THE ANATOLIAN JOURNAL OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Are angiotensin converting enzyme (ACE1/ACE2) gene variants associated with the clinical severity of COVID-19 pneumonia? A single-center cohort study

ORIGINAL INVESTIGATION

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Accepted: September 2, 2021
Available Online Date: December 8, 2021

Cite this article as: Baştuğ S, Çavdarlı B, Baştuğ A, Şencan İ, Tunçez E, Yakışık Çakır E, et al. Are angiotensin converting enzyme (ACE1/ACE2) gene variants associated with the clinical severity of COVID-19 pneumonia? A single-center cohort study. Anatol J Cardiol 2022; 26: 133-40.

DOI: 10.5152/AnatolJCardiol.2021.502

ABSTRACT

Objective: The impact of the coronavirus disease 2019 (COVID-19) pandemic has been unceasingly ongoing worldwide. Recent bioinformatics analysis and epidemiologic studies have highlighted that the functional polymorphisms on the *angiotensin converting enzyme (ACE)* gene may have an impact on the clinical progress of COVID-19. In this study, we aimed to determine the impact of the *ACE1* gene I/D polymorphism and *ACE2* peptidase-2 domain variants on disease severity.

Methods: Hundred patients with confirmed COVID-19 related pneumonia [50 patients with severe disease in intensive care unit (ICU) and 50 patients not in ICU] were compared on the basis of genetic and clinical characteristics. Genomic DNA was purified from peripheral blood lymphocytes with an automated QIA symphony DSP DNA Mini-Kit. The Sanger sequencing analysis was performed. The frequencies of *ACE1* gene polymorphism and *ACE2* PD variants were compared in patients hospitalized in ICU and those not in ICU. The Statistical Package for Social Sciences version 22.0 was used for statistical analysis.

Results: The sequencing analysis of the *ACE2* gene exon 1 and 2 revealed none of the polymorphisms investigated or any other variants in the present cohort. The frequencies of the *ACE1* ID, DD, and II genotypes were 51%, 31%, and 18%, respectively. The frequency of the D allele was similar between the ICU and non-ICU groups (50.4% versus 49.6%). Older age and the presence of advanced stage radiologic abnormalities on admission were detected as independent predictors of ICU requirement.

Conclusion: No effect of any *ACE1* gene polymorphism on predicting ICU requirement was detected. To the best of our knowledge, this is the first study investigating the impact of *ACE* gene polymorphisms on clinical severity of COVID-19 in a Turkish cohort.

Keywords: angiotensin converting enzyme, gene polymorphism, coronavirus disease 2019 severity, severe acute respiratory syndrome coronavirus 2

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection emerged in Wuhan, China, in December 2019 and spread to other countries and even continents rapidly within a short period of time. The European population not only had a higher incidence of coronavirus disease 2019 (COVID-19), but also had a higher mortality rate than the Asian population (1). The defined geographical differences in the COVID-19 pattern (in terms of prevalence and mortality) may arise from ethnic/genetic factors in addition to socio-economic, politic, and cultural (behavioral) characteristics of the populations (2).

COVID-19 has a wide range of clinical spectrum from asymptomatic to life threatening infection. Although the pathogenesis is not clear enough yet, older age, hypertension, cardiovascular disease, and diabetes mellitus have been previously defined as predictors of severity (3). The renin angiotensin aldosterone system (RAAS) was reported to have an important role in the pathogenesis of COVID-19 (4). *ACE1* and *ACE2* have a critical role in maintaining the homeostasis of RAAS (5). The down-regulation of *ACE2* expression leads to increased level of angioten-

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sin-II (Ang-II), which leads to increased vascular permeability, pulmonary edema, and apoptosis of the bronchi alveolar epithelial cells (5). Consequently, they all contribute to lung injury and fibrosis (5-7). *ACE1* gene insertion/deletion (ID) and especially deletion/deletion (DD) polymorphisms are related to increased levels of *ACE1* and Ang-II. *ACE1* DD polymorphism was also reported to be associated with hypertension and acute respiratory distress syndrome (7).

It has been suggested that some polymorphisms (S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R) located in the gene region encoding the *ACE2* peptidase domain (PD), which binds SARS-CoV-2, may increase the risk of infection with the virus (8). We hypothesized that *ACE1* DD polymorphisms and *ACE2* gene PD domain variants would increase susceptibility, and thus, lead to a genetic predisposition for severe lung injury in patients with COVID-19 in a Turkish population. To clarify this issue, *ACE1* gene I/D polymorphism and *ACE2* PD variants were examined in addition to demographic and clinical factors that may be related to disease severity in this study.

