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Angiotensin-converting enzyme 1 and voltage-gated potassium channel-interacting protein 4 gene polymorphisms in COVID-19 patients from east of Iran

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19), the infectious respiratory disease caused by a newly discovered pathogen (severe acute respiratory syndrome coronavirus 2), is a pandemic that places a burden on the health care system. Recently, most research on COVID-19 has emphasized its profound impact on specific regions and ethnic groups. A possible explanation for these variations in disease presentation and severity might be differences in the gene pool of populations. This study therefore attempted to clarify possible involvements of genetic factors affecting COVID-19 pathogenesis with a focus on voltage-gated potassium channel-interacting protein 4 (KCNIP4) and angiotensin-converting enzyme 1 (ACE1) gene polymorphisms.
Materials and methods: In this case-control study, the polymorphisms were genotyped using PCR in 194 COVID-19 patients and 194 healthy controls.
Results: COVID-19 susceptibility and severity appeared to be unaffected by these polymorphisms. However, this study supported the relevance of ACE1 II genotype frequency to a decreased number of deaths due to the infection. We found that COVID-19 patients with the ACE1 II genotype have a statistically significant better chance of survival ($p = 0.008$).
Conclusion: This study strengthens the idea that the ACE1 I/D polymorphism can be a novel prognostic factor indicating the outcome of COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic infectious problem caused by an angiotensin I converting enzyme 2 (ACE2)-tropic virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. In the history of recent developments in disease pathophysiology, COVID-19 was first regarded as a viral respiratory illness, whereas evidence suggests that it is accompanied by extra-pulmonary multi-organ failures [1]. One of the main features of this infection faced by many patients is considerable variation in disease symptoms and severity among countries, and ethnic groups [2–5]. Recently, a substantial literature has grown up around the theme of identifying the underlying mechanisms responsible for COVID-19 susceptibility and the development of severe clinical outcomes [6–7]. Such studies have heightened the need for considering various risk factors, for instance demographic factors (e.g. old age and male gender), high population density, and comorbidities (e.g. obesity, diabetes mellitus (DM), hypertension (HTN), tumors, and immunodeficiency diseases) [8–10]. In addition, researchers hypothesized that genetic vulnerabilities and predisposition in patients with comorbidities and other subjects might place them at increased risk of COVID-19 from the beginning [11]. In the light of recent events, polymorphisms in the genes encoding the renin-

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angiotensin-aldosterone system (RAAS) members that represent complex functions in COVID-19 pathogenesis, have become the center of attention [3,12–13].

Viral adsorption to susceptible target cells defined as a prerequisite for membrane fusion and host cell entry process, is predominantly mediated by ACE2 interaction with the virus S protein in COVID-19 infection [14]. ACE2 is an integral part of the RAAS that catalyzes the hydrolysis of angiotensin II (Ang II; a vasoconstrictor peptide) into the vasodilator angiotensin 1–7 (Ang 1–7), thereby counteracting the effects of ACE1 [15]. The multifunctional ACE1 enzyme is a homologue of ACE2 that controls blood pressure by converting the hormone Ang I to Ang II and consequently regulating the volume of body fluids [16]. Recent evidence suggests that ACE1/ACE2 imbalance has been suspected to underlie the pathogenesis and development of COVID-19 [17–18]. The loss of ACE2 and increased plasma concentration of Ang II (the product of ACE1 activity) have been found to heighten unopposed effects in infected individuals, for instance endothelial injury, vasoconstriction, and high blood pressure [17]. Furthermore, it has been observed that there is a strong association between the level of Ang II and lung injury in addition to the viral load as an indicator of disease severity and mortality [19–20].

