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Coronavirus Pandemic

Effects of zinc, selenium, iron, and copper on COVID-19 patients and relationship with oxidative stress-induced DNA damage

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) is a highly contagious and pathogenic viral infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, and subsequently spread globally.

Methodology: Individuals were divided into categories (positive and control) according to their polymerase chain reaction (PCR) test results, gender, and age (< 36, 37–46, and 47–56 years). Oxidative stress parameters including 8-hydroxydeoxyguanosine (8-OHdG), total oxidative stress capacity (TOS), and total antioxidant capacity (TAS) in serum samples were investigated by enzyme linked immunosorbent assay (ELISA). Trace elements iron, zinc, copper, and selenium were measured with the Thermo Scientific ICAP-Q ICP-MS device.

Results: Statistical analyses revealed significant differences in 8-OHdG, TOS, and TAS levels between the control and COVID-19 positive groups ($p < 0.01$). Men had higher 8-OHdG and TOS levels than women. Iron, zinc, copper, and selenium did not show a normal distribution in the COVID-19 group; and there was a significant difference in their levels between the control and COVID-19 groups ($p < 0.05$). There was an increase in oxidative DNA damage and a decrease in antioxidant status in COVID-19 positive individuals due to oxidative stress parameter values.

Conclusions: The results will contribute significantly to the current knowledge. Additional assessment of various oxidative stress and antioxidant markers and the use of alternative molecular testing systems are mandatory to further elucidate the effect of COVID-19 on oxidative stress. This study may have a pioneering status in the literature as such a comparative study has not been conducted before.

Key words: SARS-CoV-2; oxidative stress; 8-OHdG, antioxidant.

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Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious and virulent viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The infection first emerged in Wuhan, China, and subsequently spread across the globe. The World Health Organization (WHO) officially designated the outbreak as COVID-19 and declared it a global pandemic on 11 March 2020, when the global death toll surpassed 4,000 cases [1]. The disease is characterized by high transmissibility and a wide spectrum of clinical manifestations, including myalgia, dry cough, fatigue, fever, and dyspnea. Since its initial identification in Wuhan in late 2019, COVID-19 has rapidly disseminated throughout China and internationally [2].

A key pathogenic mechanism of COVID-19 involves excessive release of pro-inflammatory cytokines—commonly referred to as a cytokine storm—which can lead to severe complications such as acute lung injury, acute respiratory distress syndrome (ARDS), shock, and potentially death [2–4]. Previous studies have demonstrated that oxidative stress—resulting from excessive production of reactive oxygen species (ROS) and/or impairment of the antioxidant defense system—plays a critical role in viral replication and the progression of virus-induced diseases [5–7]. Free radicals are reactive chemical byproducts generated during normal metabolic processes. DNA damage refers to alterations in the molecular integrity of genetic material caused by endogenous or exogenous factors. This is a recurrent event in the cell cycle and may lead to mutations, cancer, aging, and eventually cell death. DNA undergoes continuous modification throughout life due to cellular metabolites (such as ROS) and environmental agents, which can result in cellular deterioration and aging in multicellular organisms or cell death in unicellular organisms [3,4]. Several risk factors associated with severe COVID-19 infection overlap with those known to increase oxidative stress. These include advanced age, male gender, low socioeconomic status, hyperglycemia, and obesity [5]. However, it is important to emphasize that correlation does not imply causation. It has been hypothesized that oxidative stress levels increase with

the severity of COVID-19, whereas antioxidant levels may decline in parallel [6]. Viral pneumonia caused by SARS-CoV-2 induces hyperactivation of the immune response in pulmonary tissues through viral replication, and this pathological process is almost invariably accompanied by oxidative stress [7]. ROS play a central role in generating more than 20 different types of oxidative DNA base damages [8,9]. Among these, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most sensitive and is the commonly used biomarker for oxidative DNA damage [10–12]. Guanine has the lowest ionization potential among DNA bases and is particularly susceptible to oxidation [13]. The mechanisms underlying COVID-19–related complications are complex and multifactorial, necessitating specific strategies for effective patient management.

In this study, it was observed that increased production of reactive oxygen and nitrogen species and/or excessive consumption of antioxidant molecules resulted in a shift of the cellular oxidative balance toward oxidants in COVID-19 cases. Given the critical role of cofactors in antioxidant defense, trace elements such as zinc (Zn), selenium (Se), iron (Fe), and copper (Cu)—elements amenable to laboratory testing in our setting—were also analyzed in both healthy individuals and COVID-19–positive individuals suspected of having elevated oxidative stress. One possible explanation for the reduced trace element levels observed in COVID-19 patients is their depletion due to increased oxidative damage. The combined evaluation of these parameters (8-OHdG, TAS, TOS, Fe, Zn, Cu, and Se) offered a new perspective by demonstrating that oxidative stress and the antioxidant response are complex and multidimensional processes that cannot be adequately captured by a single biomarker.

8-OHdG is a specific indicator of oxidative DNA damage and reflects direct cellular-level injury. TOS represents the overall oxidant burden in the body, whereas TAS indicates the available antioxidant defense capacity against this burden. The integrated assessment of these three measures provides a comprehensive view of the interplay among oxidant formation, conversion of this burden into cellular damage, and the defensive response generated. Furthermore, transition metals such as Fe and Cu contribute to oxidative stress by enhancing free radical formation, while trace elements such as Zn and Se serve as regulatory components of antioxidant defense systems as cofactors of key enzymes (e.g., superoxide dismutase (SOD), and glutathione peroxidase (GPx)). Therefore, including metal levels offers critical insights not only into the magnitude of oxidative stress but also into the underlying mechanisms through which this stress develops. Trace element measurements may be important for the diagnosis and management of various diseases. Therefore, assessing trace elements in serum and urine samples constitutes an essential component of accurate diagnosis and appropriate therapeutic planning. Collectively, these findings suggest that oxidative stress markers may be elevated in individuals testing positive for COVID-19. Although their precise clinical significance remains to be fully elucidated, this study provides preliminary data that may inform future research.

Methodology

Research participants

Samples were randomly collected from COVID-19 positive patients admitted to the Çankırı State Hospital's Medical Microbiology and Infectious Diseases Outpatient Clinic. The selected patients were those with moderate COVID-19. The control group was selected from randomized healthy individuals with negative polymerase chain reaction (PCR) test results. Samples were collected immediately after diagnosis and stored at – 80 °C until the time of the study. In order to investigate oxidative stress parameters and trace element levels, patient groups were divided into a control group, and a COVID-19 positive group. The participants were also grouped based on their age (36, 37–46, and 47–56 years) and gender because oxidative stress can vary in these categories. In addition to the oxidative stress parameters, trace elements were compared between control and COVID-19 positive groups.

