Original Article

The Evaluation of ACE1-I/D Polymorphism in Kurdish Patients With Severe COVID-19

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Abstract

Background: In several studies, insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme 1 (ACE1) gene is described as a genetic risk factor for coronavirus disease 2019 (COVID-19) infection. However, in some studies, this contribution is not confirmed. Therefore, this study aimed to evaluate the genotypic and allelic frequency of ACE1-D/I in Kurdish patients with severe COVID-19 in Iran.

Materials and Methods: A total of 95 patients with PCR positive-COVID-19 were enrolled in this cross-sectional study. Genomic DNA was extracted from peripheral blood leucocytes using the salting out method. All cases were genotyped for ACE1-I/D polymorphism using polymerase chain reaction (PCR). Death percentage from COVID-19 after two months' follow-up was analyzed.

Results: Of the 95 patients, 48 were female (50.5%) and 47 were male (49.5%) with a mean age of 61.9±18.7 years. The ID genotype was the most prevalent (52.6%) followed by DD (32.6%) and II (14.7%). The D and I allele frequencies were 58.9%, and 41.1%, respectively. The D allele frequency was higher in patients with SpO2≤90% (P = 0.048). The mortality percentage was 18.9% (8 females and 10 males). The frequency of the DD, ID, and II genotypes in patients who died from COVID-19 was 27.7%, 61,1%, and 11.1%.

Conclusion: Our results indicated that the ACE1- D allele can be a genetic risk factor in COVID-19 patients. Further studies on different ethnicities and geographical regions are needed to evaluate this polymorphism in COVID-19 infection.

Keywords: Coronavirus, COVID-19, Angiotensin 1 converting enzyme, ACE1, I/D polymorphism

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Please cite this article as: Karimi F, Hosseini V, Ahmadi A, Ranjbar Kermani F, Ferdowsi S. The Evaluation of ACE1-I/D Polymorphism in Kurdish Patients With Severe COVID-19. J Cell Mol Anesth. 2023;8(3):141-9. DOI: https://doi.org/10.22037/jcma.v8i3.40994

Introduction

Coronavirus is a single-stranded RNA virus that is responsible for the coronavirus disease 2019 (COVID-19) pandemic (1-3). Several risk factors such as age, sex, blood group, smoking, and hypertension are described for this infection (4-8). In addition, genetics may contribute to COVID-19 (9).

Recent studies highlighted the angiotensin-converting enzyme 1 (ACE1) gene is a genetic risk factor in this infection (10-13). The ACE1 gene consists of 26 exons that are located on the long arm of chromosome 17 (14). In intron 16 of this gene, a

polymorphism consisting of an insertion (I) or deletion (D) of a 287-bp fragment has been identified that results in three different genotypes including DD and II homozygotes or ID heterozygotes. This polymorphism may result in differences in ACE1 levels. For instance, the DD genotype leads to an increase in serum and tissue ACE-1 levels, causing an increased level of angiotensin-2 and the progression of pulmonary edema. That phenomenon further worsens the clinical course and prognosis such as acute respiratory distress syndrome (ARDS) (15, 16).

A direct correlation between DD genotype and higher risk of morbidity and mortality in COVID-19 is reported in different countries such as India (17), Spain (18), and Italy (19). However, in some studies, this relationship is not confirmed (20-24).

To our knowledge, the ACE1-D/I genotype has not been studied in COVID-19 patients in Kurdistan province (west Iran). Therefore, this study aimed to evaluate the ACE1-I/D polymorphism in Kurdish patients with severe COVID-19.

Methods

Data collection

This study was approved by the Ethics Committee of our institution (IR.TMI.REC.1400.009). Patients with inclusion and exclusion criteria were selected. Inclusion criteria were confirmation of COVID-19 by RT-PCR method on nasopharyngeal swab samples and age of more than 18 years. Pregnant patients and patients with known underlying health conditions such as malignancy, immunodeficiency, and diabetes were excluded. The demographics and medical information of the subjects were obtained via a questionnaire or their medical records.

