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ARAŞTIRMA / RESEARCH

DNA damage and inflammation in COVID-19 cases

COVID-19 vakalarında DNA hasarı ve enflamasyon

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Abstract

Purpose: The aim of this study is to see oxidative DNA damage (8-OHdG), its relationship with inflammatory mediators (IL6 and TNFA), and its reflections on laboratory findings in patients who had COVID-19 infection at different intensities.

Materials and Methods: Serum interleukin-6 (IL6), tumor necrosis factor-alpha (TNFA), and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle.

Results: In COVID-19 positive patients treated in intensive care 8-OHdG marker level is at the highest level and statistically significant. In patients receiving inpatient treatment in the hospitalized, the 8-OHdG marker level is higher than the control and outpatient groups. IL6 values were at the highest level in the patient group treated in the intensive care unit and were higher than the outpatient and control groups. There was no statistically significant difference between the control and patient groups in terms of TNFA values. Neutrophil-to-lymphocyte ratio (NLR) was lower in the control group than in all patient groups. C-reactive protein (CRP) is higher in hospitalized patients than in the control group. Lactate dehydrogenase (LDH) was found to be statistically significantly higher in hospitalized patients than outpatients.

Conclusion: As the severity of COVID-19 increases, serum 8-OHdG and IL6 levels also increase. These parameters can guide the diagnosis of COVID-19 patients in the early stages of the disease course.

Keywords:. COVID-19, DNA damage, Interleukin-6, Tumor necrosis factor-alpha, 8-hydroxy-2'-deoxyguanosine.

Öz

Amaç: Çalışmamızda, COVID-19 enfeksiyonunu farklı şiddetlerde geçiren hastalarda oksidatif DNA hasarını (8-OHdG), inflamatuar mediatörlerle (IL6 ve TNFA) ilişkisini, ve laboratuvar bulgularına yansımalarını görmeyi amaçladık.

Gereç ve Yöntem: Serum interlökin-6 (IL6), tümör nekroz faktör-alfa (TNFA) ve 8-hidroksi-2'-deoksiguanozin (8-OHdG) düzeyleri ELISA (enzyme-linked immunosorbent assay) prensibine dayalı kitler kullanılarak ölçülmüştür.

Bulgular: Yoğun bakımda tedavi edilen COVID-19 pozitif hastalarda 8-OHdG marker seviyesi en üst düzeyde ve istatistiksel olarak anlamlı idi. Yatarak tedavi gören hastalarda 8-OHdG marker düzeyi kontrol ve ayakta tedavi gruplarına göre daha yüksekti. Yoğun bakımda tedavi edilen hasta grubunda IL6 değerleri en yüksek düzeyde olup, ayaktan ve kontrol gruplarına göre daha yüksekti. TNFA değerleri açısından kontrol ve hasta grupları arasında istatistiksel olarak anlamlı fark yoktu. Nötrofil-lenfosit oranı (NLR) kontrol grubunda tüm hasta gruplarına göre daha düşüktü. Hastanede yatan hastalarda kontrol grubuna göre C-reaktif protein (CRP) daha yüksekti. Laktat dehidrojenaz (LDH) hastanede yatan hastalarda ayaktan hastalara göre istatistiksel olarak anlamlı derecede yüksek bulundu.

Sonuç: COVID-19' un şiddeti arttıkça serum 8-OHdG ve IL6 seviyeleri de artmaktadır. Bu parametreler COVID-19 hastalarının hastalık seyrinin erken evrelerinde tanınmasına rehberlik edebilir.

Anahtar kelimeler: COVID-19, DNA hasarı, Interlökin-6, Tümör nekroz faktör-alfa, 8-hidroksi-2'-deoksiguanozin

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), 2019 identified by the World Health Organization as the etiologic agent of coronavirus disease (COVID-19)[1]. This virus is highly pathogenic and causes many different diseases, especially respiratory, enteric, hepatic and neurological[2,3]. Fever, cough, muscle pain, cough, and shortness of breath are among the first symptoms of COVID-19 [4]. In the later stages, shortness of breath may turn into acute respiratory distress or multi-organ failure [5]. Cytokine storms have been reported in many infectious diseases[4]. It has been suggested that the cytokine storm seen in COVID-19 is related to the severity of COVID-19[6].

