The assessment of serum ACE activity in COVID-19 and its association with clinical features and severity of the disease

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ABSTRACT
Angiotensin-converting enzyme (ACE)/Angiotensin (Ang) II pathway has crucial regulatory effects on circulatory hemostasis and immune responses. This pathway has a major role in the development of acute lung injury and acute respiratory distress syndrome (ARDS), which is a devastating complication of SARS-CoV-2 infection. The aim of this study is to investigate the serum ACE activity and its correlation with clinical features and the disease severity in patients with COVID-19. Patients with confirmed COVID-19 by detecting SARS-CoV-2 nucleic acid RT-PCR were included in the study. Demographic data, clinical features, laboratory and radiologic investigations were recorded. Patients were classified by disease severity; asymptomatic, mild, and severe pneumonia. The serum ACE activity was evaluated with an autoanalyzer based on a spectrophotometric method. Fifty-five patients (50.9% female) and 18 healthy subjects (33.3 % female) were enrolled in the study. The median age of patients was 40 years, ranging from 22 to 81 years. Eighteen healthy subjects were served as the control group. The baseline characteristics were comparable between groups. The median serum ACE activity of patients and controls (38.00 [IQR 21] U/L and 32.00 [IQR 24] U/L, respectively) and of between patients grouped by disease severity (38.5 [IQR 19], 36 [IQR 25], and 38 [IQR 22] U/L, asymptomatic, mild and severe pneumonia group, respectively) were similar. There was no correlation between the serum ACE activity and conventional inflammatory markers. In this study, we did not find an association between serum ACE activity and COVID-19 and serum ACE activity on admission did not reflect disease severity.

Introduction
Betacoronaviruses (Beta-CoVs), primarily leading to respiratory and gastrointestinal infection in humans, are responsible for life-threatening outbreaks, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and recently identified SARS-CoV-2 infection [1–3]. SARS-CoV-2 infection, namely coronavirus disease 2019 (COVID-19), has rapidly become a global catastrophe with resulting in serious complications such as severe lung inflammation, acute respiratory distress syndrome (ARDS), thrombosis, cardiac and renal injury [4–7]. COVID-19 has a wide range of clinical presentations, ranging from asymptomatic to severe disease. The severe disease usually has been observed in patients with older age and comorbidities, including, hypertension (HTN) cardiovascular disease (CVD), heart failure (HF), chronic obstructive pulmonary disease (COPD), and diabetes mellitus (DM) [8–11].

SARS-CoV-2 possesses a non-segmented, single-stranded positive-sense RNA and lipid bilayer envelope with structural proteins, including nucleocapsid (N), envelope (E), membrane (M), and Spike (S) protein, all of which are crucial for the viral infectivity. S proteins, consisting of S1 and S2 domains, are the receptor binding proteins that are responsible for cellular attachment and entry [12,13]. S1 domain, called receptor binding domain, binds to angiotensin-converting enzyme (ACE) 2 receptors on human host cells similar to SARS-CoV. ACE2, the type 1 transmembrane protein with a carboxymonopeptidase activity, is expressed on type 2 alveolar cells in the lung as well as on cells in the gastrointestinal tract, heart, blood vessels, kidney, oral, and nasal mucosa [14–16]. SARS-CoV infection induces the down-regulation of ACE2 expression in vivo [17]. When considering to 76% identical sequence of S protein between SARS-CoV-2 and SARS-CoV, SARS-CoV-2 infection might downregulate the ACE2 expression through the internalization of the virus to cell [18].