Among the best-studied ACE1 polymorphisms, increasing attention has been paid to the rs1799752 that is defined by the presence (insertion (I)) or absence (deletion (D)) of a 287-base pair Alu repeat element in intron 16. Although DNA fragment insertion occurs in the intragenic region, it can regulate gene expression through genetic and epigenetic pathways [21]. In line with this, forty-seven percent of the total phenotypic variation in serum ACE1 enzyme has been shown to be strongly related to this type of polymorphism in general population [22]. Recently, in consideration of evidence indicating adverse effects of comorbid conditions on COVID-19, research into the most common diseases of civilization closely associated with this I/D ACE1 gene polymorphism has also shed light on the possible influence of rs1799752 and RAAS imbalance over the infection [17–18]. Furthermore, in light of recent studies describing the ACE1 gene polymorphism associations with adult respiratory distress syndrome (ARDS) and SARS, it is becoming extremely difficult to ignore the importance of developing this point about SARS-CoV-2 infection [23–25]. There is a relatively small body of literature concerned with this issue, which also includes inconsistent and contradictory findings [12,26–31]. Regarding COVID-19 symptoms, different theories exist in the literature that could have significant implications for understanding possible underlying genetic factors modulating disease symptoms [13,23–24,32]. For example, Mosley et al. [32] conducted a genome-wide association study identifying association between an intronic polymorphism in voltage-gated potassium (Kv) channel-interacting protein 4 (KCNIP4) gene located on chromosome 4 (rs145489027 G > A) and ACE inhibitor-induced cough [32]. In addition, previous research has established that KCNIP4 single nucleotide polymorphisms (SNPs) are nominally associated with both asthma and airway hyperresponsiveness (AHR) in humans [33]. KCNIP4 is a small calcium binding biomolecule that regulates neuronal excitability in response to intracellular calcium fluctuations [32–33]. Currently, there are no data on the possible relationship between this polymorphism and COVID-19. In this study, we aimed to investigate whether these ACE1 and KCNIP4 polymorphisms are associated with susceptibility and outcome in COVID-19.

2. Materials and methods

2.1. Study design

In this case-control study, one hundred and ninety-four patients with newly diagnosed COVID-19 (positive qPCR tests for SARS-CoV-2 Alpha and Delta variants) referred to *Vali al-ʿAsr* hospital of Birjand, South Khorasan province, Iran were randomly recruited (mean age 58.84 yrs., range 2–94) from June 2021 to September 2021. The patients were divided into three groups based on their disease severity. Moderate cases (n = 151) were defined as having a persistent fever or the diagnosis of pneumonia on chest CT scan images [7]. COVID-19 was regarded as severe in subjects (n = 43) who suffered from one of the following conditions: respiratory rate (RR) ≥ 30 breaths per minute, respiratory distress, Horovitz index ≤ 300 mmHg, and oxygen saturation ≤ 93% [7]. The disease was also classified as critical in patients developing life-threatening symptoms (e.g. respiratory failure, multi-organ dysfunction, and shock) and requiring noninvasive or invasive mechanical ventilation [7]. To identify detailed research data, the patients consented to participate in the study after being informed, were asked to fill in a questionnaire about their age, body mass index (BMI), and the recent history of addiction, particularly to smoking. Besides, the presence of comorbidities (including: coronary artery disease (CAD), DM, HTN, and chronic obstructive pulmonary disease (COPD)), patients' medical treatments and other clinical data were obtained from their hospital records. An age-matched control group of 194 subjects without any history of COVID-19 was also drawn from a pool of individuals who had negative results of SARS-CoV-2 qPCR test. Unwilling subjects to cooperate in this research and patients who suffered from other infectious and immune-related disorders, were excluded from our study. Some patients whose complaint was not confirmed by qPCR, were not also included in the investigation. In addition, the primary exclusion criteria for the selection of COVID-19 cases and their hospital admission were mild clinical symptoms and no abnormal radiological findings. Before undertaking the investigation, ethical clearance was sought from the Research Council of Birjand University of Medical Sciences, Birjand, Iran (Ethical code: IR.BUMS.REC.1399.061).

