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Role of Oxidative Stress in Pathogenesis and Severity of COVID-19 Infection: Case-Control Study in Iraq

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Abstract

Coronavirus disease 2019 (COVID-19) has presented a significant threat to public health and has rapidly spread across the globe since its outbreak in Wuhan, China, in 2019. Clinical evidence suggests higher oxidative stress in COVID-19 patients, and this worsening redox status may contribute to disease progression. The present study aimed to investigate oxidative stress in patients with mild and severe COVID-19. A case-control study was conducted from September 2021 to January 2022 among eighty-eight COVID-19 patients (male: female, 35:53) and eighty-eight healthy volunteers as the control group (male: female, 53:35) with ages ranging from (18-45) years in Duhok city, Kurdistan Region-Iraq. According to the severity of infection, patients were divided into two groups (mild and severe). Serum levels of malondialdehyde (MDA) and 8-isoprostaglandin F2 alpha (8-iso-PGF2α) were assessed as oxidative stress markers. In addition, serum activity of two main antioxidant enzymes, superoxide dismutase (SOD) and catalase were measured. Furthermore, their correlation with the most frequently used laboratory parameters, C-reactive protein (CRP) and D-dimer, were investigated. Serum levels of 8-iso-PGF2α and MDA were considerably higher in patients with COVID-19 compared to healthy individuals ($p < 0.001$) and between severe and mild patients ($p<0.001$). The activity of CAT was greater in COVID-19 group than in controls ($p=0.011$), but the difference between severe and mild diseases was statistically insignificant ($p>0.05$). However, SOD activity showed an insignificant difference between control and case groups ($p>0.05$), as well as between mild and severe groups ($p>0.05$). Also, a significant correlation was found between oxidative stress biomarkers and laboratory parameters CRP and D-dimer ($p<0.001$; and $p=0.020$), respectively. COVID-19 patients show significantly increased oxidative stress parameters. This may play a crucial role in the disease pathophysiology and could be considered as a predictive marker for COVID-19 severity.

Keywords: *COVID-19, Disease severity, Pathogenesis, Oxidative Stress, Cytokine Storm, Inflammation*
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I. INTRODUCTION

Coronavirus disease 2019 (COVID-19) pandemic has rapidly spread worldwide and is a significant threat to global health (Zhu *et al*., 2020). This disease was identified for the first time in December 2019 in Wuhan, China (Peng *et al*., 2020). Current statistics indicate that it has affected 189 nations and territories (Johns Hopkins University, 2022), with almost 566 million confirmed cases and over six million documented fatalities globally (WHO, 2022). COVID-19 may cause mild to severe symptoms (acute lung injury), hospitalization for 3–4 days, transfer to the intensive care unit (ICU), and even death (Valencia, 2020). ICU patients require ventilation for oxygenation and intubation to maintain life (Cascella *et al*., 2020). Approximately half of severely ill ICU patients eventually die from infection-related complications, generally following multiple organ failures (Cascella *et al*., 2020). The complications of COVID- 19 have been linked to underlying medical issues, specifically hypertension, diabetes, and other cardiovascular diseases in older persons (Alwazeer *et al*., 2021). Typically, respiratory virus infections are linked to inflammation and cytokine production (Delgado-Roche and Mesta, 2020). Patients with COVID-19 have increased serum levels of cytokines and chemokines (Huang *et al*., 2020). In other instances, cytokine storm emerged, which is considered a significant element in the development of acute respiratory distress syndrome (ARDS) and multiple organ dysfunctions (Xu *et al*., 2020) and is the leading cause of mortality among young and middle-aged people with no medical history (Peng *et al*., 2020). Oxidative stress (OS) plays a vital role in inflammatory responses; reactive oxygen species (ROS) and hydrogen peroxide (H2O2) can activate NF-κB to promote inflammatory cytokines production (Nanduri *et al*., 2008).

