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Evaluation of the Relationship Between Aquaporin-1, Hepcidin, Zinc, Copper, and İron Levels and Oxidative Stress in the Serum of Critically Ill Patients with COVID-19

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Abstract

Our study aims to determine the relationship between hepcidin, aquaporin (AQP-1), copper (Cu), zinc (Zn), iron (Fe) levels, and oxidative stress in the sera of seriously ill COVID-19 patients with invasive mechanical ventilation. Ninety persons with and without COVID-19 were taken up and separated into two groups. The first group included seriously COVID-19 inpatients having endotracheal intubation in the intensive care unit ($n=45$). The second group included individuals who had negative PCR tests and had no chronic disease (the healthy control group $n=45$). AQP-1, hepcidin, Zn, Cu, Fe, total antioxidant status (TAS), and total oxidant status (TOS) were studied in the sera of both groups, and the relations of these levels with oxidative stress were determined. When the COVID-19 patient and the control groups were compared, all studied parameters were found to be statistically significant ($p<0.01$). Total oxidant status (TOS), oxidative stress index (OSI), and AQP-1, hepcidin, and Cu levels were increased in patients with COVID-19 compared to healthy people. Serum TAC, Zn, and Fe levels were found to be lower in the patient group than in the control group. Significant correlations were detected between the studied parameters in COVID-19 patients. Results indicated that oxidative stress may play an important role in viral infection due to SARS-CoV-2. We think that oxidative stress parameters as well as some trace elements at the onset of COVID-19 disease will provide a better triage in terms of disease severity.

Keywords COVID-19 · Oxidative stress · Total antioxidant status · Total oxidant status · Trace elements · Aquaporin · Hepcidin

Introduction

Coronaviruses are positive-sense single-stranded enveloped RNA viruses belonging to the family Coronaviridae. Coronaviruses are responsible for different diseases that affect the respiratory, digestive, and central nervous systems in humans and animals. Severe acute respiratory syndrome coronavirus (SARS-CoV) infects humans and may cause mild, moderate, and severe respiratory infections [1]. After the Coronavirus incubation period (3–14 days), the infection manifests itself with mild to severe symptoms displaying pneumonia and cytokine storm with increased mortality [2].

Angiotensin-converting enzyme 2 (ACE2) is a metallopeptidase involved in the counter regulation of the functioning of the human cardiovascular renin–angiotensin–aldosterone system. It has been reported to activate angiotensin, which regulates blood pressure and binds to cell membranes of tissues in the heart, kidney, intestinal cells, brain, lung, and testicles [1, 3]. Studies have identified ACE2 as the

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entry site of the coronavirus into human respiratory epithelial cells. In addition, some studies suggests that genetic variants in the ACE2 protein may prevent infection of a person exposed to the virus [4, 5]. ACE2 is therefore the key link between COVID-19 infection, cardiovascular diseases (CVDs), and immune response [5]. Oxidative stress results from a prooxidant-antioxidant imbalance that leads to cellular damage. It mediates free radicals such as reactive oxygen species or reactive nitrogen species produced during physiological aerobic metabolism and pathological inflammatory processes [6]. Antioxidants are compounds that prevent the initiation or progression of oxidation reactions by keeping the oxygen in the environment [7]. Free radicals, which are naturally the products of oxidation reactions in biological systems, damage cells and tissues, and several chronic diseases occur [8, 9]. Free oxygen radicals can kill cells by destroying cell membrane proteins and damaging the DNA in the cell, making it vulnerable to mutations, and weakening the immune system cells by affecting them. It is suggested that patients with atherosclerosis and COVID-19 as a result of the imbalance between the free radical and antioxidant systems predispose to congenital diaphragmatic hernia [10]. Aquaporins (AQPs) are included in the major intrinsic protein (MIP) superfamily in membranes and play an important role in the regulation of membrane water permeability. AQP1, AQP2, AQP3, AQP4, AQP6, and AQP7 are water channels localized in different types and parts of renal tubule epithelium [11]. The widely expressed sites of aquaporin 1 are alveolar cells, and pulmonary endothelial cells were alterations in their permeability and increased fluid leakage and edema formation [12, 13]. The role of aquaporin 1 was observed in SARS-CoV-2 experimentally infected golden Syrian hamsters [6]. One of our aims in this study is to understand the course of the COVID-19 disease.

