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Thiol levels in mild or moderate COVID-19 patients: A comparison of variant and classic COVID-19 cases

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Abstract

Background: Various variants of the COVID-19 have started to attract attention recently. The clinical course of these variants and possible predictive parameters are being investigated. This study aimed to examine the relationship between thiol levels, which are indicators of oxidative stress, and variant COVID-19 types.

Methods: In this cross-sectional study, patients with a diagnosis of classic COVID-19 and patients with a diagnosis of variant COVID-19 with mild and moderate symptoms followed in the clinical observatory of Ankara city hospital were included in the study group. The patients were divided into two groups according to the COVID-19 type as a variant and classic COVID-19, and a healthy control group was added for comparison. A complete blood count and thiol analysis were performed from the venous blood samples. Obtained results were compared between groups, and the ROC analysis was performed.

Results: Thiol levels were significantly lower in patients with a diagnosis of COVID-19 compared with the control group. In terms of WBC, lymphocyte, neutrophil, NLR, ferritin and thiol parameters, patients with variant COVID-19 differed significantly from patients with a classic COVID-19 diagnosis. Thiol levels' cut-off values to distinguish between variant COVID-19 patients and control group from classical COVID-19 patients were almost identical (423 and 422 μmol/L, respectively).

Conclusions: It seems possible to use thiol as a sensitive, specific and cost-effective marker to suspect variant COVID-19 cases. Since this study is probably the first example in this subject, it would form a basis for further studies.

1 | INTRODUCTION

SARS-CoV-2, also known as Coronavirus-19, is the seventh member of the coronavirus family, which has members such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), following its detection in Wuhan in 2019 in a short time, it has turned into a pandemic that has taken the world under its influence.[1] Basically, four variants of concern are mentioned, namely alpha, beta, gamma and delta of the coronavirus family. It is known that the beta type, to which SARS-CoV and SARS-CoV-2 subtypes belong, causes more severe infection.[2] In the COVID-19 pandemic, variant virus forms were first reported in South Africa in May 2020, followed by variants from the United Kingdom, India and Brazil.[3-5] South Africa has named this variant 501Y.V2, because of an N501Y mutation.[5] The UK variant is called SARS-CoV-2 VOC202012/01 (Variant of Concern, the year 2020, month 12, variant 01).[5] In January 2021, the first SARS-CoV-2 UK variant was detected in Turkey followed by the South African variant.[6] Today, 10 kinds of variants of the virus are mentioned.[7] Various speculations have been made regarding the clinical course of these variant viruses. However, a clear

conclusion about the clinical significance of COVID-19 variants has not been reached yet.

On the other hand, the role of oxidative stress in COVID-19 infection emerges as an exciting research topic. It is suggested that the effect of oxidative stress and reactive oxygen species (ROS) in infection may play an essential role in the pathogenesis of the virus, and this effect is more pronounced in elderly patients.[8] Some studies reveal that the thiol-disulphide balance has a critical role in the entry of the virus into the host cell, and oxidative stress caused by free radicals may affect this balance.[9] Therefore, it is thought that reducing or eliminating oxidative stress will benefit by preventing the fusion of the virus into the cell in the early stages of COVID-19 infection.[10-12] It has been suggested that the thiol level is inversely proportional to the duration of symptoms in COVID-19 infection and can be used as a sensitive and cost-effective marker for the course of the disease.[13]

This study aimed to compare the thiol levels measured in the early phase of the infection in patients with variant and classic COVID-19 diagnosis with a mild or moderate clinical course.

What's known

Various variants of the COVID-19 have started to attract attention recently. The clinical course of these variants and possible predictive parameters are being investigated. The thiol balance has a critical role in the entry of the virus into the host cell, and oxidative stress caused by free radicals may affect this balance.

What's new

Thiol levels were significantly lower in patients with a diagnosis of COVID-19 compared with the control group. Patients with variant COVID-19 also differed significantly from patients with a non-variant COVID-19 diagnosis in terms of thiol levels. It seems possible to use thiol levels as a sensitive, specific and cost-effective marker to distinguish between variant COVID-19 patients from non-variant cases.