Study Population

A total of 95 patients who were diagnosed from November 2021 to February 2022 were enrolled in this cross-sectional study. Severe disease was defined as patients with positive RT-PCR results for COVID-19 who had infiltration on Chest X-Ray or CT scan, hypoxia (SpO2 <94%) and required hospitalization with essential oxygen therapy by face mask, noninvasive ventilation (NIV), and/or mechanical ventilation with intubation (24). After informed consent, 5 mL of peripheral blood samples were collected in tubes containing ethylene diamine tetra acetic acid (EDTA) and stored at -20°C until further use.

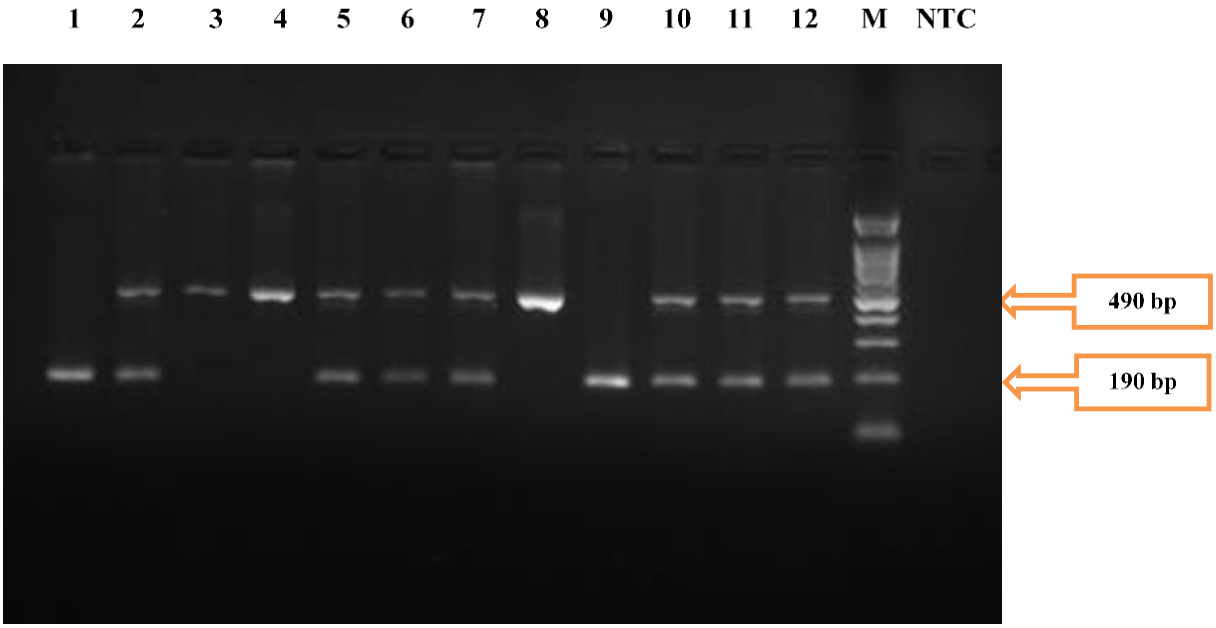

Figure 1. PCR products on agarose gel electrophoresis. I allele: 490 bp, D allele:190 bp. M: 100 bp DNA marker; P1 and P9: DD polymorphism. P2, P5, P6, P7, P10, P11, P12: DI polymorphism. P3, P4, P8: II polymorphism. NTC: non-template control.

