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European Journal of Pharmacology 907 (2021) 174306

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Effects of antiviral drug therapy on dynamic thiol/disulphide homeostasis and nitric oxide levels in COVID-19 patients

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ARTICLE INFO

Keywords:
Antiviral therapy
COVID-19
Disulfide
Nitric oxide
Thiol

ABSTRACT

The novel coronavirus disease 2019 (COVID-19) has led to a serious global pandemic. Although an oxidative stress imbalance occurs in COVID-19 patients, the contributions of thiol/disulphide homeostasis and nitric oxide (NO) generation to the pathogenesis of COVID-19 have been poorly identified. Therefore, the aim of this study was to evaluate the effects of antiviral drug therapy on the serum dynamics of thiol/disulphide homeostasis and NO levels in COVID-19 patients. A total of 50 adult patients with COVID-19 and 43 sex-matched healthy control subjects were enrolled in this prospective study. Venous blood samples were collected immediately on admission to the hospital within 24 h after the diagnosis (pre-treatment) and at the 15th day of drug therapy (post-treatment). Serum native thiol and total thiol levels were measured, and the amounts of dynamic disulphide bonds and related ratios were calculated. The average pre-treatment total and native thiol levels were significantly lower than the post-treatment values ($P < 0.001$ for all). We observed no significant changes in disulphide levels or disulphide/total thiol, disulphide/native thiol, or native thiol/total thiol ratios between pre- and post-treatments. There was also a significant increase in serum NO levels in the pre-treatment values when compared to control ($P < 0.001$) and post-treatment measurements ($P < 0.01$). Our results strongly suggest that thiol/disulphide homeostasis and nitrosative stress can contribute to the pathogenesis of COVID-19. This study was the first to show that antiviral drug therapy can prevent the depletion in serum thiol levels and decrease serum NO levels in COVID-19 patients.

1. Introduction

The novel coronavirus disease 2019 (COVID-19) is induced by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes pneumonia, cardiovascular dysfunctions, acute respiratory distress syndrome, and multiple organ failure (Parlakpinar and Gunata, 2021). These changes have been attributed to a systemic inflammatory response, a cytokine storm, and an attack by the immune system (Coperchini et al., 2020; Parlakpinar and Gunata, 2021). The maintenance of the thiol/disulphide balance is an important aspect of viral reactivity, entry, and fusion into the host cell; however, the oxidative stress produced from free radicals can disturb this balance (Lavillette et al., 2006; Suhail et al., 2020; Hati and Bhattacharyya, 2020). Recent reports demonstrate that oxidative stress plays a critical role in viral infections including the SARS-CoV-2 infection (Poe and Corn, 2020; Delgado-Roche and Mesta, 2020). It is known that the angiotensin converting enzyme 2 (ACE2) is the functional receptor used for SARS-CoV-2 to enter the host cells. In particular, the interaction between ACE2 and the viral spike proteins is an essential step in the viral replication cycle (Parlakpinar and Gunata, 2021). The receptor-binding domain of the viral spike proteins and ACE2 have several cysteine residues that participate in intra-molecular disulphide bonds (Singh et al., 2020). A recent computational study reported that the binding affinity is significantly impaired when all the disulphide bonds of both SARS-CoV-2 spike proteins and ACE2 are reduced to thiol groups (Hati and Bhattacharyya, 2020). Collectively, these findings suggest that the

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https://doi.org/10.1016/j.ejphar.2021.174306
Received 10 April 2021; Received in revised form 29 June 2021; Accepted 5 July 2021
Available online 7 July 2021

inhibition of oxidative stress may have an important effect during the early stages of viral infection by inhibiting viral protein binding on the host cells.

