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# The Status of Antioxidants and Oxidative Damage in Patients with COVID-19

## COVID-19 Hastalarında Antioksidanların ve Oksidatif Hasarın Durumu

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**ÖZ**

**Amaç:** COVID-19, son zamanlarda bir pandemiye neden olan ve insan sağlığını önemli ölçüde etkileyen bir viral hastalıktır. Bu çalışmada COVID-19'da süperoksit dismutaz, glutatyon peroksidaz, glutatyon, toplam tiyol, doğal tiyol, disülfid, oksidatif DNA hasarı ve malondialdehit düzeyleri araştırıldı.
**Araçlar ve Yöntem:** Bu çalışmaya revers transkriptaz-polimeraz zincir reaksiyonu ile COVID-19 tanısı konan 35 hasta ve 35 sağlıklı gönüllü dahil edildi. Enzim bağlantılı immünosorbent testi ile serum glutatyon, glutatyon peroksidaz, süperoksit dismutaz, doğal tiyol, toplam tiyol ve disülfid seviyeleri ve yüksek basınçlı-sıvı kromatografisi ile malondialdehit ve 8-hidroksi-2-deoksiguanozin/$10^6$ deoksiguanozin seviyeleri ölçüldü.
**Bulgular:** COVID-19 hasta grubunda serum süperoksit dismutaz, glutatyon peroksidaz, malondialdehit, 8-hydroxy-2-deoksiguanozin/$10^6$, disülfid düzeyleri sağlıklı kontrol grubuna göre daha yüksek iken, glutathione, toplam tiyol, doğal tiyol düzeyleri daha düşüktü. Ayrıca 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine ile glutatyon, doğal tiyol ve toplam tiyol arasında negatif, disülfid ile pozitif korelasyon vardı.
**Sonuç:** Bu çalışma, COVID-19 hastalarında serum süperoksit dismutaz, glutatyon peroksidaz, glutatyon, malondialdehit, 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine ve disülfid düzeylerinin arttığını ve glutatyon, toplam tiyol ve doğal tiyol düzeylerinin azaldığını ortaya koydu. Bu sonuçlar, COVID-19 hastalarında, antioksidan belirteç düzeylerinde azalma ve oksidatif stres belirteçlerinde artış olduğunu ortaya koydu.

**Anahtar Kelimeler:** GPx; SOD; oksidatif stress; tiyol/disülfit

**ABSTRACT**

**Purpose:** COVID-19 is a viral disease that has recently caused a pandemic and significantly affects human health. In this study, superoxide dismutase, glutathione peroxidase, glutathione, total thiol, natural thiol, disulfide, oxidative DNA damage and malondialdehyde levels in COVID-19 were investigated.
**Materials and Methods:** Thirty-five patients and 35 healthy volunteers were included in this study. The diagnosis of COVID-19 was made by reverse transcriptase-polymerase chain reaction. Serum glutathione, glutathione peroxidase, superoxide dismutase, natural thiol, total thiol and disulphide levels by enzyme-linked immunosorbent assay and malondialdehyde and 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine levels by high-pressure liquid chromatography measured.
**Results:** While serum superoxide dismutase, glutathione peroxidase, malondialdehyde, 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine, disulfide levels were higher in the COVID-19 patient group than in the healthy control group, glutathione, total thiol, natural thiol levels were lower. In addition, there was a negative correlation between 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine and glutathione, natural thiol and total thiol, and a positive correlation with disulfide.
**Conclusion:** This study revealed that serum superoxide dismutase, glutathione peroxidase, malondialdehyde, 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine, and disulfide levels increased and glutathione, thiol and natural thiol levels decreased in COVID-19 patients. These results revealed that there was a decrease in antioxidant marker levels and an increase in oxidative stress markers in COVID-19 patients.

**Keywords:** GPx; SOD; oxidative stress; thiol/disulphide

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## INTRODUCTION

The microorganism responsible for the COVID-19 pandemic is the novel coronavirus (SARS-CoV-2). Seriously occupied and worried the world's public opinion this disease caused numerous deaths and morbidity. In addition, considering its contagiousness, this disease gains more importance.[1] The main focus of infection in COVID-19 patients is the respiratory system. This disease is different from other coronavirus infections both in terms of contagiousness and the risk of developing respiratory failure.[2] Despite the advances in antiviral drugs, sufficient progress has not been made yet in the prevention and inactivation of this disease. Although some antiviral drugs are used, we still do not have a fully effective antiviral to prevent contagiousness and fully treat this disease. The degrees of effectiveness of the antivirals used are not fully known. Studies are continuing on both the effectiveness of the drugs used and the discovery of fully effective new antivirals. Considering the contagiousness of COVID-19, and the formation of mutant forms of the virus, the necessity of conducting extensive research on this disease and its treatment makes itself felt more.[1] The physiopathogenesis of the disease progresses rapidly in COVID-19. Major systemic disruptions are part of the disease. There are pieces of evidences that all these conditions are associated with a hyperinflammatory state called "cytokine storm". In this case, inflammatory events increase and mortality rates increase too. The increase in inflammatory events causes an increase in oxidative stress (OS). With the increase of inflammatory events, irregularities occur in iron homeostasis, the risk of coagulation and thrombus formation increases. Reactive oxygen species (ROS) occur with intracellular OS. They are produced from mitochondrial origin and cause increased release of proinflammatory cytokines. Proinflammatory cytokines affect the formation of cellular OS, ROS and cause some physiopathological conditions. The release of pro-inflammatory mediators increases. This causes mitochondrial dysfunction. An ınflammatory/oxidation vicious cycle occurs. This causes more mitochondrial damage. Finally, damage occurs in the lungs. The more severe the disease, the more hyperferritinemia occurs. Hyperferritinemia involves iron dysregulation. Iron dysregulation also causes an increase in the amount of ROS. After all these, OS increases.[2] OS is frequently encountered as a cause of physiological and metabolic changes in the body and various diseases and is also important in this viral disease and its complications.[1] Previous studies report that factors such as OS, cytokine storm, dysregulation of iron homeostasis, and apoptosis are so important in the course of Coronavirus infection.[2-4] Many studies also report an increase in pro-inflammatory cytokines with monocyte/macrophage activation.[5] Panfoli et al.[6] reported that in SARS-CoV-1 infection, viral 3C-like proteases increased the ROS, caused OS, and promoted apoptosis through Nuclear Factor kappa B (NF-κB) signalling. Angiotensin-converting enzyme type 2 (ACE2) is expressed in endothelial cells. These cells can facilitate virus entry, replication, and lung damage. Endothelial dysfunction occurs as a result of viral damage. In this case, ACE2 may be effective in generating ROS. Mitochondrial respiration and lung defence mechanisms may be adversely affected by increased OS. It also negatively affects the repair mechanisms and immune control system. OS may facilitate the thromboembolic, vasculitic events and disseminated intravascular coagulation (DIC) that can be seen in COVID-19.[5-6]