METHODS

Study design and participants

This study was approved by the Ethics Board of our institution (No. E1-20-655). All consecutive 100 patients with laboratory-confirmed COVID-19 with pneumonia admitted to the tertiary hospital from March to May 2020 were enrolled. Fifty consecutive patients hospitalized in the intensive care units (ICU) and 50 consecutive patients hospitalized in wards were included in the study. The World Health Organization interim guidance was used for the diagnosis and grouping of the patients as severe/critical and non-severe (9). Patients in need of treatment in the ICU at any time of the hospitalization (on admission or during follow-up) (ICU group) and those followed up with mild disease in the infectious disease ward (non-ICU group) were compared in terms of genetic polymorphisms. The oro/nasopharyngeal swab samples were obtained for SARS-CoV-2 reverse transcription–polymerase chain reaction (RT-PCR) test from the patients. Data including demographic, clinical, and radiological investigations of the patients were extracted from case follow-up forms and hospital electronic records.

Genetic analyses

Informed consent was obtained from patients who volunteered to participate in the study, and peripheral blood (2–3 mL) was taken into an ethylenediamine tetraacetic acid tube for genetic analyses. Genomic deoxyribonucleic acid (DNA) was purified from peripheral blood lymphocytes with an automated QIA symphony DSP DNA Mini Kit (QIAGEN Inc., Germany).

ACE2 exon 1 and exon 2 Sanger sequencing

Since 8 of the variants increasing the risk of disease and 12 of the possible protective variants were localized on exon 1 and 2 of *ACE2* gene, we sequenced only these two exons. PCR and sequencing of the exon 1 and exon 2 of *ACE2* were performed using the following primers: *ACE2* exon 1 forward primer 5′ CCCAACCCAAGTTCAAAGG 3′; and reverse primer 5′ GGAGGCAAACATCCAATCTC 3′, *ACE2* exon 2 forward primer 5′ GGGAAAACCAGGCAATAGG 3′; and reverse primer 5′ TCTTGGGATCAATGCTAAAATG 3′, respectively. PCR reaction was conducted with 15 pmol/μL of each primer, 1xTaq PCR master mix (Qiagen Inc., Germany), and 0.5 μg/μL genomic DNA in a total volume of 25 μL. PCR was performed as follows: 94°C for 2 min; 40 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and 72°C for 5 min. PCR fragments were sequenced with the forward and/or reverse primers using an ABI Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA, USA) on an ABI Genetic Analyzer 3500XL (Applied Biosystems). Sequence analysis of both exon 1 and 2 of the *ACE2* gene were evaluated by the SeqScape software® 2.0 (Applied Biosystems).

Angiotensin converting enzyme (*ACE1*) polymorphism

To determine the *ACE1* insertion/deletion genotype (rs1799752) of the patients, a genomic DNA fragment in intron 16 of the *ACE* gene was amplified. PCR amplification products were obtained using a 1xTaq PCR master mix (Qiagen Inc., Germany), 1 μg genomic DNA, 50 pmol/μl primers with 5 min of denaturation at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C. Reaction was terminated at 72°C for 2 min. The forward and reverse primers used were 5′ CTGGAGACCACTCCCATCCTTTCT 3′ and 5′ GATGTGGCCATCACATTCGTCAGAT 3′, respectively. Fragments without insertion (D allele) and with insertion (I allele) of 190 and 490 base pair (bp), respectively, were separated using capillary gel electrophoresis (QIAxcel Advanced System, Qiagen Inc., Germany).

PCR amplification using a primer pair that recognizes insertion-specific sequence identifies 4%–5% of ID genotypes that have been misclassified as DD when only a flanking primer pair was used. To increase the specificity of DD genotyping, we used another primer pair recognizing the insertion-specific sequence reported before (10). Thus, PCR amplifications were performed with an insertion-specific primer pair 5′ TGGGACCACAGCGCCCGCCACTAC 3′ and 5′ TCGCCAGCCCTCCCATGCCCATAA 3′), within 25 μl reactions; 1xTaq PCR master mix (Qiagen Inc., Germany), 0.5 μg genomic DNA, 50 pmol/μl of primers, with 1 min of denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s at 67°C, and 2 min at 72°C. Only the I allele produced a 335-bp amplicon. The 335-bp fragment was identified on capillary gel electrophoresis (QIAxcel Advanced System, Qiagen Inc., Germany). The reaction yields no products in the samples of

