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Original article

Role of vitamin D, folic acid, ferritin, inflammation and oxidative stress in the pathogenesis of COVID-19

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Abstract. The COVID-19 pandemic is one of the most devastating and significant events of recent times. COVID-19 has so far become one of the worst infectious disease outbreaks of recent times, with more than 635 million cases and more than 6.6 million deaths. Viruses cause an explosion of inflammatory cytokines and reactive oxygen types. Oxidative stress is thought to have a key role in COVID-19. vitamin D, folic acid, calcium (Ca), magnesium (Mg) and ferritin levels are thought to be associated with COVID-19. This study aims to investigate the role of oxidative stress, inflammation, vitamin D and folic acid, ferritin, Ca and Mg in the pathogenesis of COVID-19. *Materials and Methods:* 45 patients diagnosed with COVID-19 and 45 healthy persons (control group) were included in the study. Vitamin D, ferritin, folic acid, CRP, Ca, Mg and Phosphorus were measured in an autoanalyzer, and SOD, GSH-Px and MDA were spectrophotometrically measured in the serum of the participants. TNF-α, IL-1β and IL6 levels were studied by the ELISA method. *Results:* The activity of SOD, GSH-px, antioxidant enzymes, Serum vitamin D, folic acid, Ca and Mg of the COVID-19 group was found to be significantly lower than the control group ($p<0.05$). *Conclusion:* Again, the levels of MDA, TNF-α, IL-1β, IL-6, CRP and ferritin in the Covid-19 group were found to be significantly higher than in the control group ($p<0.05$).Antioxidant enzyme activities were low and oxidative stress was high in patients with COVID-19. At the same time, the levels of serum ferritin, CRP, TNF-α, IL-1β and IL6 were high, and levels of Ca and Mg were low in patients with COVID-19.According to these results, we hypothesize think that the level of oxidative stress, inflammation, vitamin D, and serum ferritin, Ca, and Mg levels play a role in the pathogenesis of COVID-19. Future clinical trials should be conducted to further clarify the pathogenesis in patients with COVID-19.

Key words: ferritin, COVID-19, Oxidative stress, vitamin D, folic acid, inflammation

Introduction

SARS-CoV-2 is the cause of Coronavirus disease (COVID-19). The SARS-CoV-2 pandemic with its rapid spread, has become a global health threat with unstable consequences all over the world. There is an urgency to understand the pathogenesis of SARS-CoV-2, which causes COVID-19 disease (1). The symptoms of COVID-19 are severe interstitial pneumonia, which can lead to fever, cough, fatigue, headache, diarrhea, arthromyalgia, acute respiratory distress syndrome, sepsis-induced coagulopathy and multi-organ dysfunction (2). Oxidative stress is an important component of critical illness (3). Oxidative stress is defined as the cause of the imbalance between ROS production and accumulation in cells and tissues and the ability of a biological system to detoxify these reactive products (4). Enzymatic antioxidants such as

SOD and GSH-px play a role in defending against the damage caused by ROS. However, increased ROS can damage cell membranes and lipoproteins by causing lipid peroxidation. This will lead to the formation of the oxidative stress biomarker malondialdehyde (MDA) compound known to be cytotoxic as well as mutagenic (4,5). It occurs with ROS as significant production of cytokines such as TNF-α, IL-1β and IL-6. This pro-oxidative proinflammatory state is referred to as a "cytokine storm" (6-9). The severe progression of COVID-19 causes a cytokine storm with excessive proinflammatory cytokine production (10). Ferritin is an iron-storing protein; The serum level of ferritin reflects the normal iron level and helps diagnose iron deficiency anemia (11). The level of serum ferritin is one of the most frequently requested research in both primary and secondary care. The most common causes of hyperferritinemia, except for iron overload, are related to inflammatory disorders, malignancy, chronic alcohol consumption, liver disease, or metabolic abnormalities (12). The level of serum ferritin could be increased during infection and inflammation (13). The pathogenesis of hyperferritinemia is thought to be cytokine-mediated, including IL-1α, IL-1β, IL-6, IL-18 and TNF-α. (12). Ferritin levels are thought to be a very important factor influencing the severity of COVID-19 (14). Vitamin D has been shown to directly inhibit viral replication, anti-inflammatory, immunomodulatory and antiviral properties (15). Some recent studies have suggested that vitamin D deficiency may increase the severity of COVID-19 and the risk of death by compromising respiratory immune function (16,17). However, reports stating that the correlation between vitamin D and COVID-19 deaths is not significant (18). It hypothesizes that Oxidative stress is an important factor that increases the severity of COVID-19, associated with viral sepsis derived from SARS-CoV-2 infection (19). Although reports characterizing the immune and inflammatory status have been published in COVID-19 patients, as far as we know, no report has been published demonstrating the link between oxidative stress and hyperinflammation, Vitamin D and ferritin in COVID-19. This study aims to investigate the role of oxidative stress, inflammation, serum vitamin D, folic acid, ferritin, Ca and Mg in the pathogenesis of COVID-19.