Although most of the patients are mildly symptomatic and some are asymptomatic, approximately 5% of patients have severe lung damage, signs of organ failure, and septic shock occurs. The incidence of sepsis in COVID-19 is higher than in the normal population. This high rate suggests that sepsis caused by this viral disease is the cause of death in mortal cases.[7] Understanding the changes in OS that facilitate the progression of this disease to life-threatening conditions such as sepsis is a requirement in follow-up and treatment. Increased oxidant activity in COVID-19 causes the progression of symptoms and increase the severity of the disease, so it is critical to investigate and determine of this balance in this situation. Clarifying the relationships between events such as inflammation and OS is important in terms of reporting the effective factors in determining the severity of the clinical condition of COVID-19. Changes in levels of Superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) enzyme, which are important endogenous antioxidants, are important in the COVID-19 infection course. In addition, it has been stated that decreases in endogenous

antioxidant levels in many viral infections and supplementation of exogenous antioxidants, especially vitamins E and C, may be beneficial in maintaining the oxidant/antioxidant balance.[7,8]

SOD, GPx and GSH are important endogenous antioxidant proteins,[9-11] and the changes in their levels in COVID-19 infection are important. Thiol/disulfide homeostasis is among the important endogenous antioxidant mechanisms in humans. Thiols have the ability to neutralize the effects of free radicals that increase OS, and they also mediate the regulation of apoptosis, detoxification, signal transduction, transcription factors, and some enzymatic activities.[11-13] Changes in thiol/disulfide homeostasis are responsible for many disease processes, especially diabetes mellitus (DM), adenoid hypertrophy, rheumatoid arthritis, cardiovascular disorders, otitis media, chronic kidney failure, acute pancreatitis and malignancy.[14-17] We also aimed to investigate their homeostasis in COVID-19.

In the literature, we did not find any studies about investigating the serum levels of SOD, GSH, GPx, native/total thiols, disulfide, 8-hidroksi-2-deoksiguanozin/$10^6$ deoksiguanozin (8-OHdG/ $10^6$ dG), and malondialdehyde (MDA), which have an important place in the oxidant/antioxidant balance in COVID-19 disease. In this study, we aimed to determine oxidative damage and antioxidant status by determining the serum levels of OS and antioxidant markers in COVID-19 patients.

## MATERIALS and METHODS

### Subjects

A total of 35 volunteer COVID-19 patients were selected from among the patients who applied to the infectious diseases and clinical microbiology polyclinic between 5 May and 30 June 2020. RT-PCR SARS-CoV-2 tests performed with nasopharyngeal and oropharyngeal swab samples of the patients were found positive. Exclusion criteria were the existence of chronic diseases such as DM, cardiovascular disease, cancer, obesity, and existence of a history of a special diet and drug use, and hospitalization due to infection in the last two quarters.

Thirty-five volunteers of similar age and sex with the COVID-19 patient group, without acute or chronic illness, did not follow a special diet and exercise regimen, and had not been hospitalized for an infection in the last six months, were selected as the control group.

### Measurement of SOD, GPx and GSH Levels

SOD, GPx, and GSH measurements were made by the ELISA method using commercially available kits (Bioassay Technology Laboratory, Shanghai, China). The detection range and sensitivity for SOD, GPx, and GSH are 0.156-40 ng/mL and 0.066 ng/mL, respectively; 0.05-20 ng/mL and 0.018 ng/mL; 0.5-150 ng/L and 0.26 ng/L.

### Measurement of Total and Native Thiol Values and Calculation of Disulfide Values

We used a commercially available kit (Rel Assay diagnostic, Turkey) to determine total thiol/native thiol levels. First, total and native thiol levels were determined. Then half of the difference between total/natural thiol levels was determined as disulfide levels. Other parameters related to thiol/disulfide homeostasis were expressed as percentages.

### Measurement of Oxidative Damage Markers

**MDA*:*** MDA levels were determined using the high-pressure liquid chromatography (HPLC) method previously described by Khoschsorur et al.[18] First 50 µl serum and then respectively 0.44 mol/L H3PO4, 42 mmol/L thiobarbituric acid and HPLC grade water were added to glass tubes for precipitation and derivatization. After incubating these tubes in a water bath for 30 minutes, they were immediately cooled and alkaline methanol was added. All samples were centrifuged (3000 rpm/3min) and the supernatant was collected in clean bottles for HPLC analysis.

**8-OHdG/$10^6$dG:** Oxidative DNA damage detection consisted of three steps; leukocyte DNA isolation, DNA hydrolysis and determination of 8-OHdG/$10^6$dG levels by HPLC. A commercially available DNA isolation kit (Nucleo Spin Blood DNA, Macherey-Nagel, Germany) was used for leukocyte DNA isolation from whole blood samples.

For hydrolysis of isolated DNA, 1 mL of isolated DNA sample was added to 0.5 mL of formic acid and incubated at 150 ºC for approximately one hour.[19] Hydrolyzed DNA samples were dissolved in mobile phase solution (0.05 M potassium phosphate buffer-pH 5.5- and acetonitrile dissolved in 97:3,v/v). 8-OHdG/$10^6$dG levels were determined using an electrochemical detector (ECD) at 600 mV. The dG concentration was monitored at 245 nm. Oxidative DNA damage was expressed as 8-OHdG/$10^6$dG.

