Medical Journal of Bakırköy

Med J Bakirkoy 2023;19:389-396
DOI: 10.4274/BMJ.galenos.2023.2023.3-21

Research

# Can Native Thiol Levels be an Indicator to Determine the Severity of COVID-19 Cases?

## Nativ Tiyol COVİD-19 Olgularının Şiddetini Tespit Etmede Belirteç Olarak Kullanılabilir mi?

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## ABSTRACT

**Objective:** To investigate the possible relationship between the severity of the disease and some oxidant-antioxidant markers in patients diagnosed with coronavirus disease-2019 (COVID-19).

**Methods:** A total of 130 cases with a diagnosis of COVID-19 were included in the study, classified as severe (group 1, n=65) and mild/moderate (group 2, n=65) and control group (group 3, n=54). Routine laboratory methods were used to analyze serum C-reactive protein, D-dimer, procalcitonin, and ferritin levels. In addition, the levels of oxidants, including malondialdehyde (MDA) and myeloperoxidase (MPO), as well as antioxidants, such as glutathione peroxidase (Gpx), superoxide dismutase (SOD), uric acid, and native thiol, were analyzed. The descriptive statistics of continuous variables were reported as the median with a range of minimum to maximum values. Furthermore, statistical tests such as the Kolmogorov-Smirnov and Mann-Whitney U tests were used. The chi-square test was used to investigate any statistical associations between groups and other categorical independent variables. To determine the significance, analysis of covariance (ANCOVA) was performed.

**Results:** The results showed that both group 1 and group 2 COVID-19 patients had considerably higher levels of routine laboratory tests than the control group ($p<0.001$). Furthermore, significantly lower levels of native thiol were found in both groups 1 and 2 compared with the control group ($p<0.001$ for both). In addition, a significant difference was observed between group 1 and group 2, with group 1 showing markedly lower levels of native thiol ($p<0.001$).

**Conclusion:** We concluded that the oxidative stress indicators MDA and MPO and the antioxidant indicators Gpx and SOD cannot be used to determine the severity of COVID-19, but decreasing natural thiol levels can be an indicator of disease severity in this population. In addition, these data may be important in explaining the mechanism of N-acetylcysteine therapy in COVID-19 cases.

**Keywords:** Native thiols, COVID-19, malondialdehyde, myeloperoxidase, superoxide dismutase, glutathione peroxidase

## ÖZ

**Amaç:** Koronavirüs hastalığı-2019 (COVİD-19) tanısı alan olgularda hastalığın şiddeti ile bazı oksidan-antioksidan belirteçler arasındaki olası ilişkinin araştırılmasıdır.

**Gereç ve Yöntem:** Toplam 130 COVİD-19 tanılı olgu çalışmaya dahil edildi, semptomlarına göre şiddetli (grup 1, n=65) ve hafif/orta (grup 2, n=65) ve kontrol grubu (grup 3, n=54) olarak sınıflandırıldı. Serum C-Reaktif protein, D-dimer, prokalsitonin ve ferritin düzeyleri rutin laboratuvar yöntemleri ile analiz edildi. Oksidan malondialdehit (MDA), miyeloperoksidaz (MPO) ve antioksidanlar glutatyon peroksidaz (Gpx), süperoksit dismutaz (SOD), ürik asit ve doğal tiyol seviyeleri de analiz edildi. Sürekli değişkenler için tanımlayıcı istatistikler medyan (minimum-maksimum) olarak sunuldu ve ayrıca Kolmogorov-Smirnov, Mann-Whitney U testleri de kullanıldı. Gruplar ve diğer kategorik bağımsız değişkenler arasındaki istatistiksel ilişkiler ki-kare testi kullanılarak test edildi. Anlamlılığın değerlendirilmesi için kovaryans analizi (ANCOVA) kullanıldı.