Protein thiols and low molecular weight plasma thiols (such as cysteinylglycine, homocysteine, cysteine, glutathione, etc.) participate in various cellular functions including the regulation of protein function, enzyme activity, apoptosis, the immune response, and mechanisms of cellular signal transduction (Moriarty-Craige and Jones, 2004; Biswas et al., 2006; Circu and Aw, 2010; Erel and Erdogan, 2020). The thiol/disulphide balance also maintains the cellular redox status (Erel and Neselioglu, 2014). Thiols can undergo oxidation reactions via oxidants and form disulphide bonds (Cremers and Jakob, 2013), which can be reduced back to thiol groups; thus, a dynamic thiol/disulphide homeostasis is maintained (Erel and Neselioglu, 2014). Although disulphide bonds are thought to be a marker of oxidative stress, thiols are considered members of the antioxidant system. There is a growing body of scientific evidence indicating that an abnormal thiol/disulphide homeostasis state is involved in the pathogenesis of several disorders (Oliveira and Laurindo, 2018; Erel and Erdogan, 2020). Measuring of a dynamic thiol/disulphide homeostasis allows determining entire dynamic thiol groups providing a benefit from determining only glutathione level or redox couples (such as reduced/oxidized glutathione, cysteine/cystine, and cysteinilglycine/cystinilglycine) individually. There is limited published data concerning the contribution of thiol/disulphide homeostasis in COVID-19 (Erel et al., 2020; Giustarini et al., 2021; Kalem et al., 2021). To the best of our knowledge, no published studies have investigated the effects of antiviral drug therapy on the thiol/disulphide status and nitric oxide (NO) levels in COVID-19 patients. Therefore, the purpose of this study was to determine the dynamic thiol/disulphide homeostasis and NO levels in COVID-19 patients and compare the pre- and post-treatment values.

2. Materials and methods

2.1. Study populations

A total of 50 adult patients hospitalized with a COVID-19 diagnosis at the Department of Infectious Diseases and Clinical Microbiology, Gaziantep University Hospital, between November 2020 and January 2021, were enrolled in this prospective study. Due to the context of the pandemic, we first received the written approval from the Turkish Ministry of Health. Diagnosis of COVID-19 was performed via RNA detection from nasopharyngeal secretions with real-time reverse transcriptase polymerase chain reactions (RT-PCR) and a chest computed tomographic (CT) scan using the diagnostic criteria described in the World Health Organization (WHO) interim guidance. Written informed consent was obtained from all participants and the study was approved by the Clinical Ethics Committee of the institution (decision no: 2020/294). This research was conducted according to the principles defined in the Declaration of Helsinki. Patients having an unconfirmed diagnosis of the SARS-CoV-2 infection with the absence of viral RNA in RT-PCR test results were excluded. A detailed history was taken from all participants, and all patients were examined extensively.

Inclusion criteria were having a confirmed case of COVID-19 and being 18 years of age or older. Exclusion criteria of the patients with COVID-19 included those with diagnosed cerebrovascular, hepatic, and renal diseases, neoplasia, younger than 18 years of age, pregnant or breastfeeding women, those who use supplemental vitamins or iron supplements, antioxidants, fish-oil, the requirement for mechanical ventilation, intensive care unit or extracorporeal membrane oxygenation. Data from 7 patients were excluded from the study due to death during therapy; therefore, 43 patients completed the study.

2.2. Drug therapy

Favipiravir (1600 mg po bid for 1 day, then 600 mg po bid for 4 days), hydroxychloroquine sulfate (200 mg po bid for 5 days), levofloxacin (750 mg for 7–14 days), and enoxaparin sodium (body mass index < 40 kg/m^2, 40 mg sc once a day; body mass index > 40 kg/m^2, 40 mg sc twice a day) were initiated after blood collection as an initial therapy to all patients, following the Turkish Ministry of Health Guideline for COVID-19 Therapy.

2.3. Blood samples

Venous blood samples were taken from each patient via venipuncture upon admission to the hospital within 24 h after diagnosis with SARS-CoV-2 infection and on the 15th day of drug therapy. Blood samples were collected in plain tubes followed by an interval of 30 min for clot formation, and sera were separated by centrifugation at 1500*g* for 10 min at 4 °C. The separated serum samples were transferred into tubes to be stored at −80 °C until analysis. Laboratory analyses were performed within 30 min after collecting the blood samples. All thiol/disulphide and NO analyses were conducted blindly.