2.2. Genotyping

In order to extract genomic DNA, 2.5 ml blood samples were obtained and collected in EDTA tubes. After collection, the peripheral blood specimens were immediately stored at −20 °C. Following this step, leukocytes' DNA was extracted using a manual salting-out method. The quantity and quality of isolated DNA were consequently checked by the spectrophotometer. After testing DNA samples, PCR and tetra-primer amplification refractory mutation system-PCR (T-ARMS-PCR) methods followed by agarose gel electrophoresis were performed to identify ACE1 (rs1799752) and KCNIP4 (rs145489027) polymorphisms respectively. The sequences of oligonucleotide primers designed by using Primer3 software were as follows: the ACE1 rs1799752, 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-GATGTGGCCATCACATTCGTCAGAT-3′; the KCNIP4 rs145489027, forward inner primer (A allele): 5′-GGTGTTTGGTTTTTGTCCTGTCA-3′, reverse inner primer (G allele): 5′-GAAACCATCATTCTCAGCAAACTCTC-3′, forward outer primer: 5′-CAGTGTGTGATGTTCCTCTTCCTGT-3′, reverse outer primer: 5′- TGTGGCACTATTCGCAATAGAAAAG-3′.

2.3. Statistical analysis

All analyses were conducted using SPSS software (version 22, SPSS Inc., Chicago, IL, USA). Groups differences were compared using the student *t*-test or Mann-Whitney *U* test for continuous variables depending on their distribution. Furthermore, One-Way - ANOVA and Kruskal-Wallis H tests were used to compare parametric and non-parametric continuous variables between several independent groups (more than two). The Chi-square test and Fisher's exact test were also carried out to provide comparisons of allele and genotype frequencies between groups. Furthermore, the odds ratio (OR) at 95% confidence interval (CI) quantifying the strength of associations was calculated by the logistic regression model. Associations between COVID-19 mortality (the survivors and non-survivors) and genotype variants were evaluated in dominant, recessive, and codominant models (significance level: 1.7%). The accordance of genotype frequencies distribution with Hardy-Weinberg equilibrium (HWE) among participants

was analyzed using the $\chi 2$ test. All significance levels in the study were also set at the 5% level. In addition, continuous data were reported as the mean ± standard deviation (SD).

3. Results

The results obtained from the preliminary analysis of demographic data are summarized in Table 1. A total of 194 COVID-19 subjects whose median age was 60.5 years [IQR: 29] were included in this study. As can be seen from the Table 1, the majority of the patients were male (57.2%) with an age range of 15–94 years at the beginning of the investigation. The second set of analyses was also conducted to examine the impacts of general characteristics on disease severity (Table 2). From the data in Table 2 we can see that the median age of critical cases is significantly higher than that of moderate participants (Mann-Whitney *U* test *p* value = 0.001, significance level: 1.7%). In addition, further analysis of the data obtained from Table 2 revealed that the percentage of COPD is significantly higher in severe to critical patients compared to the moderate ones (23.3% vs. 6%; $p = 0.002$). Critical cases also showed even a higher percentage of COPD in comparison with the other patients (moderate to severe ones) (27.3% vs. 7.6%; $p = 0.011$). The next set of analyses provided comparison of comorbidities between another two subgroups of COVID-19 participants (ICU-admitted and infectious disease ward-admitted patients) and revealed no significant differences except for a higher percentage of COPD in ICU-admitted patients (24.4% vs. 5.9%; $p = 0.001$). Before proceeding to examine the genetic polymorphisms, we found it necessary to consider influencing factors in disease mortality. The results revealed that 10.31% (20outof194) of patients died from COVID-19, which the majority of them were female (55%). Further analysis also indicated that there is a significant difference in the median age between the non-survivors (73 yrs.; IQR: 29) and survivors (59 yrs.; IQR: 29) ($p = 0.003$). Furthermore, it has been found that the mean BMI of the non-survivors (23.47 ± 4.49 kg/ m2) was significantly lower than that of patients who survived (26.69 ± 5.28 kg/ m2) ($p = 0.01$). The comparison of clinical and laboratory data between these subgroups did not also demonstrate statistically significant differences except for a higher prevalence of COPD (25% vs. 8%; $p = 0.031$) and an increase in plasma prothrombin time (PT) within the non-survivors (13 s [IQR: 2.35] vs. 12.3 s [IQR: 1.5]; $p = 0.005$).