Hepcidin is a peptide synthesized in the liver, consists of 25 amino acids, and plays a key role in the regulation of iron (Fe) metabolism [14, 15]. Both cellular Fe deficiency and Fe excess are pathological and harmful [16, 17]. In hepcidinforming tumors, severe Fe deficiency occurs because the use of Fe is impaired. Hepcidin does this by coordinating the use and storage of Fe, preventing the release of Fe into the plasma [18]. Fe is necessary for the function of hemoglobin, myoglobin, and many proteins. On the other hand, ionic Fe is toxic due to its reactivity with oxygen. On the interplay of local and systemic Fe regulation in the coronavirus research field, cytokine-mediated inflammatory processes, respiratory infections, and the possible homology problem of hepcidin and the evolutionary link between viral spike protein and hepcidin have not been evaluated [19, 20]. Zinc (Zn) is the second most abundant element in the cell. Copper (Cu) and Zn superoxide dismutase is an important factor [21]. For example, Zn deficiency is associated with decreased antibody production, dysfunction of the immune system, decreased NK cell activity, decreased cytokine production, and oxidative burst of neutrophil granulocytes. Zn binds to metallothioneins as two cations and is released as a mechanism to reduce ROS produced by viral infections [22]. Cu is another trace element that plays an important role in immunity and free radical defense [23]. This element is essential for both hosts and pathogens in infections. Therefore, Cu has been used clinically to reduce the risks of bacterial and viral contamination. Cu, like other elements, has a remarkable affinity for biological ligands and redox properties [24, 25]. The role of oxidative stress is well known in various diseases such as atherosclerosis, chronic obstructive pulmonary disease, Alzheimer's disease, and cancer. Oxidative stress may be considered as prognostic markers for evaluation of the effective treatment procedure of these diseases [26]. Thus, the determination of oxidative stress has recently gained importance in clinical practice as a complementary component. The latest evidence on coronaviruses shows that nutritional and metabolic disorders are associated with disease severity and even susceptibility to getting an infection. An adequate balance of micronutrients can enhance the host's immune response and avoid viral infections [27]. Evaluation of trace element levels in COVID-19 patients may provide a more robust and comprehensive approach to combat this devastating disease. The correlation between oxidative stress and trace elements in COVID 19 disease is still not completely investigated, and hence there is a lack of experimental data in a clinical practice. Therefore, our study focused on evaluation of the dysregulation of oxidative balance during COVID 19 infection by measuring total antioxidant status (TAS), total oxidant status TOS, and oxidative stress index (OSI) levels, and by observing their correlation with some serum trace elements levels. Thus, along with other routinely determined biochemical parameters, oxidative stress parameters and trace element profiles may provide information about the pathogenesis, diagnosis, and prognosis of COVID-19 disease and enable the evaluation of the suitability of trace element supplementation as an adjunct therapy.

In this study, we aimed to determine the relationship between AQP-1, hepcidin, Zn, Cu, and Fe levels with oxidative stress and the correlation between the studied parameters in COVID-19 patients.

Material and Methods

Selection of Study Groups

This study was conducted in patients with COVID-19. Forty-five critically ill patients (28 men and 17 women) with COVID-19 were included. The patients were diagnosed with COVID-19 by the SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) test. The inclusion criteria were: severe cases of COVID-19 adult patients at

intensive care units; they had a respiratory failure due to viral pneumonia and a threatening life condition requiring respiratory mechanical support and pharmacological medical management. The exclusion criteria were mild or moderate cases of COVID-19 patients, those having chronic or any underlying disease, and child patients. A total of 45 healthy adult people (26 males and 19 females) with negative RT-PCR tests, normal routine hematological and biochemical tests, and no history of any psychological or underlying disease were included in the study (Fig. 1).