HIGHLIGHTS

- The frequency of D allele was similar between patients in intensive care unit (ICU) and those not in ICU with coronavirus disease 2019 (COVID-19).
- *Angiotensin converting enzyme (ACE1)* gene I/D polymorphisms have no effect on predicting ICU needs in COVID-19.
- *ACE2* peptidase domain (PD) variants were not detected in the present COVID-19 cohort.
- *ACE1* gene polymorphism and *ACE2* PD variants did not have any impact on poor prognosis.
- Older age and the extensive lung involvement on admission are severity predictors.

DD genotype. All types of the genotypes are visualized on Figure 1 for explanatory information.

ACE1 polymorphism analysis results were evaluated as DD, ID, and II genotype and included in the statistical analysis according to both allele frequencies and genotype ratios.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Sciences for Windows version 22.0 software program (SPSS Inc, IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine the normality of the variables. Descriptive analysis is presented via median [interquartile range (IQR)] for non-normally distributed variables and mean ± standard deviation for normally distributed variables. Genetic, demographic, and radiologic variables were compared between the groups using the Mann-Whitney U test for non-parametric variables. The Pearson chi-squared test or the Fisher's Exact test was used for comparing categorical variables. The Kruskal Wallis test was used to compare more than two groups. Binary logistic regression analysis was used to determine the predictors of the ICU need. Odds ratios and their 95% confidence intervals (CI) were calculated for binary outcomes. Statistical significance was set as $p<0.05$.

RESULTS

Clinical and demographic characteristics

A total of 100 hospitalized patients with the diagnosis of laboratory confirmed (RT-PCR positive) COVID-19 pneumonia were enrolled. The median age was 51 years (min–max, 21–87), and 59% were men. The median duration of symptoms prior to admission was 3 days (min–max, 0–20). The most common comorbidities of the patients were hypertension, diabetes mellitus, chronic obstructive pulmonary disease, and coronary artery disease with the frequency of 33%, 29%, 16%, and 14%, respectively. The blood types were similar between the ICU and non-ICU groups (Table 1). In addition, no significant differences were detected between patients with various *ACE1* I/D polymorphisms (*ACE1* II, ID, and DD) in terms of blood types (Table 2). As expected, the sequential organ failure assessment score was significantly higher in ICU patients than in non-ICU patients ($p<0.001$). The mean acute physiology and chronic health evaluation II score was 8.33±10.4 on the day of ICU admission. Critically ill patients who were in need of ICU support were significantly older [median age 64 years (min–max, 23–75) vs. 43.5 years (min–max, 23–75), $p<0.001$] when compared with the non-ICU group. Furthermore, patients in the ICU group had significantly more underlying comorbidities [42 (84%) vs. 17 (34%); $p<0.001$]. In this study, the presence of comorbidity, in terms of hypertension, diabetes mellitus, and coronary artery disease, was all found significantly more frequent in the ICU group ($p<0.001$, $p=0.004$, and $p=0.021$, respectively). The advanced stage radiologic abnormalities, defined as bilateral multilobar ground glass opacities, mainly in the peripheral areas of the lungs and consolidation, were more frequent in the ICU group [96% (n=48) vs. 54% (n=27); $p<0.001$] (Table 1). Single logistic regression analysis revealed that older age, comorbidities including hypertension, diabetes mellitus, and coronary artery disease, and the presence of advanced stage radiologic abnormalities were all significant risk factors for ICU requirement. Older age and the presence of advanced stage radiologic abnormalities on admission were independent predictors of ICU requirement (Table 3).

Figure 1. The angiotensin converting enzyme 1 deletion/insertion polymorphisms are visualized on the electropherogram. A1 column shows the deoxyribonucleic acid ladder. Polymerase chain reaction products of deletion and insertion alleles are 190 bp and 490 bp, respectively.
bp - base pair, DD - deletion/deletion, II - insertion/insertion, ID - insertion/deletion, NC - negative control

Results of genetic analysis

ACE2 gene exon 1 and 2 Sanger sequencing analysis did not reveal either investigated polymorphisms or any other variants in any patient in this study. There is no healthy population database to determine the frequency of gene variants in the Turkish population. To evaluate the analysis result, the data of 125 patients who had undergone whole exome sequencing analysis in pre-COVID era with different indications were retrospectively analyzed for *ACE2* gene exon 1 and 2 variants. No variant was detected in any of the patients (unpublished data).

