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Evaluation of Raftlin and 8-iso-prostaglandin Levels on the Clinical Severity of Cases with Covid-19 in Türkiye: A Cross-Sectional Study

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ABSTRACT
COVID-19 is an infectious respiratory disease caused by the severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Raftlin is a large lipid raft protein found in cell membranes and plays a crucial role in inducing autoimmune and vascular inflammatory responses. Although there have been few studies on 8-iso-prostaglandin F2α (8-iso-PGF2α) as a marker of oxidative stress in COVID-19, the levels of raftlin in the disease have not been previously investigated. The objective of in this study was to evaluate the levels of Raftlin and 8-iso-PGF2α in COVID-19 patients. We analyzed clinical findings from three groups: healthy controls, patients with a mild course of COVID-19, and patients with a severe course of COVID-19. We measured the levels of Raftlin and 8-iso-PGF2α in serum samples using ELISA. This results showed that the levels of Raftlin and 8-iso-PGF2α were higher in patients with severe COVID-19 compared to the control and mild course groups. Furthermore, a significant positive correlation was found between Raftlin and 8-iso-PGF2α in the severe COVID-19 group. Raftlin was identified as a potentially important biomarker in COVID-19 patients. This study is valuable as it is the first to investigate changes in Raftlin and 8-iso-PGF2α levels in COVID-19 disease.

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Türkiye'de COVİD-19 Olgularının Klinik Şiddeti Üzerine Raftlin ve 8-izoprostaglandin Düzeylerinin Değerlendirilmesi

ÖZET
COVID-19, şiddetli akut solunum sendromu SARS-CoV-2'nin neden olduğu bulaşıcı bir solunum yolu hastalığıdır. Raftlin, hücre zarlarında bulunan büyük bir lipid sal proteinidir ve otoimmün ve vasküler inflamatuar yanıtların indüklenmesinde önemli rol oynar. COVID-19'da oksidatif stresin bir belirteci olarak 8-iso-prostaglandin F2α (8-iso-PGF2α) ile ilgili az sayıda çalışma olmasına rağmen, hastalıktaki raftlin seviyeleri daha önce araştırılmamıştır. Çalışmamızın amacı, COVID-19 hastalarında Raftlin ve 8-iso-PGF2α düzeylerini değerlendirmektir. Üç gruptan elde edilen klinik bulguları analiz edildi: sağlıklı kontroller, hafif COVID-19 seyri olan hastalar ve şiddetli COVID-19 seyri olan hastalar. ELISA kit kullanarak serum örneklerinde Raftlin ve 8-iso-PGF2α seviyelerini ölçtük. Sonuçlarımız, Raftlin ve 8-iso-PGF2α seviyelerinin şiddetli COVID-19 hastalarında kontrol ve hafif seyirli gruplara kıyasla daha yüksek olduğunu göstermiştir. Ayrıca, şiddetli COVID-19 grubunda Raftlin ve 8-iso-PGF2α arasında anlamlı bir pozitif korelasyon bulunmuştur. Raftlin, COVID-19 hastalarında potansiyel olarak önemli bir biyobelirteç olarak tanımlanmıştır. Bu çalışma, COVID-19 hastalığında Raftlin ve 8-iso-PGF2α seviyelerindeki değişiklikleri araştıran ilk çalışma olması açısından değerlidir.

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INTRODUCTION

COVID-19 is one of the most serious public health problems of recent years. Investigating the pathogenetic mechanisms of the disease and new therapeutic targets is of great importance due to high mortality as well as serious economic and social consequences (Hui ve ark., 2019). One aspect of the pathogenesis of COVID-19 that is still unclear is the great heterogeneity of response among patients, from asymptomatic course to severe symptoms (The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). Symptoms tend to be more aggressive and fatal in more vulnerable groups, including the elderly, patients with chronic diseases, patients receiving immunosuppressive therapy, and pregnant women (Hui et al., 2020; The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team, 2020). COVID-19 is believed to rarely affect infants, so body reactivity studies mainly focus on adult patients (Guan et al., 2020).