SARS-CoV-2 RT-PCR

A viral nucleic acid isolation kit (Biospeedy-Turkey) was used to isolate SARS-CoV-2 from oro-nasopharyngeal swab and tracheal aspirate samples. In accordance with the manufacturer's recommendations (Biospeedy-Turkey), a sample of 10 µl (final volume) was used. Amplification was performed on the Qiagen Rotor-Gene Q 5plex HRM instrument (Qiagen, Germany).

Collection of Blood Samples

Patient blood samples were collected from the Infectious Diseases and Clinical Microbiology Department of our hospital. Five milliliter blood samples were taken from the patients after fasting overnight. The blood tubes were centrifuged at 5.000 rpm for 10 min. Serum samples were then stored in Eppendorf tubes at − 80 °C until analyses.

Measurement of Fe, Zn, and Cu in Serum Samples

The analysis of the elements in the patient sera was performed using the Atellica device (Abbott, USA). Fe, Cu, and Zn levels were measured in these serum samples by an inductively coupled plasma optical emission spectrophotometer on the Atellica device.

Fe Working Principle

The Fe present in the samples is separated from the carrier protein transferrin by an acid and is also converted to the ferrous

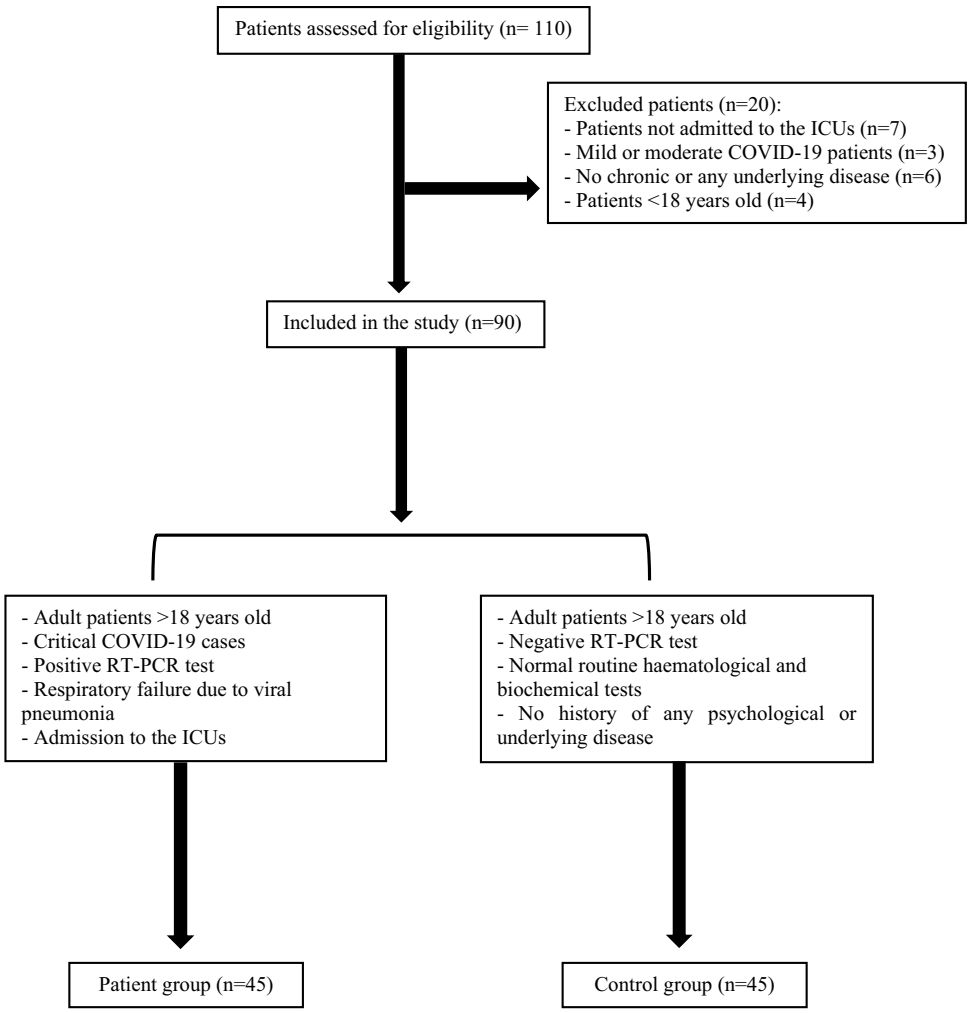

Fig. 1 A flow chart of the study preparation process

state. It is then measured spectrophotometrically by combining with ferrous iron and ferrozine, a sensitive iron indicator, to release a colored chromophore that absorbs at 571/658 nm.

Zn Working Principle

The zinc present in the samples changes the red–orange color of 5-Br-PAPS to light pink under alkaline conditions. The change in absorbance at 548 nm is proportional to the total Zn level in the sample. The assay can be calibrated with zinc sulfate dissolved in deionized water. The Zn reference range is 60–120 µg/dl.

Cu Working Principle

The Cu present in the samples changes the red–orange color of DiBr-PAESA to purple under acidic conditions. The change in absorbance at 572 nm is proportional to the total Cu concentration in the sample. The assay can be calibrated with Cu sulfated dissolved in deionized water. The Cu reference range is 70–160 µg/dl.