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**Cite as:** Kural A, Doğan M, Tekin Ş, Toker A, Doğan K. Can Native Thiol Levels be an Indicator to Determine the Severity of COVID-19 Cases?. Med J Bakirkoy 2023;19:389-396

**Received:** 18.04.2023
**Accepted:** 18.12.2023

**Bulgular:** COVİD-19 tanılı grup 1 ve grup 2 olgularda rutin laboratuvar testleri kontrol grubuna göre yüksek bulundu (p<0,001). Ayrıca hem grup 1 hem grup 2'de kontrol grubuna kıyasla daha düşük nativ tiyol düzeyleri saptanmıştır (her ikisi için de p<0,001). Buna ek olarak grup 1 ve grup 2 arasında anlamlı bir fark gözlendi. Grup 1 nativ tiyol değerleri belirgin olarak düşüktü (p<0,001).

**Sonuç:** Oksidatif stres göstergelerden MDA ve MPO'yu ve antioksidan göstergeleriden SOD ve Gpx'in COVİD-19'un şiddetini belirlemede kullanılamayacağı ancak azalan doğal tiyol seviyelerinin bu popülasyonda hastalık şiddetinin bir göstergesi olabileceği kanısına ulaştık. Ayrıca bu veri COVİD-19 olgularında N-asetilsistein tedavisinin mekanizmasını açıklamada da önemli olabilir.

**Anahtar Kelimeler:** Nativ tiyol, COVİD-19, malondialdehit, miyeloperoksidaz, süperoksit dismutaz, glutatyon peroksidaz

## INTRODUCTION

Coronavirus disease-2019 (COVID-19) was initially identified in Wuhan, China, and quickly became a global pandemic, spreading to various parts of the world. Although most COVID-19 patients do not require hospitalization, moderate or severe conditions can be detected in a minority of cases (1). In COVID-19 pathophysiology, the host's response to the infection leads to respiratory dysfunction and the activation of multisystemic inflammatory responses (2,3). The progression of COVID-19 can lead to a wide variety of clinical symptoms, ranging from no obvious symptoms to respiratory failure and dysfunction of multiple organs. It is known that some laboratory markers, such as hematological parameters (especially lymphopenia), cytokines, and liver enzymes that might be useful in indicating a progression from mild to severe disease, are used in daily practice, and some inflammatory markers have diagnostic value for disease severity and fatality (4-10). Despite extensive research, there is still debate surrounding the impact of inflammatory markers on the pathogenesis of COVID-19. The clinical course of COVID-19 depends on several factors such as cytokine storm, excessive inflammation, and low blood oxygen levels (11,12).

C-reactive protein (CRP) is an important acute phase reactant induced by IL-6. Inflammation, infection, and cellular injury cause a rapid increase in the serum levels of CRP. CRP levels are increased in COVID-19 patients and indicate a strong correlation with prognosis and disease severity (13-15).

Micronutrient iron is vital for the survival of pathogens; hence, the immune system of the host may limit the accessibility of iron during infections as a protective measure. This, in turn, leads to elevated levels of ferritin. Under inflammatory conditions caused by superoxide radicals, iron is released from ferritin, which is considered an acute phase protein and has complex functions in an inflammatory cascade (16,17).

Procalcitonin (PCT) is a hormone precursor released by thyroid parafollicular C cells and is involved in maintaining calcium homeostasis in the body. Inflammatory stimuli, primarily those of bacterial origin, cause an increase in inflammatory levels. In the context of bacterial infections, it is frequently regarded as an acute phase reactant (18). Differentiating between bacterial and viral infections can be of utmost importance, as well as other non-infectious causes of systemic inflammation (19,20).