Materials and methods

45 COVID-19 patients (case group) diagnosed with Real-Time PCR method in Dicle University Hospitals Central Laboratory and 45 healthy individuals (control group) aged over 18 and of similar gender were included in the study. The diagnosis of COVID-19 was made after the sample taken from the patients was analyzed with real-time polymerized chain reaction (PCR) method. These patients had symptoms such as difficulty breathing or shortness of breath, chest pain or chest pressure, loss of speech or movement, fever, dry cough, tiredness, headache and loss of sense of taste or smell. The diagnosis was also supported by the images of chest radiography. Healthy individuals who did not use any medication were determined as the control group. Venous blood samples were taken from the antecubital vein of the participants. After 30 minutes at room temperature, they were centrifuged at 3500 rpm for 5 minutes and the sera were taken into eppendorf tubes and stored at -80 °C until the analysis day.

Biochemical analysis

Vitamin levels such as vitamin D, ferritin, folic acid, Ca, Mg, phosphorus and CRP Cobas e 601 (Roche Diagnostics, USA) were studied in the auto analyzer device. This device uses ECL (Electro Chemi Luminescence) technology. In ECL technology, streptavidin-coated microparticles are used as solid phase, Ruthenium Chelate Complex is used for marking and TPA (TriPropilAmin) is used as substrate. Analysis of 25 parameters takes place for each sample in one run. You can access all applications, kit inserts, calibrators and All kinds of information about the controls can be accessed electronically. This information are constantly updated.

Oxidative stress markers

SOD activity determination

SOD enzyme activities were determined by the method modified by Sun et al. (1998) The principle of this method is based on the reduction of

nitrobluetetrazolium (NBT) by the xanthine-xanthine oxidase system, which is a superoxide producer (20).

GSH-Px activity determination

GSH-Px activity was studied according to the method of Paglia et al. (1967) GSH-Px catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) in the presence of hydrogen peroxide. In the presence of hydrogen peroxide, GSSG formed by GSH-Px is reduced to GSH with the help of glutathione reductase and NADPH. GSH-Px activity is calculated by reading the absorbance decrease at 340 nm during the oxidation of NADPH to NADP +. (21).

MDA determination

Lipid peroxidation will be done by applying Esterbauer method (1990), which is the applied measurement method. MDA, which reacts with thiobarbutyric acid at 90-95 ° C, creates a pink chromogen. Fifteen minutes later, the absorbances of the rapidly cooled samples were spectrophotometrically read at 532 nm (22).

Inflammation markers

Elisa method and tests:

TNF-α (Cat No. MBS267654), IL-1β (Cat No. BMS224-2), and IL6 (Cat. No: MBS021993) levels were examined by ELISA method. Elisa method is a quantitative measurement method based on the investigation of the Antigen-antibody relationship and the activity of an antibody-bound enzyme. Samples and biotinylated antibodies were added to the ELISA plate wells and washed with PBS (Phosphate Buffered Saline) or TBS (Tris Buffered Saline) after respective additions to the wells. Avidin-peroxidase conjugates were then added to the wells afterward. The TMB substrate was used for coloring after the enzyme conjugate was thoroughly washed with PBS or TBS from the wells. TMB reacts to form a blue product from peroxidase activity and finally turned yellow after addition of the stop solution (Color Reagent C). The color intensity and amount of target analyte in the sample were calculated as positively correlated.

Statistics

All data editing and statistical analyses were performed using GraphPad Prism 9.1.0 software Results were provided as mean ± standard deviation (SD) and min-max. To decide whether parametric or non-parametric analysis methods will be used in our study, the data should be subjected to a normality test and the most widely known, Kolmogorov-Smirnov and Shapiro-Wilk tests were performed. In the evaluation made, it was concluded that the data did not have a normal distribution because the p values were less than 0.05. According to this result, non-parametric analysis methods, which are an alternative to parametric analysis methods, were preferred in the different tests. Therefore, the Mann-Whitney U test was used. The Spearman non-parametric correlation was calculated. p-value <†0.05 was considered statistically significant.

Results

Of our patients diagnosed with Covid-19, 6 had hypertension, 4 had diabetes mellitus, 1 had cardiac arrhythmia, 2 had bronchial asthma, and 1 had Thyroid disease. **(Table 1)**

There was no statistically significant difference in terms of average age. Control: 45.7 ±22.6; 25/20 (M/F) Covid-19: (56.7 ±19.8), 25/20 (M/F). The activity of SOD (0.31±0.07 min:0.19 max: 0.67), GSH-Px (347.0±37.01) enzymes in the Covid-19 group was

Table 1. Comorbitidies of patients with Covid-19 (n=45).

Comorbidities	
Hypertension	6
Diabetes mellitus	4
Cerebral infarction	–
Cardiac arrhythmia	1
Prostate cancer	–
Bronchial asthma	2
Pulmonary tuberculosis	-
Claustrophobia	–
Thyroid disease	1
Liver disease	-

found to be lower than the activity of the enzymes SOD (0.52±0.15, min:0.27max: 0.85), GSH-px (435.9±63.4 min:323.1 max:542.6) in the control group and were statistically significant. And again, the levels of MDA (2.3±0.48; min :1.45, max:3.22), TNF-α (384.3±44.6; min: 229.4, max:457.6), IL-1β (37.7±6.9 min:21.57; max :49.09), IL-6 (256.5±159 min:98.5, max: 654.3), CRP (6.7±8.17 min :1.02; max: 38.98) of the Covid-19 group of the control group MDA (1.20±0.35, min: 0.78, max: 2.1), TNF–α (332.1±37; min: 212.3, max: 416.4), IL-1β (27.7±5.6; min: 25.06, max: 40.9), IL-6 (153.1±21.7; min: 149.5, max: 188.9), CRP (0.30±0.1; min: 0.1, max: 0.5) were found higher than patients and statistically significant (Figure 1 and Figure 2).