Measurement of Aquaporin-1 (AQP-1) and Hepcidin Proteins in Serum Samples

AQP1 and hepcidin levels were determined by using the enzyme-linked immunosorbent assay (ELISA) method. The SunRed ELISA kit was used in the study and was performed according to the manufacturer's instructions. Optical density (OD) values were measured spectrophotometrically at a wavelength of 450 ± 2 nm.

Measurement of TAS, TOS, and OSI

Total antioxidant status (TAS) of sera was measured using a new automated colorimetric measurement method developed by Erel [28, 29]. Test results are expressed as mmol Trolox equivalent/l. Serum TOS was determined using a new automated measurement method developed by Cao [30]. The test was calibrated with hydrogen peroxide. The results were expressed as µmol H_2O_2 equivalent/l. The ratio of TOS to TAS gives the OSI, which is an indicator of the degree of oxidative stress. The OSI value was calculated according to the formula below.

OSI = TOS (mmol H_2O_2 equiv/l) / TAS (mmol Trolox equiv/l).

Statistical Analysis

Data were analyzed by the SPSS version 24.0 package program, and $p < 0.05$ was considered statistically significant. Student t and chi-square test were performed and Spearman's test for correlation to measure the degree of association between two variables. Results of the studied parameters were expressed as mean ± standard deviation.

Results

General Characteristics

The demographic characteristics of the study population are presented in Table 1. The study group consisted of 45 patients (28 males and 17 females) with COVID-19. The mean ages of patients were 65.77 ± 7.21 years (ages between 57 and 86 years). These were elderly patients, and there were statistically significant differences by gender (The number of male patients was high than females; $p < 0.05$). No statistically significant difference was found between the patient and control groups in terms of age.