The following part of analyzes moves on to examine associations between the mentioned polymorphisms and SARS-CoV-2 infection. The results showed that both polymorphisms' genotype and allele distribution do not have any significant associations with COVID-19 susceptibility (Tables 2 and 3). Similarly, as shown in Table 2, statistical tests indicated no significant differences in genotype and allele frequencies of rs1799752 and rs145489027 polymorphisms between the three groups classified depending on their disease severity. Despite that, unexpectedly, the most striking result to emerge from the data is that the ACE1 gene polymorphism genotypes were significantly associated with mortality in the COVID-19 group ($p = 0.04$) (Table 3). The results indicated that II genotype appeared to have protective effects against COVID-19 mortality in the patients (for cohort group = the survivors: value = 1.153, confidence interval = 1.083–1.227; $p = 0.008$). In addition, ACE1 alleles tended to be distinctively distributed between these two subgroups despite the fact that it was not statistically significant (*p* value = 0.054). It was also indicated that the distribution of genotype and allele frequencies of KCNIP4 gene polymorphism was similar in all subgroups classified according to COVID-19 mortality (Table 3). The distribution of ACE1 genotypes in cases and controls was in HWE ($p = 0.924$), whereas we concluded that, for unknown reasons, the KCNIP4 genotype frequencies in this population represent a departure from HWE. In line with this finding, an excess of heterozygote carriers (AG genotype) was observed. The final section of analyses was concerned about possible associations of the polymorphisms with the COVID-19 relevant risk factors, even though no significant correlations were found in this respect.

Table 1
Demographic data of COVID-19 cases and control group.

Characteristics	COVID-19 cases (n = 194)	Control group (n = 194)	*p*
Age (years), median [IQR]	60.5 [29]	64 [27]	0.58
Sex, n (%)			
M/F	111 (57.2)/83 (42.8)	97 (50)/97 (50)	0.154
BMI (kg/ m2), mean ± SD	26.36 ± 5.28	26.34 ± 5.31	0.962
BMI classification, n (%)			0.999
Underweight	17 (8.8)	17 (8.8)	
Normal	68 (35.1)	69 (35.6)	
Overweight	55 (28.4)	54 (27.8)	
Obese	47 (24.2)	46 (23.7)	
Extremely obese	7 (3.6)	8 (4.1)	
Smoking habit, n (%)			0.586
Yes	8 (4.1)	6 (3.1)	
No	186 (95.9)	188 (96.9)	
Diabetes*, n (%)			**0.001**
Yes	17 (8.8)	3 (1.5)	
No	177 (91.2)	191 (98.5)	

IQR, Interquartile Range; SD, Standard Deviation; M, male; F, female; BMI, Body Mass Index.

4. Discussion

This study genotyped ACE1 and KCNIP4 gene polymorphisms to clarify their associations with COVID-19 susceptibility and outcome. The research has shown there is no evidence that these polymorphisms have influences on COVID-19 susceptibility and severity. However, one of the more significant findings from this study is that ACE1 II genotype frequency is negatively correlated with the number of deaths from COVID-19. Based on these results, it seems that the ACE1 gene polymorphism can be a significant prognostic factor for the outcome of COVID-19.

Genetic predisposition, demographic factors and comorbidities appear to be closely linked to COVID-19 pathogenesis, prognosis, and mortality [17]. Individual genetic profiles could disclose information on how to control virus spreading and reduce disease complications [34]. In line with this, epidemiological studies have recently considered the pathogenicity of SARS-CoV-2 in various geographic areas with genetic diversity [3,34]. The findings that the most severe cases infected with this ACE2-tropic virus are more likely to have a history of comorbidities (e.g., HTN, cardiovascular disease, and DM) and receive ACE1 inhibitor treatment shed new light on the issue that RAAS-unbalancing might have crucial roles in the disease pathogenesis [17,35]. Accordingly, previous research published genetic hypotheses on the involvements of RAAS pathway genes (i.e. ACE1 polymorphisms etc.) in COVID-19 to improve its treatment outcome and find clinical predictive factors of development [17]. So far, compared with other gene polymorphisms, ACE1 I/D polymorphism (rs1799752) has been identified as being potentially important in COVID-19 on account of its associations with ACE1 serum variations, SARS, ARDS, and different comorbidities [17–18,22–25]. Whilst some research has been carried out, there is still very little scientific understanding of that in various regions, which also includes inconsistent and contradictory findings [12,26–31]. Despite these studies, there remains a paucity of evidence on some gene polymorphisms in relation to cough, asthma, and airway hyperresponsiveness, for instance KCNIP4 ones in COVID-19 [32–33]. Therefore, this investigation seeks to obtain data which will help to address these research gaps.

