Research Article

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Oxidative stress index can be a new marker related to disease severity in COVID-19

[Oksidatif stres indeksi, COVID-19’da hastalığın şiddeti ile ilişkili yeni bir belirteç olabilir]

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Abstract

Objectives: The aim of this study was to evaluate the relationship between systemic oxidative balance, and the severity of the disease in patients with COVID 19.

Methods: Sixty-four patients were divided into three groups according to the severity of the disease: mild (n=28), moderate (n=11) and severe (n=25). Twenty-four healthy controls included to the study. Proinflammatory cytokines (IL-6 and TNF- α), D-dimer, fibrinogen, total oxidative status (TOS), total antioxidant status (TAS) were measured and oxidative stress index (OSI) was calculated.

Results: The mean age of severe group was significantly higher than the other groups (p=0.001). TAS levels were significantly decreased in all patient groups compared to controls, while serum TOS and OSI levels were significantly

different in all three stages of the disease. Serum IL-6 and TNF- α levels were significantly elevated in severe group compared to other groups. TOS and OSI levels were also significantly correlated with IL-6, CRP, ferritin, fibrinogen, LDH and D-dimer.

Conclusions: TOS and OSI levels are an indicator of systemic oxidative balance in COVID-19 and related to the disease severity. They can be an important marker for evaluating the disease severity and used in the management of patients with COVID-19.

Keywords: COVID-19; IL-6; OSI; oxidative stress; TOS.

Öz

Amaç: Bu çalışmanın amacı COVID 19 hastalarında sistemik oksidatif denge ile hastalık şiddeti arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Altmış dört hasta, hastalık şiddetine göre hafif (n=28), orta (n=11) ve şiddetli (n=25) olmak üzere üç gruba ayrıldı. Yirmi dört sağlıklı kontrol çalışmaya dahil edildi. Proinflatuar sitokinler (IL-6 ve TNF- α), D-dimer, fibrinojen, total oksidan status (TOS) ile total antioksidan status (TAS) düzeyleri ölçüldü ve oksidatif stres indeksi (OSI) hesaplandı.

Bulgular: Şiddetli grubun yaş ortalaması diğer gruplara göre anlamlı derecede yüksekti (p0,001). TAS seviyeleri kontrollere kıyasla tüm hasta gruplarında anlamlı olarak azalırken, serum TOS ve OSI seviyeleri hastalığın her üç evresinde de anlamlı olarak farklıydı. Şiddetli grupta serum IL-6 ve TNF- α seviyeleri diğer gruplara göre anlamlı derecede yüksekti. TOS ve OSI seviyeleri ayrıca IL-6, CRP, ferritin, fibrinojen, LDH ve D-dimer ile anlamlı korelasyon gösterdi.

Sonuç: TOS ve OSI düzeyleri, COVID-19’da sistemik oksidatif dengenin bir göstergesidir ve hastalık şiddeti ile

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ilişkilidir. Hastalık şiddetini değerlendirmek için önemli bir belirteç olabilir ve COVID-19 hastalarının yönetiminde kullanılabilir.

Introduction

Coronavirus Disease 2019 (COVID-19) is a global pandemic and caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affecting the whole world emerges in a broad spectrum. It can cause serious complications such as respiratory failure, sepsis, acute respiratory distress syndrome (ARDS) and organ damage especially in hospitalized patients with COVID-19 pneumonia [1].

Previous studies revealed that severely ill patients prone to have a high concentration of various inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor (TNF- α). The overproduction of these pro-inflammatory cytokines has been described as a cytokine storm. It causes an increased systemic inflammatory response and multiorgan failure due to vascular hyperpermeability and may contribute to the higher mortality rates in patients with COVID-19 [2, 3].