**Statistical Analysis**

SPSS version 22.0 (https://www.ibm.com/support/pages/how-cite-ibm-spss-statistics-or-earlier-versions-spss) was used for statistical data analysis and the results were shown as mean±standard deviation (SD). Whether the data were normally distributed or not was evaluated with the Shapiro-Wilk test and Histogram. Differences between groups were analyzed with Student's T-test. In addition, dependent variable analysis was performed with paired t-test and Wilcoxon signed-rank test. Box plots were drawn with GraphPad Prism (version 8.3). The calculation of sample size was done as described in Picemail's description (G-POWER software, 3.1.9.2).[20] When alpha, effect size, and power were selected as 0.05, 0.7, and 0.8, respectively, the sample size was calculated as 34 for each group.

Multivariate statistical analysis was performed using MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Data were loaded into the database, and markers of OS were determined by T-Test, which varied significantly between groups. Principal Component Analysis (PCA) was applied to detect segregation and clustering between groups. Images were presented in two dimensions and three dimensions. The OS markers contributing to the separation between the groups were calculated by using the Partial Least Squares Discriminant Analysis (PLS-DA) model, and Variable Importance in Projection (VIP) scores were calculated. Additionally, heat mapping was drawn by using Ward's algorithm, Euclidean distance measure, and T-test/ANOVA mode to visualize the intensities of OS markers between groups. P values of 0.05 or less were considered to be significant. In addition, the relationships between parameters were analysed using Pearson's correlation analysis.

**Ethics approval**

Ethics committee approval was obtained from Yüzüncü Yıl University Faculty of Medicine Clinical Research Ethics Committee with the decision dated 05 May 2020 and numbered 23.

**RESULTS**

All of the COVID-19 patients had complaints of fever, muscle pain, and cough. When the descriptive data was examined, there were no significant differences between the COVID-19 and the control group subjects in terms of gender, age and body mass index (BMI). The descriptive data, the results of routine tests performed on the COVID-19 patients and comorbidities were demonstrated in Table 1.

**Table 1**. The descriptive data of the COVID-19 patient and healthy control group.

| | COVID-19 Patient group (n=35) | Healthy Control Group (n=35) | P value |
|---|---|---|---|
| **Gender** | | | |
| **Female (%)** | 13 (41.9) | 18 (56.4) | 0.168 |
| **Male (%)** | 22 (58.1) | 17 (43.6) | |
| **Age (year)** | 40.9±15.6 | 41.1±12.4 | 0.822 |
| **Comorbidity** | | | |
| **Hypertension** | 8 (%22.9) | | |
| **Diabetes mellitus** | 6 (%17.1) | | |
| **No comorbidity** | 21 (% 6.0) | | |
| **BMI (kg/m2)** | 23.9±2.98 | 22.6±3.03 | 0.768 |
| **Ferritin (ng/mL)** | 156.5 (87.8-220.3) | | |
| **WBC (109/L)** | 5.1 (3.4-6.6) | | |
| **Lymphocyte (109/L)** | 1.3 (1.0-1.8) | | |
| **Neutrophile** | 2.7 (2.1-5.2) | | |
| **NLR** | 2.45 (1.29-3.55) | | |
| **D-Dimer (mg/L)** | 131 (64.6-361.1) | | |
| **CRP (mg/dL)** | 29.5 (13-68.7) | | |

Abbreviations: BMI, Body mass index; CRP, C-reactive protein; WBC, White blood cell; NLR, Neutrophile to lymphocyte ratio.
Normally distributed data are expressed as mean and standard deviation. Data that do not show normal distribution are expressed as the median and interquartile range (IQR)

While serum SOD and GPx enzyme levels in the COVID-19 patients were significantly higher than those in the control group, GSH levels, one of the important antioxidant peptides, were relatively lower. The findings showed that MDA and 8-OHdG/$10^6$dG levels were significantly higher in the COVID-19 patients than in the healthy individuals.

When thiol/disulfide homeostasis was examined, it was found that the balance shifted in favour of disulfide in the COVID-19 group. The levels of total and native thiols and the native thiol/total thiol ratio in the COVID-19 group were significantly lower than in the control group. The levels of disulfide and disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the COVID-19 group than in the control group. All detailed results are summarised in Table 2.

**Table 2.** Comparison of the levels of oxidative damage markers in the COVID-19 patients and the healthy control group. Values are given as mean±standard deviation.

| Common Name | Group (Mean±Sd) Control (N:35) | Covid-19 (N:35) | p-Value |
|---|---|---|---|
| Superoxide Dismutase (ng/mL) | 21.221±4.875 | 28.933±2.563 | 0.001 |
| Glutathione Peroxidase (ng/mL) | 8.350±1.711 | 15.548±2.721 | 0.001 |
| Glutathione (ng/L) | 19.765±4.750 | 9.015±2.713 | 0.001 |
| Malondialdehyde (µM) | 4.266±1.854 | 10.171±5.193 | 0001 |
| 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine | 1.471±0.545 | 3.843±1.198 | 0.001 |
| Total Thiol (µmol/L) | 546.726±45.541 | 386.448±65.815 | 0.001 |
| Native Thiol (µmol/L) | 519.401±43.873 | 335.875±66.465 | 0.001 |
| Disulfide (µmol/L) | 18.128±5.421 | 25.286±5.989 | 0.001 |
| Disulfide/Native Thiol (%) | 3.587±1.247 | 7.869±2.493 | 0.001 |
| Disulfide/Total Thiol (%) | 3.322±1.080 | 6.721±1.870 | 0.001 |
| Native Thiol/Total Thiol (%) | 93.354±2.161 | 86.557±3.741 | 0.001 |

SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GSH: Glutathione, MDA: Malondialdehyde, 8-OHdG/$10^6$dG: 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine TT: Total thiol, NT: Native thiol, DS: Disulfide, DS/NT: Disulfide/Native Thiol, DS/TT: Disulfide/Native Thiol, NT/TT: Native Thiol/Total Thiol