Recently, researchers have shown an increased interest in the study of ACE1 polymorphism in ARDS and SARS patients [23,25,36]. In this context, the results of our research reflect those of Jerng et al. [25] who found no statistically significant differences in the frequencies of ACE1

Table 2
Clinical and general laboratory data of COVID-19 patients and study subgroups divided according to the disease severity in addition to the allelic and genotypic distribution of ACE1 and KCNIP4 genes.

Parameter	COVID-19 cases (n = 194)	Moderate cases (n = 151)	Severe cases (n = 21)	Critical cases (n = 22)	p^1
Age*(yrs.), median [IQR]	60.5 [29]	58 [25]	70 [45]	73 [29]	**0.002**[2]
Sex, n (%)					0.272
M/F	111 (57.2)/ 83 (42.8)	91 (60.3)/ 60 (39.7)	10 (47.6)/ 11 (52.4)	10 (45.5)/ 12 (54.5)	
BMI* (kg/ m^2), mean ± SD	26.36 ± 5.28	26.55 ± 5.25	27.87 ± 5.4	23.64 ± 4.66	**0.02**[3]
BMI classification*, n (%)					**0.008**
Underweight	17 (8.8)	10 (6.6)	2 (9.5)	5 (22.7)	
Normal	68 (35.1)	56 (37.1)	2 (9.5)	10 (45.5)	
Overweight	55 (28.4)	40 (26.5)	11 (52.4)	4 (18.2)	
Obese	47 (24.2)	40 (26.5)	4 (19)	3 (13.6)	
Extremely obese	7 (3.6)	5 (3.3)	2 (9.5)	0 (0)	
Shortness of breath, n (%)	89 (45.9)	69 (45.7)	9 (42.9)	11 (50)	0.891
Smoking habit, n (%)	8 (4.1)	8 (5.3)	0 (0)	0 (0)	0.532
Hypertension, n (%)	51 (26.3)	42 (27.8)	5 (23.8)	4 (18.2)	0.608
Diabetes, n (%)	17 (8.8)	14 (9.3)	1 (4.8)	2 (9.1)	0.909
CAD, n (%)	8 (4.1)	6 (4)	0 (0)	2 (9.1)	0.402
COPD*, n (%)	19 (9.8)	9 (6)	4 (19)	6 (27.3)	**0.003**
Laboratory tests					
PT*, median [IQR]	12.3 [1.6]	12.3 [1.5]	12.7 [1.6]	13.25 [2.28]	**0.003**[4]
PTT, median [IQR]	37.25 [9.7]	37 [9]	39 [11.5]	37.5 [14]	0.387
CRP, median [IQR]	43 [35]	43 [37]	52 [24]	38.5 [24]	0.19
ESR, median [IQR]	26 [25]	26 [23]	31 [36]	28.5 [31]	0.695
rs1799752, n (%)					0.562
DD	65 (34)	51 (33.8)	6 (28.6)	8 (36.4)	
II	43 (22)	35 (23.2)	6 (28.6)	2 (9.1)	
ID	86 (44)	65 (43)	9 (42.9)	12 (54.5)	
Allele, n (%)					0.428
D	216 (55.7)	167 (55.3)	21 (50)	28 (63.6)	
I	172 (44.3)	135 (44.7)	21 (50)	16 (36.4)	
rs145489027, n (%)					0.843
GG	20 (10)	15 (9.9)	2 (9.5)	3 (13.6)	
AG	174 (90)	136 (90.1)	19 (90.5)	19 (86.4)	
AA	0 (0)	0 (0)	0 (0)	0 (0)	
Allele, n (%)					0.972
G	214 (55.2)	166 (55)	23 (54.8)	25 (56.8)	
A	174 (44.8)	136 (45)	19 (45.2)	19 (43.2)	

1. The *p* values represent the statistical significance between moderate, severe, and critical cases.
2. The Mann-Whitney U test revealed that the median age of critical cases is significantly higher than that of moderate patients ($p = 0.001$; significance level: 1.7%).
3. The Scheffé post Hoc test indicated that there is a significant difference in the mean BMI between the severe and critical cases ($p = 0.031$).
4. The results of Mann-Whitney U test showed a statistically significant difference in PT between the moderate and critical patients ($p = 0.001$; significance level: 1.7%).