D-dimer is a substance that forms when a blood clot breaks down through fibrinolysis (21). The name D-dimer comes from the two D fragments of the fibrin protein that combine to form a protein dimer. These levels are used as biomarkers to predict the occurrence of a blood disorder called disseminated intravascular coagulation, particularly in coagulation disorders associated with COVID-19 infection (22). Polyunsaturated fatty acid oxidation leads to the formation of malondialdehyde (MDA), which induces stress in cells. Hence, MDA is used as a biomarker to determine the degree of oxidative stress in an organism (23). Myeloperoxidase (MPO) is most abundantly expressed in neutrophil radical granulocytes, and it can cause some oxygen to carry out their antimicrobial activity, but these radicals may also cause oxidative damage in host tissue. This also shows that MPO is a potent oxidative stress marker (24). Superoxide dismutase (SOD) is an essential enzyme that facilitates the conversion of superoxide ($O_2$) radicals into ordinary molecular oxygen and hydrogen peroxide. This process is vital for protecting living cells exposed to oxygen radicals by acting as an antioxidant defense mechanism (25). The enzyme family with peroxidase activity is known as glutathione peroxidase (Gpx), and its primary biological function is to safeguard the organism against oxidative harm by transforming lipid hydroperoxides to their corresponding alcohols and reducing free hydrogen peroxide to water (26). Thiols, including cysteinylglycine, homocysteine, and cysteine, have various roles in cellular functions, such as regulating; apoptosis, enzyme activity, the immune response, protein function, and mechanisms of cellular signal transduction. Thiols can also react with oxidants, undergo oxidation reactions, and form disulphide bonds, which can be reduced back to thiol groups. Therefore, thiols are considered as a part of the antioxidant system (27).

Inflammation is the primary immune response to injury or infection. This complex process requires interactions among different inflammatory, oxidative, and antioxidative

mechanisms. The impact of inflammatory markers on COVID-19 remains a subject of controversy despite considerable research. However, the association of inflammatory markers with the severity of COVID-19 was identified by a meta-analysis (28). Therefore, we aimed to determine the levels of the same markers, including CRP, ferritin, PCT, D-dimer, MDA, MPO, Gpx, SOD, and native thiols, to evaluate a possible interplay with disease severity in patients with COVID-19. These results may determine the severity and treatment options of COVID-19, especially regarding the cysteine mechanism.

## METHODS

This prospective case-control study was conducted at the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital, between 2020 and September 2022 with the approval of the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (decision no: 2020-12-24, date: 08.06.2020). All participants signed written informed consent forms. A total of 125 patients were included in the study with 95% power analysis and 0.05 error level, and the G power 3.1.9.2 package program was used in the calculation.

### Patient's Selection

COVID-19 infection was identified using clinical and radiological findings along with nasopharyngeal swab polymerase chain reaction positive for severe acute respiratory syndrome coronavirus 2. The research included 130 COVID-19 patients who applied to our hospital. The 130 patients with 65 in each group were assigned from a larger patient cohort. After the follow-up observation, all patients were divided into mild-moderate and severe groups according to respiratory impairment and clinical management (Table 1). A score of five or less was considered to be mild-moderate. Those who scored six or more were classified as the severe group. As a control group, we enrolled 54 healthy volunteers. Participants with a history of renal dysfunction, hypertension, cancer, otoimmun diseases, and chronic diseases such as diabetes mellitus and patients using supplemental vitamins and antioxidant drugs were excluded from the study.

### Blood Sampling

Blood samples were collected from the patients on the first day of hospitalization. When measuring oxidant/antioxidant tests, it is important to pay attention to the impact of several factors such as diurnal variation, diet, and hormonal conditions. Therefore, blood sampling and routine laboratory measurements were performed while fasting in the early morning after hospitalization. After collection, the blood samples were centrifuged immediately. Serum samples were prepared by centrifugation for 10 min at 1600 g. They were stored at -80 °C until analysis. Hemolysed serum/plasma samples were discarded. At the time of admission, medical record data were used to confirm the patients' age, sex, and prior medical history.

### Measurement of the Serum Oxidant/Antioxidant Parameters

The levels of serum SOD and GPx were measured using an ELISA kit that was obtained from a commercial source (Bioassay Technology Laboratory, Cat No: E0918Hu, Cat No: E3696Hu respectively, Shanghai, China). The ELISA exhibited an inter-assay variability of 10% and an intra-assay variability of 8%. SOD results are expressed as U/L, and GPx levels are expressed as ng/mL.

The serum concentration of MPO was measured using an ELISA kit that was obtained from a commercial source (Bioassay Technology Laboratory, Cat No: E0880Hu, Shanghai, China). The ELISA exhibited an inter-assay variability of 10% and an intra-assay variability of 8%. Results are expressed in ng/mL.