Cytokine storm is a form of uncontrolled systemic inflammatory reaction[7]. Induction of inflammatory response a common feature of tumor necrosis factor-alpha (TNFA) and interleukin-6 (IL6) cytokines. They provide induction and secretion of additional cytokine pathways. These induce fever, general activation of C-reactive protein (CRP), fibrinogen, mononuclear phagocytes, and endothelin[8,9]. Endothelin activation triggers critical products of the inflammatory response, resulting in localized accumulation of circulating leukocytes[10,11].

A number of abnormalities in circulating cells reflecting inflammation and immune response have been identified in patients with COVID-19 infection. Some biochemical and hematological parameters are potential determinants of patient prognosis [12,13,14].

There is serious oxidative damage caused by free radicals in the pathophysiology of inflammatory processes with acute respiratory distress such as COVID-19. One of the major markers of oxidative damage is 8-hydroxy-2'-deoxyguanosine (8-OHdG)[15].

COVID-19 infection is experienced in different intensities among individuals. Investigating the mechanisms underlying some individuals greater susceptibility to SARS-CoV-2 infection and understanding why a subset of these are prone to more severe pneumonia, acute respiratory distress syndrome, and death will lead to a better approach and more effective treatments for COVID-19. There are limited studies in the literature on the relationship between oxidative DNA damage and patients with COVID-19 at different severity (outpatient, hospitalized, and intensive care). Therefore, in our study, we aimed to evaluate oxidative DNA damage and their relationship with inflammatory mediators (IL6 and TNFA) using serum 8-OHdG levels and to see their reflections on laboratory findings.

MATERIALS AND METHODS

Study population

In the current study, blood samples were taken from individuals referring to the Department of Emergency Medicine at the Training and Research Hospital affiliated to the Medical Faculty Ordu University. The laboratory phase of the study was carried out in Ordu University Faculty of Medicine, Department of Medical Biology Laboratory.

When we collected samples from our patient and healthy control groups, vaccines had not yet started in our country. Therefore, the participants of our study are unvaccinated. Pregnant women, immunosuppressed patients, and those with discordant PCR analyzes who were not diagnosed with COVID-19, who refused to participate in the study or who voluntarily terminated their emergency follow-up were excluded from the study.

105 participants were included in the study. However, 17 people refused to participate in the study. Therefore, while the minimum sample size was 89, the study was completed with 88 participants. A total of 88 participants, patient and healthy control, were included in our study. Of the participants, 47 are men and 41 are women. Our healthy control group (15 participants) consists of people over 18 years of age, without chronic disease and without COVID-19, and polymerase chain reaction (PCR) test negative. The patient group consists of those over the age of 18 who have positive COVID-19 PCR test. All patients suspected of having COVID-19 were included in the study because they had symptoms such as high fever, cough, shortness of breath, headache, sore throat, runny nose, muscle and joint pain, weakness, loss of sense of smell and taste, and diarrhea. COVID-19 positive patients were grouped as follows: Group 1 symptomatic and receiving outpatient treatment (34 patients). Group 2 receiving inpatient treatment in the hospitalized (24 patients). Group 3 receiving treatment in intensive care (15 patients).

The study has been carried out in accordance with the 1964 Declaration of Helsinki. This study was approved by the Ordu University, Faculty of Medicine Institutional Review Board Ethics

Committee (date:18.03.2021 number: 2021/KAEK 41) and Republic of Turkey Ministry of Health. Informed consent was submitted by all subjects when they were enrolled.

Measurements

In people who applied with the suspicion of COVID-19, routinely examined white blood cell count (WBC), hemoglobin (HB), platelets count (PLT), neutrophil to lymphocyte ratio (NLR), mean corpuscular volume (MCV), mean platelets volume (MPV). aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (CRE), C-reactive protein (CRP), lactate dehydrogenase (LDH). Hematological and biochemical test results for each participant were obtained from the hospital database. The clinical data of individuals with positive and negative COVID-19 PCR tests, their distribution according to the mean±standard deviation according to laboratory reference values, and statistical analyzes were determined.

The serums from the blood samples taken from the patients and controls in tubes without anticoagulant were centrifuged for 10 minutes at +4ºC. Supernatants were separated into serum separation tubes. Serum samples were stored at -80ºC until analysis.