ACE2 exhibits 61% sequence similarity to ACE. Both ACE and ACE2 play crucial roles in counter-regulatory pathways of the Renin-Angiotensin-System (RAS), which is...
responsible for the circulatory homeostasis of the human body particularly in cardiovascular and renal functions [19,20]. In addition to the circulatory effect, RAS plays an essential role in the balance of the inflammatory process. ACE converts angiotensin (Ang) I, a component of RAS, into Ang II, stimulating vasoconstriction and promoting proinflammatory, fibrotic, and thrombotic processes mediating with Ang II type 1 (AT1) receptor. In contrast to ACE, ACE2 metabolizes Ang II to Ang (1–7) and cleaves Ang I to Ang (1–9), thereby resulting in decreased inflammation, fibrosis, and thrombosis [21]. Besides, previous studies have demonstrated the importance of RAS in the development of ARDS, which is a devastating complication, seen in the severe form of SARS-CoV-2 infection. A previous animal study showed that ACE2 has protective effects on lung tissue by controlling inflammation and edema. Furthermore, the treatment with human ACE2 reduces sepsis and acid aspiration-induced acute lung injury whereas increased Ang II production mediating with ACE leads to the acute lung injury through AT1 receptor [22]. ACE expression and ACE activity have increased in mechanic ventilation-induced lung injury [23,24]. Recent research included twelve patients with COVID-19, has reported that Ang II level remarkably increased and correlated with viral load and lung injury in patients compared to healthy individuals [25].

The exact pathogenesis and factors leading to a severe form of COVID-19 have not been clearly known. According to clinical data and studies have suggested that exaggerated immune response and cytokine storm might be attributed to the severity of the disease [18]. Based on previous data from SARS-CoV infection and SARS-CoV-2 by considering disease course and outcomes, ACE/Ang II and ACE2/Ang (1–7) pathways might be related to the pathogenesis of COVID-19 and disease severity. The enhancement of ACE activity, leading to perturbation of RAS, might drive disease progression and mediate abnormal inflammatory and fibrotic processes in COVID-19. However, there has been no detailed investigation of ACE activity in SARS-CoV-2 infection. Therefore, this study aimed to assess ACE activity in COVID-19 by comparing to healthy individuals and investigate the association between disease severity and ACE activity.

Methods

Study design and participants

This study was designed as an analytic study with prospective follow-up. Consecutive patients with confirmed COVID-19 by SARS-CoV-2 nucleic acid RT-PCR in nasopharyngeal swabs or sputum were enrolled. Age and sex-matched subjects who applied for COVID-19 testing but found negative were constituted the control group. They were free of symptoms and not hospitalized. On admission, indication for hospitalization was determined with 'COVID-19 diagnosis and treatment guide' released on 12 April 2020, by the Turkish Ministry of Health [26]. These indications were the presence of dyspnea, tachypnea (>22 breaths/min), tachycardia (>125 bpm), confusion, hypotension (<90/60 mmHg), comorbid illness, including CVD, DM, HTN, malignancy, COPD, immunocompromised conditions, and age > 50 years. The study was approved by the ethics committee, and written informed consent was obtained from all participants.

Data collection

Demographic data, COVID-19 symptoms, and past medical histories such as comorbidities and active medications, were obtained by patient interviews. Laboratory findings and radiologic investigations were retrieved from electronic medical records. These data were reviewed by the team of physicians experienced in the management of COVID-19. Upon hospitalization, all patients underwent standardized workup, including complete blood count, coagulation profile and d-dimer, blood biochemistry, serum ferritin, D-dimer, C-reactive protein (CRP), procalcitonin, and interleukin (IL)-6. Chest radiography and electrocardiogram were also performed for all patients. Thorax computed tomography (CT) was performed in the case of the presence of the following conditions: dyspnea, cough, hypoxemia, comorbid disease, age > 60 years, and suspicion of venous thromboembolism (VTE). All patients were followed until hospital discharge or death. Poor outcome was defined as admission to the intensive care unit (ICU) or death.

According to the follow up clinical course, patients were divided into three subgroups with the definition of ‘COVID-19 guide of Republic of Turkey, Ministry of Health’ [27]. Based on the guide, severe pneumonia was defined as having any findings of the following: dyspnea and respiratory rate >30 breaths/min; oxygen saturation <90% at ambient air; arterial oxygen partial pressure (PaO2)/oxygen saturation concentration (FiO2) ≤300 mmHg and bilateral diffused infiltrates on chest radiography or CT. Patients with infiltrates on their chest radiography or CT, and none of the above findings were considered as mild pneumonia. Patients whose tests were positive in the contact tracing and remained asymptomatic for 14 days were formed the asymptomatic COVID-19 group.