Pathophysiological processes such as increase in cytokine release and inflammation are frequently encountered in respiratory viral infections, including COVID-19 and accordingly, may be related to oxidative stress. Recent studies described that increased reactive oxygen species (ROS) and antioxidant defense mechanisms deprivation have a very important role in the pathogenesis of SARS-CoV infection [4-6]. It has been suggested that ARDS in patients with COVID-19 depends on activation of the oxidative stress that is coupled with inflammatory machinery and resulting in an increased pro-inflammatory host response [4]. Various mechanisms have been suggested to explain increased ROS generation, including increased angiotensin II, a prooxidant and pro-inflammatory substance, due to angiotensin-converting enzyme II (ACE) II inhibition (I), respiratory burst generating superoxide radicals and H₂O₂ by activated macrophages and neutrophils (II), ROS production by heme and iron released by damaged erythrocytes (III), hypoxemia caused by alveolar edema (IV) [7]. Although previous studies have revealed a relationship between systemic oxidative stress and viral diseases such as HCV [8], there is not enough data in the literature for SARS-CoV-2 infection.

This study aimed to determine the relationship between systemic oxidative balance and inflammation and their roles in the severity of the disease at different stages of patients with COVID-19.

Materials and methods

Patients and control groups

Sixty-four patients with laboratory-confirmed and clinically-diagnosed COVID-19 admitted to Hatay Mustafa Kemal University Hospital Infectious Diseases and Chest Diseases COVID-19 Polyclinic were enrolled in the study. Ethical approval was obtained by the Republic of Turkey Ministry of Health (2020-05-15T14_35_18) and the Institutional Ethics Committee of Hatay Mustafa Kemal University (ethical no: 2020/69). WHO interim guidance for COVID-19 was used to diagnose the patients. Three groups were created depending on the severity of the disease: mild group, moderate group, and severe group. The mild group consisted of symptomatic patients without viral pneumonia or hypoxia and without computed tomography (CT) findings. The moderate group consisted of patients with CT findings and clinical signs of pneumonia and severe group consisted of patients with the moderate symptoms plus SpO₂ $\leq$ 90% and/or respiratory rate $>$ 30 breaths/min with CT findings. Patients under the age of 18 years old, missing medical data, having pregnancy and malignancy were excluded from the study. Patients having comorbidities such as diabetes mellitus (DM), hypertension, coronary heart disease (CHD), chronic kidney disease (CKD), chronic pulmonary disease (CPD) and patients with CT findings of pneumonia excluded from mild group.

In our study, 24 age- and gender-matched healthy individuals without any acute/chronic infections, chronic diseases such as CHD, DM, CKD and CPD, a rheumatological disease were included as a control group. Demographic and laboratory data were collected from the hospital information system. Written informed consent was obtained from all COVID-19 patients and control subjects prior to entry. The study was performed in accordance with the Declaration of Helsinki.

Specimen collection

Venous blood samples were collected from COVID-19 patients and healthy controls for serum separation. After centrifugation at 1,500 $\times$ g for 10 min, all serum samples were portioned and stored at -80°C .

Biochemical analysis

Serum albumin, total protein, creatinine levels, lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) activities were assayed by an autoanalyzer using spectrophotometric method (Siemens Advia 1800 Chemistry System, Newark, Germany). Ferritin levels were performed via immunometric assay (Siemens Advia Centaur XP Immunassay System, Newark, Germany). CRP levels were determined by nephelometric method (Siemens BN II System Marburg, Germany). Leukocyte, neutrophils, lymphocytes and platelet counts were determined by Mindray BC 6000 (MINDRAY Co., Shenzhen, China). D-dimer levels were studied on VIDAS D-Dimer Exclusion™ II (BioMérieux, France) and fibrinogen was measured on STA Compact Max (Stago, USA).

Serum oxidative stress parameters

Serum total oxidant status (TOS) and total antioxidant status (TAS) were measured by a colorimetric method which was described by Erel (Rel Assay Diag., Turkey) [9, 10]. TAS results were expressed as mmol Trolox Eq/L and the results of TOS were given as $\mu\text{mol H}_2\text{O}_2$ equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Eq/L). Oxidative Stress Index (OSI) was calculated as follows: $\text{OSI (arbitrary unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ Eq/L}) / \text{TAS } (\mu\text{mol Trolox Eq/L}) \times 100$ [11].