Serum IL6, TNFA, and 8-OHdG, levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle. Serum 8-OHdG was measured using the Cloud-Clone Corp ELISA Kit (Cloud-Clone, USA). Serum IL6 and TNFA were performed using the DIA Source ImmunoAssays S.A ELISA Kit (Rue du Bosquet,2, B-1348 Louvain-la-Neuve, Belgium). The protocols of the relevant kits were followed. Absorbance (450 nm) was measured using the Biotek Epoch 2 microplate reader and Gen5 software.

The amounts of 8-OHdG, IL6 and TNFA in the serum were calculated from the standard curve and the results were expressed as pg/ml. The detection range was 74.07 to 6.000 pg/ml for 8-OHdG, 0-2560 pg/ml for IL6, and 0-518 pg/ml for TNFA.

Statistical analysis

The normality of data and homogeneity of groups variances were checked using the Kolmogorov–Smirnov test and Levene's test. The laboratory parameters were analyzed one-way ANOVA or Welch's ANOVA. Tukey's test or Games-Howell test was used as multiple comparison tests. Pearson's chi-square test was used to compare the distribution of men and women in groups. Statistical analysis used the SPSS 28 (IBM Co., Armonk, NY) software. In all statistical analyses, a p value less than .05 was accepted as statistically significant.

The power analysis was performed using the G*Power 3.1.9.6 statistical program to determine the sample size. It was assumed that four study groups would be compared with one-way ANOVA. Confidence interval (1-α)=95%, test of power (1-β)=80% and effect size "large effect size (w=0.4)" recommended by Cohen (1998) were used in the calculation.

RESULTS

The clinical and laboratory data of all cases are shown in Table 1 and Table 2. There were a total of 88 subjects, including 73 patients (35 females and 38 males) and 15 healthy control subjects (6 females and 9 males). The mean ages (years±standard deviation) of intensive care, hospitalized and outpatient patients were 75.067 (±14.063), 61.708 (±20.369), and 62.176 (±20.667), respectively. The mean age (years) in the control group is 59.4 (±18.738). There were no significant group differences between patients and controls for age (p=0.102) and gender (p=0.782).

Laboratory parameters WBC (range 3.91-10.9 10^9/L), HB (range 13.5-16.9 g/dL), PLT (range 150-450 10^9 /L), NLR, MPV (range 9.3-12.1 fL), MCV (range 81.8-95.5 fL), AST (range 0-40 U/L), ALT (range 0-41 U/L), BUN (range 5-18 mg/dL), CRE (range 0.70-1.2 mg/dL), CRP (range 0-0.5 mg/dL) and LDH (range 135-225 U/L) were studied in the control and patient groups (Table 2). Accordingly, NLR was statistically significantly lower in the control group than in all patient groups (p<0.001). CRP was higher in hospitalized patients than in the control group (p=0.012). LDH was found to be statistically significantly higher in hospitalized patients than outpatients (p=0.031) (Table 2). However, there was no statistically significant difference between the patient groups and the control group in terms of WBC (p=0.198), HB (p=0.439), PLT (p=0.259), MPV (p= 0.207), MCV (p=0.187), AST (p= 0.115),

ALT (p=0.263), BUN (p=0.103), CRE (p=0.217) parameters (Table 2).

Table 1. Distribution of the number of male and female in the groups

Group	Gender				Total		p
	M		F				
	n	%	n	%	n	%	
Outpatient	16	47.1	18	52.9	34	100.0	0.782*
Hospitalized	13	54.2	11	45.8	24	100.0	
Intensive care	9	60.0	6	40.0	15	100.0	
Control	9	60.0	6	40.0	15	100.0	
Total	47	53.4	41	46.6	88	100.0	

*Chi-square test, Female (F), Male (M)

