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Angiotensin-converting enzyme-1 gene insertion/deletion polymorphism may be associated with COVID-19 clinical severity: a prospective cohort study

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BACKGROUND: Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism may play a role in the pathogenesis of coronavirus-19 disease (COVID-19).

OBJECTIVES: Investigate the relationship between ACE I/D polymorphism and the clinical severity of COVID-19.

DESIGN: Prospective cohort study.

SETTING: Tertiary care hospital.

PATIENTS AND METHODS: The study included COVID-19 patients with asymptomatic, mild, and severe disease with clinical data and whole blood samples collected from 1 April 2020 to 1 July 2020. ACE I/D genotypes were determined by polymerase chain reaction and agarose gel electrophoresis.

MAIN OUTCOME MEASURES: ACE DD, DI and II genotypes frequencies.

SAMPLE SIZE: 90 cases, 30 in each disease severity group.

RESULTS: Age and the frequency of general comorbidity increased significantly from the asymptomatic disease group to the severe disease group. Advanced age, diabetes mellitus and presence of ischemic heart disease were independent risk factors for severe COVID-19 [OR and 95 % CI: 1.052 (1.021-1.083), 5.204 (1.006-26.892) and 5.922 (1.109-31.633), respectively]. The ACE II genotype was the dominant genotype (50%) in asymptomatic patients, while the DD genotype was the dominant genotype (63.3 %) in severe disease. The ACE II genotype was protective against severe COVID-19 [OR and 95% CI: .323 (.112-.929)]. All nine patients (8.9%) who died had severe disease.

CONCLUSIONS: The clinical severity of COVID-19 infection may be associated with the ACE I/D polymorphism.

LIMITATIONS: Small sample size and single center.

CONFLICT OF INTEREST: None.

A new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus-19 disease (COVID-19). COVID-19 has a broad clinical spectrum ranging from asymptomatic course to acute respiratory distress syndrome (ARDS) accompanied by bilateral viral pneumonia and death.[1] In COVID-19 infection, the renin-angiotensin system (RAS) pathway is thought to play an important role in the development of ARDS and aggravation of the clinical picture.[2] Theoretically, in COVID-19, the angiotensin-converting enzyme-1 (ACE1)/ angiotensin-converting enzyme-2 (ACE2) balance is disturbed due to overexpression of the ACE1 receptor and down-regulation of the ACE2 receptor. Disruption of this balance results in excessive RAS activation, and lung damage occurs with excessive production of angiotensin II.[3,4]

It is well known that the RAS pathway can cause many deleterious events associated with increased angiotensin II production, such as tissue damage, edema, inflammation, apoptosis, fibrosis, thrombosis, elevated blood pressure, and decreased ACE2 expression.[4-10] The insertion/deletion (I/D) polymorphism at intron 16 of the *ACE1* gene (NM 000789.2) is associated with changes in circulating and tissue concentrations of ACE.[7-9] DD genotype causes high serum and tissue ACE1 levels.[4-9] Studies on host genetic factors that may cause this clinical difference in COVID-19 patients are quite limited. We sought to examine the effect of this ACE I/D polymorphism on the clinical severity of COVID-19.

PATIENTS AND METHODS

Reverse transcription (RT)-qPCR-positive COVID-19 patients who were followed up at Samsun Research and Training Hospital from 1 April 2020, to 1 July 2020, were included in this prospective cohort study. Clinical data and whole blood samples of were collected,and the clinical condition of the patients was classified as severe, mild or asymptomatic in line with the recommendations of the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Seventh Version, Released by National Health Commission State Administration of Traditional Chinese Medicine) and Republic of Turkey Ministry of Health COVID-19 Guidelines:[11-14]

- Severe disease was defined as patients with positive RT-PCR result for COVID-19 who had significant respiratory distress (respiratory rate >30/min), blood oxygen saturation <93%, arterial oxygen partial pressure/fraction of inspired O_2 (PaO_2/FiO_2) <300 mm Hg, respiratory failure with mechanical ventilation, shock, or other organ failure in need of intensive care.
- Mild disease group was defined as patients with positive RT-qPCR result for COVID-19 who had a fever, muscle/joint pain, cough and sore throat, respiratory rate <30/minute, SpO_2 level above 90% in room air.
- Asymptomatic patients had a positive RT-qPCR with a contact history but no clinical complaints during follow-up.