The study received approval from the Ministry of Health and the Harran University Clinical Research Ethics Committee.

Methods

The samples were collected from patient groups separated by age and gender. Healthy patients who tested negative for COVID-19 were assigned to the control group, and those who tested positive were assigned to the COVID-19 positive group. Oxidative stress parameters including 8-OHdG, total antioxidant (TAS), and TOS were measured spectrophotometrically with commercial enzyme-linked immunosorbent analysis (ELISA) kits. For trace element analysis, Fe, Zn, Cu, and Se were measured with the Thermo Scientific ICAP-Q ICP-MS (USA) device. The analysis was carried out according to the EPA 200.8 standard.

Total antioxidant test (TAS)

In order to measure TAS, the ELABSCIENCE kit (Cat No: E-B801C-K; Hubai, China) was added to the 96-well microplate with 10 mL of samples (control and COVID-positive) following the ELISA protocol provided in the kit. Standards (standard solutions included in the kit) were prepared with ethanol according to the manufacturer's protocol at concentrations of 2 mmol/L, 1.8 mmol/L, 1.6 mmol/L, 1.4 mmol/L, 1.2 mmol/L, 0.8 mmol/L, 0.4 mmol/L, and 0 mmol/L. 10 mL of sample and 10 mL of standards were added to the wells. Then 200 mL of reagent 1 was added to each well and the first calorimetric reading was taken at 660 nm with the Thermo Scientific Plate Reader spectrophotometer (USA) and recorded as A1. Then 20 □L of Reagent 2 was added and incubated at 37 °C for 5 min in a Nüve Cooled incubator (Ankara, Turkey). The second reading was taken at 660 nm upon the conclusion of the incubation period, and recorded as A2. The difference between the two readings (A2 – A1) was calculated.

Total oxidative stress test (TOS)

The ELABSCIENCE (Cat No: E-BC-K802-M) (China, Hubai) kit was used for TOS analysis. 20 □L of serum samples from the control and COVID-19 positive group were added to the 96-well microplate according to the ELISA procedure recommended by the manufacturer. Standard solutions included in the kit were prepared with distilled water at concentrations of 100 □mol/L, 80 □mol/L, 60 μmol/L, 50 □mol/L, 40 □mol/L, 20 □mol/L, 10 □mol/L, and 0 □mol/L; and added at 20 mL. 200 □L of Reagent 1 was added to each well and the first calorimetric reading was taken at 590 nm on a Thermo Scientific Plate Reader spectrophotometer (USA) and recorded as A1. Then 50 □L of Reagent 2 was added and incubated at 37 °C for 5 min in a Nüve Cooled incubator (Ankara, Turkey). At the end of the incubation period, the second reading was taken at 660 nm and recorded as A2. The difference between the two readings (A2 – A1) was calculated.

8- hydroxy-deoxyguanosine (8-OHdG)

The 96-well microplate of the BT-LAB kit (Cat No: EA0048HN; Hubai, China) was used for measuring the 8-OHdG levels. The standard solutions were prepared according to the ELISA procedure recommended in the kit, and 50 □L standard solutions of 50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL, and 800 ng/mL concentrations were added to the plate. 50 □L of serum samples from the control and COVID-19 positive groups were added in the remaining wells. Then, 50 □L biotinylated antigen was added to each well and incubated in a Nüve Cooled incubator (Ankara, Turkey) at 37 ° C for 60 min. The incubated plate was washed 5 times with 300 □L washing solution. Then 50 □L avidin HRP was added. This was incubated again at 37 °C for 60 min, and then washed 5 times with 300 □L washing solution. After washing, substrate solution A was added to each well, followed by substrate solution B and incubated at 37 °C for 10 min in the dark. After removal from the incubator, 50 □L of stop solution was added to each well and measured spectrophotometrically at 450 nm.

Trace elements analysis

The elements Fe, Zn, Se, and Cu were analyzed because they are used the most in cellular metabolism. For trace element analysis, Fe, Zn, Cu, and Se were measured with the Thermo Scientific ICAP-Q ICP-MS device (Massachusetts, USA). The study was performed using the ICP-MS device located in the Uşak University Scientific Research Center (UBATAM). After optimizing the device, the analysis was carried out following the manufacturer's protocol. After the samples and standards are prepared, the Chiller unit, autosampler and ICP-MS device are turned on (calibration standards: Inorganic Ventures, WW-CAL-5 (Lot number: V2-MEB740749), WW-CAL-3 (Lot number: V2-MEB730238), WW-CAL-4A (Lot number: V2-MEB729687)).

Table 1. Descriptive statistics of the participants in the study.

Characteristics	Frequency (n)	Percentage (%)
Gender		
Female	150	50
Male	150	50
Age (years)		
< 36	120	40
37–46	90	30
47–56	90	30
Group		
Control	100	33.3
COVID-19 +	200	66.7
8-OHdG		
SD	437.87 ± 96.60	
Min-max	311.10–596.90	
Skewness-Kurtosis	0.218, 1.349	
TAS		
SD	0.92 ± 0.66	
Min-max	0.282.90	
Skewness-Kurtosis	0.9940.384	
TOS		
SD	26.38 3.85	
Min-max	16.9733.78	
Skewness-Kurtosis	0.4890.160	

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8 hydroxy-deoxyguanosine.

Table 2. Differences between 8-OHdG, TAS, and TOS values based on gender.

	Categories	SD	
8-OHdG	Female	424.83 89.42	0.019
	Male	450.91101.93	
TAS	Female	1 ± 0.70	0.036
	Male	0.840.62	
TOS	Female	24.95 ∓ 3.54	0.001**
	Male	27.81∓3.63	

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHDG: 8-hydroxy-deoxyguanosine; N: number; ** $p < 0.001$.

Statistical analysis

IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL) was used to analyze the data. Descriptive categorical data are shown as numbers (n) and percentages (%); quantitative data comparing the OHdG, TAS and TOS values showed normal distribution; therefore, independent sample t test was used to compare the means of the two independent groups and one way analysis of variance (ANOVA) was used to compare the means of more than two groups. There was no missing data. $p < 0.05$ was determined to be the significance level.

