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Oxidative DNA Damage in Relation to the Severity of COVID-19 Infection in Duhok City, Kurdistan Region- Iraq

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Abstract

Coronavirus disease 2019 (COVID-19) 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 which may contribute to disease progression. The present study aimed to investigate the oxidative Deoxyribonucleic acid damage in patients with mild and severe COVID-19 infection and to evaluate its relationship to the disease progression and severity. A case-control study was conducted from September 2021 to January 2022 in Duhok city, Kurdistan Region-Iraq. 180 individuals have participated. Among 88 COVID-19 cases, 92 healthy volunteers as the control group, with ages ranging (18-45) years. Patients were divided into two groups according to the severity of infection (mild cases, severe cases). Serum level of 8-OHdG and malondialdehyde (MDA) were assessed as oxidative stress biomarkers. Serum levels of 8-OHdG were considerably higher in patients with COVID-19 infection in comparison to the control group, ($p<0.01$). The further statistical analysis has revealed a significantly higher 8-OHdG in blood in female cases with severe COVID-19 infection compared cases with a mild infection, ($p<0.01$). Serum MDA levels in severe cases were higher, statistically significant when compared with the control group ($p=0.007$). Severe cases had higher level of MDA than in mild case, in male cases ($p<0.05$) in female cases ($p<0.0001$). The current data suggest that patients who were infected severely with COVID-19 are under huge oxidative stress attack. Analysis of data shows that severe cases of COVID-19 infection had significantly greater level of serum 8-OHdG than in healthy control subjects.

Keywords: *Oxidative DNA Damage, Lipid Peroxidation, 8-Ohdg, Disease Severity, Prognosis*

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I. INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a respiratory infection caused by a novel coronavirus identified as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). COVID-19 infection was first detected in Wuhan (China) (Zhu *et al*., 2020). An Emergence of viral diseases represents a serious threat to global public health and spreads worldwide. Therefore, world Health Organization (WHO, 2020) stated it as a pandemic. The pathogenic agent of COVID-19 disease is an enveloped RNA betacoronavirus (Wax and Christian 2020), and cause the mild symptoms or severe symptoms, such as acute lung injury, hospitalization, and even death. Symptomatic patients with mild disease show cough, anorexia, myalgia, and fever; other non-specific symptoms such as anosmia, ageusia, sore throat, headache, nausea, vomiting and diarrhea respiratory symptoms with no signs of hypoxia or pneumonia. However, patients with severe clinical symptoms present severe pneumonia, cough, fever, dyspnea, severe respiratory distress (SpO2 <92% on room air) and respiration rate >30 breaths/minute (Agrupis *et al*., 2021). Shang *et al*., (2020), stated that mild patients show rapid recovery whereas patients with moderate or critical symptoms show poor prognosis with high mortality range. Recently, several studies have suggested the potential role of Oxidative Stress (OS) in COVID-19, causes the lung tissue damaged by viral replication experiences an immune response that is overly active as a result of viral pneumonia produced by SARS-CoV-2. Free radicals are released by activated immune cells in order to eliminate the infection. Oxidative stress is almost always present during this disease phase. Moreover, it has been proposed that oxidative stress in patients with COVID-19 could contribute to the cytokine storm and coagulopathy. Additionally, antioxidant agents have been argued to reduce oxidative stress (Nasi *et al.*, 2020; Delgado-Roche and Mesta 2020). Generally, respiratory viral infections are related with various pathophysiological processes including cytokines production, inflammation and

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apoptosis that may be linked with oxidative stress or redox imbalance (Khomich *et al.*, 2018). In COVID-19 infection receptor binding domain (RBD) in S protein of virus is tightly bind to angiotensin converting enzyme II (AGE2) receptor of the alveoli, in type-II pnuemocytes. Angiotensin 2 is converted to angiotensin-(1-7) via ACE2 (Tai *et al*., 2020). Reactive Oxygen Species (ROS) produced as a result of membrane-bound NDPH oxidase stimulation by Ang II (Suhail *et al.*, 2020). Consequently, Angiotensin 2 is degraded into Angiotensin (1-7) through ACE2, reduces OS via NDPH oxidase inhibition (Lovren *et al.*, 2008). According to Sena *et al*., (2018) emphasized that, ACE2 bounding to COVID-19 down-regulates ACE2, resulting an elevated existence of superoxide species and consequent cellular damages, that could contain protein carbonylation, DNA oxidation, and lipid peroxidation. Nucleic acid damage induced by oxidative stress and significant elevation of oxidized guanine species (OGS): 8-oxo-deoxyguanosine (8-oxo-dG) and also named 8-hydroxy-20-deoxyguanosine (8-OHdG) an oxidative, modified DNA product has intensively been assessed as a biomarker of DNA damage caused by oxidative stress due to its stability. The elevation in 8-OXO-dG level is linked with severity and poor prognosis of diabetes, cancer, cardiovascular diseases and in viral infections such as hepatitis C (HCV), hepatitis B virus (HBV), and HIV (Gunson *et al.*, 2003; Kolgiri *et al.*, 2018; Wu *et al.*, 2004). The increase in the level of oxidative species in the body could cause genomic damage (oxidative DNA damage) that may further trigger tissue damage and immune system dysfunction (Markkanen, 2017). However, Studies have been conducted about lipid, protein peroxidation, and antioxidant status in SARS-COV-2 infection patients. Therefore, this study aimed to evaluate DNA damage caused by oxidative stress assessed by serum 8-OHdG as a prognostic factor and to compare and find its relationship with MDA, CRP, and D-dimer, and the severity of the disease.

