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Aminothiols exchange in coronavirus disease 2019

Fefelova Elena Viktorovna[1*], Karavaeva Tatyana Mikhailovna[2], Parshina Anastasia Anatolyevna[1], Ma Van De Vasilina Denisovna[3], Tereshkov Pavel Petrovich[4]

Abstract

Objective: Clinical manifestation of the inflammatory process in its relation to biochemical markers (total cysteine [Cys], cysteine-glycine [CysGly], glutathione [GSH], glutamate-cysteine [Glu-Cys], homocysteine [Hcy], the ratio of reduced to oxidized glutathione [GSH/GSSG], the ratio of reduced to oxidized cysteine [CySH/CySS], malondialdehyde-oxidized low-density lipoproteins [MDA-oxLDL]) has been studied in patients with coronavirus disease 2019 (COVID-19). **Material and methods:** 48 patients with mild to severe COVID-19 and 20 healthy volunteers were included in our research. The participants were divided into 4 experimental groups according to inflammation intensity estimated based on the serum levels of interleukin 6 (IL-6). **Results:** All 4 groups showed the prevalence of male patients and elevated serum levels of IL-6 (by 54.6%). There was no comorbidity in patients with mild COVID-19 (nasopharyngitis symptoms) and in healthy control subjects. 50% of patients with lung damage had accompanying diseases. Alterations of aminoethyl metabolism were detected in COVID-19 patients: as reflected by the decreased levels of Cys, CysGly, and Glu-Cys and the increased levels of GSH as compared to the control group. **Conclusion:** Elevation of IL-6 over 7.5 pg/mL was associated with decreased GSH/GSSG and CySH/CySS ratios indicating enhanced oxidative stress and was followed by protein oxidation, specifically MDA-oxLDL.

Keywords

coronavirus disease 2019; aminothiols; oxidative stress; interleukin-6; oxidized lipoproteins

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[1]*Department of Pathological Physiology, Chita State Medical Academy, Chita 672090, Russia*
[2]*Department of Chemistry and Biochemistry, Chita State Medical Academy, Chita 672090, Russia*
[3]*Department of Faculty Therapy, Chita State Medical Academy, Chita 672090, Russia*
[4]*Laboratory of Experimental and Clinical Biochemistry and Immunology, Chita State Medical Academy, Chita 672090, Russia*

**Corresponding author Fefelova Elena Viktorovna, E-mail: fefelova.elena@mail.ru*

During the coronavirus disease 2019 (COVID-19) pandemic, nearly 500 million cases have been identified and more than 6 million people have died from the infection. Older ages and multiple chronic conditions were reported to be critical factors of increased morbidity and mortality, in contrast to younger patients who had mild symptoms and better outcomes without comorbidity. Reasons for the exceptional contagiousness and severity of clinical manifestations remain unclear.

Altered redox balance is one the recognized mechanisms of cell damage due to viral infections. Viruses disturb metabolic processes in host cells inducing over generation of reactive oxygen species (ROS) and deficiency of glutathione (GSH) leading to enhanced oxidative stress. It has been shown that virus merge with cell membrane depends on thiol-disulfide homeostasis[1-3]. Low-molecular weight aminothiols (cysteine [Cys], cysteine-glycine [CysGly], glutathione [GSH], glutamate-cysteine [Glu-Cys], homocysteine [Hcy]) are crucial for metabolic processes that maintain the development of host response in COVID-19[4].

GSH is one of the essential intracellular ROS scavengers, and glutathionylation is an important mechanism for the post-translational regulation of protein function[5]. A low level of reduced GSH is associated with an increased propensity of respiratory infections and poor cardiometabolic outcomes. Moreover, GSH level is considered a predictor of atherothrombotic events[6-8]. GSH is derived from homocysteine *via* the transsulfuration pathway and can also be synthesized from cysteine–the product of hydrolysis of extracellular proteins and GSH itself. Homocysteine, produced from methionine through intermediate products S-adenosylmethionine and S-adenosylhomocysteine, can regulate the expression of cytokines and acute phase protein-coding genes as well as the production of viral particles[9]. However, hyperhomocysteinemia induces oxidative stress, endothelial dysfunction, and thrombosis and activates type 1 angiotensin II receptors. Meanwhile, ROS inhibits methionine synthase and betaine-homocysteine

methyltransferase to increase homocysteine levels thereby creating a vicious circle[10]. Alterations of aminothiol exchange in COVID-19 remain poorly studied despite intensive research into the subject.

The aim of the present research was to study the clinical manifestation of the inflammatory process and biochemical markers (total Cys, CysGly, GSH, Glu-Cys, Hcy, ratio of reduced GSH to oxidized GSH, MDA-LDL) in their relation to interleukin 6 (IL-6) blood level in patients with COVID-19.