Numerous articles have been published regarding the involvement of OS in viral

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infections such as HIV, respiratory syncytial virus, and herpes viruses such as Epstein Barr virus (EBV) (Schwarz, 1996; Paracha *et al*., 2013). However, the general pathologic mechanisms of ROS generation and infection are the same for all viruses. Essentially, viruses disrupt redox equilibrium viral infected cells and stimulate the ROS formation in activated phagocytes (Camini *et al*., 2017; Khomich *et al*., 2018; Checconi *et al*., 2020). Moreover, studies suggested that the ROS role in the pathophysiology of SARS-CoV-2 infection and the relationship between viral infection, ROS production, and OS levels (Hosakote *et al*., 2009; Paracha *et al*., 2013; Camini *et al*., 2017; Suhail *et al*., 2020; Mironova *et al*., 2020; Beltrán-García *et al*., 2020; Delgado-Roche and Mesta, 2020). Hence, the association between OS and the severity of the disease has not been adequately studied. Therefore, we aimed to assess the OS and its effect on the pathophysiology of COVID-19 infection and determine the severity of the disease by measuring OS parameters in COVID-19 patients in Duhok city. Hence this study is considered one of the new approaches for studying COVID-19 patients and disease severity status in our country.

II. MATERIALS AND METHODS

A. Patients and study design

This case-control study was performed at Burn and Plastic Surgery Hospital in Duhok, Kurdistan Region-Iraq, designated for COVID-19 patients. The study was established from September 15th, 2021, to January 20th, 2022. Eighty-eight patients with COVID-19 (male: female, 35:53) and eighty-eight healthy subjects (male: female, 53, 35) with a mean age was 30.15±8.45 (ranging from 18 to 45) years were considered throughout this study.

B. Research tools and data collection

A questionnaire was prepared to gather essential information from participants and was filled in through direct interviews after obtaining their consent. The questionnaire included personal information (name, age, sex, work, contact number, and address), direct measurement of body features (height and weight), date of COVID-19 infection, smoking habit, diseases that were related to exclusion criteria, and history of taking medications (cytotoxic drugs) and antioxidant supplementations.

C. Inclusion criteria

1. Patients Group: Severe (ICU) and Mild to Moderate (non-ICU). The classification and diagnosis of COVID-19 were based on the WHO's Interim Guidance for Clinical Management of COVID-19 (WHO, 2020). The subjects with mild(mostly)to moderate disease were adults with a fever, fatigue, cough, myalgias, anorexia as well as other non-specific symptoms such as sore throat, nasal congestion, headache, diarrhoea, nausea, and vomiting, but showing no evidence of pneumonia or hypoxia. Or signs of mild pneumonia, indicated by SpO2 > 90% on room air, and were not admitted to the ICU. While (ICU group) severe cases further met at least one of the following criteria: SpO2 < 90% on room air, respiratory rate >30 breaths per minute, or having severe respiratory distress. All patients were confirmed to have SARS-CoV-2 infection by real-time reverse transcriptase-polymerase chain reaction assay (RT-qPCR) from nasal and pharyngeal swab specimens.

2. Control group: The participants in this category were selected as healthy subjects free of any underlying disease.

D. Exclusion criteria

Participants with a history of hypertension, diabetes militias, autoimmune diseases, malignant tumours, and smokers were excluded. In addition, participants who took antioxidant supplements or had a history of COVID-19 infection were also excluded from the study.

E. Body Mass Index (BMI)

BMI was measured using data from (Deore *et al*., 2012) by "dividing the weight in kilograms on the squares of height in meters (kg/m^2)"

F. Sample collection

Approximately 8 ml of blood was withdrawn in two tubes. The first one contained 3.2% buffered sodium citrate for preparing plasma. The obtained plasma samples were used to assess CRP and D-dimer following standard laboratory procedures using the clinical chemistry analyzer Cobas 6000 Roche. The second tube was a serum separating tube. The obtained serum samples were converted into Eppendorf tubes. Then stored in a freezer (-20) for later assessments of malondialdehyde (MDA), 8-isoprostaglandin F2 alpha (8-iso-PGF2α), catalase, and superoxide dismutase (SOD).

G. Determination of oxidative stress markers

A human (8-iso-PGF2a) ELISA kit with catalog number: SL0036HuSensitivityty: 1.8pg/ml (from SUNLONG, China), was used for quantitative detection of serum 8-iso prostaglandin F2α. In addition, Malondialdehyde (MDA) kit (Solarbio, Beijing, China) was used for spectrophotometric determination of the serum MDA concentration of patients following the manufacturer's protocol.

Serum activities of two antioxidant enzymes: Catalase activity was estimated by the spectrophotometric method using the readily available kit (Elabscience Biotechnology Inc.). SOD activity was also measured in serum using the commercial kit according to the instructions of the manufacturer (Solarbio, Beijing, China) via spectrophotometer.