Of the patients, 51% had *ACE1* ID polymorphisms, and 31% had *ACE* DD and 18% had *ACE* II polymorphisms. When the patients in the ICU and non-ICU groups were compared, there was no significant difference on the basis of any of the *ACE1* gene polymorphisms. The frequency of 'I' and 'D' allele was 43.5% and 56.5% in our study group, and it was not different between the ICU and non-ICU groups ($p>0.05$, 50.4% vs. 49.6% for the 'D' allele) (Table 1).

Distribution of demographic, clinical, and radiological variables according to *ACE1* polymorphism was similar between the groups (Table 2). There was no significant effect of *ACE1* ID, DD, and II polymorphisms on the risk of ICU need among patients in both groups (Table 4). ICU requirement was determined in 61.1% of the patients with *ACE1* II polymorphism,

Table 1. Demographic, clinical, and genetic polymorphism characteristics of the patients

			Non-ICU group, n=50	ICU group, n=50		
		Total n=100, %	n (%)	n (%)	χ^2	*P*-value
Age, years (median, IQR)		51 (28)	43.5 (15.75)	64 (25.5)	-5.607*	<0.001
Male sex		59	31 (62)	28 (56)	0.372	0.542
Time from symptom onset (days), (median, IQR)		3 (5)	3 (5.25)	3 (5)	-1.025*	0.305
Smoker		13	3 (6)	10 (20)	4.332	0.037
Presence of Comorbidity		59	17 (34)	42 (84)	25.837	<0.001
Hypertension		33	7 (14)	26 (52)	16.327	<0.001
DM		29	8 (16)	21 (42)	8.208	0.004
CAD		14	3 (6)	11 (22)	5.316	0.021
CHF		3	1 (2)	2 (4)	0.344	1.000
COPD		16	5 (10)	11 (22)	2.679	0.102
CVD		2	-	2 (4)	2.041	0.495
Malignity		3	1 (2)	2 (4)	0.344	1.000
ACE2 gene polymorphism		0	-	-		
ACE I gene polymorphism	ACE DD	31	13 (26)	18 (36)	3.284	0.194
	ACE ID	51	30 (60)	21 (42)		
	ACE II	18	7 (14)	11 (22)		
Allele frequency n=200	'I' allele (n=87)	43.5	44 (50.6)	43 (49.4)	0.020	0.887
	'D' allele (n=113)	56.5	56 (49.6)	57 (50.4)		
Blood group n=57	AB (n=9)	15.8	4 (17.4)	5 (14.7)	1.251	0.801
	B (n=9)	15.8	3 (13)	6 (17.6)		
	A (n=23)	40.4	8 (34.8)	15 (44.1)		
	O (n=16)	28	8 (34.8)	8 (23.5)		
Treatment						
ACE inhibitors		5	1 (2)	4 (8)	1.895	0.362
ACE receptor antagonists		12	2 (4)	10 (20)	6.061	0.014
Other antihypertensive agents		3	8 (16)	23 (46)	10.519	0.001
Oral antidiabetics		24	6 (12)	18 (36)	7.895	0.005
Chloroquine		63	42 (84)	21 (42)	18.919	<0.001
Favipiravir		65	15 (30)	50 (100)	53.846	<0.001
Steroid		15	-	15 (30)	25.235	<0.001
Tocilizumab		11	-	11 (22)	12.360	<0.001
Anakinra		1	-	1 (2)	1.010	1.000
IVIG		2	-	2 (4)	2.041	0.495
Supportive treatment						
MV		21	-	21 (42)	27.198	<0.001
NIMV		24	-	24 (48)	32.327	<0.001
High Flow		28	-	28 (56)	38.889	<0.001
IVIG		2	-	2 (4)	2.041	0.495
Advance stage radiologic abnormalities		75	27 (54)	48 (96)	23.520	<0.001
APACHE II score (mean ±SD)		-	-	8.33±10.4		
Q SOFA	Score 0	42	39 (78)	3 (6)	65.420	<0.001
	Score 1	37	11 (22)	26 (52)		
	Score 2	17	-	17 (34)		
	Score 3	4	-	4 (8)		
Hospital mortality		19	-	19 (38)	24.552	<0.001
28-day mortality		21	-	21 (42)	26.582	<0.001