In the case of the trace element data, the Mann Whitney U test was used to determine whether the descriptive statistics and values of Fe, Zn, Cu, and Se levels in the control and COVID-19 positive groups differed. There was no missing data. The significance level was determined to be $p < 0.05$.

Results

There were significant differences between the control group, and the COVID-19 positive group. Table 1 summarizes the descriptive statistics (n, percentage, mean, standard deviation, skewness, and kurtosis) for the study participants. It was found that 50% of the participants were female and 50% were male, 40% of the participants were under the age of 36 years, 30% were between the ages of 37–46 years, and 30% were between the ages of 47–56 years. When the control and COVID-19 positive groups were examined, it was observed that 33.3% were in the control group and 66.7% were in the COVID-19 positive group. The average 8 OHdG value (for all participants) was 437.87 ± 96.60, with minimum 311.10, and maximum 596.90. The average TAS value was 0.92 ± 0.66, with minimum 0.28, and maximum 2.90; and the average TOS value 26.38 ± 3.85, with minimum 16.97, and maximum 33.78. The skewness and kurtosis values of the 8 OHdG, TAS, and TOS parameters were in the range of ± 1.5 and were compliant with the normal distribution (Tabachnick). Therefore, parametric methods were used for comparing the means.

Independent sample *t* test was performed to examine the differences between 8 OHdG, TAS, and TOS values for men and women (Table 2). A significant difference was found between the 8 OHdG values of men and women ($p < 0.05$). Accordingly, it was found that the mean of 8-OHdG values of the men (mean = 450.91) was higher than that of the women (mean = 424.83). A significant difference was found between the mean TAS of men and women ($p < 0.05$). Accordingly, it can be said that the mean TAS of women (mean = 1) was higher than that of men (mean = 0.84) ($p < 0.001$). The mean TOS value of the men (mean = 27.81) was also higher than that of women (mean = 24.95).

One way analysis of variance (ANOVA) was performed to examine the differences between 8-OHdG, TAS, and TOS parameters according to age groups (Table 3). In the case of a significant difference, Scheffe test was used when group variances were homogeneous, and Tamhane's T2 test was used when they were not homogeneous. A significant difference was found between the mean 8-OHdG according to age

Table 3. Differences between 8-OHdG, TAS, and TOS values according to age groups.

	Years	SD	*p*	Differences between groups
8-OHdG	1	388.7452.25	0.001**	1–2, 3 2–3
	2	442.5491.34		
	3	498.72111.08		
TAS	1	1.100.73	0.001**	1-3
	2	0.900.65		
	3	0.680.48		
TOS	1	24.693.86*	0.001**	1–2, 3 2–3
	2	26.663.23*		
	3	28.363.41*		

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8-hydroxy-deoxyguanosine; N: number. The F test statistic was obtained from the ratio of the mean squares between groups to the mean squares within groups. **$p < 0.001$. [1] Under 36 years old; [2] Between 37–46 years; [3] Between 47–56 years.

Table 4. Comparison of 8-OHdG, TAS, and TOS parameters between COVID-19 positive and control groups.

	Categories	SD	*p*
8-OHdG	Control	326.9711.86	0.001**
	COVID-19 +	493.3368.42	
TAS	Control	1.790.39	0.001**
	COVID-19 +	0.480.13	
TOS	Control	22.603.40	0.001**
	COVID-19 +	28.272.41	

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8-hydroxy-deoxyguanosine; N: number; independent sample T: **$p < 0.001$.

groups ($p < 0.001$). Accordingly, the mean 8-OHdG of those under 36 years of age (mean = 388.74) was lower than those between 37–46 years of age (mean = 442.54) and those between 47–56 years of age (mean = 498.72). It was also found that the mean 8-OHdG of those between 37–46 years of age (mean = 442.54) was lower than those between 47–56 years of age (mean = 498.72). A significant difference was found in mean TAS of the different age groups ($p < 0.001$). Accordingly, it was found that the mean TAS of those under 36 years of age (mean = 1.10) was higher than those between 47–56 years of age (mean = 0.68). A significant difference was found between age groups in terms of mean TOS ($p < 0.001$). Accordingly, it was found that the mean TOS of those under 36 years of age (mean = 24.69) was lower than those between 37–46 years of age (mean = 26.66) and those between 47–56 years of age (mean = 28.36). Also, the mean TOS of those between 37–46 years of age (mean = 26.66) was lower than those between 47–56 years of age (mean = 28.36).

The 8-OHdG, TAS, and TOS values were compared between the control and COVID-19 positive groups (Table 4). Independent sample t test was used to examine the differences between the two groups. There was a significant difference between the 8-OHdG means of the control and COVID-19 positive groups ($p < 0.001$). The mean 8-OHdG of the control group (mean = 326.97) was lower than that of the COVID-19 positive group (mean = 493.33). There was a significant difference in the mean TAS values of the control and COVID-19 positive groups ($p < 0.001$). Accordingly, the mean TAS of the control group (mean = 1.79) was higher than that of the COVID-19 positive group (mean = 0.48). There was a significant difference between the control and COVID-19 positive groups in terms of TOS values ($p < 0.001$). Accordingly, the mean TOS of the control group (mean = 22.60) was lower than that of the COVID-19 positive group (mean = 28.27).

Pearson correlation analysis was performed to examine the relationship between 8-OHdG, TAS, and TOS parameters according to the groups (Table 5). There was a significant, negative, and quite strong relationship between the 8-OHdG value and TAS in the control group ($p < 0.001$). Accordingly, it can be said that the TAS values will decrease as the 8-OHdG increases for the control group. In addition, a significant, positive and strong relationship was found between the 8-OHdG value and TOS value in the control group ($p < 0.001$). Thus, the TOS value will increase as the 8-OHdG value increases in the control group. In addition, a significant, negative, and moderate relationship was found between TAS and TOS ($p < 0.001$). Thus, the TOS value decreased as the TAS increased in the control group. A significant, negative and strong relationship was found between the 8-OHdG value and TAS in the COVID-19 positive group ($p < 0.001$). The TAS value decreased as the 8-OHdG increased in the COVID-19 positive group. In addition, there was a significant, positive, and moderate relationship between the 8-OHdG and TOS values of the COVID-19 positive group ($p < 0.001$); when the 8-

Table 5. Relationships between 8-OHdG, TAS, and TOS according to the groups.