The serum concentration of MDA was measured using an ELISA kit that was obtained from a commercial source (Bioassay Technology Laboratory, Cat No: E1371Hu, Shanghai, China). The ELISA exhibited an inter-assay variability of 10% and an intra-assay variability of 8%. Results were expressed in nmol/mL. Commercial kits from Rel Assay Diagnostics in Gaziantep, Türkiye were used to measure native thiol levels, and the resulting values were expressed in μmol/L. After manual spectrophotometric optimization studies, CRP, uric acid, and ferritin levels were measured using an automatic analyzer (AU5800, Beckman Coulter,

**Table 1. The classification criteria in the COVID-19 therapeutic trial synopsis**

| | |
|---|---|
| 0 | No evidence of infection |
| 1 | No limitation of activities |
| 2 | Limitation of activities |
| 3 | Hospitalized, no oxygen therapy |
| 4 | Hospitalized, oxygen by mask or nasal prongs |
| 5 | Hospitalized, non-invasive ventilation or high flow oxygen |
| 6 | Hospitalized, intubation and mechanical ventilation |
| 7 | Hospitalized, ventilation + additional organ support-pressors |
| 8 | Death |

COVID-19: Coronavirus disease-19

Inc.). D-dimer was measured using an automatic analyzer (AU480, Beckman Coulter, Inc.) by the ımmunoturbidimetric method. PCT levels were measured using an automatic analyzer (DXI 800, Beckman Coulter, Inc., Fullerton, CA) using the paramagnetic particle chemiluminescent immunoassay method.

### Statistical Analysis

Statistical analyses were conducted using version 21 of the SPSS software. (SPSS, Inc., Chicago, IL). The normality of the variables was examined using the Kolmogorov-Smirnov test to determine their distribution pattern. Because the measured biochemical parameters were not normally distributed, the differences between the patient and control groups were investigated using the non-parametric Mann-Whitney U test. Descriptive statistics are presented as median (minimum-maximum) for continuous variables. Statistical associations between groups and other categorical independent variables were evaluated with the $\chi^2$ test. Because there was an age difference between groups ($p<0.001$), the significance of differential changes between the groups was tested by analysis of covariance (ANCOVA). In patients with patients (groups 1 and 2), the Spearman test was employed to compute correlation coefficients and determine their significance for variables that did not follow a normal distribution. A significance level of $p<0.05$ was considered statistically significant.

## RESULTS

A total of 130 patients infected by COVID-19 and 54 healthy control individuals (group 3) were included in this study. Among the disease contributors, 65 patients were assigned to a severe group (group 1) and 65 patients were allocated to a mild/moderate group (group 2). The male to female ratio did not significantly differ between the groups ($p>0.05$). The median age was significantly higher in both groups 1 and 2 than in group 3 ($p<0.0001$ for both). Table 1 shows the statistical analysis results of laboratory findings. According to laboratory findings that inflammation tests are important in the follow-up of the disease, the levels of CRP, ferritin, D-dimer, and PCT were significantly elevated in both groups 1 and 2, compared to group 3 ($p<0.001$ for both). There were no statistically significant differences in the MDA, MPO, SOD, and Gpx contents between groups ($p>0.05$). Furthermore, the native thiol levels were significantly lower in both groups 1 and 2 than in the other groups ($p<0.001$ for both). Moreover, we found significantly decreased native thiol levels in group 1 compared with group 2 ($p<0.001$).

The native thiol levels of all patients with COVID-19 were negatively correlated with CRP ($r=-0.362$, $p<0.001$), ferritin ($r=-0.279$, $p<0.001$), PCT ($r=-0.390$, $p<0.001$), and D-dimer ($r=-0.458$, $p<0.001$) levels (Table 2). In our study, a positive correlation was observed between inflammatory routine biochemical markers as expected [CRP levels and ferritin,