ACE1-I/D Genotyping

Extraction of genomic DNA was performed according to the salting-out method**.** ACE1-I/D genotype was determined by polymerase chain reaction (PCR) using specific primers (forward primer 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and reverse primer 5′-GATGTGGCCATCACATTCGTCAGAT-3′). Reactions were set up in a volume of 25 µL containing 1 µL of each primer, 12.5 µL master mix, 9.5 µL H2O, and 50 ng DNA. PCR amplification was performed under the conditions: Initial denaturation at 94 ◦C for 5 min, followed by 32 cycles of denaturation at 94 ◦C for 30 s, annealing at 60°C for 30 s, extension at 72 ◦C for 30 s and final extension at 72°C for 7 min. PCR products were checked on 2% agarose gel and visualized by the gel documentation system (BIO-RAD). 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 (Fig. 1).

Statistical analysis: Statistical analysis was performed using SPSS software (version 21). Continuous variables were expressed as mean and standard deviation (SD), and categorical variables were expressed as the 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. All 'P' values were considered statistically significant for $p<0.05$.

Results

We performed a cross-sectional study on 95 hospitalized patients with severe COVID-19, 47 males (49%) and 48 females (51%), with a mean age of 61.9±18.7 years (Table 1). Our data showed that the ID genotype was the most prevalent (52.6%) followed by DD (32.6%) and II (14.7%) (Table 2).

There was no significant relationship between genotype and sex (p=0.56). The median age of patients with the DD genotype was 68.65±15.70 years, ID genotype 57.52±18.90 years, and II genotype 62.57±20.47 years.

The frequency of D and I alleles were 58.9%, and 41.1%, respectively. The frequency of alleles was not significant according to sex (p=0.2). The median age of patients with the D allele was 63.68±17.943 years and for patients with the I allele was 59.33±19.361 years.

According to the percentage of oxygen, patients were divided into two groups, including SpO2≤90% and SpO2>90%. A total of 72 patients (75.8%) had SpO2≤90% with a mean age of 62.53±19.514 years and 23 patients (24.4%) had SpO2>90% with a mean age of 59.91±15.474 years. The relationship between the percentage of oxygen and sex was significant (p=0.04) and females had higher levels of SpO2 than males (Table 3).

In the group with SpO2>90%, the ID, DD, and II genotype frequencies were 52.2%, 21.7%, and 26.1%, respectively. In this group, 22 patients (47.8%)

Table 1: Characteristics of the studied patients

		N (%)	Total (n)
Sex	Female	48(50.5%)	95
	Male	47(49.5%)	
Median age (years)	Female	64.14	95
	Male	59.59	
	Total	61.89	

Table 2: Distribution of ACE1-I/D genotypes with I and D allele frequencies in COVID-19 patients

Genotype	Total Number	%	Sex		P value
			Female	Male	
II	14	14.7%	6	8	p= 0.56
ID	50	52.6%	24	26	
DD	31	32.6%	18	13	
*Allele I	78	41.1%	30	34	p= 0.2
*Allele D	112	58.9%	42	39	

*Alleles, the total number of chromosomes

had the D allele and 24 patients (52.2%) had the I allele.

In the group with SpO2≤90%, the ID, DD, and II genotype frequencies were 52.8%, 36.1%, and 11.1%, respectively. In this group, 90 patients (62.5%) had the D allele and 54 patients (37.5%) had the I allele. The relationship between the D allele and the percentage of oxygen (SpO2≤90%) was significant (p=0.048).

Death percentage from COVID-19 after two months interval was analyzed. A total of 18 (18.9%) patients died in the hospital (8 females and 10 males). The distribution of ID, DD, and II genotypes were 61.1% (11 cases), 27.7% (5 cases), and 11.1% (2 cases), respectively. All patients who died from COVID-19 infection had no history of underlying disease (Table 4).

Discussion

Since the pandemic of COVID-19, polymorphism in the ACE1 gene has been the subject of debate in different studies. In severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) infection, previous reports confirmed that the incidence of pneumonia was higher in patients carrying D allele (24-27). This finding has led to researchers for studying the relationship between COVID-19 and ACE1-I/D polymorphism.