Groups			8-OHDG	TAS	TOS
Control	8-OHDG	r	1	-0.818^{**}	0.802^{**}
		p		0.001	0.001
	TAS	r	-0.818^{**}	1	-0.742^{**}
		p	0.001		0.001
	TOS	r	0.802^{**}	-0.742^{**}	1
		p	0.001	0.001	
COVID-19+	8 OHDG	r	1	−0.814**	0.553**
		p		0.001	0.001
	TAS	r	−0.814**	1	−0.555**
		p	0.001		0.001
	TOS	r	0.553**	−0.555**	1
		p	0.001	0.001	

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHDG: 8-hydroxy-deoxyguanosine; N: number. Pearson correlation analysis: The correlation coefficient is shown as r and the r values range between –1 and +1. **$p < 0.001$.

Table 6. Comparison of 8-OHdG, TAS, and TOS between groups according to gender.

Gender	Parameters	Groups	SD	*p*
Female	8-OHDG	Control	324.0110.08	0.001**
		COVID-19+	475.2565.45	
	TAS	Control	1.900.44	0.001**
		COVID-19+	0.540.13	
	TOS	Control	21.233.47	0.001**
		COVID-19+	26.801.56	
Male	8-OHDG	Control	329.93 ± ∓12.83	0.001**
		COVID-19+	511.4066.83	
	TAS	Control	1.670.30	0.001**
		COVID-19+	0.420.10	
	TOS	Control	23.972.72	0.001**
		COVID-19+	29.742.21	

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8-hydroxy-deoxyguanosine; N: number; **$p < 0.001$.

OHdG values increased in the COVID-19 positive group, the TOS values also increased. There was a significant, negative, and moderate relationship between the TAS and TOS values of the COVID-19 positive group; as the TAS values of the group increased, the TOS value decreased.

Independent sample *t* test was applied to compare the 8-OHdG, TAS, and TOS parameters between the groups according to gender (Table 6). There was a significant difference in the 8-OHdG, TAS, and TOS values of the control and COVID-19 positive groups among women ($p < 0.001$). Accordingly, the mean 8-OHdG of the control group among the women (mean = 324.01) was lower than the mean of the COVID-19 positive group (mean = 475.25). The mean TAS of the women in the control group (mean = 1.90) was higher than the COVID-19 positive group (mean = 0.54). The mean TOS of the women in the control group (mean = 21.23) was lower than the COVID-19 positive group (mean = 26.80). There was a significant difference between the 8-OHdG, TAS, and TOS values between the control and COVID-19 positive groups among the men ($p < 0.001$). Accordingly, the mean 8-OHdG of the men in the control group (mean = 329.93) was lower than that of the COVID-19 positive group (mean = 511.40). The mean TAS of the men in the control group (mean = 1.67) was higher than the men in the COVID-19 positive group (mean = 0.42). The mean TOS of the men in the control group (mean = 23.97) was lower than the men in the COVID-19 positive group (mean = 29.74).

The one-way ANOVA test was performed to compare the 8-OHdG, TAS, and TOS parameters between the different age levels in the control and COVID-19 positive groups (Table 7). Post hoc analysis was performed to determine which groups differed. When the group variances were homogeneous, Scheffe test was applied; and when they were not homogeneous, Tamhane's T2 test was applied. A significant difference was found between the mean 8-OHdG of the different age groups in the control group ($p < 0.001$). The post hoc analysis was performed to determine which groups differed, and it was determined that the mean 8-OHdG of those under 36 years of age (mean = 316) was lower

Table 7. Comparison of 8-OHdG, TAS, and TOS parameters among age levels according to groups.

Groups	Parameters	Years	SD	*p* value	Differences between groups
Control	8-OHdG	1	3162.21	0.001**	1–2, 3 2–3
		2	325.643.09		
		3	342.935.72		
	TAS	1	2.110.31	0.001**	1–2, 3 2–3
		2	1.800.19		
		3	1.350.16		
	TOS	1	19.641.64	0.001**	1–2, 3 2–3
		2	23.252.40		
		3	25.892.50		
COVİD-19+	8 OHdG	1	425.119.55	0.001**	3–2, 1 2,1
		2	500.9946.47		
		3	576.619.39		
	TAS	1	0.600.10	0.001**	3–2, 1 2,1
		2	0.450.08		
		3	0.350.06		
	TOS	1	27.211.34	0.001**	3–2, 1 2,1
		2	28.362.01		
		3	29.593.14		

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8-hydroxy-deoxyguanosine; N: number; **$p < 0.001$. Post hoc analysis, F value, *p* value, Tamhane's T2. [1] under 36 years old; [2] between 37–46 years; [3] between 47–56 years.

Table 8. Descriptive statistics of the parameters of healthy and COVID-19 patient groups.

Group	Variables	SD	Median	Minimum	Maximum	Skew.	Kurt.
Control	Iron	28.05 ± 1.43	28.33	23.24	29.68	– 1.369	3.182
	Zinc	4.02 ± 0.41	4.04	3.28	4.87	– 0.322	-0.555
	Copper	88.50 ± 16.92	92.45	14.72	99.31	– 3.528	13.897
	Selenium	679.14 ± 79.86	673.50	469	790.2	– 1.007	1.815
COVID-19+	Iron	25.17 ± 5.02	26.78	4.15	29.68	– 2.490	6.877
	Zinc	3.60 ± 0.59	3.62	1.63	4.87	– 1.237	2.972
	Copper	86.34 ± 630.26	4.95	3.20	4887	7.746	60
	Selenium	482.86 ± 69.83	501.80	243.1	615	– 1.143	2.311

SD: standard deviation; TAS: total antioxidant system; TOS: total oxidative system; 8-OHdG: 8-hydroxy-deoxyguanosine; N: number; *$p < 0.001$.