**Table 2. Demographic, clinical and laboratory characteristics of study groups**

| | Group 1 (n=65) | Group (n=65) | Group 3 (n=54) | p[a] |
|---|---|---|---|---|
| Age (years) | 59.9±11.6 | 52.0±17.1 | 50.2±3.0 | **<0.001** |
| Gender (M/F) | 35/30 | 34/31 | 27/27 | >0.05 |
| CRP (mg/L) | 174.0 (1.2-609.6)[b,c] | 17.5 (0.3-223.0)[d] | 2.2 (0.3-6.2) | **<0.001** |
| Ferritin (ng/mL) | 835.1 (20.5-9700.0)[b,c] | 164.3 (4.7-1351.0)[d] | 69.0 (34.0-112) | **<0.001** |
| PCT (ng/mL) | 2.49 (0.0-1179.0)[b,c] | 0.1 (0.0-3.9)[d] | 0.0 (0.0-0.0) | **<0.001** |
| D-dimer (µg FEU/mL) | 2.46 (0.1-8.0)[b,c] | 0.3 (0.0-3.5)[d] | 0.2 (0.1-0.4) | **<0.001** |
| Uric acid (mg/dL) | 4.3 (0.9-13.6) | 4.2 (1.6-13.8) | 4.5 (2.1-7.8) | >0.05 |
| MDA (nmol/L) | 5.7 (0.7-83.9) | 5.3 (1.0-84.0) | 6.1 (1.6-83.9) | >0.05 |
| MPO (ng/mL) | 1.8 (0.9-32.1) | 1.9 (0.9-30.3) | 1.8 (0.9-33.5) | >0.05 |
| SOD (U/L) | 66.4 (4.0-1006.5) | 62.0 (4.5-924.0) | 72.0 (1.9-1004.6) | >0.05 |
| GPx (ng/mL) | 23.9 (10.7-192.0) | 24.7 (7.0-101.0) | 25.9 (1.4-99.3) | >0.05 |
| Native thiol (µmol/L) | 82.4 (4.8-387.7)[b,c] | 178.5 (22.7-438.0)[d] | 455.5 (245.0-757.3) | **<0.001** |

Laboratory data are presented as the median and minimum-maximum values. p[a]: P-value between groups, $p<0.001$ was statistically significant
[b]Shows differences with group 1 and group 2 with $p<0.001$
[c]Shows differences with group 1 and group 2 with $p<0.001$
[d]Shows differences with group 1 and group 2 with $p<0.001$
Group 1; severe, group 2; mild/moderate according to their symptoms, group 3; control group
M: Male, F: Female, CRP: C-reactive protein, PCT: Procalcitonin, MDA: Malondialdehyde, MPO: Myeloperoxidase, SOD: Superoxide dismutase, Gpx: Glutathione peroxidase

PCT, D-dimer levels in all COVID-19 patients (r=0.637, p<0.001, r=0.788, p<0.001, r=0.542, p<0.001 respectively)]. Although we could not find any significant differences in GPx, MDA, MPO, and SOD levels between the groups (Table 2), a strong positive correlation was observed among these markers in all the patients (Table 3).

## DISCUSSION

Our results revealed that severe patients with COVID-19 had higher serum CRP, ferritin, D-dimer, and PCT levels than both moderate patients and controls, in accordance with the literature findings. However, similar MDA, MPO, Gpx, and SOD levels were measured among the groups. The findings of this study indicated that serum native thiol values were lower in both severe and moderate patients with COVID-19 than in healthy controls.

The clinical course of COVID-19 depends on several factors such as cytokine storm, excessive inflammation, and low blood oxygen levels (11,12). It clearly identified the association of inflammatory markers with the severity of COVID-19 in a meta-analysis (28).

Therefore, measurement of inflammatory markers may be useful to monitor and evaluate the severity and prognosis of the disease. Serum levels of CRP, D-dimers, ferritin, and cardiac troponins are used for risk stratification in hospitalized patients (8). It has been reported that inflammation and coagulation are responsible for mortality in this population. An increased circulating level of inflammatory markers, such as CRP, is characterized during the development of a "cytokine storm". and indicates a strong correlation between prognosis and disease severity in COVID-19 patients (13-15,29-31). Our CRP results are consistent with the literature findings. The inflammatory response is not fully understood, but the innate immune response may contribute to the severity of this disease (31).