Reactive oxygen species (ROS) are formed during metabolic activity under physiological conditions in living organisms (Kurutas, 2016). Activation of antimicrobial immune cells, neutrophils, and macrophages and the production of proinflammatory cytokines depend on ROS (Miraloglu et al., 2016; Juan et al., 2021; Muhammed et al., 2021). ROS have an important role in the development of an antiviral immune response through the formation of type I interferon (Nour et al., 2020). ROS also play an important role as mediators of cell signalling pathways, but this does not require a negative effect on cell structures due to compensation by antioxidant mechanisms (Angel et al., 2018). Overproduction of ROS, including COVID-19, can stimulate inflammatory signalling cascades through the increased genomic expression of protein kinases, transcription factors, and proinflammatory regulators, leading to hyperactivation of the immune system. This may lead to the development of oxidative damage (Saeki et al.,2003).

Lipid peroxidation caused by ROS is an important pathological process involving the oxidation of polyunsaturated fatty acids in biological membranes (Bilgen et al., 2019). Oxidative stress through lipid peroxidation is thought to play an important role in the development of many diseases, including COVID-19. Isoprostanes, a family of prostaglandin-like compounds, are by-products of free radical catalysed oxidation of arachidonic acid. 8-iso-prostaglandin F2α (8-iso-PGF2α), an important isoprostane, is very stable in body fluids and tissues and is therefore considered the most ideal index for the detection of excessive chemical lipid peroxidation and the amount of free circulation (Bilal et al., 2021). 8-iso-PGF2α have been measured in various bodyfluids, such as urine, blood, bile, pericardial fluid, and cerebrospinal fluid, as indicators of lipid peroxida-tion (Milne et al., 2008; Kaviarasan et al., 2009; Pratico et al., 2004).

Lipid rafts are membrane microdomains enriched in saturated phospholipids, sphingolipids, and cholesterol (Muhammed et al., 2020). They are involved in various cellular processes, including signal transduction, endocytosis and exocytosis, entry of obligate intracellular pathogens, and production of pathological protein forms associated with various diseases (Zarkovic et al., 2022). Lipid rafts have a diverse and different protein composition. Raftlin (RFTN), the main lipid raft protein, is found in B cells and is responsible for the regulation of B cell antigen receptor (BCR) signalling. RFTN also plays an important role in the induction of autoimmune response and vascular inflammatory response. RFTN is a parameter used in the pathophysiology of a vascular inflammatory response to diagnose inflammatory diseases and to characterize the immune response (Lahaie et al., 1998).

Lipid rafts are glycoprotein (sphingolipid) and cholesterol-rich regions containing receptor proteins. Lipid rafts are membrane microdomains enriched in saturated phospholipids, sphingolipids and cholesterol. They have a diverse but distinct protein composition and are involved in a variety of cellular processes, including polarised traffic, signal transduction, endo- and exocytosis, entry of obligate intracellular pathogens and production of pathological protein forms associated with Alzheimer's and prion diseases (Lee et al., 2014).

RFTN levels have not been previously investigated in COVID-19 disease. However, there are limited studies on the use of 8-iso-PGF2α as an oxidative stress marker in COVID-19. In this study, we aimed to evaluate the changes in RFTN and 8-iso-PGF2α levels in COVID-19 patients.

MATERIALS AND METHOD

The research was designed as a cross-sectional study. Patients who applied to Tokat Gaziosmanpaşa University Faculty of Medicine Hospital between 01.05.2021 - 01.12.2021, received a definitive diagnosis of COVID-19 and received outpatient treatment and stayed in home isolation for 14 days; infectious

diseases, chest diseases, general internal medicine, ear, nose and throat, etc. Intubated patients in need of intensive care and mechanical ventilation who received inpatient supportive treatment in hospital wards were brought to the Anesthesiology and Reanimation Intensive Care Unit, and patients transferred from in-hospital internal medicine and infection wards and external centres were included. This study consisted of a total of 75 patients randomly selected and included patients who were in isolation at home (n=25), hospitalized in the ward (n=25), and receiving supportive treatment in the intensive care unit (n=25). In addition, 25 healthy individuals were selected as the control group. Sociodemographic data of the patients and control subjects included in the study are presented in Table 1. This study was approved by Tokat Gaziosmanpasa University Faculty of Medicine Local Ethics Committee (21-KAEK-099).