ELISA measurements

Serum IL-6 and TNF- α concentrations were assayed using commercially available kits by ELISA method (Thermoscientific MultiscanGo; Elabscience, catalog no, respectively: E-EL-H0109 and E-EL-H0102). The assay ranges for the TNF- α kit were 7.81–500 pg/mL, analytical sensitivity 4.69 pg/mL and the intra- and interassay coefficients of variance (CV%) were 6.22 and 5.29%, respectively. The assay ranges for the IL-6 kit were 7.81–500 pg/mL, analytical sensitivity 4.69 pg/mL and the intra- and interassay coefficients of variance (CV%) were 6.72 and 6.61%, respectively. The results were presented as pg/mL.

PCR analysis

All COVID-19 patients were confirmed positively by RT-PCR using specimens derived from oropharyngeal swabs prior to or during the hospitalization [12]. SARS-CoV-2 RT-PCR assay (Montania 4896 Real-Time PCR Instrument, Anatolia Diagnostics Inc, Istanbul, Turkey) was conducted according to the manufacturer's instructions (Bioeksen R&D Tech. Ltd, Istanbul, Turkey).

Statistical analysis

Categorical data and continuous variables were expressed as numbers/percentages and mean $\pm$ SD or SEM, respectively. The distribution of the continuous variables was studied by the Kolmogorov-Smirnov test. One Way ANOVA Test (Post hoc: LSD) was applied to analyze normally distributed data. Kruskal Wallis Test (Post hoc: Mann Whitney U Test) was applied to analyze non-normally distributed data. The Chi-square test was used for categorical data. We also used covariance analysis (ANCOVA) to assess the differences between the groups after adjusting age for the potential confounder effect. Receiver operating characteristic (ROC) analysis was performed to evaluate the potential value of oxidative stress parameters in disease severity of patients with COVID-19. Correlations were assessed using the Spearman test. All statistical procedures were studied using SPSS 24.0 (IBM Corporation, NY, USA). Values of $p < 0.05$ were considered statistically significant.

Results

The demographic characteristics of 64 patients and 24 healthy individuals participating in the study are given in Table 1. The mean age of the patients with the severe group was significantly higher than the other groups ($p < 0.001$, Table 1).

The clinical characteristics of COVID-19 patients are given in Table 2. Comorbidities including DM (17%), hypertension (12.5%), CHD (14%), CKD (4.6%) and CPD (7.8%) were observed in 22 patients (34.4%) participating in the study.

Table 1: Demographic characteristics of healthy controls and patient groups.

	Control, n=24	Mild, n=28	Moderate, n=11	Severe, n=25	Total, n=88	p-Value
Age, mean $\pm$ SD, years	38.29 $\pm$ 8.23	39.99 $\pm$ 13.58	43.82 $\pm$ 12.48	57.76 $\pm$ 13.75 ^{a,b,c}	–	<0.001 ^{a,b,c}
Sex, n, %						0.122 ^{**}
Female	11 (45.8%)	6 (21.4%)	2 (18.2%)	11 (44.0%)	30 (34.1%)	
Male	13 (54.2%)	22 (78.6%)	9 (81.8%)	14 (56.0%)	58 (65.9%)	

*One way ANOVA test (Post hoc: LSD); **Chi-square test; ^aComparison between control and severe group. ^bComparison between mild and severe group. ^cComparison between moderate and severe group. Bold values show $p < 0.05$ and considered statistically significant.

Table 2: Clinical characteristics of patient groups.