Blood samples and measurement of serum ACE activity

Blood samples for the measurement of ACE activity were taken once during COVID-19 RT-PCR testing for control subjects and on the first day of hospitalization before the start of COVID-19 treatment for patients. Samples of asymptomatic patients were taken on the next day of the positive COVID-19 RT-PCR report date. After waiting 30 min, blood samples were separated into sera and stored at −80 °C freezer until the measurement. Serum ACE activity assay was performed using commercial kits (SENTINEL, Italy) by an autoanalyzer (Beckman Coulter, AU5800, CA, USA), based on the automated kinetic assay (the traceability of ACE activity; ACE calibrator, REF 1665001). The reference interval of serum ACE activity is 13.3–63.9 U/L. In this method, Furlacryloylpenylalanine-glycylglycine (FAPGG) was hydrolyzed to Furlacryloylphenylalanine (FAP) and
Glycylglycine by ACE activity (intra-assay coefficient of variation (CV) < 2% and inter-assay CV for this method < 3%) [28].

**Statistical analyses**

Statistical Package for the Social Sciences software v16.0 (SPSS Inc, Chicago, IL) and Microsoft Excel package programs was used for statistical analysis. Categorical data were described as absolute numbers and percentages. Chi-square or Fisher’s exact tests were used to compare categorical variables. The conformity of continuous variables to the normal distribution was evaluated using visual (histogram/probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk tests). Continuous variables are expressed as median (Interquartile range (IQR)) values since they did not show normal distribution. Continuous variables of groups were compared with Mann–Whitney U and Kruskal–Wallis tests. Pairwise comparisons were evaluated with the Mann–Whitney U test. p1 represents the hypothesis between asymptomatic and mild pneumonia group; p2, between asymptomatic and severe pneumonia group; and p3, between mild and severe pneumonia group. Spearman Rho was used for correlation analyzes and interpreted as a poor (.0–.2), fair (.2–.4), moderate (.4–.6), good (.6–.8) or excellent agreements (.8–1.0) [29,30].

**Results**

Fifty-five patients and eighteen healthy controls were enrolled in the study. The disease characteristics of the patient and healthy control groups were presented in Table 1. The median age of patients was 40 (IQR 35) years, ranging from 22 to 81 years.

The distribution of baseline characteristics, including sex, comorbidities, and treatment, were similar in both groups. The median serum ACE activity of patients and controls were 38.00 (IQR 21) U/L and 32.00 (IQR 24) U/L, respectively. No significant difference in the serum ACE activity was found between patients and healthy subjects (p = .279).

According to clinical presentation, 24 (43.6%) patients had mild pneumonia, and 11 (20%) patients had severe pneumonia. Twenty patients were remained asymptomatic in their follow-up. The mean symptom duration of patients with mild and severe pneumonia before the admission was 4 ± 2.5 and 5 ± 4 days, respectively, and there is no meaningful difference in the symptom duration between the two groups (p = .4). Mild and severe pneumonia patients were hospitalized. During the follow-up, nine of these patients required ICU admission, and three of them were eventually died. The baseline characteristics of patient groups are demonstrated in Table 2. The median age of patients with severe pneumonia was significantly higher compared to the asymptomatic and mild pneumonia group (p < .001 for both). Comorbidities were present in one-third of patients with hypertension being the most common disorder in mild and severe pneumonia. Patients with mild pneumonia and severe pneumonia had considerably more comorbidities compared to asymptomatic patients (p1 = .007 and p2 = .001, respectively). Notably, CAD in the severe pneumonia group was more frequently observed compared to the asymptomatic and mild pneumonia group.

Table 3 has shown the details about the laboratory findings of each patient group. There was a significant increase in the level of inflammatory markers and biochemical parameters of organ injury towards the disease severity. The baseline lymphocyte count was prominently lower in severe pneumonia than the asymptomatic group and mild pneumonia group (p2 < .001 and p3 = .013, respectively). The median (IQR) serum ACE activity of the asymptomatic, mild, and severe pneumonia groups were 38.5 (19) U/L, 36 (25) U/L, and 38 (22) U/L, respectively (p > .05). The comparison of ACE activity with regard to disease outcome showed no significant difference between patients with good and poor outcome (p = .9). Moreover, there was no correlation between serum ACE activity and inflammatory markers, including ferritin, CRP, procalcitonin, fibrinogen, and IL-6 level, as well as cell counts.

