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Araştırma Makalesi / *Research Article*

Serum Glypican-3 and Interleukin-6 Levels in COVID-19 Patients

COVID-19 Hastalarında Serum Glipikan-3 ve İnterlökin-6 Düzeyleri

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ABSTRACT

Aim: We aimed to determine the glypican-3 levels in patients diagnosed with COVID-19, compare these values with the healthy controls, and analyze the evaluation of glypican-3 levels with inflammatory markers, including interleukin-6 (IL-6), to determine its role in disease pathogenesis.

Material and Methods: A total of 88 participants (58 patients and 30 controls) were included. Medical history and laboratory findings of the population were recorded. Serum glypican-3 and IL-6 levels were analyzed by the Enzyme-linked immunosorbent assay method.

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Results: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH) levels, and IL-6 levels were significantly higher in the COVID-19 patient group than the healthy controls, while glypican-3 levels were significantly lower in the patient group ($p<0.001$). Glypican-3 levels significantly and inversely correlated with serum AST, CRP, ferritin, IL-6, and LDH levels ($p<0.05$).

Conclusions: We found that the levels of glypican-3 decreased in COVID-19 patients. Additionally, serum glypican-3 levels are inversely correlated with CRP and IL-6 levels. Targeting glypican-3 may be useful in understanding the pathophysiology of COVID-19.

***Keywords**: Glypican-3; IL-6; COVID-19*

ÖZET

Amaç: COVID-19 tanılı hastalarda glipikan-3 düzeylerini belirlemeyi, bu değerleri sağlıklı kontrollerle karşılaştırmayı; ayrıca glipikan-3 düzeylerinin interlökin-6 (IL-6) dahil inflamatuar belirteçlerle ilişkisini ve hastalık patogenezindeki rolünü belirlemeyi amaçladık.

Gereç ve Yöntem: Çalışmaya toplam 88 olgu (58 hasta ve 30 kontrol) dahil edildi. Olguların tıbbi öyküsü ve laboratuvar bulguları kaydedildi. Serum glipikan-3 ve IL-6 seviyeleri Enzim-bağlı immünosorbent ölçüm yöntemi ile analiz edildi.

Bulgular: Alanin aminotransferaz (ALT), aspartat aminotransferaz (AST), C-reaktif protein (CRP), D-dimer, ferritin, laktat dehidrojenaz (LDH) ve IL-6 seviyeleri COVID-19 hasta grubunda sağlıklı kontrollere göre anlamlı derecede yüksek bulunurken, glipikan-3 seviyeleri hasta grubunda anlamlı derecede daha düşüktü ($p <0,001$). Glipikan-3 seviyeleri, serum AST, CRP, ferritin, IL-6 ve LDH seviyeleri ile anlamlı ve ters korelasyon gösterdi ($p<0.05$).

Sonuç: COVID-19 hastalarında glipikan-3 düzeylerinin daha düşük olduğunu bulduk. Ek olarak, serum glipikan-3 seviyeleri, CRP ve IL-6 seviyeleri ile ters ilişkilidir. Glipikan-3' ü hedeflemek, COVID-19'un patofizyolojisini anlamada faydalı olabilir.

Anahtar kelimeler: Glipikan-3, IL-6, COVID-19

INTRODUCTION

The COVID-19 pandemic is a global pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It had adverse effects on the daily lives of patients with high mortality and morbidity rates [1]. A virus surface spike S protein mediates SARS-CoV-2 entry into cells. The spike S glycoprotein binds to the angiotensin-converting enzyme-2 (ACE-2) receptor, mediating the entry of SARS-CoV-2 into cells and immune evasion of the virus. Heparan sulfate proteoglycans consist of a core protein covalently linked to glycosaminoglycan chains. They have many functions, including contribution to the basal membrane organization and mediation of adhesion and cell motility, binding to cytokines, chemokines, and growth factors [3]. Importantly, heparan sulfate proteoglycans can interact with the basic sediments of viral glycoproteins [4, 5]. Very recently it has been demonstrated that heparan sulfate proteoglycans have an important role as co-receptors by binding spike proteins for favoring SARS-CoV-2 attachment to cells; inhibition of this binding is defined as a target in treatment [6, 7].

Glypicans and syndecans are the two main classes of heparan sulfate proteoglycans and are known to be important cell surface receptors, for many viruses [8]. Glypican-3 is bound to the cell surface by a glycosylphosphatidylinositol anchor. Glypican-3 is expressed in various tissues and many cancer cells [9-11]. Interleukin-6 (IL-6) is a cytokine and has pro-inflammatory effects. It has an important role in the inflammatory response, by stimulating acute phase reactants, immune response, and hematopoiesis. In SARS-CoV-2-related infection and pneumonia, cytokine storm is the cornerstone and IL-6 also has an

essential role in this process [12]. Moreover, the prognostic role of IL-6 levels in COVID-19 infection was also shown [13]. Glypican-3 and IL-6 levels were not evaluated together in COVID-19.