Evaluation of AQP-1, Hepcidin, Fe, Cu, and Zn in Serum Samples

Serum AQP1, hepcidin, Fe, Cu, and Zn levels were shown in Table 1. The difference in hepcidin level between the groups was found to be significant ($p < 0.01$). While serum Fe and

Table 1 Trace element and oxidative stress parameters in serum samples

Parameters	COVID-19 group ($n = 45$)			Control group ($n = 45$)			
	Min	Max	$\overline{X} \pm SD$	Min	Max	$\overline{X} \pm SD$	p value
AQP-1 (U/l)	10.35	28.39	18.20 ± 5.17	1.58	11.32	5.51 ± 2.80	< 0.01
Hepsidin (ng/ml)	46.70	301.14	112.72 ± 48.05	7.81	42.63	31.65 ± 8.7	< 0.01
Fe (µg/dl)	13.0	65.0	41.23 ± 11.59	77.0	112.0	96.86 ± 9.14	< 0.01
Zn (µg/dl)	47.60	94.4	66.16 ± 11.15	54.4	105.4	90.24 ± 13.59	< 0.01
Cu (µg/dl)	71.20	160.0	116.25 ± 26.75	65.8	167.9	99.65 ± 25.98	< 0.01
TAS (mmol Trolox equiv/l)	0.57	1.04	0.88 ± 0.12	1.06	1.74	1.56 ± 0. 18	< 0.01
TOS (µmol H_2O_2 equiv/l)	13.64	22.41	17.63 ± 2.72	9.11	22.15	11.49 ± 2.47	< 0.01
OSI (arbitrary units)	1.61	2.76	2.02 ± 0.35	0.43	1.56	0.66 ± 0.29	< 0.01

AQP-1 aquaporin-1, *Fe* iron, *Zn* zinc, *Cu* copper, *TAS* total antioxidant status, *TOS* total oxidant status, *OSI* oxidative stress index

Zn levels were higher in the control group The difference in Fe and Zn levels was found to be significant between the two groups ($p<0.01$). The Cu levels revealed a significant difference ($p<0.01$) between controls and patients.

Evaluation of TAS, TOS, and OSI in Serum Samples

TAS, TOS, and OSI values in the serum of the patient and control groups were given in Table 1. The range of TAS levels in the control group was significantly high as compared to the range of the patient group. The difference between the patient and control groups was considered significant statistically ($p<0.05$).

While TOS was found to be low in the control group. it was high in the patient group, and the difference was considered significant ($p<0.01$). OSI value was not different from TOS. In other words, while OSI level was low in the control group, it was found to be high in the patient group. The difference between the two values was considered significant ($p<0.05$).

Evaluation of Correlation Between Parameters Tested

The correlations of the parameters tested in the patient and control groups were given in Tables 2 and 3 respectively. In the patient group, the relationship between AQP-1 and Fe levels was statistically significant negative one (Spearman correlation $r=-0.19$; $p<0.01$). In other words, it shows that when the AQP-1 level increases, the level of the Fe decreases (the reverse is true).

In the same group, the correlation between AQP-1 and Zn was statistically positive ($r=0.43$; $p<0.05$). In other words, it shows that when the level of AQP-1 increases, Zn will decrease. The results indicate that Fe levels ($r=-0.20$; $p<0.05$) were negatively correlated with Zn concentrations in the patient group (Table 2).

Serum TAS and Fe concentrations ($r=-0.06$) were strongly and negatively correlated in the patient group (Table 3). On the other hand, a positive correlation was found between TOS and Fe concentrations ($r=0.07$).

Although a statistically strong, negative, and moderate correlation was found between TAS and OSI ($r=-0.50$; $p<0.01$). A strong positive correlation was seen between TOS and OSI ($r=0.60$, $p<0.05$).

In the control group, APQ-1 levels were positively and moderately correlated with Fe and Zn levels ($r=0.12$, $p<0.01$, and $r=0.06$, $p<0.05$, respectively). The results indicate that Fe and/or Zn elements increase when AQP-1 increases. There was also a moderate positive correlation between Fe and Zn ($r=0.13$, $p<0.05$).

There was a strong and positive correlation between TAS and Fe levels with $r=0.02$ and $p<0.01$ (Table 3). Contrary to the patient group, there was a strong and negative relationship between TOS and Fe ($r=-0.11$, $p<0.01$) in the control group.