Pearson chi-squared, Fisher's exact test, *Mann-Whitney U analysis,
ACE - angiotensin converting enzyme, APACHE II - acute physiology and chronic health evaluation II, COPD - chronic obstructive pulmonary disease, ICU - intensive care unit, IQR - interquartile, ID - insertion/deletion, II - insertion/insertion, DD - deletion/ deletion, IVIG - intravenous immunoglobulin, MV - mechanical ventilation, NIMV - non-invasive mechanical ventilation, SOFA - sequential organ failure assessment, DM - diabetes mellitus, CAD - coronary artery disease, CHF - congestive heart failure, CVD - cerebrovascular disease

Table 2. Distribution of demographic and clinical variables according to ACE polymorphism

		ACE II	ACE ID	ACE DD		
		n (%)	n (%)	n (%)	χ^2/Z*	*P*-value
Age, years (median, IQR)		65 (27.75)	49 (26)	49 (28.75)	4.445*	0.108
Male sex		9 (50.0)	30 (58.8)	20 (64.5)	0.993	0.609
Time from symptom onset (days), (median, IQR)		3.5 (4.25)	3 (5)	3 (5.25)	0.723*	0.697
Smoker		3 (16.7)	6 (11.8)	4 (12.9)	0.498	0.861
Presence of comorbidity		14 (77.8)	26 (51.0)	19 (61.3)	4.047	0.132
Hypertension		7 (38.9)	16 (31.4)	10 (32.3)	0.351	0.839
DM		6 (33.3)	15 (29.4)	8 (25.8)	0.322	0.851
CAD		4 (22.2)	6 (11.8)	4 (12.9)	1.383	0.556
CHF		1 (5.6)	1 (2.0)	1 (3.2)	1.222	0.755
COPD		5 (27.8)	6 (11.8)	5 (16.1)	2.564	0.270
CVD		1 (5.6)	-	1 (3.2)	2.903	0.242
Malignity		1 (5.6)	1 (2.0)	1 (3.2)	1.222	0.755
Severity of illness	ICU group	11 (61.1)	21 (41.2)	18 (58.1)	3.284	0.194
	Non-ICU group	7 (38.9)	30 (58.8)	13 (41.9)		
Blood group	AB	2 (13.3)	6 (24.0)	1 (5.9)	9.143	0.166
	B	3 (20.0)	4 (16.0)	2 (11.8)		
	A	8 (53.3)	10 (40.0)	5 (29.4)		
	O	2 (13.3)	5 (20.0)	9 (52.9)		
Treatment						
ACE inhibitors		2 (11.1)	1 (2.0)	2 (6.5)	2.832	0.202
ACE receptor antagonists		4 (22.2)	4 (7.8)	4 (12.9)	2.722	0.246
Other antihypertensive agents		6 (33.3)	16 (31.4)	9 (29.0)	0.105	0.949
Oral antidiabetics		5 (27.8)	13 (25.5)	6 (19.4)	0.635	0.778
Chloroquine		7 (38.9)	34 (66.7)	22 (71.0)	5.628	0.060
Favipiravir		16 (88.9)	29 (56.9)	20 64.5	6.003	0.049
Tocilizumab		4 (22.2)	3 (5.9)	4 (12.9)	3.836	0.130
Anakinra		-	1 (2.0)	-	0.970	0.616
IVIG		1 (5.6)	1 (2.0)	-	1.908	0.428
Supportive treatment						
MV		4 (22.2)	10 (19.6)	7 (23.3)	0.292	0.945
NIMV		8 (44.4)	10 (19.6)	6 (20.0)	4.500	0.114
High flow		9 (50.0)	11 (21.6)	8 (25.8)	5.442	0.066
Advance stage radiologic abnormalities**		15 (83.3)	36 (70.6)	23 (74.2)	3.333	0.507
QSOFA	Score 0	7 (38.9)	24 (47.1)	2 (6.5)	3.940	0.703
	Score 1	6 (33.3)	19 (37.3)	6 (19.4)		
	Score 2	5 (27.8)	6 (11.8)	12 (38.7)		
	Score 3	-	2 (3.9)	11 (35.5)		
Hospital mortality		4 (23.5)	9 (17.6)	6 (20.0)	0.461	0.838
28-day mortality		4 (22.2)	10 (19.6)	7 (22.6)	0.243	0.946