	All patients, n=64	Mild, n=28	Moderate, n=11	Severe, n=25	p-Value
Any of the following					
Diabetes	11 (17%)	0	1 (9%)	10 (40%)	
Hypertension	8 (12.5%)	0	1 (9%)	7 (28%)	
Coronary heart disease	9 (14%)	0	1 (9%)	8 (32%)	
Chronic kidney disease	3 (4.6%)	0	0	3 (12%)	
Chronic pulmonary disease	5 (7.8%)	0	0	5 (20%)	
Comorbidity (total)	22 (34.4)	0	3 (27%) ^b	19 (76%) ^a	<0.001
Non-comorbidity	42 (65.6%)	28 (100%)	8 (73%) ^b	6 (24%) ^a	<0.001

^aComparison between mild and severe group. ^bComparison between mild and moderate group.

Biochemical data of the patients and the healthy subjects are shown in Table 3. Serum levels of lymphocytes in the severe group significantly decreased and LDH, CRP, D-dimer and fibrinogen levels were significantly increased compared to mild, moderate and control groups.

Oxidative stress parameters (TAS, TOS and OSI) and pro-inflammatory cytokines (IL-6 and TNF- α) are shown in Figures 1 and 2, respectively. TAS levels were significantly decreased in all study groups compared to controls (mild $p=0.026$, moderate $p=0.027$ and severe $p=0.001$). On the other hand IL-6, TNF- α , TOS and OSI levels were significantly elevated in the severe group compared to other groups ($p<0.001$). However, the significant difference of IL-6, TOS and OSI levels between severe and other groups still existed after adjusting for the age (Figures 1D–F and 2CD–FC Figures 1D–F and 2C Figures 1D–F and 2C Figures 1D–F and 2C, D).

The correlation of pro-inflammatory and oxidative stress parameters were given in Table 4. Serum levels of

TOS and OSI showed a positive moderate correlation with IL-6, CRP, ferritin, fibrinogen and D-dimer. And IL-6 levels were correlated with TNF- α , CRP, ferritin, fibrinogen and D-dimer.

We performed ROC analyses to evaluate the diagnostic potential for the disease severity and detect the optimal cut-off values of laboratory, inflammatory and oxidative stress parameters in COVID-19 patients (Table 5 and Figure 3). We detected that areas under the ROC curve for TAS, TOS, OSI, ferritin, fibrinogen, D-dimer, TNF- α , IL-6 and CRP were 0.7077, 0.9557, 0.8890, 0.8210, 0.9443, 0.9925, 0.6852, 0.8730 and 0.8812, respectively. Also 95% confidence intervals for TOS, OSI, ferritin, fibrinogen, D-dimer, IL-6 and CRP were 0.9169–0.9946, 0.8236–0.9544, 0.7343–0.9076, 0.8983–0.9904, 0.9805–1.005, 0.7981–0.9480 and 0.8125–0.9499, respectively ($p<0.0001$).

Multiple regression analyses indicating CRP, D-dimer, fibrinogen, IL-6, LDH and ferritin with OSI in patients with COVID-19 are shown in Table 6. These

Table 3: Biochemical parameters in control and patient groups.