Biochemical Analysis

Blood samples obtained from patients and control subjects were taken between 08.00 and 11.00 in the morning and after 8 to 12 hours of fasting because they were fasting venous blood samples. The samples were sent to Tokat Gaziosmanpaşa University Faculty of Medicine, Department of Biochemistry. Fasting venous blood samples (10 ml) of the study participants were placed in anticoagulant-free tubes and centrifuged at 5000 g for 10 minutes at 4°C in a device (Hettich MIKRO 220 R; Andreas Hettich GmbH & Co. KG Tuttleen, Germany). After centrifugation, sera were separated and stored in acid-washed tubes at -20°C until RFTN and 8-iso-PGF2α analyses. The levels of these parameters were analyzed in the Department of Medical Biochemistry, Tokat Gaziosmanpaşa University. RFTN and 8-iso-PGF2α levels were measured by quantitative sandwich enzyme immunoassay technique (ELISA) using a commercial kit (Mybiosource, USA) according to the manufacturer's instructions.

Statistical analysis

The data obtained at the end of the data collection phase were transferred to the computer environment and analyzed. Data were analyzed using the Statistical Package for Social Sciences (SPSS 27.0) software. Mann-Whitney U test was used to determine the degree of differences in serum levels (ng/mL) between the severe, mild, and control groups of COVID-19. Data were expressed as mean and standard deviation (SD). An Independent sample t-test was used to compare normally distributed variables. The chi-square test was used to compare the groups. Spearman correlation test was used for the relationship between variables. A receiver operating characteristic (ROC) curve was plotted to test the diagnostic performance of RFTN and 8-iso-PGF2α in patients with COVID-19. The association of RFTN and 8-iso-PGF2α with the severity of COVID-19 was visually shown by scatter plots. The significance level was accepted as **$p<0.05$**.

RESULTS and DISCUSSION

When the general gender distribution of the patients was analyzed, 40 (50.6%) were male and 35 (49.4%) were female. Sociodemographic data of the patient and control groups are given in Table 1.

Table 1 Socio-demographic characteristics of the individuals in the patient and control groups.
Çizelge 1. Hasta ve kontrol gruplarındaki bireylerin sosyo-demografik özellikleri.

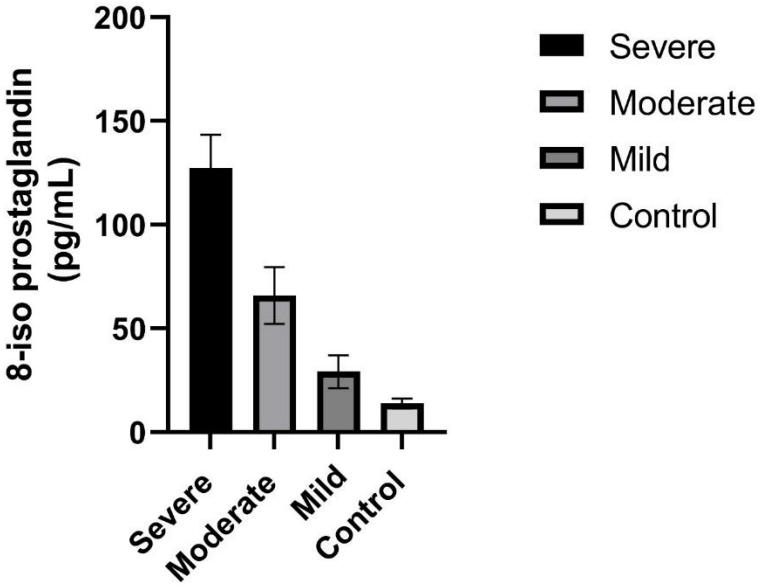
Parameters	Patient (n=75)	Control (n= 25)	P değeri
Age (years)	43.8 ± 13.8	40.2 ± 6.8	0,28
Gender			0,20
Male	40 (70%)	14 (52.4%)	
Female	35 (30%)	11 (47.6%)	
BMI(kg/m^2)	21.9 ± 2.2	21.1 ± 1.1	0,15
Symptoms			
Mild	20 (%80)	0 (%)	
Moderate	20 (%80)	0 (%)	
Severe	20 (%80)	0 (%)	
Comorbidities			
No	40 (%80)	0 (%)	
Hypertension	6(%80)	0 (%)	
Diabetes	7(%80)	0 (%)	

As shown in Table 2, Figures 1 and 2, serum RFTN and 8-iso-PGF2α levels were significantly higher in severe COVID-19 patients compared to the control group ($p<0.001$). Moreover, RFTN and 8-iso-PGF2α levels were significantly higher in severe COVID-19 patients compared to mild and moderate COVID-19 patients ($p<0.001$).