H. Statisticalcal analysis

SPSS version 20 and GraphPad Prism version 8 for Windows were utilized for all analyses. The distribution of qualitative data was characterized by frequencies and percentages and compared between three groups (control, mild, and severe) using Chi-square testing or Fisher exact tests or reported as median ($25–75^{th}$ percentile) where appropriate. The mean and Standard deviation (SD) of quantitative data were compared using a one-way Analysis of Variance (ANOVA). Moreover, the Independent-samples T-test was also used, where

appropriate. A p-value less than 0.05 was considered statistically significant in all tests. Finally, Kendall's tau-b (b) correlation test was used to determine the possible correlations between variables.

I. Ethical considerations

Before starting any research procedures, informed consent was obtained from participants. Research Ethics Committee of Duhok Polytechnic University, Ministry of health – planning department, and Duhok General Directorate of Health, Kurdistan Region-Iraq, approved the study. (Reference number: 15092021-9-16).

III. RESULTS

A. Demographic characteristics of study populations

Table 1 presents the baseline characteristics and biochemical parameters of the subjects. Regarding gender, the male-to-female ratio of case group (mild and severe) and control group were 1:1.3, 1:1.8, and 1.51:1, respectively.

In addition, the difference in the prevalence of the SARS-CoV-2 vaccine was statistically significant among study participants; among healthy controls, (37.5%) were vaccinated, while in the patient group (mild and severe), only (10.9% and 0.0%) were vaccinated in turn; p <0.001. Also, the mean value of BMI was significantly elevated in the case group (mild and severe) compared to the healthy subjects. In the group of patients (mild and severe), the BMI was (25.87 ± 7.42 and 28.68 ± 5.90) (kg/m^2), and in the healthy group was (23.99 ± 4.27) (kg/m^2), p <0.001. Referred to laboratory assessments, C-reactive protein, and D-dimer levels were greater in the severe group than in the mild group; p = 0.002 and p = 0.01.

Table 1. The laboratory and demographic characteristics of case (mild and severe) and control groups.

Variables	**Controls (mean ± SD)**	**Mild Disease (mean ± SD)**	**Severe Disease (mean ± SD)**	**P value**
Age (year)	26.57 ± 6.82	31.5 ± 8.87	36.26 ± 7.39	< 0.001
Gender				
Male	53 (60.2%)	20 (43.5%)	15 (35.7%)	0.019
Female	35 (39.8%)	26 (56.5%)	27 (64.3%)	
Vaccination status				
Vaccinated	35(37.5%)	5(10.9%)	0 (0.0%)	< 0.001
Unvaccinated	55(62.5%)	41(89.1%)	42(100%)	
BMI (kg/m2)	23.99 ± 4.27	25.87 ± 7.42	28.68 ± 5.90	< 0.001
CRP (mg/L)	2.91 ± 1.43	27.62 ± 16.35	94.03 ± 69.04	< 0.001
D-dimer (ng/mL) (IQR)	370 (293-431)	2014(540-1230)	5149 (1246-4490)	0.01

B. Evaluating oxidative stress biomarkers across the study groups

The serum levels of MDA, 8-iso-PGF2α, SOD, and catalase are reported in Table 2. Following the present results, the 8-iso-PGF2α levels in case groups (mild and severe) were substantially higher than control group: 134.16 ± 20.44, 182.58 ± 22.15, and 127.47 ± 26.83 (pg/mL), respectively; p <0.001. Also, serum levels of 8-iso-PGF2 differed significantly between mild and severe patients.; p <0.001 (Figure 1A).

Table 2. Antioxidant enzymes and markers of oxidative stress among mild, severe COVID-19 patients, and healthy people.

Parameters		Healthy Controls (mean ± SD)	Mild Disease (mean ± SD)	Severe Disease (mean ± SD)	P value
Antioxidant enzymes	SOD (U/mL)	16.95 ± 2.89	18.35 ± 3.97	17.73 ± 2.87	0.053
	Catalase (mU/L)	13.70 ± 3.62	15.15 ± 3.44	15.38 ± 2.96	0.011
Oxidative stress markers	MDA (mmol/L)	1.23 ± 0.35	1.37 ± 0.24	1.90 ± 0.20	<0.001
	8-iso-PGF2α (pg/mL)	127.47 ± 26.83	134.16 ± 20.44	182.58 ± 22.15	<0.001

The serum concentration of MDA was considerably higher in the patient's group 1.37 ± 0.24 and 1.90 ± 0.20 mmol/L than in the healthy group 1.23 ± 0.35 mmol/L; p <0.001. Interestingly, serum levels of MDA in the severe group were substantially greater than in the mild group, p <0.001 (Figure 1B). The mean serum concentration of catalase in the Control group was 13.70 ± 3.62 (mU/L), which was significantly less than in case groups 15.15 ± 3.44 in mild and 15.38 ± 2.96 (mU/L) in severe group, p =0.011. There was no significant difference in serum catalase levels between mild and severe groups, p > 0.05 (Figure 1C). The mean differences in SOD were not statistically significant among study groups, p >0.05 (Figure 1D).