Our results indicated that the frequencies of the ID, DD, and II genotypes were 52.6%, 32.6%, and 14.7%, respectively. The D and I allele frequencies were 58.9% and 41.1%, respectively. Comparison between two groups of patients (SpO2>90% and SpO2≤90% (showed a higher percentage of the D allele compared to the I allele in the group with SpO2≤90% (62.5% vs 37.5%). The mortality percentage was 18.9% which was higher in patients with the ID (61.1%) genotype than DD (27.7%) and II (11.1%) genotypes.

In Table 5, we compared our findings to other

Table 3: Percentage of oxygen (SpO2) in COVID-19 patients according to gender

Sex	SpO2>90%	SpO2≤90%	Total	P value
Female	16 (69.6%)	32 (44.4%)	48 (50.5%)	0.04
Male	7 (30.4%)	40 (55.6%)	47 (49.5%)	
Total	23 (100%)	72 (100%)	95 (100%)	

Table 4: ACE1-I/D genotype distribution in patients who died from COVID-19 infection

Patient	Age	gender	SpO2 (%)	Genotype
1	32	male	85%	ID
2	79	Female	89%	II
3	27	Female	77%	ID
4	84	Female	85%	DD
5	50	Male	65%	ID
6	67	Male	83%	DD
7	71	Female	83%	DD
8	55	Male	76%	ID
9	29	Female	84%	ID
10	80	Male	75%	ID
11	78	Female	87%	DD
12	60	Male	75%	ID
13	74	Male	78%	ID
14	75	Male	75%	ID
15	73	Female	82%	ID
16	87	Male	72%	DD
17	72	Female	87%	ID
18	87	Male	64%	II

studies in different countries and geographical regions. In a study by Akbari et al. (28), in Tehran, Iran, ACE1-I/D polymorphism was examined in two groups of COVID-19 patients (intensive care unit (ICU) and non-ICU patients). In their findings, the ID genotype was reported as a dominant genotype in both groups. However, in a study in Mazandaran, north of Iran, the frequency of the ACE1 DD genotype was higher in severe and critical COVID-19 patients (29).

In a study by Gomez et al, (18) in Spain, 67 patients with severe COVID-19 were investigated and the frequencies of the ID, DD, and II genotypes were reported as 46%, 46%, and 8%, respectively.

In men with severe disease, hypertension, hypercholesterolemia, and the ACE1 DD genotype were significantly increased. In a study by Verma et al, (17) in India, they found that DD genotype, frequency of D allele, and older age (≥46 years) were significantly higher in severe COVID-19 patients. The ID genotype was found to be statistically associated with high socio-economic COVID-19 patients. However, there was no statistical significance between the ACE1-I/D genotype and demographic characteristics such as age, gender, and marital status.

In Italy, Annunziata et al, (19) studied the frequency of the ACE1-I/D genotype in severe COVID-19 cases in the ICU and found that the ACE1 DD genotype was the dominant genotype in 73% of patients. They found that ID and II genotype frequencies were 23% and 8%, respectively.

In Turkey, the results of four studies have been reported (20, 22, 30, 31). In a study by Gunal et al (31), the DD genotype was dominant in severe COVID-19 patients (63.3 %). In their study, nine patients died,