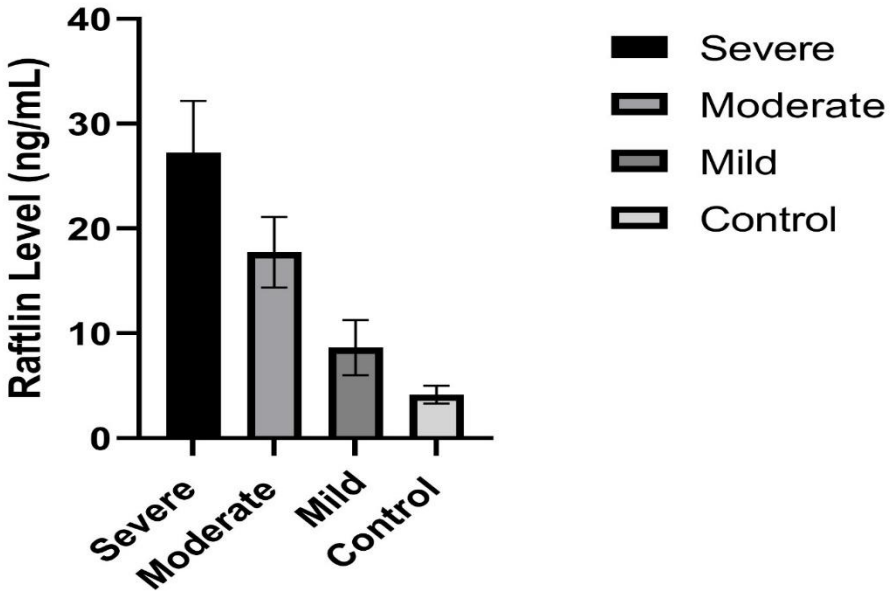
As shown in Table 3, a significant correlation was found according to the correlation analysis between the groups for RFTN levels ($p<0.001$, $r=0.691$). In Table 4, a significant positive correlation was found between the groups for 8-isoPG levels ($p<0.001$, $r=0.711$).

Table 2 The results of Raftlin and 8-iso prostaglandin levels between patient and healthy controls
Çizelge 2. Raftlin ve 8-iso prostaglandin düzeylerinin hasta ve sağlıklı kontroller arasındaki sonuçları.

Variable		Covid Severe (n=25)	Covid Moderate (n=25)	Covid Mild (n=25)	Control (n=25)	p-value
Raftlin-1 (ng/mL)	Median (Q1-Q3)	27.25 (22.37-17.96)	17.75 (14.39-21.45)	8.65 (6.02-11.27)	4.16 (3.32-5.0)	<0.001
8-iso prostaglandin (pg/mL)	Median (Q1-Q3)	127.25 (111.15-143.35)	65.9 (52.27-79.53)	29.2 (21.26-37.14)	13.90 (11.48-16.3)	<0.001



Şekil 1. Gruplar arasında 8-iso prostaglandin düzeyleri
Figure1. 8-iso prostaglandin levels between groups



Şekil 2. Gruplar arasında Raftlin düzeyleri
Figure 2. Raftlin levels between groups

Table 3 Correlation results between groups for RFTN levels
Çizelge 3. RFTN düzeyleri için gruplar arasındaki korelasyon sonuçları.

