms of blood oxygen transport are a prime target for NO and H_2S effects. Disturbances in the function of gaseous transmitter formation cause impairments in blood oxygen-binding properties and, consequently, provoke a reduction of adequate oxygen delivery

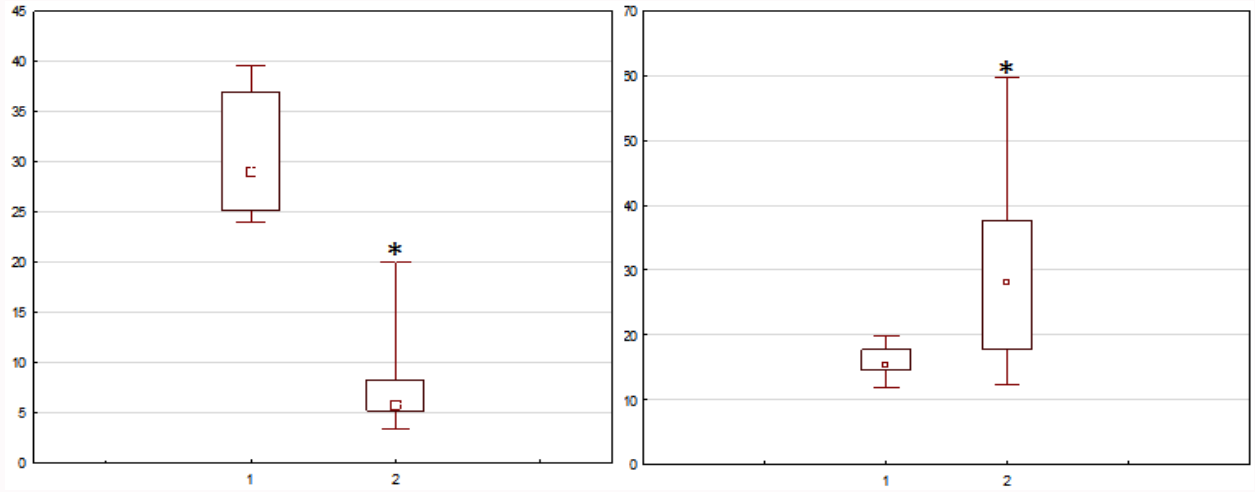

Figure 2: Blood plasma nitrite/nitrate (a) and hydrogen sulfide (b) concentrations in healthy individuals (1) and patients with COVID-19 (2). *significant differences from the group of healthy individuals

by a blood flow to meet tissue requirements, thus promoting the development of hypoxia.

Conclusion

In conclusion, this study found that patients with COVID-19 demonstrated impairments in major parameters of blood oxygenation (pO_2, SO_2) and an increase in HOA. The changes found in hemoglobin oxygen-binding properties are related to the alterations in the state of the system of gaseous transmitters (nitric oxide and hydrogen sulfide), which is significant in the pathogenesis of hypoxia developing in this pathology.

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Statement of Ethics

The Ethics Committee of Grodno Medical University approved the study protocol, and all participants provided written informed consent before initiation of study measurements (Protocol No 1 of 26.01.2021). Procedures were conducted according to the principles of the Declaration of Helsinki.

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Author Contributions

N.H. and V.Z. contributed to the design and implementation of the research. V.Z. and N.H. analyzed the data. V.Z. wrote the paper. All the authors gave the final approval of the version to be published and agree to be accountable for all aspects of the work.

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