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The relationship between serum soluble ACE 2 protein level and the clinical course of COVID-19 disease

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ABSTRACT

Aims: The angiotensin converting enzyme 2 (ACE 2) molecule, which mediates the entry of the virus into the cell, plays a very important role in the pathogenesis of COVID-19 disease. However, its effect on prognosis has not been fully explained. In this study, it was aimed to investigate the relationship between soluble ACE 2 (sACE 2) levels in the blood and the course of the disease.

Methods: sACE 2 levels at 0, 3 and 5 days were measured in patients with mild, moderate and severe COVID-19 pneumonia who were hospitalized between March 15, 2020 and August 30, 2020.

Results: 69 patients, 35 (51.5%) female and 34 (49.3%) male, with a mean age of 64.3±2.1 were included in the study. 42.0% of the patients had mild, 30.4% moderate, 27.5% severe pneumonia. Clinical follow-up of 7 patients resulted in death. There was no statistically significant difference between sACE 2 levels and gender, severity of pneumonia, initial hospitalization, presence of intubation and mortality.

Conclusion: sACE 2 levels were not associated with disease severity and inflammatory markers. Studies in larger patient populations are needed to explain the relationship between sACE 2 activity and SARS-CoV-2 infection and to develop new treatment strategies.

Keywords: COVID-19, angiotensin converting enzyme 2, prognosis

INTRODUCTION

Coronavirus-associated disease 19 (COVID-19), caused by a novel type of beta coronavirus, "Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)", has triggered a pandemic shortly after the first cases appeared in China in December 2019. The disease can cause a variety of clinical manifestations ranging from an asymptomatic course to mild upper respiratory tract infection, severe pneumonia, multiple organ failure, and death.[1-3]

Unfortunately, personal protective measures such as wearing masks, social distancing, hand hygiene, and lockdown measures have not proven effective enough in containing the outbreak. The molecule angiotensin converting enzyme 2 (ACE 2), which plays a key role in the pathogenesis of the disease and mediates viral entry into cells, has been the focus of most of the vaccine and drug development efforts, which are proceeding at a dizzying pace.[4] In the human body, ACE 2 is expressed in the central nervous system, lungs, cardiovascular system, adipose tissue, intestines, and kidneys.[5] The expression and distribution of ACE 2 in the human body may indicate potential infection pathways for SARS-CoV-2, so organs whose cells express high levels of ACE 2 should be considered high-risk areas for SARS-CoV-2 infection.[6] The large surface area of alveolar epithelial cells, particularly in the lung, may explain the susceptibility of this organ to the consequences of viral invasion. SARS CoV-2 uses the spike (S) protein to invade human alveolar epithelial cells. S protein binds to the ACE 2 receptor upon host cell invasion.[7,8] Therapeutic monoclonal antibodies targeting neutralizing epitopes on the S protein and vaccine-induced antibodies block viral binding to ACE 2, thereby preventing viral entry into cells.[9-11]

ACE 2, a transmembrane glycoprotein, is also an important component of the renin-angiotensin system (RAS).[4] ACE 2 reduces inflammation, fibrosis, and thrombosis mainly by converting Ang (angiotensin) I and Ang II to Ang 1-9 and Ang 1-7, respectively, and shows a protective effect against tissue damage in the organs where it is expressed.[12] Acute respiratory distress syndrome (ARDS) is a condition associated with high clinical mortality and ACE 2 has a protective effect on this type of acute lung injury.[13,14]

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Circulating ACE 2 levels in healthy individuals are very low and difficult to detect.[15] ACE 2 in the cell membrane can be cleaved and excreted into the circulation by both a disintegrin and metalloproteinase 17 (ADAM17) and a type II transmembrane serine protease (TMPRSS2). ADAM17 cleavage is a normal pathway that results in the production of circulating ACE 2 (soluble ACE 2, sACE 2).[5,13] Since soluble ACE 2 contains the virus binding site, it can also bind to the virus and thus facilitate virus entry.[4] Circulating ACE 2 can prevent severe pathological conditions and protect organs during SARS-CoV-2 infection. Meanwhile, cleavage by TMPRSS2 results in elevated SARS-CoV cell entry, allowing SARS-CoV-2 to invade cells in the lungs and intestines. The TMPRSS2-division pathway can block the ADAM17 division pathway. If the immune system fails to combat the virus, SARS-CoV-2 invades cellular ACE 2, where it proliferates greatly and destroys host cells in the lung and intestine.[13] The physiological and pathological effects of sACE 2 on particular organs or tissues are being investigated at present, but a good understanding of the mechanism behind these effects has not yet been established.[4]

Recent studies have demonstrated that the poor prognosis of COVID-19 patients is associated with sex (male), age (>60 years), underlying diseases (hypertension, diabetes, and cardiovascular disease), secondary ARDS, and other relevant factors. Owing to the protective effects of ACE 2 on these chronic underlying diseases and ARDS, the development of drugs that increase ACE 2 activity may be one of the most promising approaches for the treatment of COVID-19 in the future.[13,14] This subject, however, requires further research.