2.4. Thiol/disulphide detection

The serum thiol/disulphide levels were measured using the spectrophotometric method as described previously (Temel et al., 2019). We used commercial kits (Rel Assay Diagnostics, Mega Tip Ltd., Gaziantep, Turkey) to analyze the serum native thiol (reflecting only reduced thiols, –SH) and total thiol (includes both –SH and –S–S–) levels. A microplate reader (Epoch Microplate Spectrophotometer, BioTek Instruments, Winooski, VT, USA) was used to measure the total and native thiol levels. The reducible disulphide bonds were reduced to create free-form functional thiol groups. All the thiol groups were identified after reaction with 5,5′-dithiobis-(2-nitrobenzoic) acid. Half of the difference between the native and total thiols was accepted as the dynamic disulphide (–S–S–) content.

2.5. NO analysis

Serum NO was measured as described previously (Temel et al., 2019). The NO/ozone chemiluminescence technique used in this research converts nitrite (NO_2) or nitrate (NO_3) back to NO and measures the gaseous form NO. The serum samples were deproteinized with absolute ethanol at 0 °C in a 1:2 v/v mix, and incubated for 30 min at 0 °C followed by centrifugation at 21000*g* for 5 min. The supernatant was used to determine the NO levels using a NO analyzer (Model 280i NOA, Sievers Instruments, Boulder, CO, USA). Standards and samples were reacted with vanadium III chloride (dissolved in 1 M HCl at 95 °C) as the reducing agent, and the resultant gaseous form NO was analyzed under pure nitrogen. The NO concentration of the sample was calculated from a standard curve generated by sodium nitrate. The NO Analysis™ software (version 3.21, Sievers, Boulder, CO, USA) was used for collecting and analyzing data.

2.6. Routine laboratory tests and biochemical analysis

Routine laboratory tests and biochemical analysis were conducted within 24 h of admission. All biochemical tests were analyzed in an autoanalyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany) using commercially available kits. An automated hematology analyzer system (Beckman Coulter LH 780 Hematology Analyzer, Beckman Coulter, Brea, CA, USA) was used to measure the hematologic parameters. All the subsequent analyses were based on absolute cell counts.

2.7. Statistical analysis

The results are shown as the mean ± standard deviation (S.D.). The qualitative data were given as ratios with percentages. We evaluated the

normality of the data distribution using the Kolmogorov–Smirnov test. ANOVA was used to compare more than two groups when assumptions of normality and variance homogeneity were met. Then, a post hoc Bonferroni test was applied for multiple comparisons. Kruskal–Wallis test (with Dunn's multiple comparison post-test) was used to compare more than two groups when assumptions of normality were not fulfilled. Unpaired Student's *t*-test was performed to compare the average age of the control and patients' groups. The correlations were analyzed using Pearson's test. GraphPad Instat (version 3.05, GraphPad Software Inc., San Diego, CA, USA) statistical software was used, and a P value of less than 0.05 was considered statistically significant for all the analyses.

3. Results

Although 50 patients with confirmed SARS-CoV-2 infection were enrolled in the study, 43 patients completed the study. Thus, the death rate of the study was 14%. Among these patients 21 (48.8%) were female and 22 (51.2%) were male. While the average age of the patient group was 59.1 (±16.4) years old, 28 patients (65.1%) had at least one comorbidity. Hypertension (n = 22, 51.2%), diabetes mellitus (n = 13, 30.2%), and coronary artery disease (n = 10, 23.3%) were recorded as the most frequent comorbidities. The most prevalent presenting symptoms were cough (n = 33, 76.7%), fever (n = 33, 76.7%), dyspnea (n = 31, 72.1%), fatigue (n = 24, 55.8%), and myalgia (n = 21, 48.8%). The control group (n = 43) comprised sex-matched normal healthy subjects (21 females and 22 males) who were never infected with COVID-19. Individuals in the control group were among the subjects admitted to the Infectious Diseases and Clinical Microbiology Department with negative viral RNA in the RT–PCR test results. The average age of the control group was 57.5 (±7.6) years (P = 0.5677, compared to COVID-19 patients). Table 1 presents the laboratory features of the control group and the patients with COVID-19. No significant changes were observed in the white blood cells, neutrophils, and platelet counts, but the lymphocyte count was significantly reduced in the COVID-19 patients compared to controls (P < 0.001), and the drug therapy prevented this reduction (P < 0.05). There were no significant changes in hemoglobin, aspartate aminotransferase, and alanine aminotransferase levels. However, a significant decline in the albumin levels was noted in the COVID-19 patients compared to controls (P < 0.001), which was recovered following the drug therapy (P < 0.001). The creatinine levels were significantly elevated in the COVID-19 patients compared to the controls (P < 0.05) and decreased with the drug therapy, as shown in the post-treatment values (P < 0.001). Increased blood urea nitrogen levels in the COVID-19 patients were significantly suppressed with drug therapy (P < 0.001). The C-reactive protein levels were abnormally high in the COVID-19 patients compared to the controls (P < 0.001). Drug therapy reduced this increase, but this value was still high at day 15 (P < 0.001, for all, Table 1).