The correlations between TAS and OSI and between TOS and OSI were not different from the patient group. That is, the relationship between TAS and OSI was strong and

Table 2 Correlation of trace element and oxidative stress parameters in the patient group

			TAS	TOS	OSI	AQP-1	Hepcidin	Zn	Cu	Fe
Spearman's rho	TAS	Correlation coefficient	1.0	0.29	−0.50**	0.20	−0.17	−0.07	0.36*	−0.06
		Sig. (2-tailed)		0.11	0.00	0.28	0.34	0.70	0.05	0.73
	TOS	Correlation coefficient	0.29	1.0	0.60**	0.08	−0.01	0.04	0.32	0.07
		Sig. (2-tailed)	0.11		0.0	0.65	0.93	0.81	0.08	0.69
	OSI	Correlation coefficient	−0.50**	0.60**	1.0	−0.01	0.10	0.13	−0.00	0.17
		Sig. (2-tailed)	0.00	0.0		0.93	0.56	0.46	0.98	0.34
	AQP-1	Correlation coefficient	0.20	0.08	−0.01	1.0	0.13	0.14	0.00	−0.19
		Sig. (2-tailed)	0.28	0.65	0.93		0.46	0.43	0.99	0.29
	Hepcidin	Correlation coefficient	−0.17	−0.01	0.10	0.13	1.0	−0.15	−0.10	0.00
		Sig. (2-tailed)	0.34	0.93	0.56	0.46		0.41	0.58	0.97
	Zn	Correlation coefficient	−0.07	0.04	0.13	0.14	−0.15	1.0	−0.19	−0.20
		Sig. (2-tailed)	0.70	0.81	0.46	0.43	0.41		0.30	0.26
	Cu	Correlation coefficient	0.36*	0.32	−0.00	0.00	−0.10	−0.19	1.00	0.11
		Sig. (2-tailed)	0.05	0.08	0.98	0.99	0.58	0.30		0.53
	Fe	Correlation coefficient	−0.06	0.07	0.17	−0.19	0.00	−0.20	0.11	1.00
		Sig. (2-tailed)	0.73	0.69	0.34	0.29	0.97	0.26	0.53	

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 3 Correlation of trace element and oxidative stress parameters in the control group

			TAS	TOS	OSI	AQP-1	Hepcidin	Zn	Cu	Fe
Spearman's rho	TAS	Correlation coefficient	1.0	−0.01	−0.64**	−0.14	−0.20	0.02	−0.11	0.02
		Sig. (2-tailed)		0.92	0.00	0.43	0.27	0.87	0.56	0.90
	TOS	Correlation coefficient	−0.01	1.00	0.72**	0.01	0.16	−0.06	−0.10	−0.11
		Sig. (2-tailed)	0.92		0.00	0.96	0.39	0.74	0.58	0.53
	OSI	Correlation coefficient	−0.64**	0.72**	1.00	0.07	0.28	−0.06	0.05	−0.15
		Sig. (2-tailed)	0.00	0.00		0.70	0.13	0.74	0.77	0.41
	AQP-1	Correlation coefficient	−0.14	0.01	0.07	1.00	−0.01	0.06	0.03	0.12
		Sig. (2-tailed)	0.43	0.96	0.70		0.94	0.73	0.84	0.52
	Hepcidin	Correlation coefficient	−0.20	0.16	0.28	−0.01	1.00	0.01	−0.33	−0.07
		Sig. (2-tailed)	0.27	0.39	0.13	0.94		0.95	0.07	0.71
	Zn	Correlation coefficient	0.02	−0.06	−0.06	0.06	0.01	1.00	0.04	0.13
		Sig. (2-tailed)	0.87	0.74	0.74	0.73	0.95		0.80	0.47
	Cu	Correlation coefficient	−0.11	−0.10	0.05	0.03	−0.33	0.04	1.00	0.12
		Sig. (2-tailed)	0.56	0.58	0.77	0.84	0.07	0.80		0.52
	Fe	Correlation coefficient	0.02	−0.11	−0.15	0.12	−0.07	0.13	0.12	1.00
		Sig. (2-tailed)	0.90	0.53	0.41	0.52	0.71	0.47	0.52	

**Correlation is significant at the 0.01 level (2-tailed)

negative ($r = -0.64$, $p < 0.01$), while there was a positive relationship between TOS and OSI ($r = 0.72$, $p < 0.01$).