2 | METHODS

2.1 | Study design

We conducted a cross-sectional study. Between April and May 2021, patients with a diagnosis of classic COVID-19 and patients with a diagnosis of variant COVID-19 with mild and moderate symptoms followed in the clinical observatory of Ankara city hospital were included in the study group. As the control group, patients who applied to the family medicine outpatient clinic of the same hospital for routine control and had no known health problems were included.

2.2 | Participants

All participants were 18 years or older. Consent forms were obtained after informing about participation in the study. The patients were divided into two groups according to the COVID-19 type as a variant and classic COVID-19. The diagnoses of the patients who were positive for COVID-19 were confirmed by the PCR analysis made from the nasal swab, and the virus typing was done with the same method. Since all positive patients had an oxygen saturation of ≥90% and a respiratory rate of ≤25/min, and no imaging showed 50% or more lung involvement in any of the participants, they all met the CDC criteria for mild to moderate COVID-19 infection.[14] All patients diagnosed with COVID-19 were receiving the standard treatment protocol consisting of favipiravir and enoxaparin. Vitamin supplement, iron and mineral supplement, fish oil and antioxidant use were amongst the exclusion criteria from the study in both COVID-19 positive and control group patients. The patients with co-morbid conditions that may affect thiol levels, such as Alzheimer's disease, cardiovascular diseases and cancer, were also excluded from the study.

2.3 | Laboratory procedures

Venous blood samples were taken from the patients within the first 24 hours of their hospitalisation and sent to the biochemistry laboratory of Ankara city hospital for complete blood count and thiol analysis. Blood samples for thiol were taken into standard empty tubes. The samples were centrifuged at 1500 *g* for 10 minutes and stored at −80°C until the analysis. Plasma thiol levels were determined with 5,-5'-dithiobis-(2-nitrobenzoic) acid (DTNB) and were measured by an automated spectrophotometric method developed by Erel and Neselioglu.[15] The same procedures were applied to healthy volunteers who were admitted to the outpatient clinic. We also performed a complete blood count (CBC) and ferritin analysis from the blood samples. We used Siemens ADVIA 1800 chemistry analyzer (Siemens Healthcare GmbH, Erlangen, Germany) for measurements.

2.4 | Statistical analyses

The data of the participants were first transferred to the Microsoft Excel program and arranged. Then, IBM SPSS v.22 software was used for statistical analysis. The conformity of the data to the normal distribution was examined with the Kolmogorov–Smirnov test. Normally distributed data were shown as mean ± standard deviation, and non-normally distributed data were shown as median (min.–max). In descriptive statistics, numbers and percentages were used for categorical data. Student's *t*-test and

Mann–Whitney *U*-test were used to compare continuous data. Kruskal–Wallis test and ANOVA test were preferred for multi-group comparisons. The ROC curve analysis was used to calculate AUCs to evaluate cut-off points for continuous variables. In all statistical analyses, the cut-off value of significance was accepted as 0.05.

2.5 | Ethical considerations

Ethical approval no. E1-21-1965 was obtained from the local ethics committee for the study. In addition, the official approval dated 20 February 2021 was obtained from the Turkish Ministry of Health.

3 | RESULTS

A total of 430 patients, 181 of whom were diagnosed with classic COVID-19 and 249 variants of COVID-19 as well as 173 healthy volunteers, were included in the study. Variant patients were categorised as 111 (47.6%) UK variant (VOC202012/01) and 122 (52.4%) South African variant (501Y.V2). The median age of the patients was 39 (min = 18, max = 86), and 219 (50.9%) were female. The patients were older than the control group (Table 1). The age distribution between covid-19 sub-groups was similar (Table 2). The median age of the control group was 23 (min = 20, max = 64), and 118 (69%) were female.

The comparison of some inflammatory parameters and thiol levels between patients diagnosed with COVID-19 and the healthy control group is given in Table 1, and the comparison of these values according to COVID-19 types is given in Table 2.