Table 2 shows the measured dynamic thiol/disulphide parameters of the control and COVID-19 patients. We observed that the native and total thiol values were significantly attenuated in the pre-treatment group (P < 0.001, for all). Antiviral therapy prevented these attenuations, but there was still a significant reduction in the post-treatment native thiol levels compared to the control group (P < 0.01). No significant change was noted in the disulphide levels between the groups. We found that the disulphide/native thiol (P < 0.01) and disulphide/total thiol (P < 0.05) ratios were significantly augmented, but the native thiol/total thiol ratio (P < 0.05) was decreased, in the COVID-19 patients compared to the control group (Table 2).

Correlation analysis revealed a negative correlation between the NO level and total thiol during pre-treatment (Table 3). This negative correlation was lost in post-treatment. Moreover, no significant correlation was detected between the thiol levels and the age of the patients (data not shown).

Fig. 1 demonstrates the serum NO levels in the study groups. There was a significant increase in the NO levels in the pre-treatment values (120.5 ± 53.5 μmol/l) compared to the control (85.6 ± 24.4 μmol/l, P < 0.001) and the post-treatment values (92.8 ± 30.2 μmol/l, P < 0.01).

4. Discussion

We believe that this is the first study to compare the pre- and post-drug treatment levels of thiol/disulphide and NO in the COVID patients. Our data showed that the decreased serum native thiol and total thiol status in the pre-treatment values were significantly inhibited by

Table 1
Laboratory findings of the control group and the patients with COVID-19.

Parameters	Control (n = 43)	Patients with COVID-19 (n = 43)		P values
		Pre-treatment	Post-treatment	
White blood cells (x 10^3/mm^3)	6.63 ± 1.89	6.98 ± 2.78	6.37 ± 2.05	0.4480
Neutrophils (x 10^3/mm^3)	4.92 ± 1.61	4.98 ± 2.64	4.67 ± 1.93	0.7597
Lymphocytes (x 10^3/mm^3)	2.09 ± 0.79	1.37 ± 0.54	1.75 ± 0.60	<0.001[a] <0.05[c]
Platelets (x 10^3/mm^3)	240.16 ± 73.38	208.44 ± 55.94	237.49 ± 90.59	0.0960
Hemoglobin (g/dl)	13.11 ± 1.50	13.05 ± 1.88	12.71 ± 2.63	0.6156
AST (U/l)	26.51 ± 8.96	27.72 ± 11.12	26.23 ± 13.29	0.8091
ALT (U/l)	24.30 ± 12.33	24.79 ± 14.56	27.56 ± 14.20	0.4970
AST/ALT ratio	1.24 ± 0.41	1.34 ± 0.79	1.27 ± 1.16	0.0563
Albumin (g/l)	41.58 ± 4.11	35.40 ± 5.24	39.72 ± 5.14	<0.001[a] <0.001[c]
Creatinine (mg/dl)	0.77 ± 0.16	0.91 ± 0.37	0.68 ± 0.19	<0.05[a] <0.001[c]
BUN (mg/dl)	15.93 ± 5.31	19.14 ± 9.31	13.25 ± 2.21	<0.001[c]
CRP (mg/l)	1.08 ± 1.07	68.81 ± 53.08	4.56 ± 3.25	<0.001[a] <0.001[b] < 0.001[c]

[a]Values are presented as mean ± S.D. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CRP, C-reactive protein.
[a] Control versus pre-treatment.
[b] Control versus post-treatment.
[c] Pre-treatment versus post-treatment.