There was a weak negative correlation between SOD activity and CRP, TNF-α and IL-1β levels, and it was significant (respectively, r= -0.37, p<0.01; r=-0.39, p0.01; r=-0.40; p0.01). There was a weak and very weak negative correlation between GSH-px activity and CRP, TNF-α, IL-1β levels and it was significant (respectively, r= -0.37, p<0.01; r=-0.27, p=0.008; r=-0.31; p=0.002). There was a weak positive correlation between the MDA level and CRP, TNF-α, IL-1β levels and it was significant (respectively r=0.35 p=0.001; r= 0.44 p<0.01; r=0.43, p0.01) There was a weak positive correlation between the serum ferritin level and CRP, TNF-α, IL-1β, IL-6 levels and it was significant (respectively r=0.44 p<0.01;

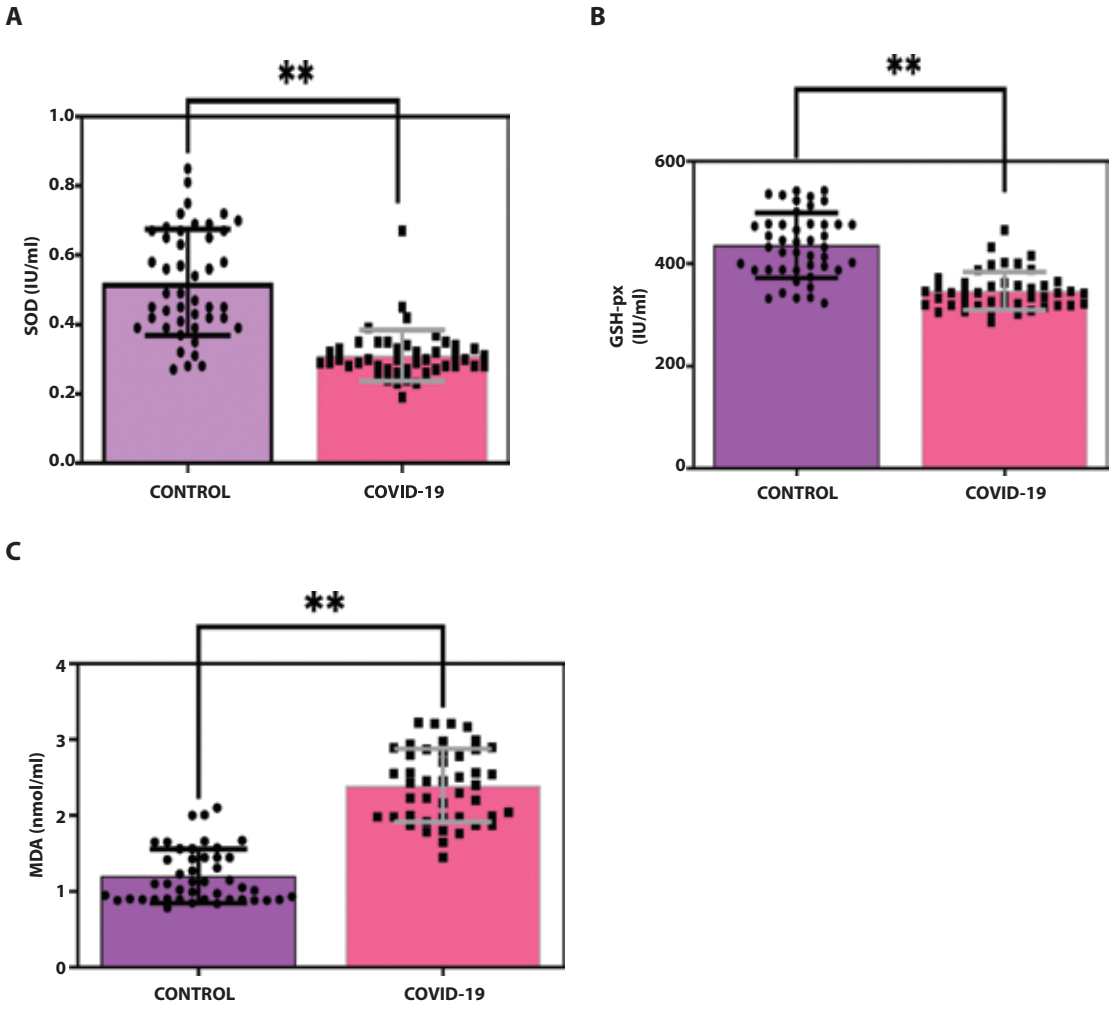

Figure-1. Comparison of SOD (A), GSH-px (B), MDA (C) parameters between Covid-19 group and Control group. ** P < 0.01 versus control.