than those between 37–46 years of age (mean = 325.64) and those between 47–56 years of age (mean = 342.93). It was also found that the mean 8-OHdG of those between 47–56 years of age (mean = 342.93) was higher than those between 37–46 years of age (mean = 325.64). A significant difference was found between the mean TAS of the different age groups in the control group ($p < 0.001$). According to the post hoc analysis conducted to determine which groups differed, the mean TAS of those under 36 years of age (mean = 2.11) was higher than those between 37–46 years of age (mean = 1.80) and those between 47–56 years of age (mean = 1.35). In addition, the mean TAS of those between 47–56 years of age (mean = 1.35) was lower than those between 37–46 years of age (mean = 1.80). There was a significant difference between the mean TOS of the different age groups in the control group ($p < 0.001$). According to the post hoc analysis conducted to determine which groups had differences, it was found that the mean TOS of those under 36 years of age (mean = 19.64) was lower than of those between 37–46 years of age (mean = 23.25) and of those between 47–56 years of age (mean = 25.89). In addition, the mean TOS of those between 47–56 years of age (mean = 25.89) was higher than of those between 37–46 years of age (mean = 23.25). There was a significant difference between the mean 8-OHdG of those in the COVID-19 positive group according to their age levels ($p < 0.001$). According to the post hoc analysis conducted to determine which groups had differences, it was determined that the mean 8-OHdG of those under 36 years of age (mean = 425.11) was lower than of those between the ages of 37–46 (mean = 500.99) and 47–56 years (mean = 576.61). In addition, it was found that the mean 8-OHDG of those between the ages of 47–56 years (mean = 576.61) was higher than of those between the ages of 37–46 years (mean = 500.99). A significant difference was found between the mean TAS of those in the COVID-19+ group according to their age levels ($p < 0.001$). According to the post hoc analysis conducted to determine which groups had differences, it was determined that the mean TAS of those under 36 years of age (mean = 0.60) was higher than those between the ages of 37–46 (mean = 0.45) and 47–56 (mean = 0.35) years. In addition, it was found that the mean TAS of those aged 47–56 years (mean = 0.35) was lower than of those aged 37–46 years (mean = 0.45). A significant difference was found between the mean TOS of those in the COVID-19 positive group according to their age levels ($p < 0.001$). According to the post hoc analysis conducted to determine which groups differed, mean TOS of those under 36 years (mean = 27.21) was lower than those aged 37–46 (mean = 28.36) and 47–56 (mean = 29.59) years.

Descriptive statistics (n, mean, standard deviation, median, minimum, maximum) for the parameters of the control and COVID-19 patient groups are in presented in Table 8. When the skewness and kurtosis values of the parameters of the control group were examined, it was determined that the Fe and Cu values did not show a normal distribution, while the Zn and Se values showed a normal distribution. The Fe, Zn, Cu, and Se values did not show a normal distribution in the COVID-19 positive group.

Therefore, the Mann Whitney U test was used to compare these values (Table 9). A significant difference

Table 9. Comparison of trace element parameters in control and COVID-19 groups.

Trace elements	Gruoup	Average ordinal numbers	*p* value
Iron (Fe)	Control	58.48	0.001*
	COVID-19+	37.98	
Zinc (Zn)	Control	58.21	0.001*
	COVID-19+	38.10	
Copper (Cu)	Control	73.5	0.001*
	COVID-19+	30.97	
Selenium (Se)	Control	71.50	0.001*
	COVID-19+	31.90	

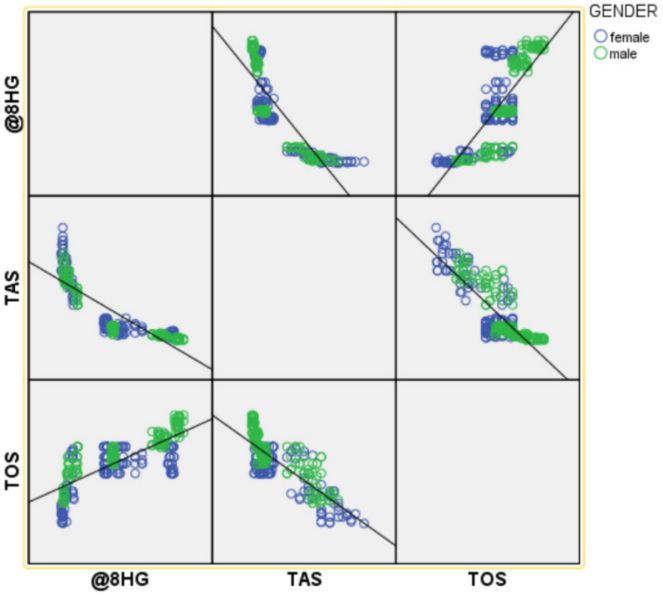
*$p < 0.001$.

was found in terms of Fe levels between the control and COVID-19 patient groups ($p < 0.05$). Accordingly, it was found that the mean Fe values of the control group (SD mean = 58.48) was higher than the value of COVID-19 patients (SD mean = 37.98). A significant difference was found in terms of Zn levels between the control and COVID-19 patient groups ($p < 0.05$). The average Zn levels (SD mean = 58.21) of the control group was higher than the level in the COVID-19 patients (SD mean = 38.10). A significant difference was found in terms of Cu levels in the control group and COVID-19 patient groups ($p < 0.05$). It was found that mean Cu level in the control group (SD mean = 73.50) was higher than mean of the COVID-19 patients (SD mean = 30.97). A significant difference was found in terms of Se levels between the control group and COVID-19 patient groups ($p < 0.05$). Accordingly, the mean Se levels in the control group (mean = 71.50) was higher than in the COVID-19 patient group (mean = 31.90).

Partial correlation analysis was applied to examine the relationships between 8-OHdG, TAS, and TOS when controlling for gender. Controlling for gender revealed a significant, negative, and strong relationship between 8-OHdG and TAS ($p < 0.001$), a significant, positive, and strong relationship between 8-OHdG and TOS ($p < 0.001$), and a negative, significant, and strong relationship between TAS and TOS ($p < 0.001$).

The scatter plot of the relationship between 8-OHdG, TAS, and TOS parameters, controlling for gender, is presented in Figure 1.

Figure 1. Scatter plot of the relationship between 8-OHdG, TAS, and TOS parameters, controlling for gender.



Discussion

Oxidative stress is continuously generated as a byproduct of normal metabolic processes. Free radicals are produced both through endogenous metabolic pathways involving oxygen and via exogenous factors such as radiation, pharmaceuticals, xenobiotics, and other toxic chemicals. The accumulation of these reactive species contributes to oxidative stress, which exerts cytotoxic and genotoxic effects. The antioxidant defense system must neutralize these reactive molecules to maintain homeostasis and ensure organismal survival. Enzymatic and non-enzymatic components of the antioxidant system perform this essential function by scavenging excess free radicals, thereby preserving cellular integrity and viability [14,15]. However, when oxidative stress exceeds the capacity of these defense mechanisms, oxidative damage occurs—a process implicated in the pathogenesis of over 100 diseases [16]. Several studies have examined gender-based differences in susceptibility to COVID-19 [17]. A systematic review reported that the pooled prevalence of COVID-19 cases was significantly higher in males (55.00%; 95% CI: 51.43–56.58) than in females (45.00%; 95% CI: 41.42–48.57), with substantial heterogeneity ($I^2 = 99.5\%$, $p < 0.001$). Similarly, a population-based study in Ontario, Canada, found that males were more likely to test positive for SARS-CoV-2 [18–25]. Consistent findings were reported in Pakistan, where 72% of confirmed COVID-19 cases occurred in males. Data from Global Health also indicate that both the incidence and mortality rates of COVID-19 are consistently higher among males across multiple countries [26].