The most elevated parameter in the patient group was Cu. It was followed by hepcidin, Fe, Zn, and AQP1. In the same group, TOS was the highest biomarker of oxidative stress, followed by OSI and TAS, respectively. In the control group, the highest increasing parameter was the Cu element, followed by Fe, Zn, hepcidin, and AQP-1, respectively. TOS was found to be the highest biomarker of oxidative stress, followed by TAS and OSI.

Discussion

It should be stated that an interaction exists between inflammation and oxidative stress. Inflammation may cause high production of free radicals at the site of infection by immune cells, especially macrophages. This mostly happens in the lungs in COVID-19 patients. Oxidative stress also increases with advancing age. The role of oxidative stress in the pathogenesis of COVID-19 viral infection has been studied by determining the status of antioxidant enzymes [6, 7].

Low levels of oxidative stress increase cellular oxidative stress and are associated with immune dysfunctions leading to higher susceptibility to viral infections such as COVID-19 infection [21]. Uncontrolled viral replication leads to oxidative damage to the lungs, which increases viral load. Thus, it increases the severity of viral infection [11].

Antioxidants are substances that can clear free radicals and prevent cell damage. In humans, antioxidants are either naturally produced by the body or taken externally and act as free radical scavengers. Therefore, they increase the effect of the defense system and reduce the risk of disease [31]. There is concern that patients with immunosuppressive miscarriage may be at higher risk of developing poor outcomes during COVID-19 infection. Age and sex have an important place in the severity of COVID-19 [25]. The study showed males patients and elderly or older patients (age ≥ 50 years) are at higher risk of developing severe COVID-19 disease. This is consistent with our sudy in which there were eldery and older patients (more than 57 years old) and there were stastitically significant differnces according to gender; it means more males than females ($p < 0.05$).

Oxidative stress affects Fe and Zn synthesis and disrupts the gene structure, and COVID-19 Fe and Zn values decrease [31, 32]. In our study, when the COVID-19 patient group was compared with the control group, serum Cu levels were found to be lower than Zn levels in patients with COVID-19 ($p < 0.001$; Table 1). A significant negative correlation was found between TAS and Zn levels in the COVID-19 patient group. However, no relationship was found between Cu levels and COVID-19. High TAS levels support the presence of increased oxidative stress in COVID-19 disease.

Researchers have shown that elevation of serum hepcidin is closely related to decrease in serum Fe and oxidative stress, suggesting that the increase in oxidative stress may be a marker in the pathogenesis of COVID-19 [16, 33]. However, in our study, serum Fe levels in the COVID-19 patient group were found to be statistically more significantly lower when compared to the control group ($p<0.01$) (Table 1). A significant negative correlation between serum hepcidin and TAS levels in the COVID-19 patient group was determined

(Table 2). This is indicative of increased oxidative stress in the COVID-19 patient group ($p<0.01$).

Trace elements play an important role in the mechanisms of the immune response; their deficiency has an important place in the pathogenesis of COVID-19 [34]. Zn has the potential to inhibit SARS-CoV RNA polymerase and thus its replication capacity. This is based on its more potent immunomodulatory and antiviral properties, suggesting the use of Zn for the potential treatment of COVID-19 [22, 35]. In our study, Zn levels in the patient group were found to be statistically significantly lowered when compared to the control group ($p<0.001$). Our study was found to support other studies.