** P < 0.01 versus control. Values are mean ± SD, Mann–Whitney U test, SD: Standard deviation. **Ns:** Nonsignificant, **(A) SOD:** Superoxide dismutase, **(B) GSH-Px:** Glutathione peroxidase, **(C) MDA:** Malondialdehyde

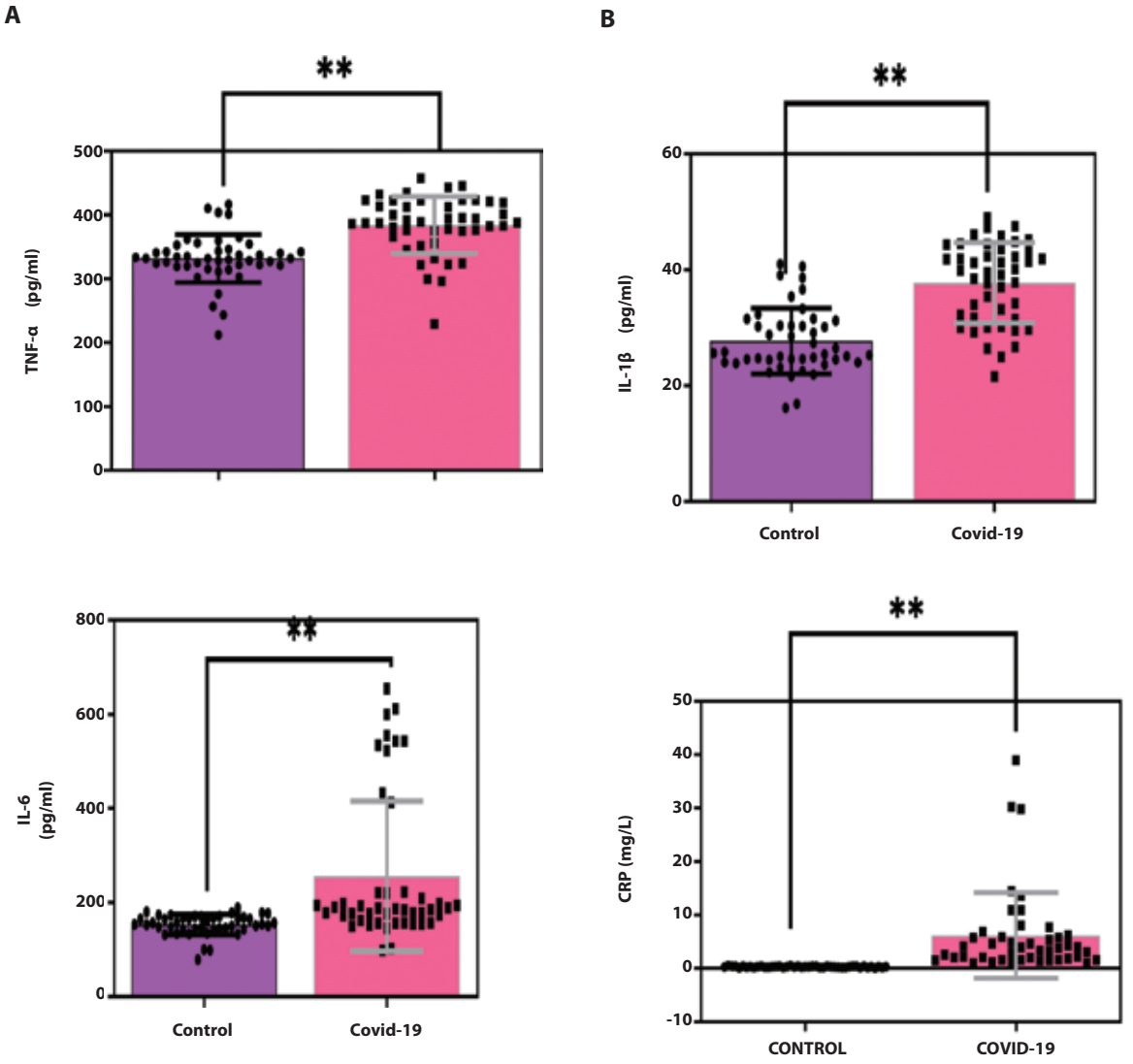

Figure-2. Comparison of TNF-α (A), IL-1β (B), IL-6 (C), CRP (D) parameters between Covid-19 group and Control group. ** p< 0.01 versus control. ** P < 0.01 versus control. Values are mean ± SD, Mann–Whitney U test, SD: Standard deviation. **Ns:** Nonsignificant. **(A) TNF-α:** Tumor necrosis factor α, **(B) IL-1β:** Interleukin 1β, **(C) IL-6:** Interleukin 6, **(D) CRP:** C-reactive protein

r= 0.42 p=0.001; r=0.44 p<0.01; r=0.43, p0.001) There was a weak negative correlation between the serum Vit D level and CRP, TNF-α, IL-1β, IL-6 levels and it was significant (respectively r=-0.66 p<0.01; r= -0.39 p=0.001; r=-0.46 p<0.01; r=-0.42, p=0.001) **(Table 2).**