Table 2
Dynamic thiol/disulphide parameters of the control and the COVID-19 patients.

Parameters	Control (n = 43)	Patients with COVID-19 (n = 43)		P values
		Pre-treatment	Post-treatment	
Native thiol (μmol/l)	279.0 ± 54.4	169.7 ± 65.9	233.4 ± 58.6	<0.001[a] <0.01[b] < 0.001[c]
Total thiol (μmol/l)	410.5 ± 69.8	282.5 ± 87.8	375.8 ± 95.1	<0.001[a] <0.001[c]
Disulphide (μmol/l)	65.8 ± 24.4	56.4 ± 30.1	71.2 ± 41.3	0.1119
Disulphide/Native thiol (%)	24.7 ± 10.5	39.4 ± 29.9	33.3 ± 21.3	<0.01[a]
Disulphide/Total thiol (%)	15.8 ± 5.0	19.8 ± 7.5	18.0 ± 8.3	<0.05[a]
Native thiol/Total thiol (%)	68.3 ± 10.0	60.5 ± 15.1	64.0 ± 16.5	<0.05[a]

Data show mean ± S.D. values.
[a] Control versus pre-treatment.
[b] Control versus post-treatment.
[c] Pre-treatment versus post-treatment.

Table 3
Correlations between NO and thiol/disulphide parameters in COVID-19 patients.

Parameters	Correlation coefficient (r)	Coefficient of determination (r^2)	P values
Pre-treatment values			
NO ↔ Native thiol	−0.2780	0.0773	0.0711
NO ↔ Total thiol	−0.3704	0.1372	0.0145
NO ↔ Disulphide	−0.2360	0.0557	0.1217
NO ↔ Disulphide/ Native thiol (%)	−0.0693	0.0048	0.6586
NO ↔ Disulphide/ Total thiol (%)	0.0070	0.0001	0.9647
NO ↔ Native thiol/ Total thiol (%)	−0.0070	0.0001	0.9647
Post-treatment values			
NO ↔ Native thiol	0.0090	0.0001	0.9548
NO ↔ Total thiol	0.0801	0.0064	0.6140
NO ↔ Disulphide	0.0922	0.0085	0.5613
NO ↔ Disulphide/ Native thiol (%)	0.1995	0.0398	0.2053
NO ↔ Disulphide/ Total thiol (%)	0.0973	0.0095	0.5399
NO ↔ Native thiol/ Total thiol (%)	−0.0619	0.0038	0.6968

NO, nitric oxide.

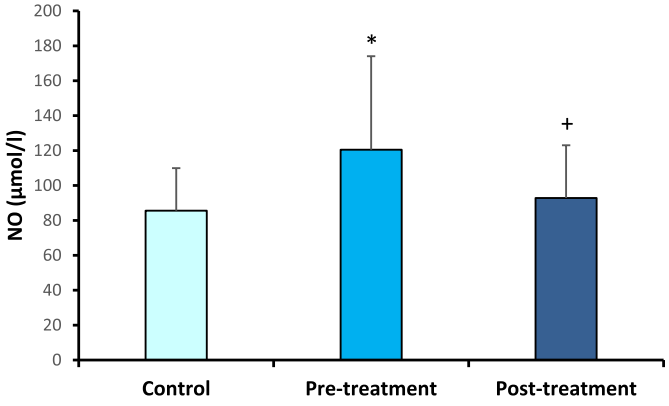

Fig. 1. Serum nitric oxide (NO) levels in control group (n = 43), and pre- and post-treatment groups in patients with COVID-19 (n = 43). Values are given as mean ± S.D., *P < 0.001 when compared to control group, $^{+}$P<0.01 when compared to pre-treatment group.

antiviral therapy. We demonstrated that the disulphide/native thiol and disulphide/total thiol ratios were significantly elevated, but the native thiol/total thiol ratio diminished in the COVID-19 patients. We unprecedentedly reported that a negative correlation exists between the increased NO level and decreased total thiol level during pre-treatment in the COVID patients.