**Discussion**

The RAS is the main contributor to physiologic circulatory homeostasis by maintaining blood pressure and electrolyte balance [20]. Two major vital enzymes, ACE and ACE2, are responsible for the regulation of RAS with balancing effects. ACE, a zinc-dependent dipeptidylpeptidase, is expressed in most of the tissues of the body, while the expression is remarkable higher in the lungs, kidneys, and duodenum [31]. The serum level of ACE is affected by genetic polymorphism and age. Children and adolescents have higher serum levels compared to adults [32–34]. In addition to the effects of the ACE/Ang II pathway on hemodynamic regulation, they have been shown to act on the immune system. Ang II retains critical functions in the inflammatory response through leukocyte recruitment with mediating the.

**Table 1.** Baseline characteristics of patients and control group.

| Comorbidities, n (%) | Patient group | Control group | p       |
|---------------------|---------------|---------------|---------|
| Asthma              | n = 55        | n = 18        |         |
| DM                  | 3 (5.5)       | 1 (5.6)       | .673    |
| COPD                |               |               | .570    |
| Angiotensin receptor blockers | | | .758    |
| Malignancy          |               |               | .926    |
| CAD                 | 4 (7.3)       | 0             | .566    |
| DM                  | 6 (10.9)      | 2 (11.1)      | 1.000   |
| HT                  | 13 (23.6)     | 5 (27.8)      | .758    |
| Smoking             | 12 (21.8)     | 2 (11.1)      | .494    |
| Comorbidities       | 17 (30.9)     | 5 (27.8)      | .802    |
| COPD                | 3 (5.5)       | 0             | .570    |
| DM                  | 6 (10.9)      | 2 (11.1)      | 1.000   |
| Male                | 32 (58.2)     | 25 (83.3)     | .007    |
| Female              | 23 (41.8)     | 5 (16.7)      | .279    |
| Malignancy, n (%)   |               |               | .150    |
| DM                  | 6 (10.9)      | 2 (11.1)      | 1.000   |
| HT                  | 13 (23.6)     | 5 (27.8)      | .758    |
| Smoking             | 12 (21.8)     | 2 (11.1)      | .494    |
| Comorbidities       | 17 (30.9)     | 5 (27.8)      | .802    |
| COPD                | 3 (5.5)       | 0             | .570    |
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| HT                  | 13 (23.6)     | 5 (27.8)      | .758    |
| Smoking             | 12 (21.8)     | 2 (11.1)      | .494    |
| Comorbidities       | 17 (30.9)     | 5 (27.8)      | .802    |
Table 2. The comparison COVID-19 patient groups for their baseline characteristics.

|                       | Asymptomatic patients | Mild pneumonia | Severe pneumonia | p1  | p2  | p3  |
|-----------------------|-----------------------|----------------|------------------|-----|-----|-----|
| Age, years (IQR)      | 29.5 (10)             | 44 (20)        | 75 (9)           | <.001 | <.001 | <.001 |
| Female                | 15 (75)               | 10 (41.7)      | 3 (27.3)         | .550 | .021 | .478 |
| Smoking               | 2 (10)                | 6 (25)         | 4 (36.4)         | .239 | .151 | .689 |
| Comorbidities         | 1 (5)                 | 11 (45.8)      | 7 (63.6)         | .007 | .001 | .471 |
| DM                    | 1 (5)                 | 2 (8.3)        | 3 (27.3)         | 1.000 | .115 | .297 |
| HTN                   | 0                     | 6 (25)         | 7 (63.6)         | .025 | <.001 | .057 |
| CAD                   | 0                     | 1 (4.2)        | 4 (36.4)         | 1.000 | .010 | .026 |
| HF                    | 0                     | 0              | 2 (18.2)         | 3 (27.3) |
| COPD                  | 0                     | 0              | 3 (27.3)         | 1.000 | .037 | .082 |
| Asthma                | 0                     | 3 (12.5)       | 1 (9.1)          | 239 | .355 | 1.000 |
| Renal diseases        | 0                     | 0              | 0                | 2 (18.2) |
| Malignancy            | 0                     | 1 (4.2)        | 3 (27.3)         | 1.000 | .037 | .082 |
| Medications           |                       |                |                  |      |      |      |
| Anti-hypertensive drugs| 0                    | 6 (25)         | 7 (63.6)         | .025 | <.001 | .057 |
| ACEI                  | 0                     | 1 (4.2)        | 2 (18.2)         | 1.000 | .118 | .227 |
| ARB                   | 0                     | 3 (12.5)       | 3 (27.3)         | .239 | .037 | .352 |
| Steroid              | 0                     | 1 (4.2)        | 0                |      |      |      |
| Statins              | 0                     | 1 (4.2)        | 1 (9.1)          | 1.000 | .355 | .536 |