Covid-19 group's Vit D (11.6±6.1 min:3.0; max:28.34), folic acid (7.27±3.23 min:2.89; max:16.04), Ca (8.41±0.60 min:7.32; max: 9.61), Mg (1.85±0.49 min:0.16; max:2.53) values of the control group Vit D (22.2±5.9, min: 13.42 max: 36.5), folic acid (12.3±2.38, min:7.43 max: 17.4), Ca (9.09±0.52, min: 8.1 max: 10.1) Mg (2.04±0.14; min:1.78 max: 2.1) decreased significantly compared to values. However, the ferritin (603,2±391.1; min:151.2 max: 2000 levels of the Covid-19 group increased significantly compared to the ferritin (l190.5 ±52.4; min:75.6 max:375.1) levels of the control group. There was no significance in the comparison of phosphorus levels between groups **(**Figure 3**).**

There was a moderate and weak positive correlation between SOD activity and Vit D and folic acid levels and it was significant (respectively, r=0.552,

Table 2. Correlation analysis between serum SOD, GSH-Px, MDA, ferritin, Vit D levels, serum CRP, TNF-α, IL-1β and IL-6 levels.

	SOD		GSH-Px		MDA		Ferritin		Vit D	
	r	p	r	p	r	p	r	p	r	p
CRP	-0.37**	<0.01	-0.37**	<0.01	0.35**	0.00	0.66**	<0.01	-0.66**	<0.01
TNF-α	-0.39**	<0.01	-0.27**	0.008	0.44**	<0.00	0.42**	<0.01	-0.39**	0.001
IL-1β	-0.40**	<0.01	-0.31**	0.002	0.43**	<0.00	0.44**	<0.01	-0.46**	<0.01
IL-6	-0.17	0.106	-0.44	0.665	0.20	**0.54**	.43**	<0.01	-0.42**	<0.01

SOD: Superoxide dismutase, **GSH-Px:** Glutathione peroxidase, **MDA:** Malondialdehyde, **TNF-α:** Tumor necrosis factor alpha, **IL-1β:** Interleukin 1 beta. Spearman correlation analysis was performed to determine the relationship between parameters. p <0.05 results were considered statistically significant. **Correation is significant at the 0.01 level (2-tailed). Correlation coefficient was shown with "r".

p<0.01; r=-0.47;p0.01). There was a moderate and weak positive correlation between GSH-px activity and Vit D and folic acid levels, and it was significant (respectively, r=0.523, <0.01; r=0.470; p0.01). There was a negative correlation between the MDA level and Vit D levels between the intermediate levels and it was significant (r= -0.589, p<0.01, respectively). **(Table 3).**

Discussion

In this study, serum vitamin D, ferritin, folic acid, inflammation and oxidative stress parameters of patients hospitalized due to COVID-19 infection were compared with healthy population (control group). Based on searching the literature, no studies were found to such comparison. Therefore, this study could be the first report evaluating oxidative stress and serum vitamin D, folic acid, ferritin levels and other findings in patients with COVID-19. This study has shown that the level of serum ferritin, oxidative stress and inflammation has increased, and the activity of antioxidant enzyme has decreased in patients with COVID-19. In addition, it was shown that Vitamin D, Ca, Mg and folic acid were lower in patients with COVID-19 compared to healthy people. It is well known that oxidative stress plays an important role in critical diseases characterized by an intense systemic inflammatory response such as tissue ischemia-reperfusion injury, sepsis, and acute respiratory distress syndrome. Oxidative stress can exacerbate organ damage and thus the overall clinical outcome (23). Oxidative stress is thought to have a key role in the pathogenesis of COVID-19 (24). Oxidative stress is an imbalance between increased ROS in cells and tissues and a biological system that will eliminate or reduce (4,5, 25). SOD and GSH-px play a key role due to their antioxidant protective properties against the free radical attack of biological systems (26). Violi et al. (2020) have shown that NADPH oxidase-2 is overexpressed in COVID-19 patients and causes an increase in oxidative stress (27). In our study, it was found that while MDA levels, which are an oxidative stress marker, of Covid-19 patients were higher than healthy individuals, the primary enzymes SOD and GSH-px activities of the antioxidant defense system were found to be lower than those the healthy ones **(Figure 1)** SARS-CoV-2 infection can generate more than one free radical such as H_2O_2, O2• - and • OH. SARS-CoV-2 proteins, it causes loss of host cell membrane integrity and mitochondrial dysfunction, resulting in a more severe increase in ROS production. Increasing ROS causes a decrease in SOD and GSH-px activity and an increase in MDA. This suggests that oxidative stress plays a role in Covid-19 (28, 29). Excessive ROS production by mitochondria and NADPH oxidase of leukocytes and endothelial cells that are not compensated by antioxidant systems can cause severe cellular and tissue damage and promote chronic inflammation that underlies many neurodegenerative, cardiovascular and metabolic diseases (30, 31). The different temporal regulation of IL-1 (early response) TNF and IL-6 (late response) indicates that different cytokines will be differently affected by mitochondrial ROS production (32). In a virus-induced mouse model, oxidative stress has been shown to trigger lung damage by increasing the production of NF-κB-driven pro-inflammatory cytokines and adhesion molecules