IQR, Interquartile Range; M, male; F, female; BMI, Body Mass Index; SD, Standard Deviation; CAD, Coronary Artery Disease; COPD, Chronic Obstructive Pulmonary Disease; PT, Prothrombin Time; PTT, Partial Thromboplastin Time; CRP, C Reactive Protein; ESR, Erythrocyte Sedimentation Rate.

genotypes between ARDS patients and control groups [25]. In line with this, comparison of the findings with those of other studies also confirms consistency with data obtained on SARS cases [23,36]. This also accords with earlier observations, which showed similar ACE1 alleles distribution between COVID-19 cases and controls, even though the previous study's findings did not support the same distribution of ACE1 genotypes [37]. It has been suggested by Akbari *et al.* that ACE1 rs1799752 genotypes might affect the risk of SARS-CoV-2 infection [37]. Likewise, previous research evaluating the influence of ACE1 I/D polymorphism over COVID-19 susceptibility also observed inconsistent results [12,26,37,28–31]. Several possible explanations for this might be that these studies have a small sample size, an ecological design or use statistical models not including confounding factors, such as an adequate number of laboratory diagnostic tests [29–31]. Regarding disease severity, the insights gained from other studies differ from the findings presented here [26,28]. This discrepancy could be attributed to different geographical regions, covariant effects, and sample selection bias. Concerning the research questions, the results corroborate the ideas of researchers (2006), who suggested that ARDS patients with II genotype have a statistically significant better chance of survival [25]. In the same vein, other researchers who undertook ecological studies into COVID-19 also confirm our finding [27,38]. The contributions of this previous research have been to illustrate that ACE1 II genotype may serve as a predictive marker of COVID-19 severity [27,38]. However, in the literature on this issue, the influence of ACE1 gene polymorphism is debated [12,29,31]. As was pointed out in the introduction to this paper, in reviewing the literature, no data was found on the association between KCNIP4 gene polymorphism and COVID-19. This study lays the groundwork for future research into this issue.

Furthermore, our study found that the median age of critical patients is significantly higher than that of moderate cases. Further analysis also showed the same result between the non-survivors and survivors (73 years vs. 59). These results are likely to be related to the associations of comorbid conditions with advanced age. The comparison of comorbidities between the subgroups also demonstrated a higher prevalence of COPD in the non-survivors as well as severe to critical cases, which their combination provides some support for the crucial roles of inflammation and lung conditions in the outcome of COVID-19.

In conclusion, this study enhanced our understanding of genetic factors and their relevance to SARS-CoV-2 infection. Taken together, the findings suggest a prognostic value for ACE1 gene I/D polymorphism in COVID-19 among Iranian population. More broadly, to develop a full picture of this topic in other regions, future studies are recommended.

CRediT authorship contribution statement

Hamid Abbaszadeh: Conceptualization, Resources, Methodology, Project administration, Funding acquisition. **Fariba Mohammadi:**

Table 3
The allelic and genotypic distribution of ACE1 and KCNIP4 genes in control group and COVID-19 patients divided according to the disease mortality in addition to their medical treatments.

Parameter	Controls	COVID-19 cases (n = 194)		p^1
		Non-survivors (n = 20)	Survivors (n = 174)	
rs1799752*, n (%)				**0.04**
DD	52 (26.8)	8 (40)	57 (32.8)	
ID	101 (52.1)	12 (60)	74 (42.5)	
II	41 (21.1)	0 (0)	43 (24.7)	
Allele frequency, n (%)				0.054
D	205 (53)	28 (70)	188 (54)	
I	183 (47)	12 (30)	160 (46)	
rs145489027, n (%)				0.44
GG	24 (12.4)	3 (15)	17 (9.8)	
AG	170 (87.6)	17 (85)	157 (90.2)	
AA	0 (0)	0 (0)	0 (0)	
Allele frequency, n (%)				0.753
G	218 (56)	23 (57.5)	191 (55)	
A	170 (44)	17 (42.5)	157 (45)	
Medical treatments*, n(%)				**0.0001**
Anti-malarial drugs[2]	–	0 (0)	18 (10.3)	
Antiviral drugs[3]	–	0 (0)	115 (66.1)	
Interferon-beta	–	0 (0)	17 (9.8)	
Corticosteroids[4]	–	11 (55)	21 (12.1)	
Combination drug therapy	–	9 (45)	3 (1.7)	