Thiol levels were significantly different between the classic COVID-19, variant COVID-19 and control groups (Figure 1). In the ROC analysis performed to investigate the usability of thiol levels in predicting the diagnosis of COVID-19, it was observed that thiol could be a sensitive and specific marker, especially amongst classic COVID-19/variant COVID-19 and classic COVID-19/control patients (Table 3, Figure 2).

4 | DISCUSSION

Thiols (-SH containing compounds), which are critical antioxidant molecules in the body, play an important role in neutralising free oxygen radicals.[11] In this context, the role of thiol levels in the course of COVID-19 infection has recently attracted attention. This is the first study to evaluate thiol status in variant COVID-19 infection to the best of our knowledge.

Our study revealed that the thiol levels of patients with a diagnosis of COVID-19 were significantly lower than the control group. It has been reported in the literature that thiol levels decrease dramatically in patients with severe COVID-19 pneumonia.[16] Similarly, Erel et al revealed that the thiol level was significantly low in patients with a diagnosis of COVID-19, the thiol levels were negatively associated with the severity of the disease, and thiol status could be a useful biomarker in predicting the severity of COVID-19.[17] Since severe COVID-19 patients were excluded in our study, the design of our study was not suitable for investigating the relationship between COVID-19 severity and thiol. However, the significantly lower thiol levels in the patient group supported the hypothesis that thiol was reduced because the immune system used it during COVID-19 infection. When examined in terms of some inflammatory markers other than thiol, we observed that WBC, lymphocyte and neutrophil values decreased, and ferritin values increased in the patient group compared with the control group. This situation was consistent with the relevant literature.[18] Numerous studies show that ferritin, D-dimer and IL-6 levels are also associated with the severity of COVID-19.[19,20] However, the cost of thiol analysis per test was found to be 22.6 times cheaper than IL-6.[13] In this respect, we can infer that thiol is not only a specific and sensitive indicator but also a very cost-effective assay, which is promising for its broad use in COVID-19.

Another key finding of our study was that inflammatory markers such as WBC, lymphocyte, neutrophil, NLR, ferritin and thiol values were significantly different when classic COVID-19 patients were compared with variant COVID-19 patients. Amongst these parameters, lymphocyte count and thiol level were higher in variant COVID-19 patients than classic COVID-19 cases, whereas

TABLE 1 Comparison of age, some inflammatory markers and thiol levels between COVID-19 patients and control group

	COVID-19 patients (n = 430) Median (IQR)	Control group (n = 173) Median (IQR)	*P* value
Age (y)	39 (23)	23 (19)	<.001
WBC count (/mm^3)	5300 (2515)	6450 (3210)	<.001
Lymphocyte count (/mm^3)	1350 (910)	1900 (830)	<.001
Neutrophil count (/mm^3)	3120 (1833)	3700 (1990)	<.001
NLR	2.02 (1.99)	1.96 (1.02)	NS
Ferritin (μg/L)	86 (156)	25 (58)	<.001
Thiol (μmol/L)	410 (133)	502 (132)	<.001

Abbreviations: IQR, interquartile range; NLR, neutrophil to lymphocyte ratio; NS, not significant; WBC, white blood cell.

TABLE 2 Comparison of age, some inflammatory markers and thiol levels between classic COVID-19 patients, UK variant COVID-19 patients and South Africa variant COVID-19 patients

	COVID-19 type					
	Classic (C)	UK variant (UK)	South Africa variant (SA)			
	median (IQR)	median (IQR)	median (IQR)	*P* value[a]	Comparison of groups	Post hoc *P* value[b]
Age (y)	37 (22)	40 (21)	41.50 (22)	NS	NA	NA
WBC count (/mm^3)	5970 (2620)	4975 (1825)	4930 (1945)	<.001	C vs UK C vs SA UK vs SA	.004 <.001 NS
Lymphocyte count (/mm^3)	1160 (790)	1480 (1003)	1400 (830)	<.001	C vs UK C vs SA UK vs SA	<.001 .001 NS
Neutrophil count (/mm^3)	3580 (2070)	2715 (1370)	2840 (1380)	<.001	C vs UK C vs SA UK vs SA	<.001 <.001 NS
NLR	3.03 (3.12)	1.66 (1.41)	1.93 (1.33)	<.001	C vs UK C vs SA UK vs SA	<.001 <.001 NS
Ferritin (μg/L)	140 (243)	68 (110)	73 (124)	<.001	C vs UK C vs SA UK vs SA	<.001 <.001 NS
Thiol (μmol/L)	327 (139)	460 (78)	492 (97)	<.001	C vs UK C vs SA UK vs SA	<.001 <.001 NS

Abbreviations: IQR, interquartile range; NA, not available; NLR, neutrophil to lymphocyte ratio; NS: not significant; WBC, white blood cell.