In this study, we aimed to investigate the effects of serum soluble ACE 2 levels on the clinical course of COVID-19 disease.

METHODS

The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 11/09/2020, Decision No: 2020/432). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was designed retrospectively. It was planned to study serum soluble ACE 2 protein levels by using Elisa kits from the samples taken for routine examinations on the day of hospitalization, on the 3rd and 5th days of hospitalization from patients with the mild, moderate, and severe clinical course of COVID-19 pneumonia, who were hospitalized in the infectious diseases pandemic ward and intensive care unit of Afyonkarahisar Health Sciences University Hospital between March 15, 2020, and August 30, 2020, as well as to retrospectively analyze the patient records, to examine the relationship of serum soluble ACE 2 protein levels with laboratory and clinical parameters affecting prognosis and to evaluate the effect of serum soluble ACE 2 protein levels on patient prognosis.

Participants

Patients who were found SARS-CoV-2 positive using the reverse transcriptase polymerase chain reaction (RT-PCR) method and who were found to be compatible with viral pneumonia in the lung tomographic examination were included in the study. According to the chest CT interpretation of the patients at the time of presentation, the presence of a ground glass density of less than 3 cm in all three foci was classified as mild pneumonia, while the presence of ground glass density or consolidation in more than three foci or greater than 3 cm was classified as moderate pneumonia and when there was involvement in all lobes of both lungs with at least three of the lesions larger than 3 cm were classified as severe pneumonia.[16]

Laboratory Method

Venous blood samples were collected from the patients in a 10 ml gel Vacutainer SST tube (Becton Dickinson, France). After waiting for 30 min, it was centrifuged at 2000 g for 10 min at 4°C. The serum samples remaining after centrifugation were separated into Eppendorf tubes and stored at -80°C until the tests were performed.

ACE 2 was measured in serum using BT-LAB Human ACE 2 Elisa kit (BT Lab Bioassay Technology Laboratory, Zhejiang, China). Absorbance reading was performed on a ChemWell 2910 Elisa reader (Awareness Technology, Inc. Martin Hwy. Palm City, USA). Results were expressed in ng/ml.

Statistical Analysis

In descriptive statistics, mean, standard deviation, median, and distance between quartiles were used for continuous variables, while numbers and percentages were used for nominal variables. Normality distributions of continuous variables were analyzed via Kolmogorov Smirnov and Shapiro-Wilk tests. The differences between the measurements of ACE 2 and other continuous variables on days 0, 3, and 5 were determined by Friedman's analysis of variance test and posthoc Dunn test (Bonferroni correction) in dependent measures. Differences in ACE 2 values between independent groups were analyzed via Mann-Whitney U-test in paired groups and Kruskall-Wallis tests in groups of more than two. The correlation between age and ACE 2 levels was determined by Spearman Correlation analysis. The results were considered significant at $p<0.05$. Statistical analyzes were conducted via SPSS 28.0 software.

RESULTS

A total of 69 patients were included in the study. The mean age of the patients was 64.3±2.1 years and 35 (51.5%) were female. Sixty-five percent of the patients had a history of chronic disease. The first hospitalization place of 79.7% of the patients was the ward, and 6 patients were transferred from the ward to the intensive care unit (ICU). Of the patients, 42.0% had mild pneumonia, 30.4% had moderate, and 27.5% had severe pneumonia. Ten patients had been intubated and seven patients had died. The characteristics of the patients are presented in Table 1.

Table 1. Characteristics of the patients

Characteristics **Age (n=69)**	**(Mean±SD)** **64.3±2.1** **n**	**Median (IQR)** **69 (23)** **%**
Sex (n=69)		
Female	35	50.7
Male	34	49.3
Place of first hospitalization (n=69)		
Ward	55	79.7
Transferred from ward to ICU	6	8.7
ICU	14	20.3
Pneumonia Severity (n=69)		
Mild	29	42.0
Moderate	21	30.4
Severe	19	27.5
Intubated (n=69)	10	14.5
Prognosis (n=69)		
Healing	62	89.9
Death	7	10.1
Smoker (n=68)	7	10.3
Comorbidities (n=69)		
Any chronic disease	45	65.2
Hypertension	33	47.8
Diabetes	32	46.4
Heart failure	17	24.6
Renal failure	14	20.3
COPD	10	14.5
Malignancy	10	14.5
Liver failure	2	2.9

No significant difference was found between ACE 2 levels. However, there was a significant decrease in Hgb (p=0.036), and Neu/Lym (0.004) levels on day 5 compared to baseline values (Posthoc Dunn test with Bonferroni adjustment). On the other hand, no significant difference was found between the measurements according to days in terms of other parameters (Table 2).