Table 5: ACE1-I/D polymorphism in COVID-19 patients in different countries

Country (Reference)	severe COVID-19				Non-sever COVID-19			
	II , n (%)	ID, n (%)	DD, n (%)	Total patients	II, n (%)	ID, n (%)	DD, n (%)	Total patients
Iran, Kurdistan (Present study)	14 (14.7%)	50 (52.6%)	31 (32.6%)	95	-	-	-	-
Iran, Tehran, Akbari, et al (28)	0 (0)	31 (83.8%)	6 (16.2%)	37	4 (7.4%)	39 (72.2%)	11 (20.4%)	54
Iran, Tehran, Rezaiezadeh et al(37)	18 (18%)	45 (45%)	37 (37%)	100	30 (19.9%)	87 (57.6%)	34 (22.5%)	151
Iran, Mazandaran, Najafi et al(29)	4 (9.09)	33 (75.0)	7 (15.91)	44	15 (14.71)	44 (43.14)	43 (42.16)	102
Italy, Annunziata et al (19)	2 (8%)	6 (23%)	19 (73%)	27	-	-	-	-
Italy, Calabrese et al (38)	3 (12%)	4 (16%)	18 (72%)	25	2 (4.7%)	21 (48.8%)	20 (46.5%)	43
India, Verma et al (17)	42 (35.0%)	48 (40.0)	30 (25.0)	120	74 (49.7)	58 (38.9)	17 (11.4)	149
Spain, Gómez et al(18)	5 (8%)	31 (46%)	31 (46%)	67	17 (12%)	76 (56%)	44 (32%)	137
Turkey, Gunal et al (31)	9 (30%)	2 (6.7%)	19 (63.3%)	30	7 (23.3%)	8 (26.7%)	15 (50%)	30
Turkey, Aladag et al (30)	٠	10 (31.3%)	2 (8%)	12	8 (100%)	22 (68.7%)	23 (92%)	53
Turkey, Baştuğ et al(22)	11 (22)	21 (42)	18 (36)	50	7 (14)	30 (60)	13 (26)	50
Czech Republic, Hubacek et al(23)	71 (29%)	123 (50.2%)	51 (20.8%)	245	36 (22.1%)	87 (53.4%)	40 (24.5%)	163

with a percentage of 8.9%; however, 5 cases (55.6%) had the DD genotype and 4 (44.4%) cases had the II genotype. However, in a study by Aladag et al, (30) patients with ID genotype (31%) had more severe pneumonia than patients with DD (8%) and II (0%) genotypes (p=0.021). The mortality in patients with the

ID genotype (6.8%) was higher than in the DD (2.2%) and II (0%) genotypes. The findings of the Aladag et al, (30) study are consistent with our results. In another study on the Turkish population, by Celik et al, (20) a different result was reported. A total of 155 patients were divided into three groups, mild (78 cases), moderate (42 cases), and severe (35 cases). They reported no association between ACE1-I/D polymorphism and the severity of infection. In addition, they showed no association between groups according to allele frequencies and genotype. The same finding was reported by Baştuğ et al, (22) in Turkey.

On the other hand, an epidemiological study in the Asian population showed that the ACE1 D allele is associated with COVID-19 infection and mortality rate (12). But a study by Hubacek et al, (23) in the Czech Republic, reported the opposite result. They suggested that the II genotype is a predictive marker for COVID-19 severity. In addition, Delanghe et al, (32) showed an increased prevalence of COVID-19 cases with the ACE1 I allele. These findings suggest that the course of COVID-19 differs in the background of ethnicity.

Different results in the association of genetic factors with COVID-19 susceptibility and severity have been reported. The HLA-A*25:01, -B*15:27, and -C*07:29 alleles have been identified as high-risk alleles, while HLA-A*02:02, -B*15:03, and -C*12:03 have been identified as low-risk alleles (33, 34). In addition, several variants in ACE2 and TMPRSS2 could be related to this infection (18, 35-37). Identifying genetic factors associated with this infection could provide more knowledge for the detection of susceptible individuals and the design of therapeutic strategies.

This study has some limitations. First, the sample size is limited to a single center. Nevertheless, our sample size is similar to some recent investigations, one with Spanish Caucasians (18) and another with Turkish (30). Second, we just investigated patients with severe diseases, and patients with mild diseases were not included in the study.

Conclusion

Our results indicated that the ACE1 D allele can be a genetic risk factor in COVID-19 patients. Therefore, this study suggests that genotyping for ACE1-I/D polymorphism could be used to better prognosis and management in COVID-19 patients.

However, further studies on different ethnicities and populations are needed to evaluate this polymorphism and other genetic factors.

Acknowledgment

The authors acknowledge the Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran, and Kurdistan University of Medical Sciences, Sanandaj, Iran.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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