[a]Kruskal–Wallis test.

[b]Mann–Whitney *U*-test with Bonferroni correction.

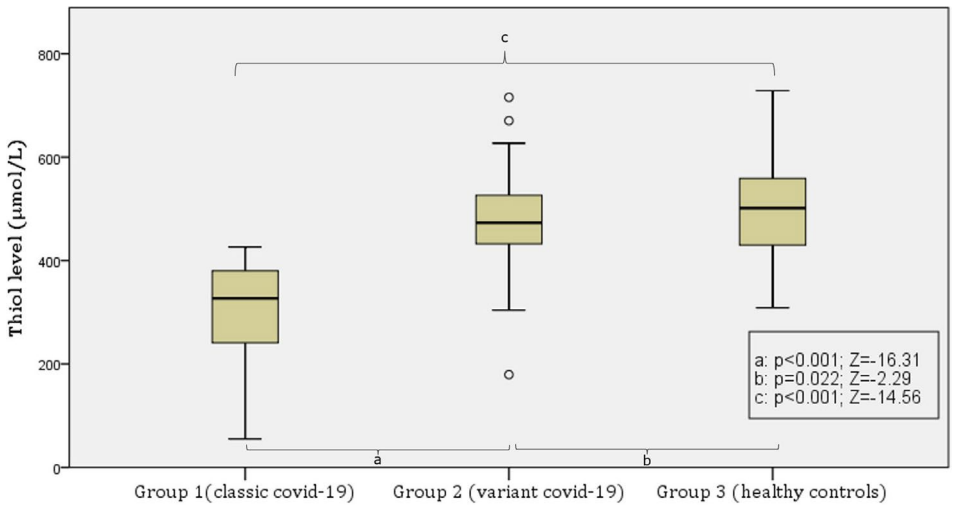

FIGURE 1 Box plot graph of thiol levels between groups and binary comparisons

lower levels were observed in other parameters. However, similar levels were observed between the United Kingdom and the South African variants in terms of these parameters. We believe that these results can be interpreted as variant COVID-19 infection causes less oxidative stress compared with classic COVID-19 infection, whereas UK and South African variants cause similar levels of oxidative stress. However, we could not find enough literature to reach a more straightforward interpretation of this issue.

Finally, according to the results of the ROC analysis, we found that if the cut-off value of thiol was taken as 423 μmol/L as the predictive value in distinguishing classic COVID-19 patients from

TABLE 3 ROC analysis results of thiol levels to predict COVID-19 patients

	Between classic COVID-19 patients and control group	Between variant COVID-19 patients and control group	Between classic and variant COVID-19 patients
Cut-off value of thiol levels (μmol/L)	423	627	422
AUC (95% CI)	0.95 (0.93-0.97)	0.57 (0.51-0.62)	0.96 (0.94-0.98)
Sensitivity	0.78	0.11	0.80
Specificity	0.99	0.99	0.99
P value	<.001	.022	<.001