Parameters	Control, n=24	Mild, n=28	Moderate, n=11	Severe, n=25	p-Value
Leukocyte, $\times 10^9/L$	6.13 ± 0.92	6.29 ± 1.64	6.34 ± 1.29	7.75 ± 6.05	0.923*
Neutrophils, $\times 10^9/L$	3.72 ± 0.93	4.13 ± 1.75	4.29 ± 0.84	5.56 ± 5.22	0.173**
Lymphocytes, $\times 10^9/L$	2.15 ± 0.60	1.43 ± 0.53^a	1.28 ± 0.41^b	1.18 ± 0.48^c	<0.001* ^{a,b,c}
Platelet, $\times 10^9/L$	241.87 ± 69.11	218.82 ± 51.37	208.81 ± 132.14	199.16 ± 63.37	0.233**
ALT, U/L	21.62 ± 8.54	24.00 ± 8.24	28.45 ± 16.99	33.49 ± 39.21	0.280**
AST, U/L	19.91 ± 4.90	28.50 ± 9.96	29.09 ± 10.53	$44.40 \pm 37.16^{c,e,f}$	<0.001* ^c 0.008** ^{e,f}
Creatinine, mg/dL	0.70 ± 0.11	0.75 ± 0.15	0.77 ± 0.11	$0.97 \pm 0.49^{c,e}$	0.001* ^c 0.005** ^e
Albumin, g/L	41.4 ± 3.9	41.1 ± 4.2	40.6 ± 2.0	39.1 ± 3.3	0.138**
Total protein, g/L	66.6 ± 6.0	65.3 ± 4.7	65.1 ± 3.3	64.4 ± 4.9	0.504**
CK, U/L	72.85 ± 21.37	88.75 ± 32.23	94.72 ± 22.98	$158.40 \pm 203.36^{c,e}$	0.008* ^c 0.025** ^e
LDH, U/L	144.41 ± 17.4	204.35 ± 28.00^a	244.09 ± 40.65^b	$348.16 \pm 150.76^{c,e,f}$	0.012** ^a 0.002** ^b <0.001* ^{c,e,f}
Ferritin, mg/dL	34.25 ± 24.99	78.11 ± 24.17^a	186.70 ± 125.63^b	$369.24 \pm 333.98^{c,e,f}$	0.035** ^{a,b} <0.001* ^{c,e} 0.012** ^f
CRP, mg/L	32.2 ± 2.8	50.3 ± 24.3^a	134.3 ± 173.0^b	$623.2 \pm 590.6^{c,e,f}$	<0.001* ^{a,c,e} 0.001* ^b 0.002* ^f
D-dimer, ng/dL	108.41 ± 19.10	249.32 ± 78.12^a	$618.03 \pm 471.02^{b,d}$	$1,091.72 \pm 1,276.72^{c,e}$	<0.001* ^{a,b,c,e} 0.001* ^d
Fibrinogen, mg/dL	233.62 ± 25.6	325.10 ± 63.80^a	408.09 ± 126.11^b	$462.16 \pm 194.84^{c,e}$	0.007** ^a <0.001* ^{b,c,e}

The data are expressed as mean $\pm$ SD. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; CRP, C-reactive protein. *Kruskal Wallis Testi (Post hoc: Mann Whitney U Testi); **One way ANOVA test (Post hoc: LSD).

^aComparison between control and mild group. ^bComparison between control and moderate group. ^cComparison between control and severe group. ^dComparison between mild and moderate group. ^eComparison between mild and severe group. ^fComparison between moderate and severe group. Bold values show $p<0.05$ and considered statistically significant.

Figure 1: One way ANOVA test (Post hoc: LSD) was applied for figure 1a, 1b and 1c. Data were expressed as mean $\pm$ SD. a) TAS, total antioxidant status. * $p=0.026$, ** $p=0.027$, *** $p=0.001$. b) TOS, total oxidant status. * $p<0.001$ c) OSI, oxidative stress index. * $p<0.001$, ** $p=0.007$, *** $p=0.014$. The levels of TAS, TOS and OSI adjusted for the age were presented in figure 1d, 1e and 1f. Data were expressed as mean $\pm$ SEM. d) * $p=0.004$, e) * $p<0.0001$, ** $p=0.001$, *** $p=0.005$, **** $p=0.031$, f) * $p>0.0001$, $p=0.002$, $p=0.025$.

Figure 2: One way ANOVA test (Post hoc: LSD) was applied for figure 1a and 1b. Data were expressed as mean $\pm$ SD. a) IL-6: Interleukin-6. * $p=0.014$, ** $p=0.016$, *** $p<0.001$. b) TNF- α , tumor necrosis factor- α . * $p<0.001$, ** $p=0.018$, *** $p=0.010$, **** $p=0.025$. The levels of IL-6 and TNF- α adjusted for the age were presented in figure 1c and 1d. Data were expressed as mean $\pm$ SEM. d) * $p<0.0001$, ** $p=0.003$.