II. Materials And Methods

A. Subjects and study design

To achieve the aims of this study, a case-control design was performed from September 2021 to January 2022. All participants were adult, nonsmokers, had no history of taking cytotoxic agents, and had no history of chronic disease such as cardiovascular disease, neurodegenerative disorder, cancer, chronic infammation, as well as they had no chemotherapy or radiation or any other condition that causes oxidative stress or DNA damage. 210 individuals were participated in this study, (age 18– 45 years) living in Duhok city, Kurdistan Region, Iraq. 30 participants excluded because of pathologic conditions that lead to oxidative stress, smoking, lack of enough sample, and death of many intensive care unit ICU participants before sampling. A total of 92 healthy subjects with no history of COVID-19 infection and 88 COVID-19 patients (n=48) with mild symptoms and (n=40) severe cases admitted to Intensive Care Unit (ICU) at Burn and Plastic Surgery Hospital in Duhok.

All cases were confirmed to have COVID-19 infection by real-time reverse transcriptase-polymerase chain reaction assay (RT-PCR) from nasal and pharyngeal swab specimens. Healthy subjects were those who did not have the infection before. Therefore, according to the WHO guidelines the study population was stratified into healthy subjects, mild cases (home recovery), and severe cases (admitted to ICU) (World Health Organization 2020).

B. Sample collection

All enrolled participants had their blood drawn by venipuncture into 5 mL to the gel tube for serum separation and the blue-capped tube containing 3.2% buffered sodium citrate to seperate plasma for measuring D-dimer. The samples were centrifuged immediately after venipuncture, and the resulting serum was stored in an Eppendorf tube and frozen at (-20° C) for further investigations. D-dimer and CRP measured by Cobas 311. MDA was measured using a spectrophotometer kit (SolarBio), and serum 8-OHdG was determined using an ELISA kit with catalogue number: SL2044Hu, Sensitivity: 16pg/ml (from SUNLONG).

C. Statistical analysis

Using IBM SPSS Statistic 20, all data were analyzed. Categorical data were summarized as frequency (percentage) according to the kind of normalcy of distribution and variables, and results were given as the mean and standard deviation. Statistical hypotheses were examined using an independent T-test between two groups. Kendall's tau-b (τb) correlation coefficient was used to determine the correlation between variables. The data were shown graphically using Graph-pad Graph-pad Prism version 5. A P-value less than 0.05 (typically < 0.05) is statistically considered significant.

D. Ethical aspects

Both Ethical Committee of Duhok General Directorate of Health of Kurdistan Region- Iraq, and Scientific Committee of Duhok Polytechnic University examined and approved the study protocol on September 15th, 2021. *Reference number: 15092021-9-15*.

III. Results

A. Study samples distributions

A total of one-hundred eighty people were enrolled in this study. Participants were divided into two groups, the COVID-19 cases were eighty-eight (35 male, 53 female) with mean ± SD of age (34.79 ± 1.562 and 33.20 ± 1.143 years), respectively, and the ninety-two control group were ninety two (51 male, 41 female) with mean ± SD (27.39 ± 0.9961 and 25.59 ± 1.116), in turn. The 8-OHdG level and BMI of patients were significantly higher compared with control group. Although the case group had greater levels of MDA than the control group, statistically, there were no significant differences (Table 1). As for the clinical condition, patients were classified as mild cases n=48 and severe cases n=40

admitted to ICU. In accordance with current findings, the CRP, D-Dimer, MDA, and 8-OHdG levels and BMI for both genders were significantly higher in severe group than mild group, besides in male patients, 8-OHdG and CRP showed no significant difference between patient groups (Table 2).