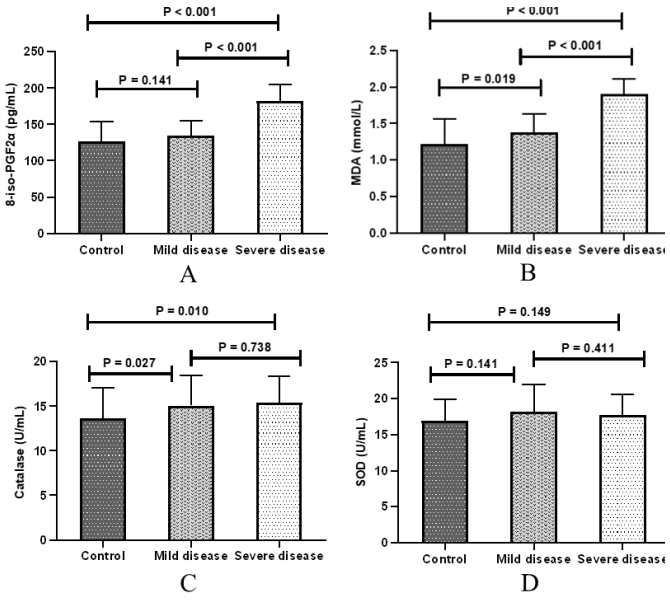

Figure 1. Box plots showing the antioxidant enzymes activity and oxidative stress biomarkers across study groups.

Table 3. Correlation between 8-iso-PGF2 and other variables among study participants.

Parameters	Study subjects	
	Correlation (r)	P value
Age (years)	0.300**	< 0.001
BMI (kg/m2)	0.328**	< 0.001
Catalase (mU/L)	0.085	0.095
SOD (U/mL)	0.032	0.524
MDA (mmol/L)	0.417**	< 0.001
D-dimer (ng/mL)	0.267*	0.020
CRP (mg/L)	0.498**	< 0.001

C. Correlations with clinical and oxidative stress parameters against 8-iso-PGF2α levels

The Kendall's tau-b (τb) correlation test was conducted to determine the correlation coefficient between 8-iso PGF2α levels and all other inflammation and oxidative stress parameters in the study population. The results revealed a remarkable positive correlation for MDA (r = 0.417, p<0.001), CRP (r = 0.498, p <0.001), also for age and BMI (r = 0.300, p < 0.001) (r = 0.328, p <0.001), respectively, however, catalase (r = 0.085, p =0.095) and SOD (r = 0.032, 0.524) values presented no significant relation with 8-iso-PGF2α levels. Other positive association between 8-iso-PGF2α levels noted is D-dimer (r = 0.267, p =0.020) (Table 3).

IV. DISCUSSION

The findings from this study contribute to clinical evidence that OS level is higher in COVID-19 patients than in healthy individuals. This is recognized by a significant increase in serum MDA and 8-iso-PGF2α levels; this altered redox status could contribute to the course of the disease. Similar results were reported by Pincemail *et al*. and Alamdari *et al*. who demonstrated that the OS levels were significantly elevated in patients with COVID-19 compared to control groups (Alamdari *et al*., 2020; Pincemail *et al*., 2021). The increased MDA levels in patients with COVID-19 indicate an overproduction of free radicals, which damages lipid membranes, leading to the generation of MDA and 8-iso-PGF2 as by-products. In addition, there is a strong relationship between OS biomarkers and respiratory viral infection, especially RNA viruses (Komaravelli *et al*., 2014). In the current research, the levels of 8-iso-PGF2α and MDA were substantially higher in the severe group compared with the mild group. This finding aligns with the results of a cross-sectional study done by Mehri *et al*. (2021) who demonstrated that the serum MDA levels in ICU patients were substantially greater than in patients with mild disease. In addition, the retrospective cohort study by Zheng *et al*. (2021) revealed that the serum levels of 8-iso-PGF2α were higher in severe patients with more extended hospital stays than those with mild disease.