Many studies have reported that COVID-19 infection may affect iron metabolism. Higher concentrations of serum ferritin in severe cases were found to be associated with poor prognosis versus milder cases (32). COVID-19 is one of the rare "hyperferritinemic" diseases characterized by increased ferritin levels and cytokine storm (33). Similar to these study results, we found higher ferritin levels in a patient with severe disease when compared with other groups. This could result from the potential impact of impaired iron metabolism or may be increased as acute phase reactants.

According to a meta-analysis, PCT levels are higher than CRP levels in distinguishing bacterial infections from both viral infections and non-infectious causes of systemic inflammation (20). This distinguishing feature makes PCT a valuable diagnostic marker. On the other hand, viruses can increase serum PCT levels. Especially during coronavirus and influenza A, infections had higher PCT levels than the other studied ones (34). In addition to these findings, elevated PCT levels are reported in patients with COVID-19, and higher levels are positively associated with disease severity (7,35). In a meta-analysis, it was reported that ~5-fold increased PCT levels are related to a higher risk of severe disease. In addition, a progressive increase in PCT levels may predict a worse prognosis (36). Therefore, serial PCT measurements could be important to predict the evolution toward a more severe form of COVID-19. In our study, we found higher PCT levels in both severe and mild/moderate patients with COVID-19 when compared with control patients, consistent with the literature (7,35,37). The underlying mechanism of increasing PCT levels and disease severity in patients with COVID-19 is not fully understood.

**Table 3. Correlation analysis between characteristics and biochemical parameters in patients with COVID-19**

| Variables | Ferritin | Native thiol | PCT | D-dimer | GPx | MDA | MPO | SOD | Age |
|---|---|---|---|---|---|---|---|---|---|
| C-reactive protein (mg/L) | **0.637**** | **-0.362**** | **0.788**** | **0.542**** | **-0.206*** | **-0.174*** | **-0.231**** | -0.172 | **0.194*** |
| Ferritin (ng/mL) | - | **-0.279**** | **0.681**** | **0.446**** | -0.103 | -0.084 | -0.49 | -0.035 | 0.151 |
| Native thiol (µmol/L) | - | - | **-0.390**** | **-0.458**** | 0.062 | 0.090 | 0.001 | -0.030 | 0.102 |
| Procalcitonin (ng/mL) | - | - | - | **0.681**** | -0.087 | -0.048 | -0.072 | -0.080 | **0.206*** |
| D-dimer (µg FEU/mL) | - | - | - | - | -0.064 | -0.038 | -0.011 | -0.096 | 0.102 |
| Glutathione peroxidase (ng/mL) | - | - | - | - | - | **0.843**** | **0.860**** | **0.829**** | **-0.252**** |
| Malondialdehyde (nmol/L) | - | - | - | - | - | - | **0.883**** | **0.886**** | -0.901 |
| Myeloperoxidase (ng/mL) | - | - | - | - | - | - | - | **0.882**** | **-0.198*** |
| Superoxide dismutase (U/L) | - | - | - | - | - | - | - | - | **-0.180*** |

*p<0.05, **p<0.001
PCT: Procalcitonin, MDA: Malondialdehyde, MPO: Myeloperoxidase, SOD: Superoxide dismutase, Gpx: Glutathione peroxidase, COVID-19: Coronavirus disease-19

It could be associated with concomitant bacterial infection during moderate disease because the co-infection rate is similar to the rate of increased PCT levels in this population. However, especially in severe and critical patients, the co-infection rate is different from the PCT increasement (37).

Another test increasing during COVID-19 is D-dimer, which has demonstrated a poor prognosis for coagulopathy, especially in severe patients, similar to our study result (4,6,38,39). D-dimer indicates both the activation of coagulation and fibrinolytic system. The D-dimer consists of two D fragments of fibrin and shows a demolished fibrin (21). Several mechanisms, such as inflammatory response and endothelial dysfunction, may increase D-dimer levels in patients with COVID-19. In addition, hypoxia, age, the existence of concomitant disease, and long-term hospitalization may result in coagulation disorders in this population (22). In the present study, we found significantly higher D-dimer levels in the severe group than in the mild/moderate and control groups.