In the correlation analysis, we found a significant negative correlation between SOD and CRP levels. In addition, there was a significant negative correlation between SOD and ferritin levels. SOD negatively correlated with both MDA and 8-OHdG/$10^6$dG. We found a positive correlation between MDA-ferritin and MDA-CRP. MDA levels indicated a negative correlation with both total thiols and native thiols. In addition, while 8-OHdG/$10^6$dG levels were positively correlated with disulfide, 8-OHdG/$10^6$dG was negatively correlated with both total thiols and native thiols. There was a positive correlation between 8-OHdG/$10^6$dG-CRP, 8-OHdG/$10^6$dG-Ferritin and 8-OHdG/$10^6$dG-D-dimer. The total thiol levels indicated a negative correlation between CRP and ferritin. Likewise, native thiol levels were negatively correlated with both CRP and ferritin. The other detailed data are expressed in Table 3 and Figure 1.

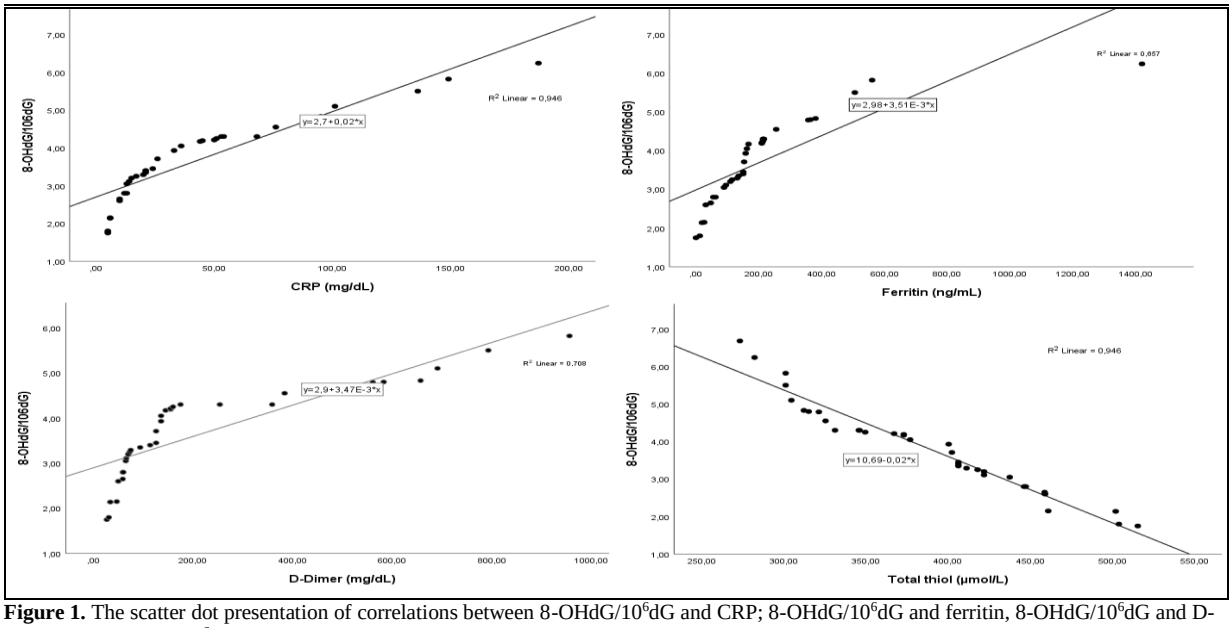


**Figure 1.** The scatter dot presentation of correlations between 8-OHdG/$10^6$dG and CRP; 8-OHdG/$10^6$dG and ferritin, 8-OHdG/$10^6$dG and D-dimer; and 8-OHdG/$10^6$dG and total thiol.

**Table 3.** The correlation analysis in all subjects.

| Pearson's r | Ferritin | WBC | Lymphocyte | D-Dimer | Neutrophile | NLR | SOD | GPx | GSH | MDA | 8-OHdG/$10^6$dG | Total thiol | Native thiol | Disulfide |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CRP (mg/dL) | 0,911* | -0,072 | -0,163 | 0,691 | 0,180 | 0,409 | -0,960* | 0,021 | -0,211 | 0,884* | 0,923* | -0,875* | -0,854* | 0,886* |
| Ferritin (ng/mL) | | -0,236 | -0,264 | 0,914* | 0,161 | 0,589 | -0,841* | 0,101 | -0,231 | 0,826* | 0,811* | -0,733* | -0,713* | 0,775* |
| WBC ($10^9$/L) | | | 0,300 | -0,246 | -0,094 | -0,229 | 0,152 | -0,055 | -0,151 | -0,211 | -0,147 | 0,145 | 0,160 | -0,121 |
| Lymphocyte ($10^9$/L) | | | | -0,268 | -0,177 | -0,516 | 0,148 | -0,190 | -0,081 | -0,233 | -0,213 | 0,180 | 0,168 | -0,250 |
| D-Dimer (mg/dL) | | | | | 0,151 | 0,708* | -0,572* | 0,232 | -0,279 | 0,588* | 0,842* | -0,440 | -0,420 | 0,507 |
| Neutrophile ($10^9$/L) | | | | | | 0,682* | -0,116 | 0,249 | 0,089 | 0,092 | 0,070 | -0,059 | -0,075 | -0,020 |
| NLR | | | | | | | -0,269 | 0,432 | -0,133 | 0,264 | 0,214 | -0,141 | -0,135 | 0,145 |
| SOD (ng/mL) | | | | | | | | 0,067 | 0,130 | -0,933* | -0,975* | 0,964* | 0,944 | -0,945* |
| GPx (ng/mL) | | | | | | | | | 0,097 | -0,076 | -0,135 | 0,149 | 0,124 | -0,122 |
| GSH (ng/L) | | | | | | | | | | -0,015 | -0,075 | 0,039 | -0,029 | -0,068 |
| MDA (µM) | | | | | | | | | | | 0,936* | -0,933* | -0,907* | 0,936* |
| 8-OHdG/$10^6$dG) | | | | | | | | | | | | -0,977* | -0,957* | 0,980* |
| Total thiol (µmol/L) | | | | | | | | | | | | | 0,982* | -0,966* |
| Native thiol (µmol/L) | | | | | | | | | | | | | | -0,950* |
| Disulfide (µmol/L) | | | | | | | | | | | | | | |