		Raftlin	Groups
Raftlin	r value	1,000	,691**
	p-value	.	,0001
	N	85	85
Groups	r value	,691**	1,000
	p-value	,0001	.
	N	85	85

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4 Correlation results between groups for 8-isoPG levels
Çizelge 4. 8-isoPG levels *düzeyleri için gruplar arasındaki korelasyon sonuçları.*

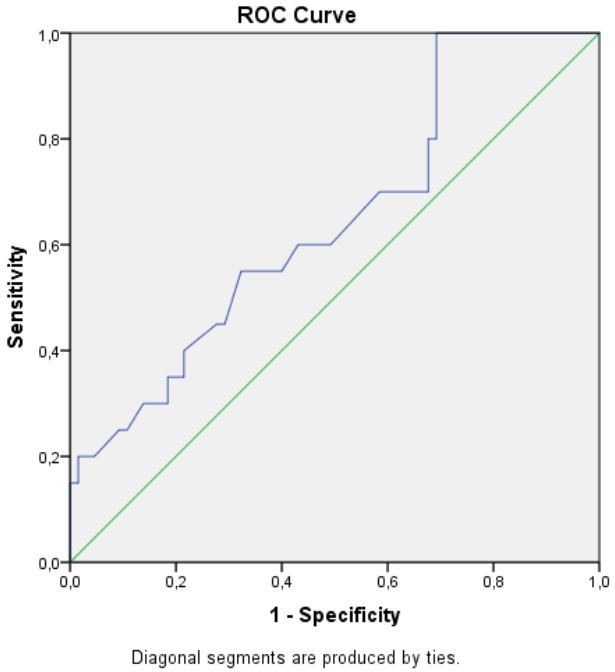
		8-isoPG	Groups
8-isoPG	r value	1,000	,711**
	p-value	.	,0002
	N	85	85
Groups	r value	,711**	1,000
	p-value	,0002	.
	N	85	85

**. Correlation is significant at the 0.01 level (2-tailed).

The ROC curve AUC, cut-off value, specificity, and sensitivity values of RFTN are shown in Figure 3 and Table 5. Accordingly, the cut-off value of RFTN was found to be 9.5 ng/mL, sensitivity 85, and specificity 79. In addition, ROC curve AUC, cut-off value, specificity, and sensitivity values for 8-iso-PGF2α are shown in Figure 4 and Table 6. The cut-off value of 8-iso-PGF2α was found to be 97.8 pg/mL, sensitivity 88, and specificity 85. (P<0.05).

Table 5 ROC curve AUC cut-off, specificity, and sensitivity values for Raftlin
Çizelge 5. Raftlin için ROC eğrisi AUC kesme, özgüllük ve duyarlılık değerleri.

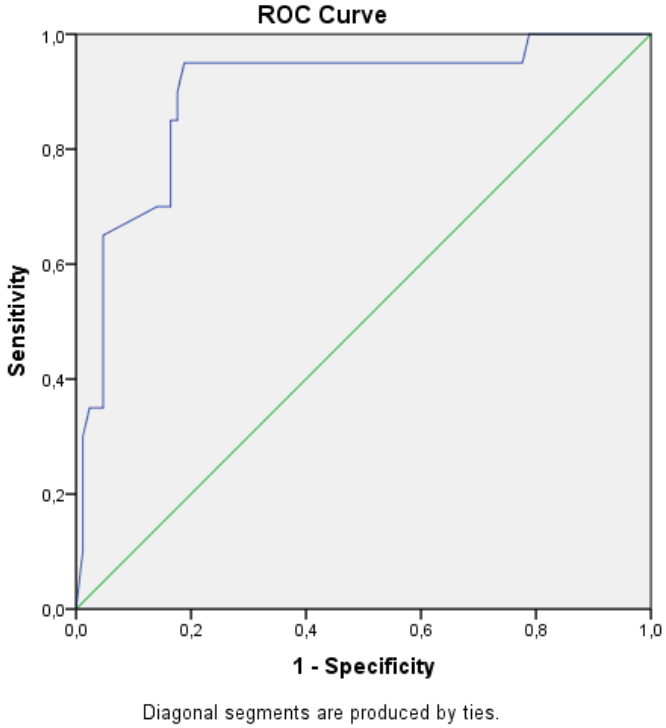
Risk Factor AUC 95%	*P*	Cut off	Sensitivity (%)	Specificity
0.846 (0.702-0.889)	<0.001	9.5	85	79



Şekil 3. Bu eğri, doğru-pozitif oranı ve doğru-negatif oranı bilgilerini birleştirir ve AUC, Raftlin'in genel ayırt edici gücünün bir ölçüsüdür.
Figure 3. This curve combines the information of the true-positive rate and the true-negative rate, and the AUC is a measure of the overall discriminative power of Raftlin.