Experimental studies using animal models suggest that estrogen may exert a protective effect against coronavirus infections by modulating immune responses. Specifically, estrogens have been shown to attenuate the acute inflammatory phase and suppress cytokine release [27]. One study demonstrated that female mice treated with estrogen receptor antagonists exhibited mortality rates comparable to male mice [28]. Estrogens activate manganese superoxide dismutase and GPx expression via estrogen receptors, as well as through the mitogen-activated protein kinase (MAPK) and NF-κB pathways. Early initiation of estrogen therapy has been shown to mitigate oxidative and metabolic stress [29].

This study supports the observation that oxidative stress is more pronounced in males with COVID-19, compared to females. While higher infection rates among males may be partly attributable to greater social

exposure, the elevated mortality observed in this group could result from oxidative stress-induced inflammation and apoptosis. Qin *et al.* reported that COVID-19 activates phagocytic cells, leading to excessive ROS production that overwhelms the antioxidant defense system [30]. Another study confirmed increased oxidative stress in COVID-19 patients, accompanied by altered levels of glutathione (GSH), SOD, and GPx [31].

Furthermore, levels of 8-OHdG measured via high performance liquid chromatography (HPLC) were significantly higher in COVID-19 patients compared to healthy controls. Although serum SOD and GPx levels were elevated, GSH levels were markedly reduced. Both malondialdehyde (MDA) and 8-OHdG levels were significantly higher in COVID-19 patients and positively correlated with disulfide levels [32]. In this study, 8-OHdG was measured using ELISA kits to evaluate its diagnostic and prognostic utility in COVID-19. The results were consistent with previous reports: 8-OHdG levels were lowest in healthy individuals and significantly higher in PCR-positive COVID-19 patients.

COVID-19 induces infection, inflammation, increased oxidative stress, ROS accumulation, and impairment of the antioxidant defense system. The findings reinforce the critical role of oxidative stress in COVID-19 pathogenesis and highlight its contribution to oxidative DNA damage. One study assessed MDA and TOS as markers of oxidative load, alongside TAS, SOD, catalase (CAT), and glutathione reductase (GR) as indicators of antioxidant capacity, particularly in relation to smoking. The results indicated that smoking exacerbated oxidative damage and significantly diminished antioxidant defenses [33]. Another study examining the combined effects of smoking and COVID-19 found that antioxidant capacity decreased by 27.4% among non-smokers diagnosed with COVID-19, compared to healthy controls ($p < 0.001$); whereas in smokers, the reduction reached 33.8% ($p < 0.001$). Comparisons of SOD and CAT levels across non-smokers and smokers with or without COVID-19 demonstrated significant reductions, with the most pronounced decreases observed in COVID-19 patients who smoked. GR levels were similarly reduced, suggesting that both smoking and SARS-CoV-2 infection exacerbate oxidative stress [34].

Acute respiratory distress in COVID-19 involves severe oxidative damage, with 8-OHdG serving as a key biomarker of such damage. COVID-19 exhibits a spectrum of severity across individuals, and understanding why some patients are more susceptible to severe outcomes could inform more effective therapeutic strategies. Notably, there is a paucity of studies investigating oxidative DNA damage across different disease severity levels (outpatient, hospitalized, and ICU patients).

Oxidative stress induces the oxidation of lipids, proteins, and DNA, leading to cellular damage and death, thereby contributing to aging and the development of chronic diseases [35]. Older adults are particularly susceptible to severe forms of COVID-19. One study reported that MDA and protein carbonyl (PC) levels increase with age, suggesting that aging and age-related oxidative stress exacerbate disease severity [36]. Aging is also associated with a decline in glutathione synthesis and overall antioxidant defense capacity, resulting in elevated levels of ROS and the establishment of a pseudohypoxic state characterized by reduced NAD^+/NADP levels [37,38]. Notably, NAD^+ levels are lowest in the elderly and highest in children [26,36]. Oxidative stress further activates NAD^+-dependent enzymes such as poly (ADP-ribose) polymerase 1 (PARP1), whose excessive activation leads to NAD^+ and ATP depletion, energy failure, and cell death, thereby initiating a pro-inflammatory cascade [39].

Trace element levels in both patient and healthy groups were measured to support the findings. Trace elements play vital roles in metabolic pathways. COVID-19 has been shown to affect oxidative stress and alter trace element levels. For instance, inflammation in COVID-19 patients generates superoxide radicals, especially in the lungs. These are converted to hydrogen peroxide by SOD (which contains Zn), and then detoxified by catalase and peroxidase [39]. Therefore, Zn is a crucial cofactor in antioxidant defense [40]. A study involving 135 patients and 26 healthy individuals found that critically ill patients were older than those with milder symptoms. Zn levels were significantly lower in moderate, severe, and critical patients than in mild cases and controls. Moreover, Zn levels negatively correlated with inflammatory markers such as C reactive protein (CRP), ferritin, D-dimer, and fibrinogen [40]. Zn's immunomodulatory and antiviral effects suggest that it may be a useful supportive treatment. Some studies indicate that Zn supplementation can enhance the effects of treatments like hydroxychloroquine and reduce respiratory symptoms [41]. The findings demonstrated that Zn levels were higher in the healthy control group, aligning with previous reports in the literature. Reduced Zn concentrations highlight the immunomodulatory roles of Zn, Cu, and Mg in host

defense [44]. In their study involving 150 COVID-19 patients and 50 healthy controls, serum Zn and Cu levels were significantly lower in patients than in controls. Notably, Zn concentrations were particularly reduced in severe cases, whereas Cu levels exhibited a paradoxical pattern—elevated in severe cases but overall lower than in healthy individuals [42]. Similarly, in this study, Cu levels were higher among healthy participants. Although copper is an essential trace element, its imbalance during inflammatory conditions may exacerbate oxidative stress. Fe is another vital element whose metabolism is affected during infection. Fe deficiency has been associated with impaired immune function, including decreased neutrophil and macrophage activity, and altered cytokine production [43,44]. COVID-19 infection disrupts Fe homeostasis, particularly in the elderly, potentially exacerbating disease severity [45]. The findings confirm that Fe levels were higher in the healthy group. Optimizing Fe intake may help prevent immune complications caused by SARS-CoV-2. Se, though toxic in high doses, is an essential trace element and a cofactor for various antioxidant enzymes, including GPx and thioredoxin reductase. Se also plays a role in immune and endocrine regulation [46]. Se deficiency may compromise antioxidant defenses, suggesting that supplementation could be beneficial during the COVID-19 pandemic [47,48]. The Zn-to-Cu ratio thus represents a critical determinant in mitigating Cu-induced oxidative effects. While Cu levels are known to fluctuate during infectious processes, including viral infections, specific data on Cu homeostasis in the context of COVID-19 remain limited [48]. This study confirmed that Se levels were higher in the healthy group, aligning with the literature. Se's antioxidant and immunoregulatory properties make it a candidate for adjunctive therapy [49].