Pearson chi-squared, Fisher's Exact test, *Z, Kruskal Wallis H analysis, **Bilateral multi-lobar ground glass opacities and consolidation
ACE - angiotensin converting enzyme, APACHE II - acute physiology and chronic health evaluation II, COPD - chronic obstructive pulmonary disease, ICU - intensive care unit, IQR - interquartile, ID - insertion/deletion, II - insertion/insertion, DD - deletion/ deletion, IVIG - intravenous immunoglobulin, MV - mechanical ventilation, NIMV - non-invasive mechanical ventilation, SOFA - sequential organ failure assessment, DM - diabetes mellitus, CAD - coronary artery disease, CHF - congestive heart failure, CVD - cerebrovascular disease

whereas it was 58.1% for *ACE1* DD and 41.2% for *ACE1* ID (p>0.05) (Table 2). In patients who had *ACE1* ID gene polymorphism, the presence of hypertension was significantly more common in ICU patients [p=0.002, Exp (B)=8.67, 95% CI=2.22–33.83]. Older age was a significant predictor of ICU requirement in patients with *ACE1* ID and DD polymorphism (p<0.001 and p=0.008, respectively). Furthermore, there was no difference in terms of age and comorbidities (hypertension, diabetes mellitus, and coronary artery disease) between the ICU and non-ICU groups in patients with *ACE1* II polymorphism (Table 5).

Main interventions and treatment

For antiviral treatment, favipiravir was administered to 65 patients, and chloroquine was given to 63 patients (Table 1). Favipiravir was given to all critically ill patients in the ICU group and to 30% of the non-ICU group (n=15). Steroid and anticoagulant treatment were not given to patients in the

Table 3. Binary logistic regression analysis for the effect of variables on ICU requirement

	OR	95% CI	*P*-value
Single binary logistic regression analysis			
Age, years	1.09	1.05–1.13	<0.001
Hypertension	6.65	2.52–17.6	<0.001
DM	3.80	1.48–9.75	0.005
CAD	4.42	1.15–16.97	0.030
Advanced stage radiologic abnormalities	20.44	4.47–93.47	<0.001
Multivariate binary logistic regression analysis			
Step 2			
Age, years	1.08	1.04–1.13	<0.001
Advanced stage radiologic abnormalities	17.72	3.24–96.79	0.001
Constant	0.00		<0.001
Variables not in the equation			
			P-value
DM		2427.00	0.119
Hypertension		0.827	0.363
CAD		0.376	0.540
Overall Statistics		2961.00	0.398

OR - odds ratio, CI - confidence interval, DM - diabetes mellitus, CAD - coronary artery disease

Table 4. Binary logistic regression analysis for the effect of ACE polymorphism on ICU requirement

	Odds ratio	95% CI	*P*-value
ACE II			0.197
ACE ID	0.45	0.15–1.34	0.149
ACE DD	0.88	0.27–2.89	0.834
ACE II vs. ID+DD	0.58	0.2–1.64	0.301
ACE ID vs. II+DD	2.07	0.93–4.6	0.073
ACE DD vs. II+ID	0.62	0.27–1.47	0.281

ACE - angiotensin converting enzyme, ICU - intensive care unit, ID - insertion/deletion, II - insertion/insertion, DD - deletion/ deletion

non-ICU group as there was no known recommendation for their administration at that point in time. It was given only in 41.7% (15/50) of the ICU patients for critical illness related corticosteroid insufficiency solely on the basis of the clinical decision of the ICU specialist. Anticoagulant treatment was implemented in all the patients in the ICU group routinely. Tocilizumab and anakinra were administered in 22% (n=11) and 2% (n=1) of the patients, respectively, in the ICU group as an immunomodulatory therapy. Oral antidiabetic treatment and antihypertensive agent usage were significantly higher in the ICU group (p=0.005 for antidiabetic agents, p=0.014 for *ACE1* receptor antagonists, and p=0.001 for other anti-hypertensives).