Table 4: Correlations between oxidative stress parameters, inflammation markers and coagulation markers.

Parameters		TAS, n=88	TOS, n=88	OSI, n=88	IL-6, n=88	TNF- α , n=88	CRP, n=88	Ferritin, n=88	Fibrinogen, n=88	D-dimer, n=88	LDH, n=88
TAS	r-Value		−0.360	−0.597	−0.266	NS	−0.253	NS	NS	−0.326	−0.261
	p-Value		0.001	0.000	0.012	NS	0.018			0.002	0.014
TOS	r-Value	−0.360		0.899	0.469	NS	0.569	0.447	0.463	0.646	0.587
	p-Value	0.001		0.000	0.000		0.000	0.000	0.000	0.000	0.000
OSI	r-Value	−0.597	0.899		0.447	NS	0.532	0.414	0.392	0.619	0.506
	p-Value	0.000	0.000		0.000		0.000	0.000	0.000	0.000	0.000
IL-6	r-Value	−0.266	0.469	0.447		0.592	0.647	0.511	0.485	0.591	0.587
	p-Value	0.012	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
CRP	r-Value	−0.253	0.569	0.532	0.647	0.262		0.649	0.696	0.724	0.702
	p-Value	0.018	0.000	0.000	0.000	0.014		0.000	0.000	0.000	0.000
Ferritin	r-Value	NS	0.447	0.414	0.511	NS	0.649		0.570	0.656	0.669
	p-Value		0.000	0.000	0.000		0.000		0.000	0.000	0.000
Fibrinogen	r-Value	NS	0.463	0.392	0.485	0.220	0.696	0.570		0.608	0.743
	p-Value		0.000	0.000	0.000	0.039	0.000	0.000		0.000	0.000
LDH	r-Value	−0.261	0.587	0.506	0.587	0.284	0.702	0.669	0.743	0.767	
	p-Value	0.014	0.000	0.000	0.000	0.007	0.000	0.000	0.000	0.000	

TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein; LDH, lactate dehydrogenase; CK, creatinine kinase.

Table 5: Area under the receiver operating characteristic curve and optimal cut-off values of TAS, TOS, OSI, ferritin, fibrinogen, D-dimer, TNF- α , IL-6 and CRP in patients with COVID-19.

Variables	AUC (95% CI)	Sensitivity %	Specificity %	Cut-off	p-Value
TAS	0.7077 (0.5848–0.8306)	66.7	67.2	1.61	=0.0028
TOS	0.9557 (0.9169–0.9946)	89.1	87.5	14.68	<0.0001
OSI	0.8890 (0.8236–0.9544)	79.7	79.2	9.15	<0.0001
Ferritin	0.8210 (0.7343–0.9076)	71.9	70.8	45.50	<0.0001
Fibrinogen	0.9443 (0.8983–0.9904)	87.5	87.5	265.50	<0.0001
D-dimer	0.9925 (0.9805–1.005)	96.9	95.8	143	<0.0001
TNF- α	0.6852 (0.5514–0.8190)	64.1	62.5	35.3	=0.008
IL-6	0.8730 (0.7981–0.9480)	73.4	75.0	27.9	<0.0001
CRP	0.8812 (0.8125–0.9499)	81.3	83.3	3.25	<0.0001

AUC, area under the receiver operating characteristic curve; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein.

results revealed that CRP and IL-6 might explain and the regression model was statistically significant ($p < 0.001$ and $p = 0.022$, respectively).

Discussion

This is the first study evaluating the systemic oxidative stress parameters in COVID-19 patients and their relationship with inflammation and disease severity. We found a significant deprivation in antioxidant defense mechanism and an increase in total oxidative stress in COVID-19 patients. TOS levels were also significantly

different between study groups and related to disease severity. We also found a significant correlation in TOS and calculated OSI levels among IL-6, ferritin, D-dimer, CRP and fibrinogen. Serum pro-inflammatory cytokine levels were significantly different in mild, moderate and severe groups and IL-6 were significantly correlated with TNF- α , CRP, ferritin, fibrinogen and D-dimer.