*: Indicated statistical significance p<0.05. Pearson's r: the correlation coefficient, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GSH: Glutathione, TT: Total thiol, NT: Native thiol, DS: Disulfide, MDA: Malondialdehyde, 8-OHdG/$10^6$dG: 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine; NLR: Neutrophile to lynmphocyte ratio.
The blue colour indicates a positive correlation. The red colour indicates a negative correlation. An increase in colour intensity indicates an increase in the correlation coefficient.

The configuration of the parameters in our study in the patient and control groups was demonstrated in two-dimensional space by PCA. While the first dimension explained 67.1% of the original inter-variable variation, the second dimension explained 12.7%. The variance explanation rate of the two dimensions together was found to be 79.8%. In other words, 79.8% of the variation between the original variables for both groups could be explained by two dimensions (two principal components) (Figure 2). Thus, it can be said that the patient and control groups differed significantly according to 11 variables or when 11 variables were taken together. As a result, PCA1 and PCA2 data according to PCA analysis were 67.1% and 12.7%, respectively. As a result of the analysis, a significant separation and clustering were found between the groups. All the OS markers that were analysed were found to be important and successful in distinguishing between the COVID-19 and control groups (Figure 2).

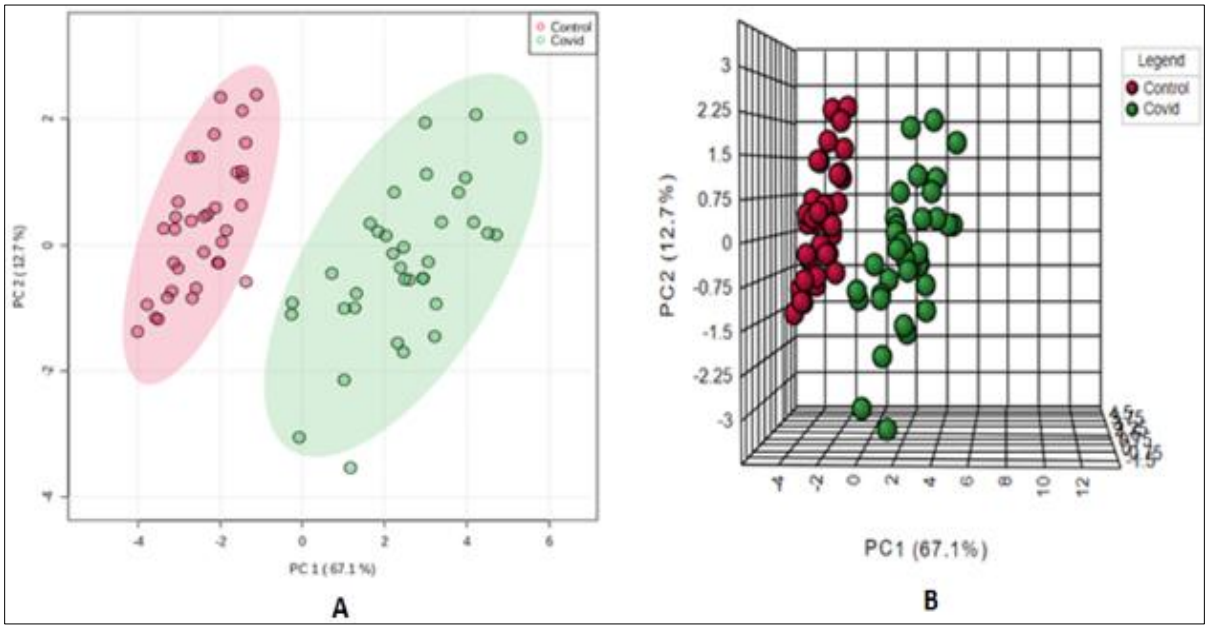


**Figure 2.** Two-dimensional (A) and three-dimensional (B) PCA score graphs of groups.

A visual representation of the effectiveness of the variables in separating the patient and control groups in two-dimensional space is given in Figure 3. VIP graph was created, which ranks the OS markers according to their ability to distinguish COVID-19 patients from healthy controls (Figure 3). An increase in the VIP score will be more decisive in distinguishing COVID-19 patients from controls.

The first two OS markers with the highest VIP scores were NT and GPx. As seen in Figure 3, the variables DS, MDA, SOD, DS/NT, DS/TT, 8OHdG and GPx showed an increasing trend in the patient group, while other variables showed a decreasing trend.