Table 1. Demographic and biochemical features of study population

Variables		Control (mean ± SD)	Cases (mean ± SD)	P value
Age (year)		31.95 ± 0.93	34.29 ± 0.91	0.0758
Gender	Male (n)	51	35	
	Female (n)	41	53	
BMI (kg/m2)		24.20 ± 0.46	27.40 ± 0.78	< 0.001
MDA (mmol/L)		1.583 ± 0.12	1.677 ± 0.06	0.4650
8-OHdG (pg/mL)		1072 ± 145.2	1466 ± 44.67	< 0.001

Table 2. Biochemical variables in case group

variable		Mild (mean ± SD)	Severe (mean ± SD)	P-value
CRP	Male	25.30 ± 11.28	174.7 ± 93.87	0.34
	Female	16.85 ± 4.45	86.03 ± 15.93	<0.05
D-Dimer	Male	366.3 ± 40.03	1778 ± 496.9	<0.05
	Female	618.0 ± 87.28	1951 ± 361.0	<0.05
MDA	Male	1.477± 0.10	1.697 ± 0.21	<0.05
	Female	1.358 ± 0.11	2.090 ±0.73	< 0.0001
8-OHdG	Male	1419 ± 110.9	1491 ± 69.71	0.625
	female	1326 ± 36.80	1574 ± 44.68	<0.01

B. *Serum 8-OHDG levels in study participants*

The mean ± SD of serum 8-OHdG in patients was (1466 ± 44.67) significantly higher than in control subjects (1016 ± 143.0), *P*=0.0084 (Figure1). The mean of Serum 8-OHdG in mild cases was not significantly higher than that in control subjects, 1378 ± 63.16, 1016 ± 143.0 respectively, p=0.073, while its level was significantly greater in severe cases compared to that in control subjects, 1556 ± 60.58, 1016 ± 143.0 respectively p= 0.008 (Figure 2).

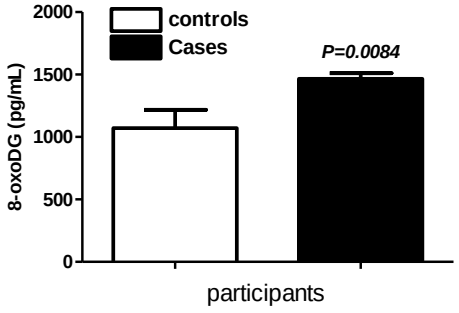

Figure 1. Serum 8-OHdG levels in the study participants' cases and controls. Analyzed by Graph-pad Prism.

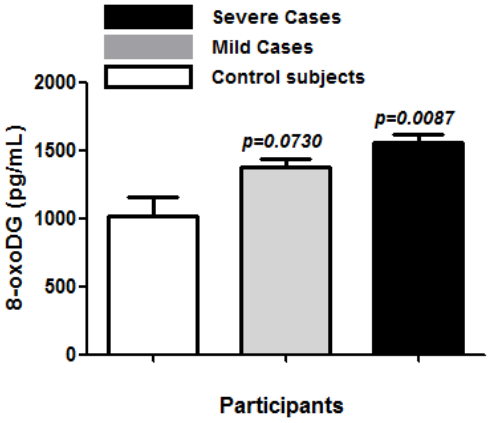

Figure 2. Difference between the mean of serum 8-OHdG in control subjects and case groups (mild and severe) of COVID-19 infection. Analyzed by Graph-pad Prism.

C. *Serum MDA levels in study participants:*

Serum MDA level in severe cases was statistically significant when compared with control group p=0.007, mean ± SD (1.970 ± 0.07366, 1.583 ± 0.1275) respectively, while no significant difference between mild cases and control was found mean ± SD (1.412 ± 0.04011,1.583 ± 0.1275) respectively (Figure 3).

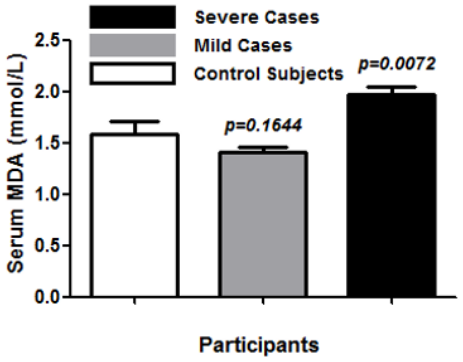

Figure 3. Comparison of serum MDA levels in cases (mild and severe) with controls. Analyzed by Graph-pad Prism.

D. *Association of Serum 8-OHdG level and other parameters in patients with COVID-19*

According to Kendall rank correlation coefficient (r), there was a significant positive correlation (p-value< 0.05) between serum 8-OHDG level in COVID-19 cases with Age, increased level of 8-OHdG found in older participants. Furthermore 8-OHdG was positively correlated with obesity, patients with elevated BMI had high level of oxidative DNA damage (Kendall r=0.140, p<0.05). Additionally, inflammatory biomarker CRP and coagulation biomarker D-dimer were significantly correlated to high 8-OHdG (Kendall r=0.302, p<0.05), and (Kendall r=0.213, p<0.05) respectively. Moreover, oxidative DNA damage and lipid peroxidation biomarker serum MDA were statistically positively correlated (Kendall r=0.167. p<0.05) (Table.3).