Abbreviations: AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

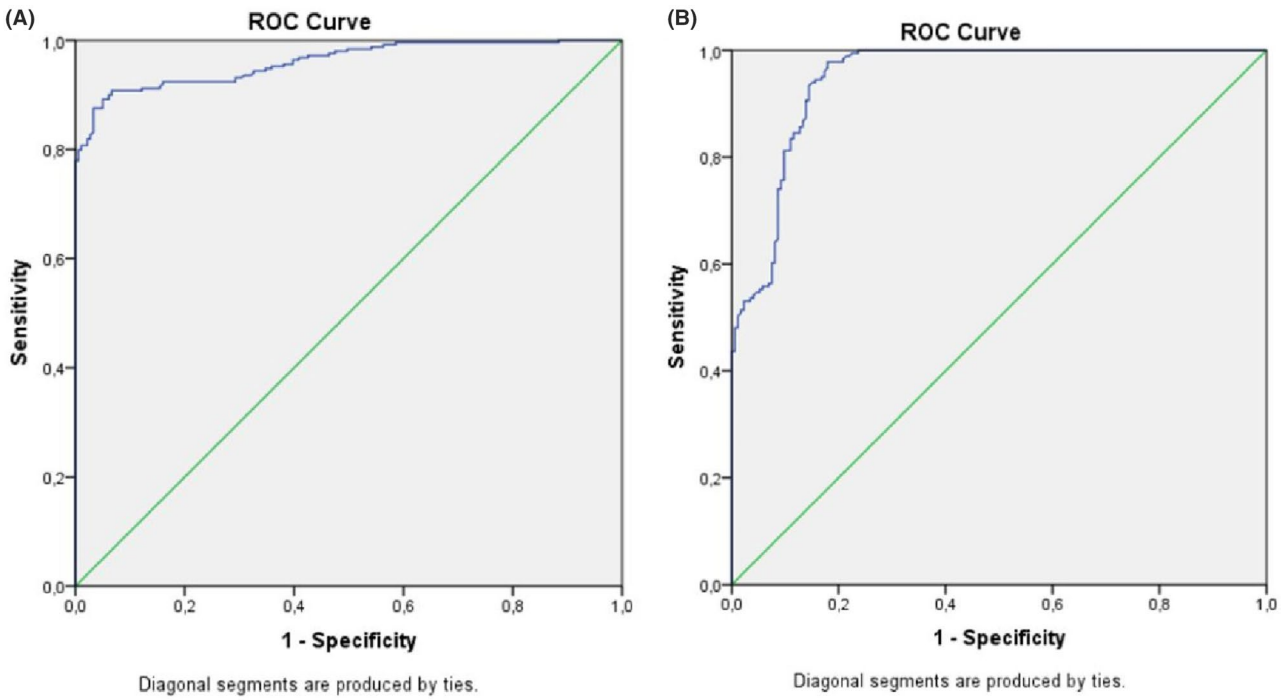

FIGURE 2 AUC of thiol in the distinction between various groups ((A) between classic and variant COVID-19 patients, (B) between classic COVID-19 patients and control group). AUC, area under the curve; ROC, receiver operating characteristic

the healthy control group, it could work as a highly sensitive and specific marker. In another remarkable study, Erel et al calculated the optimal cut-off value of the thiol level as 323 μmol/L between the control group and the patient group, which they had formed by 517 COVID-19 patients of all severity.[17] It seems highly probable that the higher cut-off value in our study is since our patients had a diagnosis of COVID-19 with only mild and moderate clinical course. Interestingly, we calculated that thiol could also be used in differentiating classic and variant COVID-19 patients, with a very similar cut-off value. The fact that we could not find a similar study on this subject prevents us from reaching a conclusive opinion about the external validity of this data. However, we consider it exciting that thiol can be a valuable and cost-effective biomarker in predicting variant patients amongst classic COVID-19 patients.

4.1 | Limitations

Although our study included a specific group of patients with variant COVID-19 diagnosis, it was carried out with a relatively high number of participants. However, the generalisability of the findings is not clear, as it still only includes patients followed in a single centre. As another limitation, the mean age of healthy volunteers in the control group was lower than in the patient group, which may have affected some biochemical parameters, including thiol. In the literature, it is seen that enoxaparin, which is used in the routine treatment of the patients in our study, has no effect on the antioxidant system, and the effect of favipiravir is uncertain.[21] The major factors such as age distribution, disease severity and the medication protocol were similar between patient groups. However, further studies are still needed to reveal the clinical use of thiol levels

to identify patients with the variant compared with the classic COVID-19, as there may be some unknown confounding factors.

DISCLOSURE

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data are not shared because of legal restrictions.

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