COVID-19 laboratory confirmation was defined as a positive result on RT-qPCR (Bioeksen, Istanbul, Turkey) (Bio-speedy SARS-Cov-2 RT-qPCR detection kit) of nasal and pharyngeal swab specimens. The local medical ethics committee (Decision no: KAEK2020/4/4) and the Turkish Ministry of Health (2020-05-05T14_19_43) approved the present prospective cohort study in accordance with human and animal rights and complied with the principles of the Helsinki Declaration. Informed consent was obtained before collecting samples from all patients.

DNA extraction and genotyping

DNA was extracted from the peripheral blood samples of COVID-19 patients using a commercial spin column (QIAamp DNA Blood Mini Kit; QIAGEN, Hilden, Germany) protocol as recommended by the manufacturer. ACE I/D genotypes were determined by polymerase chain reaction (ABI RT-PCR 7500, Applied Biosystems, USA) amplification. The forward and reverse primers were 5'- CTG GAG AGC CAC TCC CAT CCT TTC T-3' and 5'- GGG ACG TGG CCA TCA CAT TCG TCA G-3', respectively. Reactions were set up in a volume of 25 µL containing 1.5 µL of each primer, 12.5 µL master mix, 7,5 µL H2O, 2 µL deoxyribonucleic acid (DNA). After initial denaturation at 95°C for 7 minutes (min), 94°C for 45 seconds (sec); the reaction mixtures were subjected to 35 cycles of 60°C for 30 sec, 72°C for 45 sec, and a final extension at 72°C for 7 min. This method yielded amplification products of 490 base pairs (bp) for the II genotype, 190 bp for the DD genotype and 490 bp + 190 bp for the ID Genotype. The products were electrophoresed and visualized in 2% agarose gels with ethidium bromide for 30 min.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 25 software for Windows (Armonk, New York, United States: IBM Corp). Continuous variables were expressed as mean and standard deviation (SD), and categorical variables were expressed as a number of patients (n) and percentage (%). The Pearson chi-square test and Fisher's Exact test were used for comparisons of the categorical variables. One-way ANOVA and the

post-hoc Tukey test were used for comparisons of the continuous variables. Ordinal regression analysis was used to determine risk factors that could affect the severity of the disease. A *P* value <.05 was considered statistically significant.

Power analysis was calculated using G*power as .80 (input: effect size w=0.6, β/α ratio=1.069056, total sample size=90, df=5).

RESULTS

Ninety patients were included in the study, with 30 patients in each group. The statistically significant difference in gender distribution was due to the difference between the asymptomatic and mild disease groups (**Table 1**). The difference in mean age was due to the difference between the severe group compared with the asymptomatic and mild disease groups. The ACE II genotype was more frequent in the asymptomatic compared with the mild disease group, the ACE ID genotype was more frequent in the mild compared with the severe group, and the ACE DD genotype was more frequent in the severe compared with the asymptomatic group (**Table 2**). Overall, comorbidities were more frequent in the severe disease group. The frequency of diabetes mellitus, hypertension, chronic obstructive pulmonary disease, ischemic heart disease and Alzheimer's disease were statistically different between the groups (**Table 3**). Only the frequency of hypertension was statistically different between the mild and the asymptomatic groups. The frequencies of the other four comorbid conditions (except for Alzheimer's disease) were statistically different between the mild and severe disease groups. The frequencies of three other comorbid conditions (except for Alzheimer's disease and chronic obstructive pulmonary disease) were statistically different between the asymptomatic and the severe disease groups (**Table 4**). Nine patients (8.9%) died; all had severe disease. The remaining 81 patients were discharged. No statistically significant difference was found between the group who died and the discharged group in terms of ACE genotypes (**Table 5**). In the risk factor analysis, age, the presence of ischemic heart disease and diabetes were independent risk factors for severe disease. The presence of ACE II genotype was a preventive factor for severe disease (**Table 6**).