This can be attributed to an excessive amount of ROS, therefore, triggers a cascade of pathological events and would elevate OS levels and lead to the development of a severe phase of the disease, as well as organ failure and even death (Polonikov, 2020; Pincemail *et al*., 2021). Therefore, both markers might be strong and reliable prognostic factors. According to in vitro and in vivo studies, some viruses can change the cell redox balance. The initiation of OS is due to a virus infection (such as a respiratory syncytial virus) required to activate innate immunity via the generation of cytokines (Kim *et al*., 2013). In addition, OS generated by several viruses promotes virus replication within the cell (Gjyshi *et al*., 2014). ROS can be generated because of the respiratory burst function of macrophages in response to COVID-19 infection (Jain *et al*., 2020). Acute respiratory infections can cause damage to lung tissue and epithelial barrier disruption due to excessive ROS/RNS generation (Ivanov *et al*., 2017). NADPH oxidase 2 is crucial in ROS generation, vascular dysfunction, and thrombosis (induced by platelet activation) (Fuentes *et al*., 2018). In the epithelial cells of the small airway and alveolar type 2-like epithelia, viruses can disrupt antioxidant systems, such as SOD, peroxidase, glutathione peroxidase, and glutathione S-transferase, and catalase (Poggi and Dani, 2014). According to the findings of this research, a high activity of catalase in serum was observed as an antioxidant enzyme; this could be a remedial mechanism against OS.

The results of the current investigation supported those of Mehri *et al*. (2021) and Goud *et al*. (2021), who found a correlation between elevated catalase levels and clinical progression in viral infections. However, Muhammad *et al*.

(2021) observed that COVID-19 patients had a greater OS rate and decreased antioxidant status, as well as deteriorating catalase activity. Consequently, OS levels change in infection with SARS-CoV-2 and other viruses, but the parameters and pathophysiological mechanisms underlying these changes are still unclear; however, the parameters that change and the pathophysiological mechanisms behind these alterations are unclear. While in the present research, there were no significant changes between the SOD enzyme activity of patients infected with COVID-19 and those of healthy controls. Similarly, the results of this investigation matched those of a recent study by Yaghoubi *et al*. (2022). Therefore, it is reasonable to conclude that the enzyme activity has not significantly changed. However, a minor difference was noticed, this may be due to the quick development of COVID19 illnesses, and there is insufficient time to alter the enzyme activities. In addition, compensatory mechanisms can maintain enzyme levels near normal. SOD and catalase significantly neutralize free radicals, such as RNS and ROS (Pham-Huy *et al*., 2008).

Consistent with current findings, Zhu *et al*. (2020) found higher inflammatory biomarkers levels, such as CRP, in patients with COVID-19, particularly in patients requiring ICU care, compared to mild cases. It is well accepted that CRP is a sign of severe systemic inflammation and infections which are severe. As an acute-phase reactant, CRP can be bound to phosphocholine in host cell membranes and pathogens and act as an opsonin to aid phagocytosis and clearance (Ballou and Kushner, 1992). Additionally, ligand-bound CRP efficiently stimulates the classical route of the complement system, a crucial component of the innate host defense (Volanakis, 2001). According to our findings, significantly more individuals with severe COVID-19 infection who required hospitalization had elevated D-dimer levels than those with mild illness. Other studies demonstrated similar findings (Lehmann *et al*., 2021). Increased D-Dimer is most possibly a result of acute lung injury or the increased rate of complications of thromboembolism, which caused by an imbalance in endothelial damage, procoagulant agents, natural coagulation inhibitors, and inflammation in patients with the COVID-19 virus (Voicu *et al*., 2021). In the long-term follow-up, the D-Dimer level may significantly predict thromboembolic events (Cosmi *et al*., 2008; Jara-Palomares *et al*., 2018).

V. Conclusion

Our findings revealed that the patients with COVID-19 experience high levels of OS, which may worsen their condition. One of the benefits of this study is that it excludes any underlying condition that may have an impact on OS levels. Overproduction of free radicals and defects in the antioxidant system plays a significant role in the SARS-COV-2 pathogenesis. Conversely, the interaction between OS and cytokine storm may have a crucial role in the severity of infection due to COVID-19. Therefore, OS levels could be considered a predictive marker of COVID-19 severity. Consequently, it appears that the methods aimed at reducing or preventing OS could aid in managing COVID-19. However, for further clarity of the involvement of OS parameters in COVID-19, a larger sample size comprising patients with varying degrees of disease severity and antioxidant measurements such as vitamins and trace elements is required.

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