1 Material and methods

This study was approved by the Medical Ethics Commission of Chita State Medical Academy (No.104, 2020-11-11), and informed consent in writing was provided by the legal guardians.

The experimental group was composed of 48 patients with newly diagnosed mild to severe COVID-19 according to the Ministry of Health's temporary guidelines "Prevention, diagnosis, and treatment of novel coronavirus infection, version 8.1, 01.10.2020" and received inpatient treatment in Chita city hospital No.1[10]. Including criterion was laboratory-confirmed severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Excluding criteria were HIV, hepatitis B, hepatitis C, exacerbations of chronic cardiovascular diseases, terminal stages of malignancy, decompensated liver, and renal failure. 20 healthy volunteers were included in the control group.

The participants were divided into 4 subgroups according to the serum levels of IL-6. The first subgroup was composed of healthy subjects with IL-6 levels of 0-7 pg/mL; the second > 7-15 pg/mL; the third > 15-25 pg/mL; and the fourth greater than 25 pg/mL. According to Chest computer tomography severity score, patients of the third and fourth subgroups suffered from moderate to severe pneumonia: 1-2 and 3-4, respectively.

Total serum levels of reduced and oxidized aminothiols were assessed with a highly effective liquid chromatography method. Concentrations of MDA-oxLDL and IL-6 were measured using ELISA (Biomedica Gruppe, Germany; Vector Best, Russia).

Statistical analysis was performed using Mann-Whitney U-test and the Kruskal-Wallis test. Data obtained are presented as median and 25th percentile to 75th percentile; $P < 0.05$ was considered as significant.

2 Results

Baseline characteristics of the patients are presented in Table 1.

Among the patients, the prevalence of male subjects was uncovered for all subgroups. Moderate COVID-19 pneumonia with elevation of IL-6 plasma level from 13 to 25 pg/mL was diagnosed in more than a half of patients (54.6%). Severe pneumonia was associated with more than 25 pg/mL concentration of IL-6. Normal body temperature was detected

Table 1 Baseline characteristics of the patients with COVID-19

Indicator	1st subgroup (*N* = 20)	2nd subgroup (*N* = 10)	3rd subgroup (*N* = 26)	4th subgroup (*N* = 12)	Kruskal-Wallis test
Interleukin 6, pg/mL	2.25 (1.11, 3.55)	8.96 (7.17, 12.86)	15.48 (13.25, 23.46)[b]	44.98 (35.50, 65.91)[a,c]	$\chi^2 = 16.98$ $P = 0.000\ 01$
Gender (female/male), *N*	10/10	2/8	8/18	5/7	-
Age, years	43.00 (30.65, 50.20)	31.00 (27.75, 41.00)	46.00 (33.75, 52.20)	49.00 (44.00, 50.60)	$\chi^2 = 5.38$ $P = 0.15$
Chest computed tomography severity score	0 (0, 0)	0 (0, 0)	2 (1, 2)[a]	3 (1, 4)[a]	-
Body temperature, ℃	36.6 (35.5, 36.8)	36.8 (36.6, 37.65)[a]	38.5 (37.68, 38.62)[a,b]	38.6 (37.80, 38.92)[a,b]	$\chi^2 = 13.20$ $P = 0.004$
Blood oxygen Saturation, %	99.80 (98.50, 99.90)	97.00 (96.25, 97.75)[a]	95.50 (93.75, 97.20)[a,b]	94.00 (93.00, 96.40)[a]	$\chi^2 = 14.54$ $P = 0.002$
MDA-oxLDL	0 (0, 0.12)	8.66 (3.78, 11.60)[a]	1.81 (0.50, 7.50)[a]	0.49 (0.34, 2.87)[a]	$\chi^2 = 15.34$ $P = 0.002$
Systolic blood pressure, mm Hg	120 (120, 130)	130 (115, 130)	130 (120, 145)	150 (150, 155)[a,c]	$\chi^2 = 13.48$ $P = 0.004$
Diastolic blood pressure, mm Hg	80 (75, 85)	80 (75, 85)	90 (80, 95)	90 (90, 95)[a,b]	$\chi^2 = 13.03$ $P = 0.005$
Atherosclerosis, %	-	-	20	17	-
Arterial hypertension, %	-	-	10	20	-

N, the number of patients; [a], $P < 0.05$, *vs.* control group; [b], $P < 0.05$, the 1st subgroup *vs.* the 2nd and the 3rd subgroups; [c], $P < 0.05$, the 3rd subgroup *vs.* the 4th group; COVID-19, coronavirus disease 2019.