DISCUSSION

The exact etiopathogenesis of COVID-19 clinical severity remains unclear. In the present study, we tried to shed some light on this question by comparing different clinical severity groups. We found that the ACE II genotype was the dominant genotype (50%) in asymptomatic pa-

Table 1. Age and gender for ACE I/D genotypes by COVID-19 severity.

	Asymptomatic (n=30)	Mild (n=30)	Severe (n=30)	Overall *P*
Gender (male)	25 (83)	15 (50)	19 (63.3)	**.03**
Age (years)	40.9 (19.6)	47.1 (13.7)	67 (16.4)	**<.001**
ACE I/D genotype				
II	15 (50)	7 (23.3)	9 (30)	.08
ID	4 (13.3)	8 (26.7)	2 (6.7)	.09
DD	11 (36.7)	15 (50)	19 (63.3)	.12

Data are n (%) or mean (standard deviation).

***P* values from multiple comparisons for male gender:** *P*<.01 for asymptomatic vs mild. *P*=.30 for mild vs severe and *P*=.08 for asymptomatic vs severe. ***P* values from multiple comparisons for age:** *P*<.001 for asymptomatic vs severe. *P*<.001 for mild vs severe and *P*=.336 for asymptomatic vs mild. Please see **Table 2** for *P* values from multiple comparisons of ACE I/D genotypes.

Table 2. Statistical comparisons (*P* values) for ACE I/D genotypes by COVID-19 severity.

	Asymptomatic vs mild	Mild vs severe	Asymptomatic vs severe
ACE II	**.032**	.559	.114
ACE ID	.197	**.038**	.671
ACE DD	.297	.297	**.039**

Post-hoc Tukey test for multiple comparisons

Table 3. Frequency of comorbid conditions by COVID-19 severity.

	Asymptomatic (n=30)	Mild (n=30)	Severe (n=30)	Overall *P*
Comorbidity (Overall)	9 (30)	16 (53.3)	25 (83.3)	**<.001**
Diabetes mellitus	1 (3.3)	2 (6.7)	12 (40.0)	**<.001**
Hypertension	5 (16.7)	13 (43.3)	22 (73.3)	**<.001**
Chronic obstructive pulmonary disease	3 (10.0)	1 (3.3)	8 (26.7)	**.024**
Ischemic heart disease	1 (3.3)	2 (6.7)	11 (36.7)	**<.001**
Atrial fibrillation	0	1 (3.3)	3 (10.0)	.160
Congestive heart failure	0	0	1 (3.3)	.364
Alzheimer disease	0	0	3 (10.0)	**.045**
Chronic renal failure	0	0	2 (6.7)	.129
Solid organ tumor	3 (10.0)	2 (6.7)	0	.227
Hypothyroidism	1 (3.3)	2 (6.7)	0	.355
Chronic liver disease	0	1 (3.3)	0	.364
Ankylosing spondylitis	0	1 (3.3)	0	.364

Data are n (%)