Table 1. Correlation between serum 8-OHDG level with other parameters

Parameters	Study subjects	
	Correlation (r)	P-value
Age (Years)	0.218**	< 0.001
BMI (Kg/M2)	0.140*	<0.05
D-Dimer (ng/mL)	0.213*	<0.05
CRP (Mg/L)	0.302**	<0.05
MDA (Mmol/L)	0.167*	<0.05

IV. DISCUSSION

Our result on severity of infection, the relationship of oxidative stress parameters with inflammatory biomarkers, gender, and BMI, contribute a further understanding about the mechanism of COVID-19 infection. The most striking findings of the present study shows a significantly higher level of oxidative DNA damage and lipid peroxidation in severe COVID-19 cases compared with mild cases of patients and in healthy subjects. 8-OHdG is a potential biomarker of oxidative genomic damage of DNA. Among SARS-CoV2 infected patients our findings show significantly increased levels of serum 8-OHdG compared to healthy subjects; and the higher levels of serum 8-OHdG was positively correlated to severity of the infection. Consistent with current findings a prospective cohort study by Cao *et al*. (2022) found higher serum level of 8-OHdG in admitted patients with pneumonia. In addition, a higher level of serum 8-OHdG was also found in community acquired pneumonia with longstanding ICU admitted patient compared to healthy subjects. Oxidative stress is associated with COVID-19 disease severity and even death. Evaluation of urinary 8-OHdG as a noninvasively biomarker of oxidative DNA damage by Tantry US *et al*. (2021) found that greater level of 8-OHdG was associated with longer hospitalization and greater mortality. Moreover, female cases had significantly higher level of serum 8-OHdG than in male cases; the same result had been reported by Mihaljevic *et al*. (2022). They found a greater level of DNA damage in COVID-19 infected females than in male cases as measured by Alkaline Comet Assay Technique (Mihaljevic *et al.*, 2020). BMI has an impact on the oxidative stress status. It has been found an exacerbation of oxidative stress in individuals with increased BMI (Włodarczyk and Nowicka 2019). In consistent to the proposed mechanism, our data showed a significantly greater level of serum 8-OHdG in COVID-19 infected patients with higher BMI. Since, the Kendall rank correlation coefficient showed that the higher serum 8-OHdG level is significantly correlated to BMI and the severity of COVID-19 infection. Currently CRP and D-Dimer are considered as prognostic blood biomarkers of COVID-19 infection. It was also investigated that and CRP and D-Dimer levels in COVID-19 patients too. Data analysis revealed that CRP and D-dimer positively correlated to the disease severity (higher levels of both markers were found in severe cases among males and females of the studied group). The relationship between increased level of CRP and D-dimer with the severity and mortality of COVID-19 infection was also reported in the literature (Ali *et al.*, 2022). Concerning lipid peroxidation parameter in patient group; male severe cases had higher level of MDA than male mild cases, same in female group. In addition, lipid peroxidation level reported greater in the severe cases in comparison to control group. Martín-Fernández M, *et al* found greater level of lipid peroxidation is associated a higher risk of disease severity or intubation/death at 28 days in COVID-19 cases (Martín-Fernández *et al.*, 2021). In addition, further analysis of correlations between oxidative DNA damage level and laboratory biomarkers indicates that 8-OHdG is positively correlated with MDA, CRP, and D-dimer in COVID-19 infected patients. These observations may support the hypothesis that oxidative DNA damage is positively correlated with disease severity and poor prognosis in COVID-19 infected patients. One good point of the study was the investigation and comparing the biomarkers of oxidative stress (including lipid peroxidation and oxidative DNA damage biomarkers) in severe and mild cases of COVID-19 infection and in healthy individuals. In addition, excluding most conditions that may affect the oxidative stress status such as elderly, chronic diseases, taking cytotoxic agents, and smoking were considered. The different degrees of disease severity (mild, moderate, severe, and critically severe), as well as a larger sample size, are required for further illustration (the limitations of the study).

V. CONCLUSION

our study data showed increased biomarkers of lipid (MDA) and DNA (8-OHdG) oxidative damage in COVID-19 patients, the level of oxidative DNA damage (8-OHdG) was furtherly increased as the disease more progressed and advanced. Most importantly, the current study provides further evidence for COVID-19 disease progression (mild to severe), gender, and BMI. Interestingly, the main findings were greater oxidative genomic damage and more lipid peroxidation and their association to the severity of the infection. These findings may support further understanding of the role of 8-OHdG as a biomarker for the severity of COVID-19 infection to further evaluate this relationship, more biochemical and epidemiological studies are needed.

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