Table 6 ROC curve AUC cut-off, specificity, and sensitivity values for 8-iso-PGF2α
Çizelge 6. 8-iso-PGF2α için ROC eğrisi AUC kesme, özgüllük ve duyarlılık değerleri.

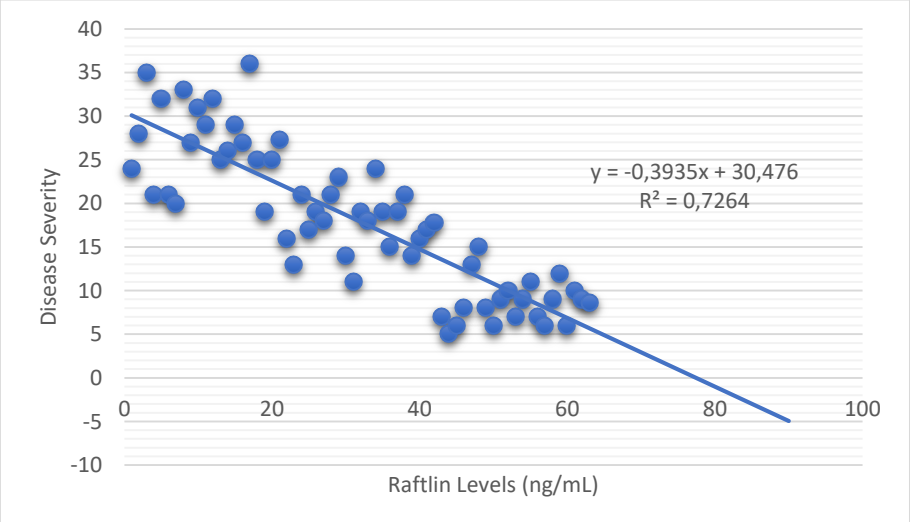
Risk Factor AUC 95%	*P*	Cut off	Sensitivity (%)	Specificity
0.896 (0.813-0.978)	<0.001	97.8	88	85



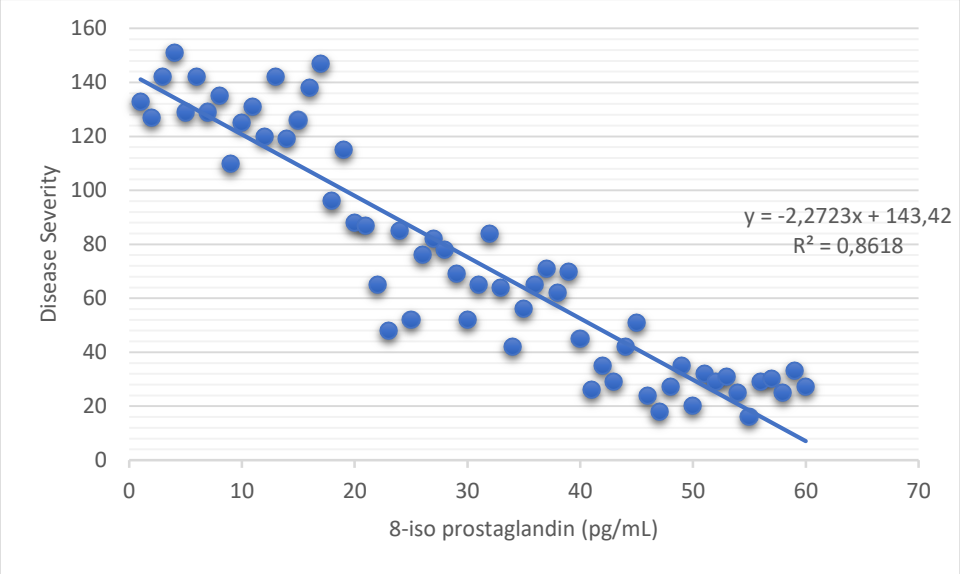
Şekil 4. Bu eğri, doğru-pozitif oranı ve doğru-negatif oranı bilgilerini birleştirir ve AUC, 8-iso prostaglandin 'in genel ayırt edici gücünün bir ölçüsüdür.
Figure 4. This curve combines the information of the true-positive rate and the true-negative rate, and the AUC is a measure of the overall discriminative power of 8-iso prostaglandin.