tients, while ACE DD genotype was the dominant genotype (63.3%) severe patients. There are few published data to confirm this result. Annunziata et al studied the frequency of ACE I/D genotype in a series of 26 severe COVID-19 cases followed in the intensive care unit and found the ACE DD genotype was the dominant genotype in 73% of the patients.[15] They found ACE ID and ACE II genotype frequencies of 23% and 8%, respectively.[15] In another studyof 204 patients, 67 with severe disease and 137 with mild disease, Gomez et al found that the ACE DD genotype was statistically higher in the severe disease group than in the mild disease group (46% vs 32%, respectively).[9] However, in their risk factor analysis, they found that the ACE DD genotype was not a risk factor for severe disease.[9] Several meta-analyzes of the effect of ACE I/D genotype and allele frequencies on COVID-19 prevalence and mortality contain conflicting results.[7,8,10] In a meta-analysis including studies published from 26 Asian countries, a significant positive correlation was found between ACE D allele frequency and the COVID-19 infection rate and mortality rate (r values: .52 and .620, respectively).[7] Similarly, a detailed systematic review published by Pabalan et al noted that the ACE DD genotype might be an important prognostic marker for mortality in Asian COVID-19 patients with acute lung injury/ARDS.[16] In contrast, another meta-analysis with data from 25 different European countries revealed a negative correlation between the frequency of the ACE D allele and the COVID-19 infection rate and mortality.[10] These findings may indicate that the course of COVID-19 may differ geographically. In our study, we found that the ACE II genotype is a protective factor for severe disease (OR and 95% CI: .323 and .112-.929). In a meta-analysis containing results consistent with our study, Yamamoto et al found a negative correlation between ACE II genotype frequency and COVID-19 infection rate and mortality.[8]

In our study, we found that age increased significantly from the asymptomatic disease to the severe disease group. In addition, our study revealed that age is a significant risk factor for severe disease. Advanced age is a significant risk factor for mortality from COVID-19. An important reason why advanced age contributes to morbidity is the increased frequency of comorbid conditions.[17] In our study, we found that the frequency of general comorbidity increased significantly from the asymptomatic disease group to the severe disease group, concurrent with age. We also found that DM and ischemic heart disease are significant risk factors for severe disease among all comorbid conditions. Recent studies have found that advanced age and the presence of comorbid conditions increase COVID-19-related mortality rates.[18-20] All of the patients who died in our study were in the severe disease group. We did not find any significant difference in ACE I/D genotype frequencies between the patients who died and those who were

Table 4. Statistical comparisons (*P* values) of comorbid conditions by COVID-19 severity.

	Asymptomatic vs mild	Mild vs severe	Asymptomatic vs severe
Comorbidity	.067	**.012**	**<.001**
Diabetes mellitus	.999	**.002**	**.001**
Hypertension	**.024**	**.018**	**<.001**
Chronic obstructive pulmonary disease	.612	**.013**	.095
Ischemic heart disease	.999	**.005**	**.001**
Alzheimer disease	-	.237	.237

Table 5. ACE I/D genotype distribution in patients who died and discharged patients.

Genotype	Discharged patients (n=81)	Died patients (n=9)	*P*
ACE II	27 (33.3)	4 (44.4)	.506
ACE ID	14 (17.3)	0 (0)	.175
ACE DD	40 (49.4)	5 (55.6)	.725

Data are n (%).

Table 6. Ordinal regression risk factor analysis for severe COVID-19.

	Estimate	SE	Wald	Odds ratio	95 % CI	*P*
Age	.05	.02	1.93	1.052	1.021-1.083	<.001
Ischemic heart disease	1.78	.85	4.33	5.922	1.109-31.633	.037
Diabetes mellitus	1.65	.84	3.87	5.204	1.006-26.892	.049
ACE II genotype	-1.13	.54	4.39	.323	.112-.929	.036

R square : 0.48, Model fit information: - 2 Log likelihood: 140.36, Chi-square: 50.59 and *P*<.001

discharged. We think that the main reason for this is the low number of patients who died (n=9). However, as mentioned above, the relationship between ACE I/D allele frequencies and COVID-19-related mortality is inconclusive.[7,10,16] In our study, the only significant difference in gender frequency was between the asymptomatic and mid disease patients. In the risk factor analysis, gender had no significant effect on disease severity. In a series of 1099 patients in China, there was no significant difference between severe disease and mild disease in terms of gender distribution.[21]

Our preliminary results show that despite the relatively small sample size, there was a close correlation between the clinical severity of COVID-19 infection and the ACE I/D polymorphism. The ACE II genotype was a protective factor against the development of severe COVID-19. In addition, advanced age, diabetes and ischemic heart disease were independent risk factors for the development of severe COVID-19. We believe that the ACE DD genotype in COVID-19 patients should be re-evaluated in terms of severe disease development with more extensive studies.

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