There is strong evidence that deterioration of the antioxidant defense mechanism leads to increased oxidative stress [13]. Previous studies have suggested that the imbalance in oxidant and antioxidant systems plays an important role in the pathogenesis of COVID-19 [4, 14]. There is also a strong relationship between oxidative stress

Figure 3: AUC, area under the receiver operating characteristic curve; TAS, total antioxidant status; TOS, total oxidan status; OSI, oxidative stress index; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; CRP, C-reactive protein.

and inflammation caused by innate and adaptive immune systems in the disease progress [3, 15]. In the current study a significant increase was observed in TOS and OSI levels of study groups compared to controls (Figure 1). We also found that TOS and OSI levels were significantly different between severe moderate and mild groups (Figure 1). TOS levels measurement is based on the oxidation of Fe^{2+} to Fe^{3+} in acidic medium due to the presence of oxidant species [10]. And it is used to evaluate total oxidative stress. However OSI level is a better indicator for systemic balance because it is calculated as TOS and TAS ratio [11]. Up to now, there is no study in literature evaluating systemic oxidative balance using OSI in COVID-19. There are several mechanisms explaining increased oxidative stress in COVID-19. It has been shown that SARS-CoV-2 virus enters into alveolar epithelial cells by binding to ACE II receptors and blocks its activity. Diminished ACE II activity leads to increased angiotensin II levels, which has oxidant and pro-inflammatory properties [16]. The lungs are the primary affected organs in COVID-19. Virus infection also causes alveolar edema together with inflammation and leads to

decreased oxygen saturation, which is another well-known contributor factor in ROS production [17]. Our results indicate that increased systemic oxidative stress is related to disease severity in patients with COVID-19. We also found that TOS and calculated OSI levels were correlated with ferritin, CRP, IL-6, fibrinogen and D-dimer levels. Additionally, area under the ROC curve for TOS and OSI were 0.9169–0.9946 (at cut-off value of $14.68 \mu\text{mol H}_2\text{O}_2 \text{ Eq/L}$) and 0.8236–0.9544 (at cut-off value of 9.15 arbitrary

Table 6: Multiple regression analysis indicating CRP, D-dimer, fibrinogen, IL-6, LDH and ferritin with OSI in patients with COVID-19.

Parameters	<i>B</i>	Std. error	p-Value	<i>F</i>	Adjusted R^2
CRP	0.073	0.021	0.001	5.49	0.236
D-dimer	−0.001	0.001	0.339		
Fibrinogen	−0.008	0.004	0.066		
IL-6	0.107	0.046	0.022		
LDH	0.006	0.008	0.501		
Ferritin	−0.004	0.003	0.193		

Model significance: $p < 0.001$.

unit), respectively ($p < 0.0001$). The area under the ROC curve for TOS and OSI were similar to D-dimer, fibrinogen and CRP (Table 5) and also the changes in calculated OSI levels were due to TOS levels. Therefore, TOS and OSI levels can be a new predictor marker for the disease severity and used in the management of the disease.

We also evaluated inflammatory cytokine levels. Serum IL-6 and CRP levels were significantly increased in the patient groups compared to the controls (Figure 2 and Table 3). IL-6 were also significantly different between severe, moderate and mild groups. Serum IL-6 levels seem related to disease severity. There is a moderate correlation between IL-6 and CRP (Table 4) and IL-6 were correlated with OSI values ($r = 0.447$, $p < 0.001$). Area under the ROC curve for IL-6 and CRP were 0.7981–0.9480 and 0.8125–0.9499, respectively ($p < 0.0001$). Moreover, multiple regression analyses showed that 23% of the alterations in OSI values might be explained by CRP and IL-6, and the regression model was statistically significant ($p < 0.001$ and 0.022, Table 6). These results implicate that IL-6 and CRP significantly contributed to the changes in the OSI levels. Besides, increased pro-inflammatory cytokines are related with systemic oxidative balance and play a role together in the progression of the disease. These findings also support the previous studies suggesting a positive feedback loop between inflammation and oxidative stress in COVID-19.