A scatter plot was drawn to further reveal the relationship between disease severity and RFTN (Figure 5). According to the scatter plot, as the severity of the disease decreases, RFTN levels also decrease. The R2 value of 0.72 reveals a significant relationship between this results.

A scatter plot was drawn to further reveal the relationship between disease severity and 8-iso-PGF2α (Figure 6). According to the scatter plot, as the severity of the disease decreases, the level of raftlin also decreases. The R^2 value of 0.86 reveals that there is a significant relationship between this results.



Şekil 5. Hastalık şiddeti ve raftlin arasındaki dağılım grafiği ilişkisi
Figure 5. Scatter plot relationship between disease severity and raftlin.



Şekil 6. Hastalık şiddeti ve 8-izoprostaglandin arasındaki dağılım grafiği ilişkisi
Figure 6. Scatter plot relationship between disease severity and 8-iso prostaglandin.

To this knowledge, this is the first study to evaluate serum RFTN and 8-iso-PGF2α levels in patients with COVID-19. We believe that the groups we compared were homogeneous enough to make a valid assessment of these biomarkers for COVID-19 patients. Four main findings were observed in this study: First, elevated serum levels of RFTN and 8-iso-PGF2α were detected in severe COVID-19 patients compared to mild COVID-19 patients and control subjects. Second, RFTN and 8-iso-PGF2α levels showed good diagnostic performance in severe COVID-19 patients. Third, we found a positive and significant correlation between RFTN and 8-iso-PGF2α levels in the COVID-19 patient group.

In areas where free radicals are formed, membrane lipids may be sensitive to peroxidation and this may lead to the formation of isoprostanes. Isoprostanes are released by the action of phospholipases and are stable products in circulation. High isoprostane levels in plasma and other body fluids indicate the presence of oxidative stress and are frequently used as biomarkers of damage in various cells and tissues (Muhammed et al.,2020; Zarkovic et al., 2022). However, circulating isoprostanes may also function as intracellular signalling molecules. They generally cause vasoconstriction in most vascular systems and constrict smooth muscles in the lymphatic, uterine, gastrointestinal, and tracheobronchial systems (Lahaie et al., 1998; Derouiche et al., 2020). In this study, COVID-19 infection alone caused a 2-3-fold increase in the 8-iso-PGF2α level mediated by the Fenton reaction. This finding showed that 8-iso-PGF2α may be an important predictive marker of cumulative oxidative stress and worsening of the condition of the patient infected with COVID-19. The increase in free radicals followed by an increase in 8-iso-PGF2α is the first evidence that 8-iso-PGF2α, a marker of oxidative stress, may play a role in the pathogenesis of COVID-19 infection. There are also studies showing that decreased antioxidant levels in severe SARS-CoV-2 patients are accompanied by increased oxidative stress, as evidenced by higher levels of reactive oxygen and nitrogen species as well as lipid peroxidation (Muhammed et al., 2021; Muhammed et al., 2020; Noonnog et al., 2023). This may indicate a poor prognosis in patients with COVID.

Lipid rafts play a very important role in cell signalling mechanisms. In the literature, the role of lipid rafts using a G-protein-coupled receptor system or tyrosine kinase receptor system in signalling mechanisms is discussed (Saeki et al., 2003). RFTN is an important lipid raft protein identified from Raji B cells. It is required for the regulation of lipid rafts and signal transduction of B cell antigen receptors (Saeki et al., 2003). In addition, RFTN plays a role in the stimulation of the nucleocapsid complex during TLR3 activation and autoimmune responses (Schmidt et al., 2009; Saeki et al., 2009; Watanabe et al., 2011). RFTN also plays a role in the pathophysiology of sepsis and vascular inflammatory response (Bae et al., 2008; Bae et al., 2004; Pike, 2003). In this study, it was shown that RFTN levels were significantly higher in the COVID-19 group compared to the control group. No study on RFTN in COVID-19 was found in the literature. Therefore, we could not compare this RFTN results obtained from COVID-19 patients. Increased RFTN levels in COVID-19 may result from increased inflammation due to COVID-19. The factors that trigger the disease in SARS-CoV-2-infected individuals are not fully understood, and the severe development of the disease does not appear to be related only to viral load and may also include an inadequate interferon response (Saeki et al., 2003).