1. The *p* values represent the statistical significance between the survivors and non-survivors.
2. E.g., hydroxychloroquine.
3. E.g., remdesivir.
4. E.g., dexamethasone.

Formal analysis, Writing – original draft, Visualization. **Mahdieh Rajabi-Moghaddam:** Investigation, Resources. **Hamid Kabiri-Rad:** Formal analysis, Writing – original draft. **Shokouh Ghafari:** Investigation, Resources. **Farshid Abedi:** Investigation, Resources. **Ebrahim Miri-Moghaddam:** Conceptualization, Validation, Resources, Data curation, Methodology, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M. Cascella, M. Rajnik, A. Aleem, S.C. Dulebohn, R. Di Napoli, Features, evaluation, and treatment of coronavirus (COVID-19), Statpearls [internet]. (2022).
[2] A. Iwasaki, N.D. Grubaugh, Why does Japan have so few cases of COVID-19? EMBO Mol. Med. 12 (5) (2020), e12481.
[3] C. Kenyon, ACE-1 I/D polymorphism associated with COVID-19 incidence and mortality: An ecological study, 2020.
[4] S. Rubino, N. Kelvin, J.F. Bermejo-Martin, D. Kelvin, As COVID-19 cases, deaths and fatality rates surge in Italy, underlying causes require investigation, J. Infect. Devel. Count. 14 (03) (2020) 265–267.
[5] A. Saglietto, F. D'Ascenzo, G.B. Zoccai, G.M. De Ferrari, COVID-19 in Europe: the Italian lesson, The Lancet. 395 (10230) (2020) 1110–1111.
[6] A.G. Harrison, T. Lin, P. Wang, Mechanisms of SARS-CoV-2 transmission and pathogenesis, Trends Immunol. 41 (12) (2020) 1100–1115.
[7] J. Mi, W. Zhong, C. Huang, W. Zhang, L. Tan, L. Ding, Gender, age and comorbidities as the main prognostic factors in patients with COVID-19 pneumonia, Am. J. Translat. Res. 12 (10) (2020) 6537.
[8] A. Sanyaolu, C. Okorie, A. Marinkovic, R. Patidar, K. Younis, P. Desai, et al., Comorbidity and its impact on patients with COVID-19, SN Comprehen. Clin. Med. 2 (8) (2020) 1069–1076.
[9] X. Fang, S. Li, H. Yu, P. Wang, Y. Zhang, Z. Chen, et al., Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis, Aging (albany NY). 12 (13) (2020) 12493.
[10] Y.d. Gao, M. Ding, X. Dong, Z. Jj, A. Kursat Azkur, D. Azkur, et al., Risk factors for severe and critically ill COVID-19 patients: a review, Allergy 76 (2) (2021) 428–455.
[11] R. Öztürk, COVID-19: pathogenesis, genetic polymorphism, clinical features and laboratory findings, Turkish J. Med. Sci. 50 (SI-1) (2020) 638–657.
[12] A. Pati, H. Mahto, S. Padhi, A.K. Panda, ACE deletion allele is associated with susceptibility to SARS-CoV-2 infection and mortality rate: An epidemiological study in the Asian population, Clinica Chimica Acta. 510 (2020) 455–458.
[13] N. Yamamoto, N. Nishida, R. Yamamoto, T. Gojobori, K. Shimotohno, M. Mizokami, et al., Angiotensin-Converting Enzyme (ACE) 1 Gene Polymorphism and Phenotypic Expression of COVID-19 Symptoms, Genes. 12 (10) (2021).
[14] P. Verdecchia, C. Cavallini, A. Spanevello, F. Angeli, The pivotal link between ACE2 deficiency and SARS-CoV-2 infection, Eur. J. Intern. Med. 76 (2020) 14–20.
[15] F. Hikmet, L. Méar, Å. Edvinsson, P. Micke, M. Uhlén, C. Lindskog, The protein expression profile of ACE2 in human tissues, Mol. Syst. Biol. 16 (7) (2020), e9610.