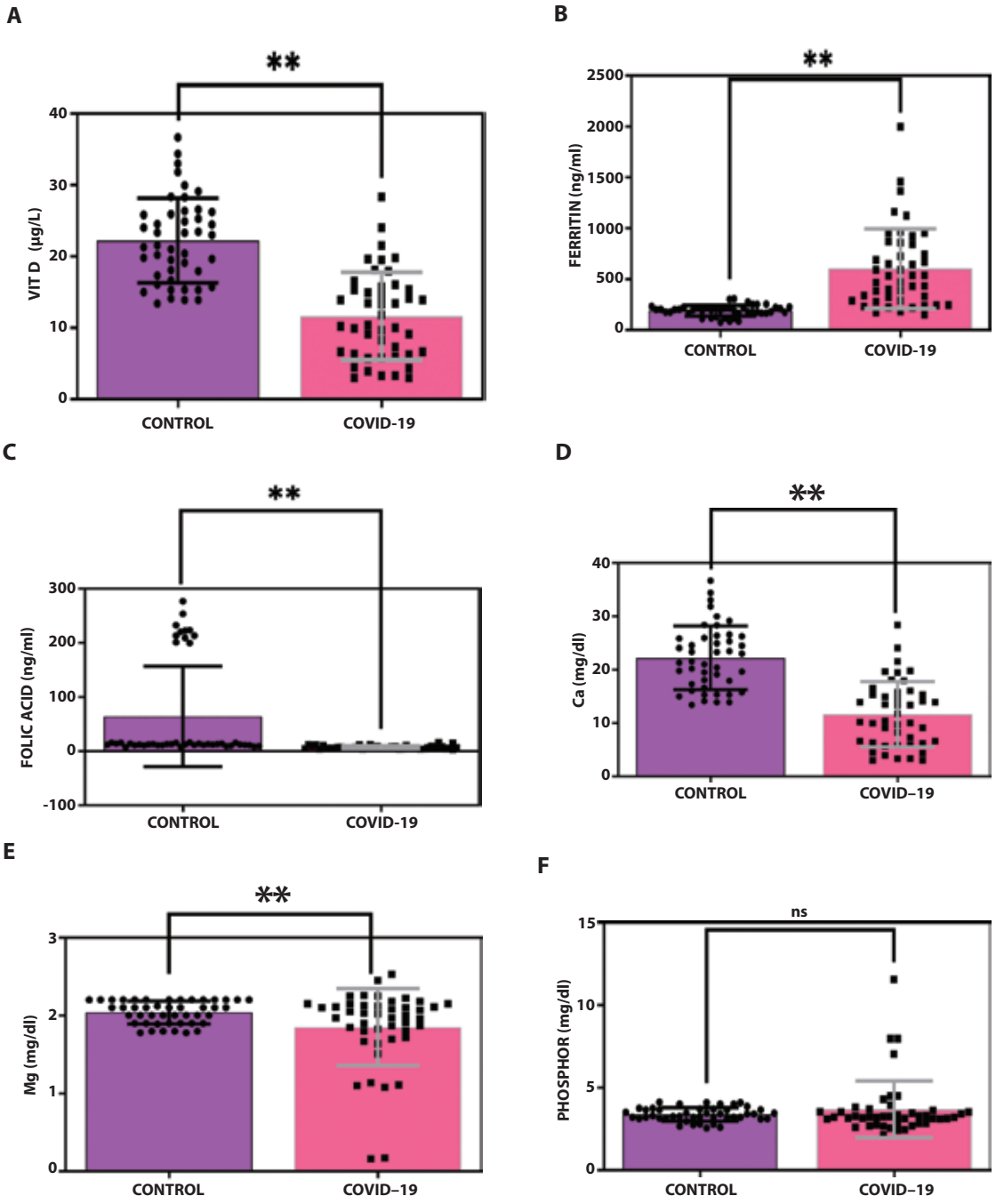

Figure-3. Comparison of Vit D (A), ferritin (B), folic acid (C), Ca (D), Mg (E) and Phosphorus (F) parameters between Covid-19 group and Control group. ** P < 0.01 versus control.** P < 0.01 versus control. Values are mean ± SD, Mann–Whitney U test, SD: Standard deviation. **Ns:** Nonsignificant

such as TNF-α, IL-1β or IL-8 (33). SARS-CoV-2 can actively induce the 'cytokine storm' by mediating the vigorous production and release of proinflammatory cytokines, confirming high levels of inflammatory markers. The most important of these is the CRP biomarker used for the diagnosis of sepsis. IL-1β, IL-6 and TNF-α are inflammatory mediators in COVID. It is thought that high CRP levels may be associated

Table 3. Correlation analysis between serum SOD, GSH-Px, MDA levels, serum Vit D, ferritin, folic acid levels.

	SOD		GSH-Px		MDA	
	r	**p**	**r**	**p**	**r**	**p**
vit D	0.55**	<0.01	0.523**	<0.01	-0.589**	<0.01
ferritin	-0.42**	<0.01	-0.428**	<0.01	0.544**	<0.01
folic acid	0.478**	<0.01	0.470**	<0.01	0.19	**0.061**

SOD: Superoxide dismutase, **GSH-Px:** Glutathione peroxidase, **MDA:** Malondialdehyde, **Vit D:** Vitamin D**,** Spearman correlation analysis was performed to determine the relationship between parameters. p <0.05 results were considered statistically significant. **Correation is significant at the 0.01 level (2-tailed). Correlation coefficient was shown with "r".

with the overproduction of inflammatory cytokines in severe and geriatric patients and with COVID-19. In this case, it has been suggested that high CRP and IL-6 levels may be a valuable early marker in predicting the probability of disease progression in severe and patients geriatric with COVID-19. (34-35). High inflammatory cytokine and chemokine levels have been associated with COVID-19 severity and mortality (6, 33-40). In the first published reports at the onset of the disease, Severe COVID-19 patients had significantly higher TH1 cytokine levels (IL-6 and TNF-α) and have been reported to have a higher incidence of ARDS (41). In a study suggesting that serum IL-6 and TNF-α levels are therapeutic options for COVID-19 patients, high levels of IL-6, TNF-α and IL-1β were found in the serum of patients with Covid-19 (42). In another study, it was reported that IL-6 and TNF-α increased during illness and decreased during recovery in individuals infected with SARS-CoV-2 (6).