In summary, COVID-19 disrupts the balance between oxidants and antioxidants, leading to significant oxidative damage. Maintaining optimal levels of trace elements such as Zn, Cu, Fe, and Se may mitigate disease severity and improve clinical outcomes.

Conclusions

It is well established that the excessive production of reactive oxygen and nitrogen species, together with the increased consumption of antioxidant molecules in COVID-19 patients, disrupts the cellular redox balance in favor of oxidants. Consequently, the role of cofactors in antioxidant defense mechanisms becomes increasingly important. In this study, 8-OHdG, TAS, and TOS levels were compared between healthy and COVID-19 positive individuals, stratified by gender. Significant differences were observed, indicating that oxidative damage was higher in men than in women. Moreover, higher average oxidative damage was detected among men in both the healthy and COVID-19 positive groups.

The analysis also revealed that metabolic cofactors were present at lower levels in COVID-19 patients, regardless of gender. One possible explanation for the reduced levels of trace elements observed in patients is the increased utilization of antioxidant molecules due to elevated oxidative damage. Collectively, these findings suggest that oxidative stress markers may be elevated in individuals infected with COVID-19, although the extent of their impact remains to be fully elucidated.

This study serves as a preliminary investigation that may contribute to and support future research in this field.

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Ethics approval and consent to participate

The Ministry of Health and Harran University Clinical Research Ethics Committee provided ethics committee permission/approval on 07.02.2022 (approval number 22/03/28).

Authors' contributions

SS, FK, AK, research design; SS, research; FK, AK, help and advice on COVID-19 and biochemical study; SS, FK, data analysis; SS, manuscript writing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

No conflict of interest is declared.

References

1. Akpınar EE (2020) Coronavirus disease 2019 (COVID-19) and the lungs: What pulmonologists need to know. Eurasian J Pulmonol 22 Suppl 1: S8–11. [Article in Turkish]. doi: 10.4103/ejop.ejop_54_20.
2. Singhal T (2020) A review of coronavirus disease-2019 (COVID-19). Indian J Pediatr 87: 281–286. doi: 10.1007/s12098-020-03263-6.
3. Sancar A, Lindsey-Boltz LA, Ünsal-Kaçmaz K, Linn S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73: 39–85. doi: 10.1146/annurev.biochem.73.011303.073723.
4. Rupp WD, Gordienko I (1994) UvrABC cutting at 3′ recessed termini of undamaged DNA may be a model for 5′ incision at damaged sites. Ann N Y Acad Sci 726: 321–323. doi: 10.1111/j.1749-6632.1994.tb52839.x.
5. Williams GM, Jeffrey AM (2000) Oxidative DNA damage: endogenous and chemically induced. Regul Toxicol Pharmacol 32: 283–292. doi: 10.1006/rtph.2000.1433.
6. 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. doi: 10.1016/j.arcmed.2020.04.019.
7. Park YJ, Walls AC, Wang Z, Sauer MM, Li W, Tortorici MA, Bosch BJ, DiMaio F, Veesler D (2019) Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. Nat Struct Mol Biol 26: 1151–1157. doi: 10.1038/s41594-019-0334-7.
8. Mach WJ, Thimmesch AR, Pierce JT, Pierce JD (2011) Consequences of hyperoxia and the toxicity of oxygen in the lung. Nurs Res Pract 2011: 260482. doi: 10.1155/2011/260482.
9. Nicholson LB (2016) The immune system. Essays Biochem 60: 275–301. doi: 10.1042/EBC20160017.
10. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D (2020) Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181: 281–292. doi: 10.1016/j.cell.2020.02.058.
11. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H (2002) Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 32: 1102–1115. doi: 10.1016/S0891-5849(02)00826-2.
12. De Martinis BS, De Lourdes Pires Bianchi M (2002) Methodology for urinary 8-hydroxy-2′-deoxyguanosine analysis by HPLC with electrochemical detection. Pharmacol Res 46: 129–131. doi: 10.1016/S1043-6618(02)00080-4.
13. McDorman KS, Pachkowski BF, Nakamura J, Wolf DC, Swenberg JA (2005) Oxidative DNA damage from potassium bromate exposure in Long-Evans rats is not enhanced by a mixture of drinking water disinfection by-products. Chem Biol Interact 152: 107–117. doi: 10.1016/j.cbi.2004.11.001.
14. Yokuş B, Çakır DÜ (2002) In vivo oxidative DNA damage biomarker: 8-hydroxy-2′-deoxyguanosine. Turkiye Klinikleri J Med Sci 22: 535–43. [Article in Turkish]. doi: 10.5336/medsci.2002.535.
15. Raghu MB, Deshpande A, Chintu C (1981) Oral rehydration for diarrheal diseases in children. Trans R Soc Trop Med Hyg 75: 552–555. doi: 10.1016/0035-9203(81)90197-8.
16. Halliwell B (1991) Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 91: 14S–22S. doi: 10.1016/0002-9343(91)90279-7.
17. Cochrane CG (1991) Cellular injury by oxidants. Am J Med 91: 23S–30S. doi: 10.1016/0002-9343(91)90280-B.
18. Stocker R, Keaney JF Jr (2004) Role of oxidative modifications in atherosclerosis. Physiol Rev 84: 1381–1478. doi: 10.1152/physrev.00047.2003.
19. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39: 44–84. doi: 10.1016/j.biocel.2006.07.001.
20. Tarpey MM, Wink DA, Grisham MB (2004) Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol 286: R431–R444. doi: 10.1152/ajpregu.00361.2003.
21. Ghiselli A, Serafini M, Natella F, Scaccini C (2000) Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med 29: 1106–1114. doi: 10.1016/S0891-5849(00)00394-4.
22. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37: 277–285. [Article in Turkish]. doi: 10.1016/j.clinbiochem.2003.11.015.
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38: 1103–1111. [Article in Turkish]. doi: 10.1016/j.clinbiochem.2005.08.008.
24. Muhamed PK, Vadstrup S (2014) Zinc is the most important trace element. Ugeskr Laeger 176: V11120654. [Article in Turkish]. doi: 10.3410/f.25096007.
25. Scheiber I, Dringen R, Mercer JF (2013) Copper: effects of deficiency and overload. In Sigel A, Sigel H, Sigel RKO, editors. Interrelations between Essential Metal Ions and Human Diseases. Metal Ions Life Sci 13: 359–387. doi: 10.1007/978-94-007-7500-8_11.
26. Miller R, Wentzel AR, Richards GA (2020) COVID-19: NAD+ deficiency may predispose the aged, obese and type 2 diabetics to mortality through its effect on SIRT1 activity. Med Hypotheses 144: 110044. doi: 10.1016/j.mehy.2020.110044.
27. Taghizadeh-Hesary F, Akbari H (2020) The powerful immune system against powerful COVID-19: a hypothesis. Med Hypotheses 140: 109762. doi: 10.1016/j.mehy.2020.109762.
28. Cutolo M, Sulli A, Seriolo B, Accardo S, Masi A (1995) Estrogens, the immune response and autoimmunity. Clin Exp Rheumatol 13: 217–226. doi: 10.1002/art.23385.
29. Viña J, Gambini J, García-García FJ, Rodríguez-Mañas L, Borrás C (2013) Role of oestrogens on oxidative stress and inflammation in ageing. Horm Mol Biol Clin Investig 16: 65–72. doi: 10.1515/hmbci-2013-0039.
30. Qin M, Cao Z, Wen J, Yu Q, Liu C, Wang F (2020) An antioxidant enzyme therapeutic for COVID-19. Adv Mater 32: e2004851. doi: 10.1002/adma.202070321.
31. Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty AA, Kabir MB, Bindawa KU, Ahmed A (2021) Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: a cross-sectional comparative study in Jigawa, Northwestern Nigeria. SAGE Open Med 9: 2050312121991246. doi: 10.1177/2050312121991246.
32. Davran F, Hacıoğlu C, Alpay M, Yıldız Gülhan P, Öztürk CF, İnce N (2023) Evaluation of free radical and antioxidant enzyme levels in smoking and non-smoking patients with possible/confirmed COVID-19 diagnosis. Journal of Research and Thought in Health Sciences 13: 93–98. [Article in Turkish]. doi: 10.33631/sabd.1184661.
33. Robbins RA, Grisham MB (1997) Nitric oxide. Int J Biochem Cell Biol 29: 857–860. doi: 10.1016/S1357-2725(96)00167-7.