Table 2. Laboratory parameters of the patient and control groups

	Outpatient			Hospitalized			Intensive care			Control			p
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	
AGE (years)	34	62.176	20.667	24	61.708	20.369	15	75.067	14.063	15	59.400	18.738	0.102*
WBC (10^9/L)	34	9.743	7.095	24	8.347	3.263	15	8.415	5.087	15	6.202	2.126	0.198*
HB (g/dL)	34	12.718	2.157	24	13.071	1.615	15	12.067	1.816	15	12.753	1.415	0.439*
PLT (10^9/L)	34	246.647	87.229	24	218.125	63.155	15	205.733	71.731	15	219.267	60.309	0.259*
NLR	34	4.381^A	3.313	24	6.549^A	5.981	15	5.218^A	2.944	15	2.398^B	1.389	<0.001 **
MCV (fL)	34	85.232	7.259	24	86.454	5.755	15	89.600	7.188	15	86.713	3.623	0.187
MPV (fL)	34	10.141	.806	24	10.292	.882	15	10.507	1.114	15	9.847	0.770	0.207
AST (U/L)	34	19.706	8.332	24	24.458	10.738	15	35.267	40.842	15	27.200	17.379	0.115**
ALT (U/L)	34	17.941	11.045	24	21.125	15.804	15	36.000	41.991	15	23.467	14.412	0.263**
BUN (mg/dL)	34	19.726	14.392	24	20.675	11.526	15	35.827	27.662	15	16.646	9.347	0.103**
CRE (mg/dL)	34	1.056	0.618	24	0.980	0.372	15	1.187	0.475	15	0.827	0.212	0.217*
CRP (mg/dL)	34	3.846^{AB}	5.347	24	7.768^A	7.874	15	5.179^{AB}	6.198	15	1.879^B	2.971	0.012**
LDH (U/L)	34	208.38^B	75.089	24	270.250^A	99.092	15	256.000^{AB}	78.981	15	224.200^{AB}	67.450	0.031*

*: ANOVA, **: Welch's ANOVA, White blood cell count (WBC), Hemoglobin (HB), Platelets count (PLT), Neutrophil to lymphocyte ratio (NLR), Mean corpuscular volume (MCV), Mean platelets volume (MPV), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Blood urea nitrogen (BUN), Creatinine (CRE), C-reactive protein (CRP), Lactate dehydrogenase (LDH).

The serum 8-OHdG levels of the control and patient groups are shown in Table 3. According to this; In COVID-19 positive patients treated in intensive care 8-OHdG marker level (mean 5111.112±591.732 pg/ml) was at the highest level and statistically significant. In patients receiving inpatient treatment in the hospitalized, the 8-OHdG marker level (mean 2088.833±800.607 pg/ml) was statistically significantly higher than the control and outpatient groups (p=0.000). However, no significant difference was found between the outpatients (mean 1094.904±385.044 pg/ml) and the control group (mean 795.452±234.080 pg/ml).

There was no statistically significant difference between the control and patient groups in terms of TNFA values (p=0.332) (Table 3). IL6 values were at

the highest level in the patient group treated in the intensive care unit (1788.252±260.026) and are statistically significantly higher than the outpatient (1520.129±277.401) and control groups (1406.801±155.043) (p=0.001). However, there was no significant difference between the intensive care patient group and the hospitalized patient group and between the outpatients and the control group (Table 3).

Table 3. Descriptive statistics and comparison results for 8-OHdG, IL6 and TNFA

Group		n	Mean	SD	p
8-OHdG	Outpatient	34	1094.904 ^{C}	385.044	0.000**
	Hospitalized	24	2088.833^{B}	800.607	
	Intensive care	15	5111.112 ^{A}	591.732	
	Control	15	795.452 ^{C}	234.080	
IL6	Outpatient	34	1520.129 ^{BC}	277.401	0.001*
	Hospitalized	24	1659.743 ^{AB}	324.460	
	Intensive care	15	1788.252 ^{A}	260.026	
	Control	15	1406.801 ^{C}	155.043	
TNFA	Outpatient	34	18.409	20.117	0.332*
	Hospitalized	24	23.787	54.518	
	Intensive care	15	38.280	46.316	
	Control	15	17.685	9.319	

*: ANOVA, **: Welch's ANOVA,
Means that do not share a letter are significantly different (p<0.05)

DISCUSSION

In our study, we aimed to determine the levels of 8-OHdG, a biomarker of oxidative DNA damage, and IL6 and TNFA, which are markers of inflammation, and to explain the possible relationship of these markers with clinical parameters in patients experiencing COVID-19 disease at different severity. To the best of our knowledge, there are very few studies examining serum 8-OHdG biomarker and TNFA and IL6 levels together in patients with different intensities of COVID-19 infection. Our study is one of the first studies on this subject.