All values are presented as median (IQR) or numbers (%). p1: comparison of asymptomatic patients and mild pneumonia group, p2: comparison of asymptomatic and severe pneumonia group, and p3: comparison of mild and severe pneumonia groups with Mann–Whitney U test. ACE: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CAD: coronary artery disease; DM: diabetes mellitus; HTN: hypertension; HF: heart failure; COPD: Chronic obstructive pulmonary disease.

Table 3. Laboratory results of patients and comparisons between patient groups.

|                       | Asymptomatic patients | Mild pneumonia | Severe pneumonia | p1  | p2  | p3  |
|-----------------------|-----------------------|----------------|------------------|-----|-----|-----|
| Hemoglobin, g/dL      | 13.5 (1.63)           | 14 (1.9)       | 12.6 (1.9)       | .257 | .072 | .007 |
| WBC, 10⁹/L            | 7.56 (2.6)            | 6 (1.6)        | 5.5 (2.56)       | .451 | .640 | .563 |
| Lymphocyte, 10⁹/L     | 1.9 (1.13)            | 1.34 (1)       | 0.75 (0.97)      | .56  | <.001 | .013 |
| Lymphopenia ≤0.8 10⁹/L| 0                     | 5 (20.8)       | 6 (5.45)         | .053 | .001 | .002 |
| Thrombocyte, 10⁹/L    | 245 (85)              | 222 (72)       | 194 (100)        | .41  | .029 | .493 |
| Creatinine, umol/L    | 53 (12)               | 79.5 (17.6)    | 95 (97)          | <.001 | <.001 | .043 |
| ALT, U/L              | 17 (11)               | 26.5 (36)      | 27 (15)          | .012 | .008 | .847 |
| AST, U/L              | 21 (7.5)              | 25 (14)        | 36 (27)          | .002 | <.001 | .022 |
| LDH, U/L              | 216 (45)              | 233 (104)      | 407 (285)        | .346 | <.001 | .001 |
| D-dimer, ng/mL        | 0.2 (0.24)            | 0.36 (1)       | 0.92 (1.47)      | .011 | <.001 | .020 |
| Ferritin, µg/L        | 17 (26)               | 143 (241)      | 229 (698)        | <.001 | <.001 | .097 |
| CRP, mg/L             | 2.14 (12.3)           | 9.7 (17.5)     | 97 (118)         | <.001 | <.001 | <.001 |
| Fibrinogen, g/L       | 3.15 (1.23)           | 3.93 (1.77)    | 5.54 (1.76)      | .019 | <.001 | <.001 |
| Procalcitonin, µg/L   | 0.02 (0.01)           | 0.053 (0.03)   | 0.175 (0.2)      | <.001 | <.001 | <.001 |
| Troponin, ng/L        | n.d.                  | 6 (9)          | 25 (26)          | <.001 |      |      |
| IL-6, pg/mL           | 1.5 (37.8)            | 11 (29.6)      | 208 (586)        | .001 | .001 | <.001 |
| Serum ACE activity, U/L| 38.5 (19)            | 36 (25)        | 38 (22)          | .234 | .317 | .930 |