A few studies have investigated the role of thiol/disulphide and NO levels in the pathogenesis of COVID-19. Our findings showed decreased serum native thiol and total thiol status in the pre-treatment values in the COVID-19 patients. This data supports the results presented by Erel et al. (2020), who reported that thiol status is depleted in COVID-19 patients. Kalem et al. (2021) also demonstrated that COVID-19 patients had lower native thiol and total thiol levels compared to healthy controls. Thiols are an independent risk factor for COVID-19 severity (Erel et al., 2020). The glutathione status was significantly altered downward in the COVID-19 patients (Pincemail et al., 2021). The decrease in the thiol/disulphide ratio of the extracellular fluids could play an important role in promoting the physical (protein–protein) interaction of SARS-CoV-2 and the host cell in the airways. Therefore, this redox-dependent interaction is expected to modify the risk of severe infection age-dependently (Giustarini et al., 2021). Since native and total thiols are considered antioxidant molecules found in the body that neutralize the effects of free radicals (Erenler and Yardan, 2017; Erel and Erdogan, 2020), our data suggest that antioxidant defense is impaired in COVID-19. No significant change was found in the disulphide levels between the pre- and post-treatment values. A similar finding reported no significant change in the disulphide levels in severe COVID-19 patients compared to healthy controls (Kalem et al., 2021). Collectively, these findings imply that endogenous thiol contents are depleted and dynamic thiol/disulphide homeostasis is modified by COVID-19.

The low levels of native thiol and total thiol observed in our study also explain the benefits of antioxidants in COVID-19 treatments. Indeed, several studies have attempted to increase thiol reserve in COVID-19 patients. Polonikov (2020) investigated the effects of glutathione levels on an individual's ability to recover from COVID-19 infection and found that a high reactive oxygen species/glutathione ratio appears to strongly correlate with slower recovery times and worsened symptoms. Oral supplementation of N-acetylcysteine as a precursor of glutathione could act as a potential therapeutic agent to treat COVID-19 infection due to its role in the synthesis of glutathione, modulating inflammation, and improving T cell response (Poe and Corn, 2020; De Flora et al., 2020). Overall, thiol-based drugs can be included among the possible strategies both in the prevention and therapy of COVID-19.

In this study, one possibility was that the drugs used in COVID-19 therapy may produce antioxidant activity and contribute to the elevation of native thiol and total thiol levels. Although the antiviral drug favipiravir suppresses SARS-CoV-2, its antioxidant activity is unknown. Although hydroxychloroquine can reduce neutrophil-derived oxidants and suppress inflammation (Hurst et al., 1988; Jančinová et al., 2015), it can cause systemic oxidative stress, and this could be problematic in treating COVID-19 patients (Klouda and Stone, 2020). Levofloxacin can also induce more reactive oxygen species. Talla and Veerareddy (2011) showed that there is a significant and gradual elevation of lipid peroxide levels, and decreased plasma antioxidant status in patients treated with levofloxacin. Serum antioxidant enzymes (superoxide dismutase and glutathione peroxidase) and plasma malondialdehyde levels did not significantly change with enoxaparin treatment (Cavdar et al., 1999). Collectively, these data suggest that the drugs used in this study had no direct effect on free radical scavenging or antioxidant activity.

Reactive nitrogen species may also play a critical role in COVID-19. NO, one of the most important reactive nitrogen species, is produced from L-arginine by nitric oxide synthase enzymes, and it rapidly interacts with superoxide to form peroxynitrite. Peroxynitrite, another reactive nitrogen species, can oxidize various biomolecules, including thiols (Demiryürek et al., 1998). We found a significant increase in NO levels in patients with COVID-19, and this increase was suppressed with antiviral therapy. Alamdari et al. (2020) reported increased serum NO levels in COVID-19 patients; nitrite and nitrate (the metabolites of NO) levels were measured by the Griess reaction assay and were found to be significantly increased in COVID-19 patients admitted to the intensive care unit. We used the chemiluminescence method in this study, which is more sensitive. In this NO/ozone chemiluminescence technique, nitrate and nitrite were reconverted to NO, and the NO level was measured in a gaseous form. Since endothelial dysfunction is evident in COVID-19 (Green, 2020), increased NO levels can be attributed to macrophage activation and the inflammatory processes observed in COVID-19 disease (Merad and Martin, 2020; Iqubal et al., 2021).