Ferritin is another inflammatory marker and several studies have shown that ferritin levels were increased in COVID-19 [18]. In the current study we found higher ferritin levels in all study groups compared to controls (Table 3). Ferritin is an acute phase reactant and plays an important role in iron metabolism. Free iron is highly toxic for tissues because it causes oxidative damage through fenton reaction. In a recent study, Liu W et al. suggested that viral proteins attack hemoglobin beta chains and lead to iron release, which make contribution to the tissue damage [19]. Ferritin is used to storage free iron in tissues to prevent oxidative stress. Therefore, increased ferritin levels in COVID-19 is an expected result and can be related to prevention of iron toxicity [18]. However, in our study, ferritin levels were more prominent in patients with severe disease ($p < 0.001$) and correlated with systemic oxidative (TOS and OSI) and inflammatory (IL-6, CRP) parameters (Table 4).

Another contributing factor responsible for disturbed oxidative balance is excessive consumption of antioxidant substrates. It has been shown in many disorders including pulmoner diseases, HIV infections, and age related disorders [20, 21]. The excessive consumption of antioxidants can contribute to the disease progression in SARS CoV-2

infection [14]. Previous studies showed that SARS-CoV-2 virus reduce glutathione production [14, 22, 23]. But there is no study evaluating TAS levels in COVID-19. In this study, we evaluated serum TAS levels and found significant decrease in mild, moderate and severe groups compared to healthy controls (Figure 1). TAS assay shows total antioxidant capacity and briefly based on the principle of initiating colored dianisidyl radicals due to hydroxyl radicals produced by fenton reaction. Potential antioxidant substrates in serum such as vitamin C, reduced glutathione, bilirubin and uric acid are suppressed the color formation depending on their concentrations as an indicator of serum total antioxidant capacity [9]. It can be suggested that decreased TAS levels may be due to the consumption of serum antioxidants including glutathione in COVID-19 patients. And this consumption is initiated from the early stage of the disease (Figure 1).

It is well known that fibrin/fibrinogen degradation products and D-dimer increase in COVID-19 [3, 15]. We found significant elevation in levels of fibrinogen and D-dimer in patient groups and confirmed the previous studies (Table 3). However, we found significant correlations between fibrinogen and D-dimer levels with TOS, OSI, IL-6, CRP and ferritin. These results implicate that fibrinogen and D-dimer levels are related to the increased oxidative stress and inflammation in COVID-19.

COVID-19 patients with comorbidities have a poor prognosis and underlying diseases such as hypertension, cardiac disease etc. have been reported as risk factors for severe disease and death. Of the 64 patients included in our study, 22 (34.4%, Table 2) patients had comorbidities including DM, hypertension, CHD, CKD, and CPD. Consistent with the literature, the rate of comorbidities in patients in the severe group was higher than other groups [24, 25]. Serum AST, CK, LDH activities and creatinine levels were significantly higher only in severe group compared to controls. But only CK and LDH levels were above the reference ranges. LDH enzyme levels indicate heart, lung, kidney, muscle tissue and erythrocyte damage. It increases due to multiple organ damage in COVID-19 patients [3, 26]. In this study, LDH enzyme activities were correlated with oxidative stress and inflammatory parameters (Table 4). Our study results support that the increasing LDH levels were associated with disease severity in COVID-19.

The small sample size due to single-center design is one of the limitations of this study. Another limitation is that critically ill patients were not included in the study group.

Conclusions

In the present study, TOS and OSI levels were increased due to the disease severity and showed a positive correlation with other prognostic markers, including inflammatory and coagulation markers. The measurement of TOS levels and calculated OSI values, an inexpensive and automated method, can be an important marker for evaluating the disease severity and used in the management of COVID-19 patients.

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