All values are presented as median (IQR) or numbers (%). p1: comparison of asymptomatic patients and mild pneumonia group, p2: comparison of asymptomatic and severe pneumonia group, and p3: comparison of mild and severe pneumonia groups with Mann–Whitney U test. ACE: angiotensin-converting enzyme, ALT: Alanine aminotransaminase, AST: aspartate aminotransferase, CRP: C-reactive protein, IL-6: interleukin 6, LDH: Lactate dehydrogenase, n.d.: not determined, WBC: white blood cell.

upregulation of adhesion molecule expression in endothelial cells, promoting the maturation of immune cell subsets, and increasing the production of reactive oxygen species. [31]. Another critical effect of Ang II on the immune response is the stimulation of IL-6 production, which remarkably increases in COVID-19, particularly in severe disease and strongly relates to lung injury [18,35,36]. Furthermore, the increased ACE activity promotes macrophages and neutrophil functions independently of Ang II effects. It is well-known that ACE level increases in numerous autoimmune or infectious disorders characterized by the granulomatous reaction [31].

Several studies and reports have revealed that COVID-19 is characterized by the dysregulation of immune cell subsets, exaggerated inflammation, and hypercytokinemia, all of which have been correlated with disease severity and lung injury [18]. The specific factors that drive progressive disease have not been clarified yet. A previous study has demonstrated that the downregulated ACE2 expression and increased Ang II production by SARS-CoV infection resulted in severe lung injury [17]. This result might be associated with disequilibrium between ACE and ACE2 activity. However, this condition yet to be demonstrated for SARS-CoV-2 considering similarities and dissimilarities between two viruses [37,38]. Another finding from an acute lung injury model is the enhanced disease progression with ACE/Mediated Ang II production [22]. Besides, ACE deletion (D) polymorphism, which is associated with a higher ACE activity, is frequently observed in ARDS [39]. Moreover, a considerably higher frequency of D allele polymorphism was observed in SARS patients who require oxygen treatment compared to non-hypoxemic counterparts [40].

There is a lack of knowledge regarding the relationship between ACE activity and SARS-CoV-2 infection. This study has set out with the aim of assessing the ACE activity in
COVID-19 and its association with disease severity. Contrary to expectations, this study did not show a significant difference in ACE activity between patients with COVID-19 and healthy individuals. Another surprising finding was that levels of ACE activity were similar in patients with different disease severity. However, these findings have supported that the role of ACE2 might be more constitutive for the pathogenesis and disease progression of SARS-CoV-2 infection rather than ACE.

As observed in previous studies, the severity and mortality of the disease is associated with advanced age [11, 41]. Besides, comorbid conditions such as CAD, HF, DM or HTN constitute significant risk factors for poor clinical outcomes in COVID-19. In this study, comorbidities were observed approximately one-third of patients with COVID-19 and the most prevalent comorbidities were HTN, DM, and CAD, which of them are associated with RAS. In accordance with the present results, previous studies and meta-analyses have demonstrated that HTN followed by DM was the most frequently seen comorbidity in patients with COVID-19 [42–45]. The use of ACE inhibitors has a favorable effect on the disease prognosis [10]. ACE inhibitors are known to reduce the levels of serum ACE and can introduce ‘artificially’ reduced levels of serum ACE in patients using these drugs [46]. However, we could not demonstrate a difference between ACE and ARB users and others with regard to serum ACE activity, which might be related to a small number of subjects.

This study has several limitations. The major limitation of the study is the size of the population; particularly severe pneumonia group was relatively small to assess the serum ACE activity. Second, the serum ACE activity was measured only at the admission, and serial measurement with different disease states might better reveal the association between ACE activity and disease severity. Third, in addition to serum ACE activity, the evaluation of Ang II level might provide more robust evidence for the activity of ACE on the disease outcome.

In conclusion, serum ACE has a similar activity between COVID-19 patients and healthy individuals. Interestingly, serum ACE activity has not been related to disease severity and did not correlate with inflammatory markers. However, further studies with a larger sample size are needed to confirm these findings and concurrently evaluation of ACE2 activity and Ang II to determine the exact role of ACE in the pathogenesis of COVID-19.

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No potential conflict of interest was reported by the author(s).

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