Among the ICU patients, invasive mechanical ventilation was administered to 21 patients (42%), non-invasive mechanical ventilation was needed in 24 patients (48%), and high flow nasal oxygen therapy was required in 28 patients (56%). Hospital mortality occurred in 19 patients (38%) and

Table 5. Binary logistic regression analysis for the effect of variables on ICU requirement in the patients with ACE II, ACE ID, and ACE DD polymorphisms

	OR	95% CI	*P*-value
ACE II			
Age	1.06	0.99–1.14	0.096
Hypertension	0.76	0.11–5.28	0.783
DM	5	0.44–56.62	0.194
CAD	0.56	0.06–5.24	0.608
ACE ID		–	
Age	1.1	1.04–1.16	<0.001
Hypertension	8.67	2.22–33.83	0.002
DM	3	0.86–10.41	0.083
CAD	9.06	0.97–84.46	0.053
ACE DD		–	
Age	1.1	1.02–1.17	0.008
Hypertension	2625146654.61	0–	0.999
DM	7.64	0.81–72.4	0.076
CAD	1500083802.98	0–	0.999

ACE - angiotensin converting enzyme, ICU - intensive care unit, ID - insertion/deletion, II - insertion/insertion, DD - deletion/ deletion, OR - odds ratio, CI - confidence interval, DM - diabetes mellitus, CAD - coronary artery disease

28-day mortality in 21 patients (42%) in the ICU group (Table 1). All the remaining patients clinically improved and were discharged.

DISCUSSION

ACE2 protein is expressed by the *ACE2* gene consisting of 18 exons (Xp22.2) located on the short arm of the X chromosome (11). It was suggested that the cause of higher risk of severe COVID-19 in men may be owing to the lower expression of *ACE2* as they have one copy of X-linked *ACE2* gene (4). However, there are conflicting results regarding the effect of sex on the clinical severity of COVID-19 (3, 12). In this study, no significant difference was found between ICU and non-ICU patients in terms of sex.

Older age, the presence of advanced stage radiologic abnormalities, and comorbidities were significantly higher in severe patients as it had been defined previously (3). The blood types were similar between the ICU and non-ICU groups. The effect of blood types on clinical severity found in this study was not in line with the results reported in the literature (13). The *ACE2* gene has polymorphic nucleotide changes that can vary in frequency between individuals and populations, and alter the function of the protein expressed. It is thought that these polymorphisms in the *ACE2* receptor gene may bind the virus to the host cell and consequently alter its virulence (8). In this study, no polymorphism on exon 1 and 2 of the *ACE2* gene was detected.

The polymorphisms of the *ACE1* gene, which has 42% amino acid similarity with *ACE2*, also affect the expression of *ACE2* (14). The *ACE1* gene is located on the long arm of chromosome 17 and shows the insertion/deletion (ID) polymorphism in the 287 bp Alu repeat region in the 16th intron (15). The frequency of D allele was reported significantly higher in patients with hypoxemic SARS than in non-hypoxemic patients in a small

cohort of Vietnamese cases (16). Pati et al. (17) conducted an epidemiological study to examine whether a functional I/D polymorphism in the *ACE* gene is associated with SARS-CoV-2 infection via the prevalence of *ACE* I/D polymorphism reported in 148 studies from various Asian countries. They involved 11 studies (with a total of 1090 individual participants) investigating the prevalence of the *ACE1* gene polymorphism in healthy populations in Turkey and reported the frequencies of *ACE1* ID, DD, and II genotypes as 46.1%, 31.8%, and 22%, respectively. In addition, the frequency of the 'D' allele was reported as 54.9% (n=1197) and 'I' allele as 45.1% (n=983) in the same study (17). They concluded that there was a positive correlation between the prevalence of 'D' allele and the mortality rate of COVID-19 in the population (r=0.620, p=0.002, n=22). However, they suggested further evaluations in hospital-based studies (17). In our cohort, the prevalence of *ACE1* ID, DD, and II genotypes were found to be similar to that previously reported in a healthy Turkish population as 51%, 31%, and 18%, respectively. In this study, the frequency of the D allele was 50.4% in the ICU group, whereas it was 49.6% in the non-ICU group, and the difference was not statistically significant (p>0.05).

The I/D polymorphisms in the intron 16 of *ACE1* are associated with changing concentrations of *ACE* in circulation and tissues. Factors affecting the *ACE2* expression may have an impact on COVID-19 outcome (4). There are conflicting data regarding the association of *ACE1* DD genotype and an increased risk of respiratory distress syndrome (16, 18). Zheng and Cao (5) suggested that the presence of *ACE1* DD polymorphism may be responsible for severe lung injury. Yamamoto et al. (1) reported that the prevalence of *ACE1* II genotype in the population was negatively correlated with the mortality because of COVID-19. Similarly, *ACE1* DD polymorphism was reported to be associated with severe COVID-19, whereas *ACE2* polymorphism did not have any effect (4).