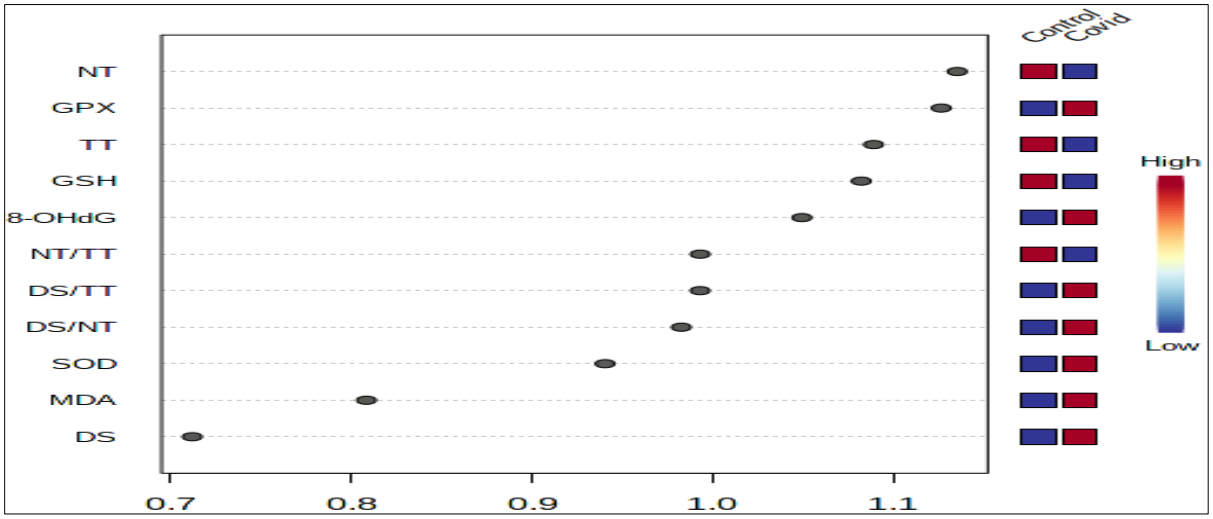


**Figure 3.** VIP score graph: Strengths of OS markers that allow for the separation of groups. SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GSH: Glutathione, TT: Total thiol, NT: Native thiol, DS: Disulfide, MDA: Malondialdehyde, 8-OHdG: 8-OHdG/$10^6$dG: 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine

In addition, a heat map was drawn to visualize the distribution of measured values for 11 OS markers in groups (Figure 4). In the heat map, columns represent individual samples, and rows represent markers. Brown bands indicate that the markers are up-regulated in groups, and blue bands indicate that they are down-regulated. The increase or decrease in the hues of the brown and blue bands determines the level of regulation of the markers in the groups. According to the heat map, 4 of 11 markers (GSH, NT/TT, TT, NT) can highly distinguish COVID-19 patients.

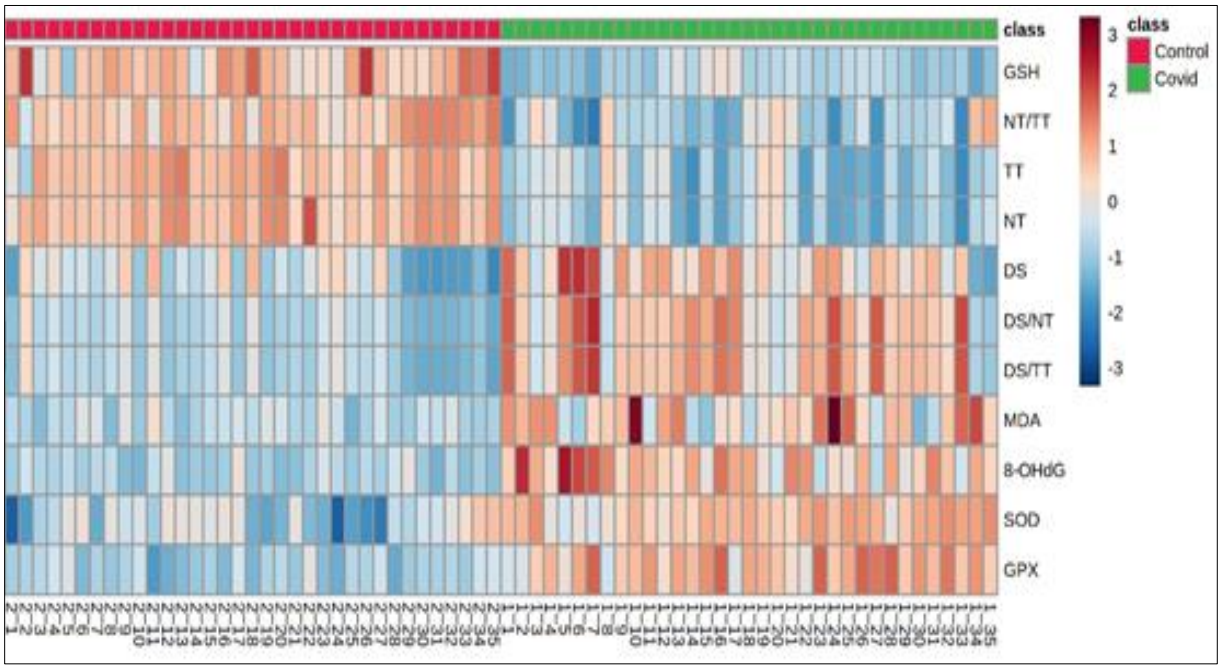


**Figure 4.** Heat map of OS markers. SOD: Superoxide dismutase, GPx: Glutathione peroxidase, GSH: Glutathione, TT: Total thiol, NT: Native thiol, DS: Disulfide, MDA: Malondialdehyde, 8-OHdG: 8-OHdG/$10^6$dG: 8-hydroxy-2-deoxyguanosine/$10^6$ deoxyguanosine

## DISCUSSION

The lungs are our most oxygenated organ and one of the target organs in viral infections and COVID-19. Hypoxemia as a result of alveolar hypoventilation and vasoconstriction of the pulmonary artery, which may occur due to infections, leads to raise in amount of the ROS. OS is an important condition that can cause many changes and diseases in the human organism. An attack of COVID-19 causes an inflammatory reaction that causes the release of pro-inflammatory cytokines seen in acute lung injury. The raised amounts of ROS and pro-inflammatory mediators are linked with inflammatory events and OS.[5] The studies in SARS-CoV-infected lung tissue have revealed that there is explicit production of oxidized phospholipids following the production of ROS in damaged alveoli, pneumocytes, and alveolar macrophages.[21] In hepatitis C virus (HCV), [22] and hepatitis B virus (HBV)[23] infections, there is a relationship among the intensity of the disease and OS has been confirmed in many studies. However, studies showing the link among the intensity and progression of SARS-CoV-2 infection and OS are limited.[24] The continued high death numbers and rates from SARS-CoV-2 motivate scientists to continue investigating factors associated with disease severity and progression. Silvagno et al.[25] reported that GSH deficiency may be linked with inflammation and respiratory distress in some common chronic disease conditions including DM and various viral infections including HIV. Taylor and Radding expressed the therapeutic importance of the GSH and GPx in COVID-19 and the diseases that with similar virus etiology. They stated that disruption of GSH synthesis may be linked with a raise in clinical findings of COVID-19.[26]