in 20% of patients, low-grade fever in 34%, moderate fever in 38%, and high-grade fever in 8% (P = 0.000 1). 57% of patients developed arterial hypertension, predominantly in the 4th subgroup (P = 0.000 3). Patients with IL-6 levels of > 15-25 pg/mL developed low-grade fever, moderate lung injury (CTS 1-2), and decreased arterial blood oxygen saturation to 96%-97%. Patients with IL-6 levels over 25 pg/mL developed moderate fever, severe lung injury (CTS 3-4), decreased arterial blood saturation down to < 96%, and increased systolic blood pressure over 150 mmHg and diastolic blood pressure over 90 mmHg.

There were no accompanying diseases in patients with mild COVID-19 and in controls. Patients with lung damage demonstrated approximately equal percentage of comorbidity (Table 1).

IL-6 level was within the normal range in controls. Patients who had no clinical manifestations or had been diagnosed with mild COVID-19 (nasopharyngitis symptoms) were assigned to the 2nd subgroup according to the IL-6 levels of > 7-15 pg/mL. Patients with IL-6 levels of > 15-25 pg/mL and higher had moderate to severe COVID-19 pneumonia and were composed of the 3rd and 4th subgroups.

Assessment of total aminothiols revealed decreases in Cys, CysGly, and Glu-Cys levels coupled with an elevation of plasma glutathione, regardless of the severity of COVID-19 (Table 2, Fig.1).

In patients with IL-6 levels ranging from > 15-25 pg/mL to > 25 pg/mL, glutathione was increased by 1.8 (P = 0.035) and 2.5 (P = 0.000 4) times, respectively. There was decrease of GSH/GSSG ratio in patients with IL-6 level more than 7.2 pg/mL. Rise in MDA-oxidized LDLP was detected in all examinated patients: the highest level was revealed in the 2nd subgroup (P < 0.05), the lowest – in the 4th subgroup (P = 0.002) (Table 2).

3 Discussion

Decreases in Cys and CysGly could be considered a sign of enhanced synthesis of glutathione and an indirect marker of intensive ROS production.

The development of oxidative stress in COVID-19 could be explained by the following mechanisms:

Respiratory viruses induce mitochondrial dysfunction and ROS generation *via* activation of NADPH oxidase, xanthine oxidase, and iNO-synthase[11-14]; SARS-CoV-2 activates ATP, which causes an elevation of AT-II level and then activates NADPH oxidase and peroxynitrite thereby exaggerated ROS production[15]; SARS-

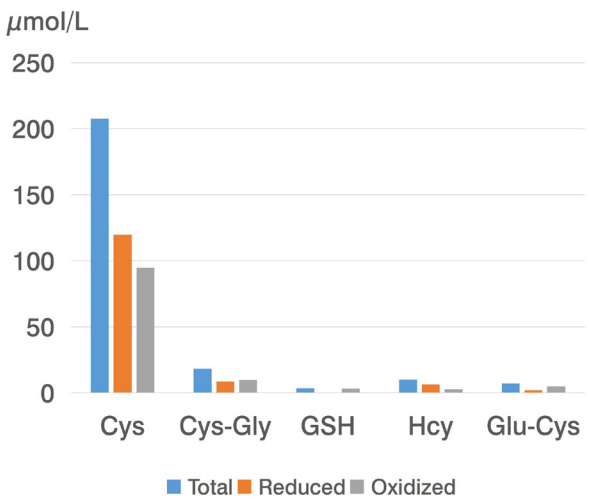

Fig. 1 Aminothyols in mild COVID-19

Cys, cysteine; CysGly, cysteine-glycine; GSH, glutathione; Hcy, homocysteine; Glu-Cys, glutamate-cysteine; COVID-19, coronavirus disease 2019.