ACE2 facilitates rapid viral replication, whereas the depletion of *ACE2* from the cell membrane enhances the damaging effects of Ang-II in the lung (19). Delanghe et al. (19) reported a negative correlation between the frequency of 'D' allele and COVID-19-related mortality. However, it was reported that more studies are required to evaluate the clinical outcome of COVID-19 infection in *ACE1* DD, ID, and II carriers and to investigate the precise role of *ACE1*.

To determine the effect of *ACE1* gene polymorphisms on COVID-19 severity, ICU and non-ICU patients were compared in this study. There was no statistically significant effect of the DD genotype compared with the II + ID genotypes (p>0.05). None of the *ACE1* genotypes had any effects on severity.

In addition, the *ACE1* DD genotype was detected statistically more frequently in hypertensive male patients with severe COVID-19 (4). Therefore, we evaluated the impact of comorbidities such as hypertension, coronary artery disease, diabetes mellitus in relation with the *ACE1* II, ID, and DD genotype in the present study. Single logistic regression analysis was performed to analyze the effect of older age and comorbidities on ICU requirement in patients with *ACE1* II, ID, and DD polymorphisms. Older age was a significant risk factor for ICU requirement in patients with *ACE1* ID and DD polymorphisms. The presence of hypertension was associated with 8.67 times higher risk of ICU requirement in patients with *ACE1* ID polymorphism, although there was no association with other comorbidities. Although *ACE* receptor antagonists and other antihypertensive agents were more frequently used in patients in the ICU, these differences were thought to be the consequences of higher percentage of hypertension in ICU patients. Reynolds et al. (20) reported no positive association between the use of *ACE* receptor antagonists/*ACE* inhibitors with severe COVID-19. The generally accepted scientific knowledge is that the use of *ACE* inhibitors and *ACE* receptor antagonists does not increase susceptibility to SARS-CoV-2 infection and has no effect on the severity of COVID-19 (21, 22). When the frequency of antihypertensive use was evaluated in patient groups with different gene polymorphisms, no difference was found between groups.

Study limitations

The small size of the study population is the limitation of our study. Large cohorts are needed to define the exact role of *ACE* gene polymorphism on the severity of COVID-19 pneumonia. Further hospital-based large cohort studies are necessary to assess the exact role of the *ACE* polymorphism on clinical outcome of COVID-19 pneumonia.

CONCLUSION

1. *ACE1* gene polymorphisms including II, ID, and DD genotypes had no impact on predicting ICU requirement in patients with COVID-19 pneumonia.
2. Single logistic regression analysis revealed that older age is a predictor of ICU requirement in patients with *ACE1* ID and DD polymorphisms. The presence of hypertension predicted severity only in patients with *ACE1* ID genotype.
3. *ACE2* PD variants on exon 1 and 2 were not detected in the study population. We suggest that predictions made with bioinformatics analysis need to be verified with real life experiences and data analysis in different ethnic groups.
4. Older age and the presence of advanced stage radiologic abnormalities on admission were independent predictors of ICU requirement.

Further hospital-based large cohort studies are necessary to assess the exact role of the *ACE* gene polymorphisms on clinical outcome of COVID-19 pneumonia.

Acknowledgments: We thank the Turkey Infectious Diseases and Clinical Microbiology Specialty Society for their support in conducting this study.

Declaration: The oral presentation of the manuscript was presented at the "9th Congress of Infectious Diseases and Clinical Microbiology Specialty Society of Turkey on 20th to 23rd May, 2021" held online.

Institutional and financial support: This research received a specific grant from Turkey Infectious Diseases and Clinical Microbiology Specialty Society (EKMUD) (Grant number: 08-30.07.2020).

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Author contributions: Concept – S.B., B.Ç., A.B., H.B.; Design – S.B., B.Ç., A.B., H.B.; Supervision – S.B., B.Ç., A.B., H.B.; Fundings – S.B., A.B.; Materials – S.B., B.Ç., A.B.; Data collection &/or processing – S.B., B.Ç., A.B., İ.Ş., E.T., E.Y.Ç., N.K., C.S., D.E., G.G.C., I.Ö.T., S.K., H.B.; Analysis &/or interpretation – S.B., B.Ç., A.B., E.T.; Literature search – S.B., B.Ç., A.B., E.Y.Ç., N.K., C.S.; Writing – S.B., B.Ç., A.B.; Critical review – S.B., B.Ç., A.B., İ.Ş., E.T., E.Y.Ç., N.K., C.S., D.E., G.G.C., I.Ö.T., S.K., H.B.

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