In our literature search, we could not find any studies that investigated the thiol/disulfide, GSH or GPx levels in patients with COVID-19. The data of this study showed that GSH was highly suppressed and GPx was elevated in the COVID-19 patients. NT levels were found to be relatively higher in the control group compared to the COVID-19 patients. Rise in the amount of ROS is effective in the formation of the clinical picture of this viral disease. Therefore, it is reported that appropriate and sufficient levels of GSH may be valuable in preventing the effects of mechanisms that increase the progression of organ failure in this disease. It has been reported that there is a decrease in total free thiol levels and an increase in OS, especially in inflammatory diseases where thiol/disulfide homeostasis is examined.[25] Similarly, Zinellu et al.[27] showed that systemic thiols are important in the infection process and OS steps. Hati et al.[28] emphasized that the situation of the endogenous local thiol/disulfide balance is effective in viral infections. It specifically affects the functions of the glycoproteins of the virus. We studied serum native/total thiol levels.

They may be associated with COVID-19 that is a viral inflammatory disease. Our study revealed that in the COVID-19 group, the antioxidant-effective native/total thiol levels were lower than in the control group, and in parallel with this result, the disulfide levels were also quite high. Zhao et al.[29] showed that the COVID-19 patients had lower levels of albumin, total protein and lymphocyte compared to the healthy control group. The same study found a significant reduction in total protein and lymphocyte levels and SOD activity in the severe patients of COVID-19 than in the moderate patients of COVID-19.

The COVID-19 patients in our study were uncomplicated and serum SOD levels were found to be relatively higher than the control group. 8-OHdG/$10^6$dG, which indicates oxidative damage to DNA, and MDA, which indicates lipid peroxidation are among the most important oxidative damage markers.[30,31] Chuma et al.[32] showed increased levels of 8-OHdG in chronic HCV infection and associated hepatocellular carcinoma (HCC). They also stated that 8-OHdG levels may be useful to monitor the development of HCC in patients with chronic hepatitis. Wong et al.[33] found that hepatitis infection and alcohol consumption increased 8-OHdG levels. As with hepatitis virus infection, we investigated the impact of COVID-19 on 8-OHdG levels in this study, as this may be effective for monitoring the prognosis and treatment of COVID-19. Our findings showed that 8-OHdG/$10^6$dG and MDA levels were relatively higher in the COVID-19 patients than in the healthy control, similar to the results of the studies mentioned above.

This study found that COVID-19 infection significantly oxidized thiol groups in proteins or peptides, resulting in a decrease in antioxidant enzyme levels; we showed that it

causes an increase in oxidant activity with markers showing oxidative DNA damage and lipid peroxidation.

This study revealed a negative correlation between natural/total thiol values and oxidative damage-related lipid peroxidation and DNA damage in COVID-19. In addition, we found that all the OS markers in our study were successful in distinguishing both groups.

NT and GPx with the highest VIP scores were the most successful parameters in distinguishing the COVID-19 patients from the controls. In addition, before-treatment levels of CRP, D-Dimer and ferritin were higher than the after-treatment levels. The before-treatment levels of WBC and lymphocyte levels were lower than the after-treatment levels.

COVID-19 represents associated with infection, inflammation, a cellular prooxidative action, an increase in ROS, OS and loss of the antioxidant system. The findings discussed in this study suggest that OS is an important risk factor for COVID-19 and also important in the pathogenesis of COVID-19.

In order to turn the oxidant/antioxidant balance in favour of antioxidants, increase antioxidant efficiency and prevent toxicity during infection, providing substances that strengthen the antioxidant system as well as prevent oxidative damage and also using molecular techniques to direct antioxidants to target tissues may be beneficial.

In addition, the examination of some markers showing OS and damage and antioxidant-acting factors (such as low serum TT/NT levels and increased DS levels) is guiding in terms of the balance between oxidant effect and antioxidant defence mechanism. Further studies are needed to find target mechanisms or agents associated with the oxidant/antioxidant system and disease prevention or reduction in severity.

**Conflict of Interest**

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

**Ethics Committee Permission**

Ethics committee approval was obtained from Yüzüncü Yıl University Faculty of Medicine Clinical Research Ethics Committee with the decision dated 05 May 2020 and numbered 23.

**Authors' Contributions**

Concept/Design: İB, HHA, EG, HİA, ŞA, ZH, MKK, HG. Data Collection and/or Pro-cessing: EG, İB, HG, MKK, ŞA, HHA, ZH, HİA. Data analysis and interpretation: İB, HHA, SA, EG, ZH, HG, MKK. Literature Search: İB, HHA, HİA, HG, EG, ŞA. Drafting manuscript: İB, ZH, HHA, EG, HG, HAİ. Critical revision of manuscript: İB, HHA, ZH, MKK Supervisor: MKK.