Similar to other studies, in our study, CRP, TNF-α, IL-1β and IL-6 values of COVID-19 patients were found to be higher than the control group. **(Figure 2)** Ferritin is a ubiquitous and highly conserved iron-binding protein (43). Ferritin is released into the bloodstream; plasma (or serum) ferritin concentrations typically reflect iron stores (44). The serum ferritin protein itself is not harmful. However, high level of serum ferritin is associated with a large number of inflammatory and degenerative diseases (45,46). Serum ferritin is a well-known acute phase reactant, and reflects the degree of acute and chronic inflammation in infectious, rheumatological, hematological and malignant diseases (47). Serum ferritin is generally considered to be a good marker of inflammation rather than an assessment of iron status (44). Garcia et al. (2007) found a relationship between hyperferritinemia (ferritin> 500 μg/L) and disease severity in children with severe sepsis and septic shock (48). A recent study reported that there is a relationship between hyperferritinemia and disease severity in COVID-19 patients (8). In this study, serum ferritin levels in patients with COVID-19 was found to be higher than in the control group **(Figure 3).** Moreover, Lin et al. (2020) reported that severe COVID-19 patients had relatively higher serum ferritin levels than non-Severe COVID-19 patients (49). In another study, when they examined the clinical features of 99 patients, they reported that the serum ferritin of 63 patients was above the normal range (50). Wu et al. (2020) demonstrated that a high level of serum ferritin is an independent risk factor associated with developing ARDS in patients with COVID-19, and in patients with pneumonia who developed acute respiratory distress syndrome or died. As an inflammatory indicator, serum ferritin was positively associated with elevated levels of IL-1β, IL-6, and TNF-α (51). It is claimed that extreme hyperferritinemia is a key mediator of immune dysregulation through direct immunosuppressive and proinflammatory effects. Therefore, considering the relationship between serum ferritin levels and the degree of systemic and pulmonary inflammation, we can reasonably think that hyperferritinemia is high in COVID-19 patients (52). Cellular damage from increased inflammation can increase intracellular ferritin leakage, raising serum ferritin (50). In our study, IL-lβ, TNF-α and IL-6 levels of the COVID-19 group were found to be higher than the control group **(Figure-2).** These findings are consistent with previous findings**.** (49, 51). We argued that proinflammatory cytokines caused by SARS-CoV-2 increases the production of

IL-6, TNF-α and therefore increase the level of serum ferritin. Henry et al. (2020) conducted a meta-analysis study and suggested that serum ferritin and IL-6 as candidate variables can serve as serious and fatal clinical predictors of COVID-19 (54). Vitamin D deficiency causes deregulation of calcium metabolism and redox cell signaling pathways. Also playing a central role in phosphorus homeostasis, Ca^{2+} and functions by regulating the cell signal mediated by ROS (55). Vitamin D has been suggested as one of the critical controllers of oxidative stress and systemic inflammation. Besides, vitamin D has an anti-inflammatory effect by controlling the adaptive immune system. When vitamin D status is adequate, most activities related to intracellular oxidative stress are downregulated. Vitamin D induces the expression of several molecules involved in the antioxidant defense system, including SOD, GSH-px, CAT, and GSR, increases reduced glutathione levels, and suppresses NADPH oxidase expression, thus contributing to the reduction of cellular oxidation. These actions related to vitamin D collectively reduce the intracellular ROS load (19, 56-59).

Higher mortality has been reported in COVID-19 patients with low vitamin D concentrations (609. In a report published in Switzerland, it was reported that patients with positive SARS-CoV-2 test results had significantly lower plasma 25-hydroxy vitamin D concentrations compared to those who tested negative (61). However, data have shown that vitamin D supplementation is effective in preventing other respiratory infections (15, 60, 62,63). Jain et al (2020) In their study reported that vitamin D deficiency significantly increased the chance of developing disease after infection with SARS Cov-2, and that vitamin D deficiency increased morbidity and mortality in COVID-19 patients (64).