Correlation results constitute an important finding of this study. The correlation coefficient is indicated by the symbol 'r'. While the 'r' value ≤0.35 represents a weak correlation, values between 0.36 and 0.67 indicate a moderate correlation. Values between 0.68 and 0.90 indicate a high correlation and values between 0.90 and 1.0 indicate a very high correlation(Taylor, 1990). In this study, a strong positive correlation was found between the severity of COVID-19 disease and RFTN and 8-iso-PGF2α levels. No study investigating the relationship between RFTN and 8-iso-PGF2α levels in COVID-19 was found in the literature. Therefore, we could not compare this results. A high correlation coefficient may form the basis for the detection of biomarkers. In conclusion, increased levels of 8-iso-PGF2α and RFTN in COVID-19 may play a role in the pathogenesis and progression of the disease.

Currently, new diagnostic biomarkers need to be identified to aid in the diagnosis of COVID. In ROC analysis, the discrimination of a value is assessed according to the area under the curve (AUC). The ROC curve is categorized as: 0.9-1 = very good, 0.8-0.9 = good, 0.7-0.8 = moderate, 0.6-0.7 = poor, and <0.6 = failure(Demirhan et al., 2023). The diagnostic values of biomarkers have been studied in some recent clinical studies and bacterial and viral infectious diseases. Tekin et al. showed that increased serum CRP levels may be a diagnostic biomarker in various bacterial and viral infectious diseases (Tekin et al., 2017). In their study, they calculated the sensitivity and specificity of CRP parameters in detecting bacterial infection as 60.26% and 45.61%, respectively, and AUC: 0.534 in ROC analysis(Karahan et al., 2021). Determined the cut-off value of plasma cytomegalovirus (CMV) virus load detected by PCR to predict the diagnosis of cytomegalovirus gastrointestinal disease (CMV-GIHD) by ROC analysis. Accordingly, they obtained AUC: 0.88, 78% sensitivity, 100% specificity, 100% positive predictive value, and 87% negative predictive value in ROC analysis in CMV-GIH positive and CMV-GIH negative solid organ transplant recipients carrying CMV virus (Karahan et al., 2021). To this knowledge, this is the first study to test the diagnostic value of the ROC curve RFTN and 8-iso-PGF2α levels in patients with COVID-19. In this study, the AUC for RFTN was 0.846 and the AUC for 8-iso-PGF2α was 0.896. This finding indicates that serum levels of RFTN and 8-iso-PGF2α have very good diagnostic value. We do not claim that the levels of RFTN and 8-iso-PGF2α are new methods that should be used as diagnostic biomarkers in patients with COVID-19. We believe, that further studies should be performed in larger and more homogeneous groups to test the diagnostic value of RFTN and 8-iso-PGF2α in patients with COVID-19.

CONCLUSION and RECOMMENDATIONS

However, this study has several limitations, such as the sample size, because it may not be easy to find associations between variables, and the small size may not provide a representative distribution of the population, and this was due to the presence of only 75 patients. Second, free radicals could have been directly determined but this was not possible due to their high reactivity, short half-life, and labile nature; instead, their most reliable and stable metabolite, 8-iso-PGF2α, was measured as a marker of oxidative stress. The results of RFTN and 8-iso-PGF2α, which we present for the first time in COVID-19 patients, suggest that RFTN and 8-iso-PGF2α may be a determinant biomarker in the prognosis of this disease. We think that the results of this study will lead to other studies and will be more supportive of new data.

Ethics Committee Approval Number: 21-KAEK-099

Summary of Researchers' Contribution Rate Declaration

The authors declare that they have contributed equally to the article.

Conflict of Interest Statement

The authors declare that there is no conflict of interest between them.

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