There was no significant correlation between sex, severity of pneumonia, place of first hospitalization, presence of intubation, mortality status, and all three ACE 2 measurements (Table 3). There was no significant correlation between the ACE 2 measurement made during hospitalization and the patient's age, whereas a significant negative correlation was determined in the measurements on the 3rd and 5th days.

DISCUSSION

In this study, sACE 2 levels were evaluated in blood samples taken on the day of admission, on the 3rd and 5th days of hospitalization of 69 patients with COVID-19 pneumonia who were hospitalized in the pandemic ward and intensive care unit of Afyonkarahisar Health Sciences University Hospital between March 15, 2020, and August 30, 2020.

Two recent studies have shown that sACE 2 binds to SARS-COV-2 and then mediates its entry into cells.17,18 These findings suggest that ACE 2 scattering and sACE 2 have a role in SARS-CoV-2 infection. However, it is not fully understood what effect they have on the clinical course of the disease, either positively or negatively.[4] ADAM17 inhibition is predicted to reduce ACE 2 trimming, viral entry, and cytokine production. Clinical grade recombinant human ACE 2 (rhACE 2), a type of exogenous soluble form of ACE 2, binds to SARS-COV-2 in human tissues and inhibits virus infection.[19] Thus, it would be very useful to develop treatment regimens for the inhibition of ADAM 17, which is involved in the producer mechanism of sACE 2 in COVID-19, and for the therapeutic use of rhACE 2 in COVID-19. In the literature, there are very few studies that address this topic.

Table 2. ACE 2 level measurements, complete blood counts, and some biochemical values of the patients

Day		**P***	**(Mean±SD)**	**Median (IQR)**		**P***	**(Mean±SD)**	**Median (IQR)**
0	ACE 2	0.933	2.48±1.87	1.82 (1.77)	Neu/Lym	0.005	10.50±22.39	3.97 (5.08)
3			2.72±2.24	1.96 (2.07)			9.18±17.41	4.28 (5.26)
5			2.71±2.21	1.91 (1.77)			6.58±6.85	3.79 (5.00)
0	Wbc	0.796	8.21±5.16	6.68 (6.03)	D-Dimer	0.694	3.14±8.53	1.20 (2.23)
3			7.97±4.53	6.83 (4.86)			2.13±3.04	1.22 (2.32)
5			7.63±4.39	7.04 (4.59)			1.95±2.20	1.54 (2.16)
0	Hgb	0.028	11.03±2.53	10.50 (4.40)	Crp	0.339	54.91±68.62	24.36 (78.99)
3			10.82±2.45	10.30 (3.90)			48.97±63.41	24.40 (69.55)
5			10.43±2.61	10.20 (3.25)			40.94±48.31	17.94 (62.99)
0	Plt	0.148	209.42±102.17	200.00 (88)	Ferritin	0.137	445.79±524.33	272.80 (417.05)
3			206.14±116.68	185 (121)			440.68±453.37	314.90 (395.80)
5			221.25±125.33	195 (143)			515.05±529.78	345.30 (511.35)
0	Lym	0.694	1.15±0.66	0.93 (0.91)	Procalcitonin	0.152	4.99±20.45	0.08 (0.24)
3			1.20±0.72	1.07 (0.89)			2.79±10.77	0.09 (0.33)
5			1.37±1.45	1.12 (1.05)			1.34±4.59	0.07 (0.32)