Table 2 Blood serum level of aminothiols in COVID-19 patients

Indicator	1st subgroup (N = 20)	2nd subgroup (N = 10)	3rd subgroup (N = 26)	4th subgroup (N = 12)	Kruskal–Wallis test
Cys, μmol/L	212.60 (167.70, 264.80)	207.67 (200.21, 235.45)[a]	294.00 (271.72, 357.35)	173.24 (146.51, 329.17)[a]	χ^2 = 10.49 P = 0.01
CysGly, μmol/L	43.20 (39.50-53.90)	18.39 (13.74, 36.92)[a]	17.37 (14.59, 33.37)[a]	15.60 (10.43, 44.68)[a]	χ^2 = 9.57 P = 0.04
GSH, μmol/L	3.10 (2.60, 3.80)	3.25 (2.49, 4.35)	5.60 (4.71, 6.21)[a]	7.85 (7.20, 9.24)[a,c]	χ^2 = 13.93 P = 0.003
Glu-Cys, μmol/L	3.20 (2.40-3.90)	6.99 (5.64, 7.84)[a]	5.50 (5.12, 6.48)[a]	3.89 (3.51, 8.00)[c]	χ^2 = 12.21 P = 0.007
Hcy, μmol/L	6.75 (5.77, 7.55)	9.97 (9.31, 10.22)[a]	16.18 (13.28, 21.55)[a,b]	15.94 (15.27, 19.61)[a]	χ^2 = 16.36 P = 0.000 01
GSH/GSSG	9.00 (8.97, 9.18)	0.10 (0.05, 0.19)[a]	0.23 (0.14, 0.37)[a]	0.25 (0.17, 0.27)[a]	χ^2 = 12.08 P = 0.004
CySH/CySS	1.67 (1.02, 1.88)	1.35 (1.22, 1.56)	0.59 (0.37, 0.69)	0.14 (0.06, 0.34)	χ^2 = 15.06 P = 0.002

N, the number of patients; [a], P < 0.05, *vs.* control group; [b], P < 0.05, the 1st subgroup *vs.* the 2nd and the 3rd subgroups; [c], P < 0.05, the 3rd subgroup *vs.* the 4th group; Cys, cysteine; CysGly, cysteine-glycine; GSH, glutathione; Hcy, homocysteine; Glu-Cys, glutamate-cysteine; COVID-19, coronavirus disease 2019.

CoV-2 particles bind to CD147 on erythrocytes surface and enter cells, leading to the destruction of hemoglobin and release of iron, which can induce oxidative damage[16]; Activated phagocytes produce ROS and initiate an inflammatory process[17].

The lowest precursors of glutathione have been associated with an increased concentration of IL-6 to more than 25 pg/mL, an indicator of exacerbated oxidative stress in patients with more severe lung injury.

Increasing serum levels of glutathione and homocysteine could be caused by their leakage during exocytosis of virus-containing vesicles, which does not depend on inflammation intensity.

Significantly reduced GSH/GSSG ratios in COVID-19 patients compared to controls had no correlation with disease severity, which could presumably be a sign of antioxidants insufficiency. Decreased CySH/CySS ratio was, in contrast, significantly different in all investigated subgroups. The value of the ratio reflects redox balance in biological fluids and can be considered as one of the important vital signs[18]. Cysteine and methionine oxidation end with the formation of disulfides[19]. It is particularly dangerous if such a process involves proteins enriched with cysteine residues such as mitogen-activated protein kinases, receptors of insulin and insulin-like growth factor, and ion channels. Hence, dimerization or autoactivation of these proteins without specific signaling molecules can cause cell dysfunction. It has been suggested that thiol and disulfide groups at cell-virus interphase could act as donors or acceptors of electrons, which are required for conformation changes promoting a fusion of viruses and host cells[20].

Free radicals, especially ROS, cannot be eliminated from the cells, due to their signaling function. However, the human body has intrinsic ROS-buffering capacities to maintain the functionalities of proteins under moderate oxidative stress conditions. Nonetheless, some proteins, for example, low-density lipoproteins, are much less resistant to oxidation damage and subsequent degradation[21]. Our data demonstrated an elevation of MDA-oxLDL in all COVID-19 patients with the highest values in the 2nd subgroup (P = 0.000 01), which could be related to higher saturation of the ROS-buffering mechanism and greater impairment of the cellular respiration, thereby enhanced ROS production.

4 Conclusions

Inflammatory process caused by SARS-CoV-2 alters aminothiols metabolism along with rise in serum level of IL-6 which is associated with development of more severe lung injury, declining of arterial blood O_2-saturation and increase in blood pressure. Reduced antioxidant capacity could worsen disease course due to propagated inflammatory process, expanded tissue damage and, thus provide unfavorable outcome.

Ethical approval

This study was approved by the Medical Ethics Commission of Chita State Medical Academy (No.104, 2020-11-11), and informed consent in writing was provided by the legal guardians.

Author contributions

Fefelova E V and Tereshkov P P designed the research, developed theoretical framework, performed statistical analysis. Ma Van De V D and Karavaeva T M arranged patients screening, collecting of samples and carried out laboratory measurements. Fefelova E V, Karavaeva T M and Parshina A A wrote the manuscript. Parshina A A was in charge for the compilation of the manuscript. Fefelova E V and Tereshkov P P revised the manuscript and approved it for publishing. All authors discussed the results and contributed to the final manuscript.

Conflicts of interests

Authors declare no conflict of interests.

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