Oxidative stress is an important and possible mechanism in COVID-19 pathogenesis (40). Viral replication results in oxidative damage, which is related to the severity of the infection. Moreover, antioxidants may prevent the virus from replicating efficiently, and milder symptoms are observed in clinical practice (41). Therefore, an understanding of the molecular mechanisms of oxidative stress in COVID-19 is required to improve therapies (42). Although clinical evidence suggests that the redox profile could be an important factor in the severity of COVID-19 pathogenesis, there is limited detailed descriptive data on oxidative stress during the progression of COVID-19 (42,43). Several studies have suggested that the overproduction of reactive oxygen species and decreased antioxidant function could be important in regulating COVID-19 pathogenesis (42-44). On the contrary, it was recently reported similar oxidant production and antioxidant capacity during disease progression. Gadotti et al. (45) performed this study only in patients with COVID-19 without control individuals. Our study also includes controls. In our study, patients with COVID-19 (both severe and moderate) exhibited counterpart MDA, MPO, SOD, and Gpx levels compared with those of control individuals. These conflicting results between studies may be related to the measurement methodology of biochemical markers, sampling time, and number of cases investigated.

In our study, a significant decrease in native thiol levels was observed in severe COVID-19 patients compared with the moderate and control groups. The plasma thiol pool primarily comprises albumin and protein thiols, as well as low-molecular-weight thiols such as cysteine, cysteinyl glycine, glutathione, homocysteine, and gamma-glutamylcysteine, albeit in smaller amounts (46). Thiols are the major component of the total antioxidant mechanisms and defense against oxidative stress (47-49). Recently, Kalem et al. (50) reported that both native and total thiol levels in COVID-19 patients were lower than those in the control group. They also postulated that the native thiol level is an indicator of the presence of the disease and a predictor of disease severity, similar to our study results. We found a statistically significant negative correlation between native thiol and disease severity markers, such as CRP, ferritin, PCT, and D-dimer, in patients with COVID-19. It has been speculated that native thiols could play an important role in the elimination of increased production of ROS, and thus levels of this marker may decrease. In other words, the lower native thiol levels may be due to their conversion to disulphides under inflammatory conditions in our study. However, we did not measure the disulphide levels in our study participants. Kalem et al. (50) also reported higher disulphide levels in patients with mild to moderate COVID-19 than in controls. Interestingly, similar disulphide levels between severe patients with COVID-19 and controls have been reported. The exact mechanism behind why disulphide levels increased in mild patients compared with the control group but not in severe ones is unclear. Therefore, more research is necessary on this topic. It has been shown in many animal and human studies that N-acetylcysteine (NAC) is beneficial for treating COVID 19 (51-55). We believe that understanding the relationship between thiol and COVID-19 can also provide information on whether NAC treatment will be effective or not via the cysteine mechanisms. However, more extensive studies are required on this subject. Our study limitations are that we could not measure iron and disulphide levels in our study participants due to economical problems with small study groups.

## CONCLUSION

In severe COVID-19 cases, while CRP, ferritin, D-dimer and PCT increase, native thiol levels decrease in line with the literature results. We believe that the thiol mechanism should be investigated, especially in larger study groups, to develop the prognosis and treatment protocols of these COVID-19 patients.

### ETHICS

**Ethics Committee Approval:** This prospective case-control study was conducted at the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and

Research Hospital, between 2020 and September 2022 with the approval of the University of Health Sciences Türkiye, Bakırköy Dr. Sadi Konuk Training and Research Hospital Clinical Research Ethics Committee (decision no: 2020-12-24, date: 08.06.2020).

**Informed Consent:** All participants signed written informed consent forms.

### Authorship Contributions

Concept: A.K., Design: A.K., Data Collection or Processing: M.D., A.T., Analysis or Interpretation: A.T., Literature Search: K.D., Writing: A.K., K.D.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The study is supported by the Scientific Research Projects Coordinatorship of the University of Health Sciences, Türkiye (Project no: 2020/063).

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