One of the important features of SARS-CoV-2 infection is increased free radical production. Increasing ROS has the capacity to change macromolecules over time. The resulting macromolecular damage contributes to many mechanisms underlying the progression of COVID-19 disease[16]. Therefore, biomarkers of DNA damage are extremely important. In their study, Kosanovic et al followed up COVID-19 positive patients for 7 and 14 days. They determined that the level of 8-OHdG increased on the 14th day of the study compared to the 7th day[15]. In our study, we also found 8-OHdG levels to be high in patients in the intensive care unit and hospitalized. In addition, we did not find any difference between the outpatients and the control group. This shows that there may be a direct correlation between the severity of the disease and 8-OHdG.

In this study, IL6 was at the highest level in the patient group treated in the intensive care unit. Lorente et al. compared IL6 levels in non-surviving and surviving patients in their study. As a result of the study, they found high IL6 levels in non-surviving patients[17]. Similar results were found in another study and there is a positive association between COVID-19 mortality and increased IL6 level[18]. In the meta-analysis study of Helia Mojtabavi et al., it was concluded that there is a reliable relationship between IL6 and COVID-19 severity[19]. Increased IL6 signaling causes T cell maturation, expression of vascular endothelial growth factor (VEGF) increases vascular permeability and decreases myocardial contractility through multi-organ damage[18]. In older adults, high IL6 levels are associated with cognitive decline, obesity, diabetes, cardiovascular disease, cancer, and increased risk of death[20]. The results of our study are compatible with the literature.

Rajendra Karki et al., in their study on mortality in SARS-CoV-2 infection, reported that TNFA activates the JAK/STAT1/IRF1 axis in mice, inducing nitric oxide production, causing a fatal cytokine shock [10]. Later in the study, it treated the mice with TNFA neutralizing antibodies. And they

concluded that it protects against death during SARS-CoV-2 infection, sepsis, and cytokine shock[10]. In our study, although TNFA levels were high in intensive care patients, there was no statistically significant difference. We think that this may be due to the numerical difference between the patient groups.

Cytokines are essential mediators in the formation of acute phase proteins in inflammation. TNFA and IL6 are among the cytokines associated with inflammation. TNFA; increases the activation of T-helper cells in acute inflammation. IL6 is a cytokine with synergistic effects with TNFA[21].

In our study, we found that CRP, an inflammation parameter, was statistically significantly higher in hospitalized patients compared to the control group. However, CRP levels are also high in patients receiving intensive care and outpatient treatment. In a meta-analysis study by Ghahraman et al. reported that CRP level was high in patients with severe COVID-19 infection[22].

In our study, we found the NLR value higher in all of our patient groups than in the control group. In many studies, NLR level was found to be high in COVID-19 positive patients[10,15,22,23]. Acute respiratory distress syndrome, a type of respiratory failure characterized by the onset of inflammation in the lungs, is the major cause of mortality in COVID-19 patients[23]. NLR is an important laboratory finding to see the prognosis of pneumonia and tumor patients and to determine the severity of infection[24,25]. COVID-19 is associated with increased inflammation from the early to late stage of infection. In particular, the increase in the release of cytokines such as IL-2, IL6, IL-7, G-CSF, and TNFA is observed in patients with severe infection[19].

In our study, we found that the LDH level was statistically significantly higher in hospitalized patients than in outpatients. In the literature, there is a positive relationship in studies showing the relationship between the level of LDH and the severity of the disease in COVID-19 infection[15,17,22].

In this study, we also did not find a relationship with the severity of the disease in the levels of WBC, HB, PLT, MCV, MPV, AST, ALT, BUN, CRE. There are also studies in the literature that do not have a statistically significant relationship between these laboratory parameters and COVID-19, like our study[15,17]. However, there are studies showing an increase in AST, ALT, BUN, and CRE levels[18,22], as well as meta-analysis studies showing a decrease in PLT and HB levels[22]. No data on MCV and MPV were found in the literature.

This study had one limitations. 99% of the targeted sample size has been reached. Only the patient's findings at the time of admission to the Emergency Department could be evaluated. The small sample size due to single-center design is one of the limitations of this study.

As a result, this study; provides potential data on both laboratory test results and levels of DNA damage (8-OHdG) and inflammation parameters IL6 and TNFA in patients with COVID-19 infection of varying severity and adds to the ongoing difficulty in understanding the mechanisms of SARS-CoV-2 infection. Studies in larger patient populations are needed to explain the differences in the severity of the disease and to better understand the prognosis of the disease.

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