*Friedman's Two Way Analysis of Variance

Table 3. ACE 2 levels by different parameters

	ACE 2 (Baseline)		ACE 2 (Day 3)		ACE 2 (Day 5)	
	(Mean±SD)	Median (IQR)	(Mean±SD)	Median (IQR)	(Mean±SD)	Median (IQR)
Sex						
Female	2.59±2.08	1.82 (1.75)	2.84±2.43	2.09 (2.32)	2.66±2.23	1.90 (1.77)
Male	2.36±1.65	1.78 (1.79)	2.58±2.05	1.91 (1.93)	2.76±2.21	2.01 (1.86)
P*	0.810		0.783		0.924	
Age						
p, r**	0.094, -0.203		0.009, -0.313		0.025, -0.270	
Severity of pneumonia						
Mild	2.29±1.70	1.68 (1.18)	2.47±2.18	1.91 (1.36)	2.33±1.88	1.63 (0.98)
Moderate	2.68±2.28	2.10 (2.54)	3.00±2.43	2.51 (4.26)	2.85±2.40	2.42 (2.42)
Severe	2.53±1.69	1.98 (1.86)	2.78±2.20	2.22 (2.41)	3.14±2.46	2.66 (1.70)
p***	0.724		0.777		0.354	
Place of the first hospitalization						
Ward	2.53±1.94	1.83 (1.72)	2.73±2.19	1.96 (1.83)	2.76±2.24	1.91 (1.62)
ICU	2.27±1.60	1.76 (1.98)	2.67±2.51	1.88 (2.57)	2.50±2.14	2.16 (2.11)
p*	0.676		0.633		0.665	
Intubation						
No	2.54±1.91	1.83 (1.72)	2.69±2.14	2.02 (1.91)	2.70±2.19	1.91 (1.63)
Yes	2.13±1.66	1.75 (1.49)	2.84±2.90	1.66 (2.93)	2.78±2.40	2.25 (2.63)
p*	0.485		0.733		0.959	
Prognosis						
Healing	2.54±1.88	1.83 (1.70)	2.82±2.30	2.06 (1.99)	2.81±2.28	1.94 (1.81)
Death	1.88±1.77	1.24 (1.13)	1.76±1.34	1.27 (2.83)	1.79±0.98	1.26 (2.04)
p*	0.218		0.190		0.330	

*Mann-Whitney U test, **Spearman Correlation, ***Kruskall-Wallis test

Many of the symptoms of COVID-19 patients are associated with the interaction of the virus with the ACE 2 protein at relevant sites, including cardiovascular and pulmonary complications.[20] There have been numerous proposals for therapeutic options related to the novel coronavirus, and a substantial number of them are based on the interaction between ACE 2 and the viral spike protein. Hydroxychloroquine, for example, one of the first medications approved for COVID-19, blocks the binding of the virus' spike protein by altering part of the ACE 2 molecule. Using soluble ACE 2 (sACE 2) to block the ACE 2 receptor and to bind to and neutralize viral spike protein are further examples of how this interaction can lead to therapeutic strategies. Moreover, changes in ACE 2 expression due to diabetes, cardiac and renal disease, and aging may also contribute to the greater susceptibility of these patient populations to SARS-CoV-2.[21] In the present study, although 65.2% of the patients had any chronic disease, no significant difference was observed between ACE 2 levels in those with comorbidities.

In 38 patients followed up with the diagnosis of COVID-19 in Iran, sACE 2 levels were measured on the 0^{th}, 3^{rd}, and 7^{th} days of hospitalization. It was determined that sACE 2 levels could be useful in predicting the need for mechanical ventilation, but it was underscored that further studies with a larger sample size are needed to make a definite conclusion on this issue.[21]

In a study, it was suggested that upregulation of ACE 2 expression may reflect the severity of the disease in COVID-19, and it was also reported that sACE 2 activity at the time of admission did not reflect disease severity.[21] Consistent with these findings, in our study, no significant difference was found between the sACE 2 levels of patients with mild, moderate, and severe COVID-19 pneumonia on the day of hospitalization. The fact that ACE activity levels were similar in patients with different disease severity is quite remarkable in this respect.

In the study, no correlation was found between biochemical parameters and sACE level and our findings are consistent with the existing literature data. Meanwhile, in the study by Robertson et al.[22] CRP and ferritin levels showed an inverse correlation with sACE 2 concentration, and no significant difference was observed in sACE 2 levels for any of the comorbidities. In a study conducted in Turkey, sACE 2 levels were examined in 55 patients and 18 healthy control subjects, and no significant difference was found in serum ACE 2 activity between patients and healthy subjects. In the same study, a comparison of sACE 2 activity according to disease outcome showed no significant difference between patients with good or poor prognosis, and no correlation was found between serum ACE activity and inflammatory markers including ferritin, CRP, procalcitonin, fibrinogen, and IL-6 levels.[23]

There is a major limitation to this study, in that there are a limited number of patients, which can be countered by conducting studies with larger patient populations to obtain more reliable results.

CONCLUSION

Serum sACE 2 levels were not associated with disease severity and did not correlate with inflammatory markers. Further studies in this area are needed to address the lack of information on the relationship between sACE 2 activity and SARS-CoV-2 infection and to develop novel treatment strategies based on this information.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Afyonkarahisar Health Sciences University Clinical Researches Ethics Committee (Date: 11/09/2020, Decision No: 2020/432).

Informed consent: Because the study was designed retrospectively, no written informed consent from was obtained from the patients.

Referee Evaluation Process: Externally peer reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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