In our study, Vitamin D levels of COVID-19 patients were low. Our findings are consistent with previous findings. In addition, our findings remind us that vitamin D deficiency increases the risk of getting COVID-19. Additionally, there likely that there appears to be a relationship between decreased vitamin D levels and oxidative stress in the COVID-19 group. In our study, there was a significant negative correlation between MDA levels and vitamin D in the COVID-19 group. However, there was a significant positive correlation between SOD and GSH-px enzyme activities and vitamin D in this group **(Table 3).** Vitamin D deficiency may have decreased the activity of SOD and GSH-px enzymes and increased cellular oxidation. These findings suggest that there's a link between SARS-CoV-2 and vitamin D and Oxidative stress. It has been reported that vitamin D deficiency could cause oxidative stress in vivo or in vitro studies (65-67). The antioxidant properties of vitamin D suggest that vitamin D may protect against COVID-19. Calcium has critical physiological roles such as neural, muscle contraction, electrophysiology of the heart, intracellular signal transduction, and coagulation (68). Hypocalcemia is a common laboratory abnormality in viral infection and pneumonia (69). During septic shock, defects in Ca regulation occurs frequently in critically ill adult and children patients (70). Serum Ca has been associated with the clinical severity and prognosis of COVID-19 patients (71). Hypokalemia is widespread among critically cases of COVID-19. A report from China showed that 93% of severely and critically ill patients suffering from COVID-19 have hypokalemia. The Ca value of the COVID-19 group in our study was found to be significantly lower than the control group (72). Liu et al. (2020) found that almost two-thirds of patients with severe COVID-19 had hypocalcemia on admission to the hospital. They reported that patients presenting with hypocalcemia were more severely ill at admission and had worse results (73). These findings are consistent with the low Ca value in COVID-19 patients in our study. Hypokalemia is a common finding in patients with hypomagnesaemia (74). Mg deficiency is thought to be associated with immune dysfunction, including acute and chronic infection (75). Mg is the fourth most common cation in the body and the second most abundant cation in the cell. Approximately 70% of the ionized Mg in serum plays an important role in maintaining internal homeostasis through actions in the musculoskeletal system, endocrine, nervous and cellular messenger systems (76,77). Mg deficiency may worsen the course of COVID19 by inducing endothelial dysfunction. In this context, it should be reminded that Mg deficiency increases the tendency to thrombosis, which is an important complication in Covid-19 (78,79). In our study, the Mg levels of Covid-19 patients were

found to be low. Dimitrios et al. (2015) In a review they wrote, they reported that many studies showed a high prevalence of hypomagnesemia in critically ill ICU patients, and that there was a relationship between increased mortality and hypomagnesemia in critically ill patients with sepsis (80). In a prospective observational study conducted by Limaye et al. (2011) with 100 patients hospitalized in an intensive care unit, they found hypomagnesemia in 52% of the patients, normal serum Mg level in 41% and hypermagnesemia in 7% of the patients at admission to the intensive care unit (81). Furthermore, it has been shown that an inflammatory response characterized by increases in plasma hs-CRP and IL-6 levels was experimentally shown in rodent models with induced Mg deficiency. Additionally, cross-sectional studies indicate an inverse relationship between magnesium intake and serum IL-6 concentration (82-84). A study consisting of 488 adolescents aged 10-13 years in Mexico found a significant association between low Mg levels and CRP (85). In this study, the CRP level was found to be high in the COVID-19 group with low Mg levels, which concurs with the existing literature. Folate, DNA and protein synthesis and adaptive immune response are vital for the synthesis of red blood cells. Furin is an enzyme associated with bacterial and viral infections and is a promising target for the treatment of infections. One of the reasons for the high contagiousness of SARS-CoV-2 is the furin cleavage site in Spike. Furin is a type of proprotein convertase (PC) and furin inhibitor with positive effects and high specificity has not been found. Recently, it has been noted that folic acid can inhibit furin, prevent binding by the SARS-CoV-2 spike protein, prevent cell entry and virus turnover. Therefore, it has been suggested that folic acid may be useful in the treatment of respiratory disease associated with COVID-19 in the early stages (86-89). Elias et al. (2020) They suggested that Pregnant women who took folic acid supplements were less likely to get COVID-19 infection, and those who were infected had a higher chance of becoming asymptomatic (90). Low level of folic acid in patients with COVID-19 compared to the control group suggested that folic acid deficiency increases the possibility of being infected with SARS-CoV-2.

Conclusion

Antioxidant enzyme activities were low and oxidative stress was high in patients with COVID-19. At the same time, level of serum ferritin, CRP, TNF-α, IL-1β and IL6 were high, and level of Ca and Mg were low in patients with COVID-19. Hyperferritinemia suggests it may be the acute phase reactant used by clinicians as an indication for therapeutic intervention, aimed at controlling inflammation in COVID-19 patients. Folic acid and vitamin D supplementation could potentially prevent and reduce the symptoms caused by SARS-CoV-2. However, results of large-scale randomized controlled folic acid and vitamin D studies are required. We think that destructive ROS production resulting in oxidative stress plays a role in cell damage that leads to COVID-19. We think that antioxidant supplements (Vitamins D, E, C, Melatonin, selenium, CAPE) will reduce oxidative stress in COVID-19 patients. These therapeutic strategies should aim at strengthening the immune system as well as reducing oxidative stress. Also, We think that hypokalemia and hypomagnesemia play a role in the pathogenesis of COVID-19.

Compliance with the ethical standards: All human studies have been approved by the appropriate ethics committee and have therefore been performed by the ethical standards laid down in the 1964 Declaration of Helsinki. All persons gave their informed consent before their inclusion in the study. The study was conducted according to the Helsinki Declaration rules and was approved by the Institutional Ethics Committee of Dicle University Faculty of Medicine. (No: 2020/324)

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