The main limitation of this study is that the severity of COVID-19 patients remains unclassified. Therefore, correlation analysis between COVID-19 severity versus thiol levels was not conducted.

In conclusion, the data presented in this study strongly suggest that thiol/disulphide homeostasis is disturbed in patients with COVID-19. Our results show that reduced and oxidized thiol levels play a critical role in the pathogenesis of COVID-19, and measuring thiol levels might be beneficial for evaluating the treatment response in COVID-19 patients. Our findings may also generate a basis for further studies

evaluating the effect of adjuvant thiol-based therapy as a complementary therapy for COVID-19 patients.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data

All the datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Ayşe Özlem Mete: Investigation, Data curation, Methodology, Writing – review & editing. **Kübra Koçak:** Investigation, Data curation. **Ahmet Saracaloglu:** Investigation, Data curation. **Seniz Demiryürek:** Investigation, Data curation. **Özgür Altınbaş:** Investigation, Data curation. **Abdullah T. Demiryürek:** Conceptualization, Methodology, Formal analysis, Visualization, Supervision, Writing – original draft, Project administration, All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

References

Alamdari, D.H., Moghaddam, A.B., Amini, S., Keramati, M.R., Zarmehri, A.M., Alamdari, A.H., Damsaz, M., Banpour, H., Yarahmadi, A., Koliakos, G., 2020. Application of methylene blue -vitamin C -N-acetyl cysteine for treatment of critically ill COVID-19 patients, report of a phase-I clinical trial. Eur. J. Pharmacol. 885, 173494.

Biswas, S., Chida, A.S., Rahman, I., 2006. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem. Pharmacol. 71, 551–564.

Cavdar, C., Camsari, I.S., Gönenç, S., Açikgöz, O., Oztüre, H., Onvural, B., Kavukçu, S., 1999. Effects of heparin and enoxaparin on lipids and oxidant system during a single hemodialysis session. Nephron 82, 81–83.

Circu, M.L., Aw, T.Y., 2010. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic. Biol. Med. 48, 749–762.

Coperchini, F., Chiovato, L., Croce, L., Magri, F., Rotondi, M., 2020. The cytokine storm in COVID-19: an overview of the involvement of the chemokine/chemokine-receptor system. Cytokine Growth Factor Rev. 53, 25–32.

De Flora, S., Balansky, R., La Maestra, S., 2020. Rationale for the use of N-acetylcysteine in both prevention and adjuvant therapy of COVID-19. Faseb. J. 34, 13185–13193.

Delgado-Roche, L., Mesta, F., 2020. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. Arch. Med. Res. 51, 384–387.

Demiryürek, A.T., Cakici, I., Kanzik, I., 1998. Peroxynitrite: a putative cytotoxin. Pharmacol. Toxicol. 82, 113–117.

Erel, Ö., Erdogan, S., 2020. Thiol-disulfide homeostasis: an integrated approach with biochemical and clinical aspects. Turk. J. Med. Sci. 50, 1728–1738.

Erel, Ö., Neşelioglu, S., Ergin Tuncay, M., Firat Oguz, E., Eren, F., Akkus, M.S., Güner, H. R., Ates, I., 2020. A sensitive indicator for the severity of COVID-19: Thiol. Turk. J. Med. Sci. (in press).

Erel, Ö., Neselioglu, S., 2014. A novel and automated assay for thiol/disulphide homeostasis. Clin. Biochem. 47, 326–332.

Erenler, A.K., Yardan, T., 2017. Clinical utility of thiol/disulfide homeostasis. Clin. Lab. 63, 867–870.

Giustarini, D., Santucci, A., Bartolini, D., Galli, F., Rossi, R., 2021. The age-dependent decline of the extracellular thiol-disulfide balance and its role in SARS-CoV-2 infection. Redox Biol. 41, 101902.

Green, S.J., 2020. Covid-19 accelerates endothelial dysfunction and nitric oxide deficiency. Microb. Infect. 22, 149–150.

Hati, S., Bhattacharyya, S., 2020. Impact of thiol-disulfide balance on the binding of Covid-19 spike protein with angiotensin-converting enzyme 2 receptor. ACS Omega 5, 16292–16298.