In the current study, we aimed to evaluate the serum glypican-3 levels in patients diagnosed with COVID-19, compare these values with the healthy controls, and analyze the evaluation of glypican-3 levels with inflammatory markers to determine the role of glypican-3 in disease pathogenesis.

MATERIALS and METHODS

Patients and design:

A total of 88 participants, 58 adult patients with confirmed COVID-19 infection and 30 healthy control cases were enrolled in the current study. Written informed consent was obtained from all participants. All patients admitted to the internal medicine department with the symptoms of COVID-19 including fever, headache, cough, shortness of breath, nausea, vomiting, or diarrhea were analyzed. COVID-19 diagnosis is assessed using Reverse transcriptase–polymerase chain reaction (RT-PCR) [14]. In the control group, healthy cases admitted to the internal medicine department for check-up purposes, without any symptoms of COVID-19 infection, who were not diagnosed with any kind of infection and agreed to participate in the study, were included. The COVID-19 RT-PCR test was not performed on the control group. All patients with a history of any chronic diseases (diabetes mellitus, hypertension, thyroid diseases, liver or renal failure), under any medical treatment, or who refused to participate were excluded.

Demographic and laboratory findings of the participants at admission were recorded. The laboratory tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), D-dimer, ferritin, lactate dehydrogenase (LDH) levels] were recorded. Complete blood count was studied by Mindray BC6800 analyzer (China), coagulation with STA Compact Max 2 (United States), hormone tests with Roche Cobas e601, and biochemical tests were studied with Roche Cobas c501 analyzer (United States).

The study was approved by Prof. Dr. Cemil Taşcıoğlu City Hospital Ethics Committee (02/8/2021- 238).

Analysis of serum glypican-3 levels

Enzyme-linked immunosorbent assay (ELISA) was used for the analysis of glypican-3 (Human glypican-3 ELISA Kit, BT LAB, catalog no: E1431Hu, China). The linear analytical measurement range was 0.05-10 ng/mL. The detection limit was 0.01 ng/mL. The intraassay and interassay variation coefficients were < 6.7 % and <10 %, respectively.

Analysis of IL-6 levels

ELISA was used for the analysis of IL-6 levels (IL-6 ELISA Kit, BT LAB, catalog no: E0090Hu, China). The analytical range was 2-600 ng/L. The detection limit was 1.03 ng/mL. The intraassay and interassay coefficients were <5.1 and < 10 %, respectively.

Statistical analysis

Statistical analyzes were made using SPSS 20.0 software. The ki-square test was performed to compare categorical variables. Mean values for continuous variables were compared using independent samples t-test when the data were normally distributed. Pearson correlation was performed to determine the association of glypican-3 and IL-6 levels with other laboratory data.

RESULTS

A total of 88 participants (58 patients and 30 control cases) were included. The demographic features of the participants are summarized in (Table 1). The two groups were similar regarding gender, age, BMI (body mass index), and smoking history.

The comparison of laboratory data between groups is summarized in (Table 2). ALT, AST,

CRP, D-dimer, ferritin, and LDH levels were significantly higher in the patient group than in the controls (p<0.001). Serum IL-6 levels were significantly higher in the patient group, while glypican-3 levels were significantly lower in the patient group compared with the control cases (p<0.001).

Correlation analysis was performed to determine the association of glypican-3 and IL-6 levels with other laboratory data (table III). IL-6 did not show any significant association with any other laboratory data (p>0.05); however, glypican-3 levels significantly and negatively correlated with serum AST, CRP, ferritin, and LDH levels (p<0.05). Moreover, there was a negative significant correlation between glypican-3 and IL-6 levels (r= -0.457, p=0.001).

Table 1. Demographic features of study participants
Tablo 1. Çalışmaya katılanların demografik özellikleri

	Control group (n: 30)	Patient group (n: 58)	p
Gender (F/M)	14 /16	21 /37	0.36
Age (years)	47.13±15.97	49.58±14.01	0.46
Smoking	6/30 (20%)	9/58 (15.5%)	0.51
BMI (kg/m²)	26.15±4.66	27.21±4.32	0.31

F: female, M: male, BMI: body mass index

Table 2. Comparison of laboratory data between groups
Tablo 2. Laboratuvar verilerinin gruplar arasında karşılaştırılması

	Control group (n: 30)	Patient group (n: 58)	p
ALT (U/L)	16.88±8.66	51.31±19.68	<0.001
AST (U/L)	17.43±4.62	35.419±11.33	<0.001
CRP (mg/L)	3.39±1.91	32.45±3.14	<0.001
D-dimer (ng/mL)	0.38±0.24	0.87±0.64	<0.001
Ferritin (ng/mL)	67.86±22.81	541.09± 224.52	<0.001
LDH (U/L)	183.78±35.36	322.22±92.62	<0.001
IL-6 (ng/mL)	54.82±35.74	74.91±49.30	<0.001
Glypican-3 (ng/mL)	1.79±1.15	1.01±0.24	<0.001