**REFERENCES**


1. Fakhri S, Nouri Z, Moradi SZ, Farzaei MH. Astaxanthin, COVID-19 and immune response: Focus on oxidative stress, apoptosis and autophagy. Phytother Res. 2020;34(11):2790-2792.
2. Saleh J, Peyssonnaux C, Singh KK, Edeas M. Mitochondria and microbiota dysfunction in COVID-19 pathogenesis. Mitochondrion. 2020;54:1-7.
3. Zhang R, Wang X, Ni L et al. COVID-19: Melatonin as a potential adjuvant treatment. Life Sci. 2020;250:117583.
4. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Med. Hypotheses. 2020;143:110102.
5. Derouiche S. Oxidative Stress Associated with SARS-Cov-2 (COVID-19) Increases the Severity of the Lung Disease - A Systematic Review. J Infect Dis Epidemiol. 2020;6(3):1-6.
6. Panfoli I. Potential role of endothelial cell surface ectopic redox complexes in COVID-19 disease pathogenesis. Clin Med. 2020;20(5):146-147.
7. Garcia JB, Verdegal RO, Pallardo FV et al. Oxidative Stress and Inflammation in COVID-19-Associated Sepsis: The Potential Role of Anti-Oxidant Therapy in Avoiding Disease Progression. Antioxidants. 2020;9(10):936.
8. Kalyanaraman B. Do free radical NETwork and oxidative stress disparities in African Americans enhance their vulnerability to SARS-CoV-2 infection and COVID-19 severity? Redox Biol. 2020;37: 101721.
9. Huyut Z, Sekeroglu MR, Balahoroglu R, Huyut MT. Characteristics of resveratrol and serotonin on antioxidant capacity and susceptibility to oxidation of red blood cells in stored human blood in a time-dependent manner. J Int Med Res. 2018;46(1):272-283.
10. Sekeroglu MR, Huyut Z, Cokluk E et al. The susceptibility to autoxidation of erythrocytes in diabetic mice: Effects of melatonin and pentoxifylline. J Biochem Mol Toxicol. 2017;31(12):.e21976.
11. Sekeroglu MR, Huyut Z, Him A. The susceptibility of erythrocytes to oxidation during storage of blood: effects of melatonin and propofol. Clin Biochem. 2012;45(4-5):315-319.

12. Simsek E, Biçer CK, Mazlumoglu MR et al. Is otitis media with effusion associated with oxidative stress? Evaluation of thiol/disulfide homeostasis. Am J Otolaryngol. 2019;40(2):164-167.
13. Güzelcicek A, Çakırca G, Erel Ö, Solmaz A. Assessment of thiol/disulfide balance as an oxidative stress marker in children with beta-thalassemia major. Pak J Med Sci. 2019;35(1):161-165.
14. Özdamar K, Şen A, Koyuncu İ. The use of the thiol-disulfide homeostasis as an indicator of oxidative stress in pediatric adenoid hypertrophy patients. Sanamed. 2019;14(1):37-43.
15. SY Durmus , Sahin NM, Ergin M et al. How does thiol/disulfide homeostasis change in children with type 1 diabetes mellitus? Diabetes Res Clin Pract. 2019;149:64-68.
16. Uyanıkoğlu A, Sabuncu T, Yıldız R et al. Impaired thiol/disulfide homeostasis in patients with mild acute pancreatitis. Turk J Gastroenterol. 2019;30(10):899-902.
17. Uyanıkoğlu H, Sak ME, Tatli F et al. Serum ischemia modified albumin level and its relationship with the thiol/disulfide balance in placenta percreta patients. J Obstet Gynaecol. 2018;38(8):1073-1077.
18. Khoschsorur G, Winklhofer-Roob B, Rabl H, Auer T, Peng Z, RJ Schaur. Evaluation of a sensitive HPLC method for the determination of malondialdehyde, and application of the method to different biological materials. Chromatographia. 2000;52:781-184.
19. Kaur H, Halliwell B. Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection. Biochem. J. 1996;318(1):21-23.
20. Pincemail J, Cavalier E, Charlier C et al. Oxidative Stress Status in COVID-19 Patients Hospitalized in Intensive Care Unit for Severe Pneumonia. A Pilot Study. Antioxidants (Basel). 2021;10(2):257.
21. Imai Y, Kuba K, Neely GG et al., Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell. 2008;133(2):235-249.
22. Cardin R, Saccoccio G, Masutti F, Bellentani S, Farinati F, Tiribelli C. DNA oxidative damage in leukocytes corre lates with the severity of HCV-related liver disease: validation in an open population study. J. Hepatol. 2001;34(4):587-592.
23. Bolukbas C, Bolukbas FF, Horoz M, Aslan M, Celik H, Erel O. Increased oxidative stress associated with the severity of the liver disease in various forms of hepatitis B virus infection. BMC Infect Dis. 2005;5(1):1-7.
24. Delgado-Roche L, Mesta F. Oxidative stress as key player in severe acute respiratory syndrome coronavirus (SARS-CoV) infection. Arch. Med. Res. 2020;51(5)384-387.
25. Silvagno F, Vernone A, Pescarmona GP. The Role of Glutathione in Protecting against the Severe Inflammatory Response Triggered by COVID-19. Antioxidants (Basel). 2020;9(7):624.
26. Taylor EW, Radding W. Understanding Selenium and Glutathione as Antiviral Factors in COVID-19: Does the Viral M(pro) Protease Target Host Selenoproteins and Glutathione Synthesis? Front Nutr. 2020;7:143.
27. Zinellu A, Zinellu E, Sotgiu E et al. Systemic transsulfuration pathway thiol concentrations in chronic obstructive pulmonary disease patients. Eur J Clin Invest: 2020;50(8):13267.
28. Hati S, Bhattacharyya S. Impact of Thiol-Disulfide Balance on the Binding of Covid-19 Spike Protein with Angiotensin-Converting Enzyme 2 Receptor. ACS omega. 2020;5(26):16292-16298.
29. Zhao G, Su Y, Sun X et al. A comparative study of the laboratory features of COVID-19 and other viral pneumonias in the recovery stage. J Clin Lab Anal. 2020;34(10):23483.
30. Cobanoglu U, Demir H, Cebi A et al. Lipid peroxidation, DNA damage and coenzyme Q10 in lung cancer patients--markers for risk assessment? Asian Pac J Cancer Prev. 2011;12(6):1399-1403.
31. Avci V, Ayengin K, Alp HH. Oxidative DNA Damage and NOX4 Levels in Children with Undescended Testes. Eur J Pediatr Surg. 2019;29(6):545-550.
32. Chuma M, Hige S, Nakanishi M et al. 8-Hydroxy-2'-deoxy-guanosine is a risk factor for development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection. J Gastroenterol Hepatol. 2008;23(9):1431-1436.
33. Wong RH, Yeh CY, Yu MH, Jung DW, Yu CL, Tsun JC. Association of hepatitis virus infection, alcohol consumption and plasma vitamin A levels with urinary 8-hydroxydeoxyguanosine in chemical workers. Mutat Res. 2003;535(2):181-186.