High Cu levels can attack microbes through Cu toxicity, especially during lung infections [21]. Almost all of the Cu (95%) content of serum is due to ceruloplasmin, whose levels increase in response to inflammation, trauma, or infection to facilitate Cu delivery to sites of infection. We did not measure ceruloplamin since we have not got ceruloplasmin kit. Elevated Cu levels may only be a marker of disease and inflammation [24]. However, due to its potent antiviral activities, greater research is required before using Cu as a therapeutic option for COVID-19 [25]. Serum Cu levels of the patient group were found to be statistically significant compared to the control group ($p<0.001$).

Hepcidin secretion from the liver is a reaction to Fe overload or administration as well as inflammation, affecting cellular Fe concentration by blocking the Fe uptake mechanism in the cell [36]. In the study of Rab et al., hepcidin levels were examined in serum samples of 111 patients with COVID-19. As a result of the study, Fe concentration was lower than normal values in 93.7% of patients, while hepcidin levels were significantly increased in 61.3% of the patients [18].

AQPs are molecular water channels that facilitate water transport across the cell membrane in response to osmotic gradients. Disruption in alveolar fluid clearance due to altered functional expression of respiratory AQPs underscores their pathophysiological importance in respiratory disease associated with pulmonary edema. The functional characterization of AQPs along with their tissue, cell-specific distribution has gained significant scientific interest to investigate their involvement in pathological conditions [34, 35]. In this study, we investigated AQP-1 levels in the serum of patients and healthy individuals. Serum AQP-1 levels were found to be statistically higher in the patient group when compared to the control group.

Our study shows that the absence or reduction of oxidative stress will have a significant effect on the host cells in the early stage of viral infection by inhibiting its binding to a viral protein. By finding that Fe and Zn are structural components of many enzymes in the cell, Zn supplementation may be helpful in the treatment and prophylaxis of COVID-19. In addition, Cu deficiency is an important factor to be considered especially in COVID-19 patients. In this case, Cu toxicity, possible more serious reactions related to Cu dose and prolongation of Cu imbalance should be considered.

However, we can say that this study is an important study that evaluated serum AQP-1, hepcidin, zinc, copper, and iron levels, and oxidative status in COVID 19 patients. Studies with larger sample size are needed for further clarification of the role of oxidative stress parameters and trace elements in severly ill patients with COVID-19.

Conclusions

We have presented results for oxidative stress parameters (TAS, TOS, and OSI) and their correlations with several trace elements in patients with COVID-19. Compared to the healthy group, the oxidative balance was found impaired in patient group, and AQP-1, hepcidin, Cu levels were found to be higher, showing increased inflammation in the patients compared to healthy controls. The Zn and Cu supplementation may be an important preventive or therapeutic strategy in combating COVID 19 infection. Serum levels of Zn and Cu need to be done more extensively before using them as a therapeutic tool for COVID-19. It also appears that strategies to reduce or prevent oxidative stress may be helpful in the management of COVID-19. Our aim is to help clinicians and other researchers in the deficiency of trace elements in our research in biological samples of COVID-19 patients and healthy individuals. Extensive future studies on a larger population of oxidative stress and trace element levels determinations are needed, and they will be valuable and helpful in evaluating critically ill patients with COVID-19.

Author Contribution All authors participated in the development and design of the study. **NB**, **MB**, and **AO** were involved in data collection and analysis of data and also in ethics and prepared the article. **MB** and **AO** made statistical analyses. **AO** and **BI** were involved in the analysis of the data and proofread the manuscript.

Data Availability If further information about this study is required, please contact the authors.

Declarations

Ethics Approval This study was approved by the local ethics committee of Harran University and the Ministry of Health of Turkey (18/01/2020-HRU/210231).

Consent to Participate All patients signed an informed consent for inclusion of personal records in the local database and for use to scientifc research purposes.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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