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, LDH: lactate dehydrogenase, IL-6: interleukin-6

Table 3. Correlation analysis between the IL-6 and glypican-3 levels with other laboratory data
Tablo 3. IL-6 ve glipikan-3 seviyeleri ile diğer laboratuvar verileri arasındaki korelasyon analizi

	IL-6		Glypican-3	
	r	p	r	P
ALT (U/L)	0.058	0.59	-0.141	0.19
AST (U/L)	0.007	0.94	**-0.299**	**0.005**
CRP (mg/L)	0.001	0.995	**-0.270**	**0.042**
D-dimer (ng/mL)	0.067	0.540	-0.130	0.229
Ferritin (ng/mL)	0.104	0.346	**-0.310**	**0.004**
LDH (U/L)	0.05	0.96	**-0.272**	**0.014**
IL-6 (ng/mL)	-	-	**-0.457**	**0.001**

r: correlation coefficient, p: significance level, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, LDH: lactate dehydrogenase, IL-6: interleukin-6

DISCUSSION

Glypican-3 is heparan-sulfate proteoglycan and the main functions of glypican-3 comprise endocytosis and degradation of cell surface receptors [15, 16]. It is known that many viruses interact with proteoglycans on the surface of cells for binding. The core proteins of the viruses attach heparan sulfate covalently, meaning that these proteins are also viral receptors [8, 17]. Although ACE-2 is the main co-receptors of SARS-CoV-2, recently, heparan sulfate proteoglycans are shown to be necessary for the binding of SARS-CoV-2 on the cell surface [11, 18]. Previously, in a few viral diseases, serum glypican-3 levels were studied [5, 19]. In a study, it was reported that glypican-3 levels tended to be higher in patients with hepatitis C virus-related hepatocellular carcinoma than in patients with hepatitis B virus-related hepatocellular carcinoma or in those without virus infection [19].

In the current study, we found that glypican-3 levels were lower in COVID-19 patients for the first time in literature. This decrease may be due to the virus interacting with glypican-3 while entering the cells and is consumed it due to the intracellular catabolization of glypican-3. Our study is a preliminary study on this subject. We were not able to directly compare our findings. However, we reviewed studies investigating ACE-2 levels as a main receptor. Chen et al, found that low serum ACE levels associated with delayed virus elimination, hyperinflammatory, and impaired antiviral immune responses contribute to COVID-19 disease progression [20]. Zhu et al, found that subjects in the severe COVID-19 group had the lowest baseline serum ACE-2 activity [21]. Zank et al, found that the poor prognosis in patients with SARS-CoV-2-infected diabetes may be due to low circulating ACE-2 levels [22]. Since both ACE-2 receptors and proteoglycans have similar roles on cell surfaces in COVID-19 infection, the decrease in glypican-3 levels determined in the current study may also have similar pathogenesis with a decrease in ACE-2 levels in COVID-19 patients. We think that scientific findings about decreased ACE-2 levels support the decreased glypican-3 levels in the current study.

The disease course and the complications of COVID-19 are associated with hyper-induction of proinflammatory cytokine production [23, 24]. IL-6 is a proinflammatory cytokine regulating inflammation and oxidative stress. Elevated IL-6 levels reflect the severity of inflammation and are a feature of a 'cytokine storm'. For this reason, IL-6 levels are regarded as the prognostic marker and treatment target in COVID-19 patients [12]. In the current study, we also determined higher levels of IL-6, CRP, and ferritin levels in COVID-19 patients. Moreover, serum glypican-3 levels negatively correlated with all those inflammatory markers. So, with an increase in the inflammatory response, serum glypican-3 levels were decreased. In light of these findings, we can suggest that glypican-3 may be having an essential role in COVID-19 pathogenesis, and targeting glypican-3 may be useful for understanding the pathophysiology of COVID-19. Our findings will shed light on future research.

The small sample size is among some of the limitations of the study. Secondly, we did not categorize the COVID-19 patients regarding the disease severity. And lastly, we did not analyze ACE-2 levels in COVID-19 patients.

CONCLUSIONS

We found that the levels of serum glypican-3 decrease in COVID-19 patients. Additionally, serum glypican-3 levels are inversely correlated with CRP and IL-6 levels. Targeting glypican-3 may be useful in understanding the pathophysiology of COVID-19.

Highlights

This is the first study to evaluate glypican-3 levels in COVID-19 patients

We found that glypican-3 levels decrease in COVID-19 patients

Glypican-3 levels is inversely correlated with IL-6 and CRP levels

Competing Interests: Authors have no conflict of interests.

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