Hurst, N.P., French, J.K., Gorjatschko, L., Betts, W.H., 1988. Chloroquine and hydroxychloroquine inhibit multiple sites in metabolic pathways leading to neutrophil superoxide release. J. Rheumatol. 15, 23–27.

Iqubal, A., Hoda, F., Najmi, A.K., Haque, S.E., 2021. Macrophage activation and cytokine release syndrome in COVID-19: current updates and analysis of repurposed and investigational anti-cytokine drugs. Drug Res. 71, 173–179.

Jančinová, V., Pažoureková, S., Lucová, M., Perečko, T., Mihalová, D., Bauerová, K., Nosáľ, R., Drábiková, K., 2015. Selective inhibition of extracellular oxidants liberated from human neutrophils–A new mechanism potentially involved in the anti-inflammatory activity of hydroxychloroquine. Int. Immunopharm. 28, 175–181.

Kalem, A.K., Kayaaslan, B., Neselioglu, S., Eser, F., Hasanoglu, I., Aypak, A., Akinci, E., Akca, H.N., Erel, O., Guner, R., 2021. A useful and sensitive marker in the prediction of COVID-19 and disease severity: Thiol. Free Radic. Biol. Med. 166, 11–17.

Klouda, C.B., Stone, W.L., 2020. Oxidative stress, proton fluxes, and chloroquine/hydroxychloroquine treatment for COVID-19. Antioxidants 9, 894.

Lavillette, D., Barbouche, R., Yao, Y., Boson, B., Cosset, F.L., Jones, I.M., Fenouillet, E., 2006. Significant redox insensitivity of the functions of the SARS-CoV spike glycoprotein: comparison with HIV envelope. J. Biol. Chem. 281, 9200–9204.

Merad, M., Martin, J.C., 2020. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat. Rev. Immunol. 20, 355–362.

Moriarty-Craige, S.E., Jones, D.P., 2004. Extracellular thiols and thiol/disulfide redox in metabolism. Annu. Rev. Nutr. 24, 481–509.

Oliveira, P.V.S., Laurindo, F.R.M., 2018. Implications of plasma thiol redox in disease. Clin. Sci. (Lond.) 132, 1257–1280.

Parlakpinar, H., Gunata, M., 2021. SARS-COV-2 (COVID-19): cellular and biochemical properties and pharmacological insights into new therapeutic developments. Cell Biochem. Funct. 39, 10–28.

Pincemail, J., Cavalier, E., Charlier, C., Cheramy-Bien, J.P., Brevers, E., Courtois, A., Fadeur, M., Meziane, S., Goff, C.L., Misset, B., Albert, A., Defraigne, J.O., Rousseau, A.F., 2021. Oxidative stress status in COVID-19 patients hospitalized in intensive care unit for severe pneumonia. A pilot study. Antioxidants (Basel) 10, 257.

Poe, F.L., Corn, J., 2020. N-Acetylcysteine: a potential therapeutic agent for SARS-CoV-2. Med. Hypotheses 143, 109862.

Polonikov, A., 2020. Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients. ACS Infect. Dis. 6, 1558–1562.

Singh, J., Dhindsa, R.S., Misra, V., Singh, B., 2020. SARS-CoV2 infectivity is potentially modulated by host redox status. Comput. Struct. Biotechnol. J. 18, 3705–3711.

Suhail, S., Zajac, J., Fossum, C., Lowater, H., McCracken, C., Severson, N., Laatsch, B., Narkiewicz-Jodko, A., Johnson, B., Liebau, J., Bhattacharyya, S., Hati, S., 2020. Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) infection: a review. Protein J. 39, 644–656.

Talla, V., Veerareddy, P., 2011. Oxidative stress induced by fluoroquinolones on treatment for complicated urinary tract infections in Indian patients. J. Young Pharm. 3, 304–309.

Temel, M.T., Demiryurek, S., Saracaloglu, A., Ozer, C.H., Kahraman, H.N., Coskun, M.E., Baspinar, O., Demiryurek, A.T., 2019. Determination of dynamic thiol/disulphide homeostasis in children with tetralogy of Fallot and ventricular septal defect. Cardiol. Young 29, 499–504.