34. Davran F, Hacıoğlu C, Alpay M, Yıldız Gülhan P, Öztürk CE, İnce N (2023) Evaluation of free radical and antioxidant enzyme levels in smoking and non-smoking patients with possible/confirmed COVID-19 diagnosis. Value Health Sci [Article in Turkish]. doi: 10.33631/sabd.1184661.
35. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA (2019) Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. Oxid Med Cell Longev 2019: 3085756. doi: 10.1155/2019/3085756.
36. Mueller AL, McNamara MS, Sinclair DA (2020) Why does COVID-19 disproportionately affect older people? Aging (Albany NY) 12: 9959–9981. doi: 10.18632/aging.103344.
37. Blacker TS, Duchen MR (2016) Investigating mitochondrial redox state using NADH and NADPH autofluorescence. Free Radic Biol Med 100: 53–65. doi: 10.1016/j.freeradbiomed.2016.08.010.
38. Guarente L (2013) Calorie restriction and sirtuins revisited. Genes Dev 27: 2072–2085. doi: 10.1101/gad.227439.113.
39. Sönmez D, Özcan O (2021) The role of oxidative stress in the pathogenesis of COVID-19. In Yücel D, editor. The Increasing Role of Medical Biochemistry in the COVID-19 Pandemic. 1st Edition. Ankara: Turkey Clinics. 68–74. [A1.1] [A1.2]. [Article in Turkish].
40. Jomova K, Valko M (2011) Advances in metal-induced oxidative stress and human disease. Toxicology 283: 65–87. doi: 10.1016/j.tox.2011.03.001.
41. Doğan S, Bal T (2022) Investigation of zinc levels in COVID-19 patients. MKU Medical Journal 13: 68–73. [Article in Turkish]. doi: 10.17944/mkutfd.992280.
42. Shakoor H, Feehan J, Al Dhaheri AS, Ali HI, Platat C, Ismail LC, Stojanovska L (2021) Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: could they help against COVID-19? Maturitas 143: 1–9. doi: 10.1016/j.maturitas.2020.08.003.
43. Kiran Kumar PVSN, Tomo S (2023) Comparative analysis of serum zinc, copper and magnesium levels and their relations in association with severity and mortality in SARS-CoV-2 patients. Biol Trace Elem Res 201: 23–30. doi: 10.1007/s12011-022-03124-7.
44. Gomes AC, Moreira AC, Mesquita G, Gomes MS (2018) Modulation of iron metabolism in response to infection: twists for all tastes. Pharmaceuticals (Basel) 11: 84. doi: 10.3390/ph11030084.
45. Kumar V, Choudhry VP (2010) Iron deficiency and infection. Indian J Pediatr 77: 789–793. doi: 10.1007/s12098-010-0120-3.
46. Bangash MN, Patel J, Parekh D (2020) COVID-19 and the liver: little cause for concern. Lancet Gastroenterol Hepatol 5: 529–530. doi: 10.1016/S2468-1253(20)30084-4.
47. Prabhu KS, Lei XG (2016) Selenium. Adv Nutr 7: 415–417. doi: 10.3945/an.115.010785.
48. Tek NA, Koçak T (2020) The role of nutrition in supporting the immune system in the fight against Coronavirus (COVID-19). Gazi Health Sciences Journal, Special Issue: 18–45.
49. Anuk AT, Polat N (2020) The relation between trace element status (zinc, copper, magnesium) and clinical outcomes in COVID-19 infection during pregnancy. Biol Trace Elem Res 199: 3608–3617. doi: 10.1007/s12011-020-02496-y.