[16] D. Coates, The angiotensin converting enzyme (ACE), Int. J. Biochem. Cell Biol. 35 (6) (2003) 769–773.
[17] N. Yamamoto, N. Nishida, R. Yamamoto, T. Gojobori, K. Shimotohno, M. Mizokami, et al., Angiotensin-Converting Enzyme (ACE) 1 Gene Polymorphism and Phenotypic Expression of COVID-19 Symptoms, Genes. 12 (10) (2021) 1572.
[18] N. Yamamoto, R. Yamamoto, Y. Ariumi, M. Mizokami, K. Shimotohno, H. Yoshikura, Does genetic predisposition contribute to the exacerbation of COVID-19 symptoms in individuals with comorbidities and explain the huge mortality disparity between the East and the West? Int. J. Mol. Sci. 22 (9) (2021) 5000.
[19] Y. Liu, Y. Yang, C. Zhang, F. Huang, F. Wang, J. Yuan, et al., Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury, Science China Life Sciences. 63 (3) (2020) 364–374.
[20] J. Fajnzylber, J. Regan, K. Coxen, H. Corry, C. Wong, A. Rosenthal, et al., SARS-CoV-2 viral load is associated with increased disease severity and mortality, Nat. Commun. 11 (1) (2020) 1–9.
[21] M. Li, L. Schifanella, P.A. Larsen, Alu retrotransposons and COVID-19 susceptibility and morbidity, Human genomics. 15 (1) (2021) 1–11.
[22] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, F. Soubrier, An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels, J. Clin. Investig. 86 (4) (1990) 1343–1346.
[23] S. Itoyama, N. Keicho, T. Quy, N.C. Phi, H.T. Long, V. Van Ban, et al., ACE1 polymorphism and progression of SARS, Biochem. Biophys. Res. Commun. 323 (3) (2004) 1124–1129.
[24] R.P. Marshall, S. Webb, G.J. Bellingan, H.E. Montgomery, B. Chaudhari, R. J. McAnulty, et al., Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome, Am. J. Respir. Crit. Care Med. 166 (5) (2002) 646–650.
[25] J.-S. Jerng, C.-J. Yu, H.-C. Wang, K.-Y. Chen, S.-L. Cheng, P.-C. Yang, Polymorphism of the angiotensin-converting enzyme gene affects the outcome of acute respiratory distress syndrome, Crit. Care Med. 34 (4) (2006) 1001–1006.
[26] H. Saad, K. Jabotian, C. Sakr, R. Mahfouz, I.B. Akl, N.K. Zgheib, The Role of Angiotensin Converting Enzyme 1 Insertion/Deletion Genetic Polymorphism in the Risk and Severity of COVID-19 Infection, Front. Med. 8 (2021).
[27] A.K. Aung, T. Aitken, B.M. Teh, C. Yu, R. Ofori-Asenso, K.L. Chin, et al., Angiotensin converting enzyme genotypes and mortality from COVID-19: An ecological study, J. Infect. 81 (6) (2020) 961–965.
[28] O. Gunal, O. Sezer, G.U. Ustun, C.E. Ozturk, A. Sen, S. Yigit, et al., Angiotensin-converting enzyme-1 gene insertion/deletion polymorphism may be associated with COVID-19 clinical severity: a prospective cohort study, Annals Saudi Med. 41 (3) (2021) 141–146.
[29] J.R. Delanghe, M.M. Speeckaert, M.L. De Buyzere, COVID-19 infections are also affected by human ACE1 D/I polymorphism, Clinical Chemistry and Laboratory Medicine (CCLM). 58 (7) (2020) 1125–1126.
[30] J.R Delanghe, M.M Speeckaert, M. De Buyzere, The host's angiotensin-converting enzyme polymorphism may explain epidemiological findings in COVID-19 infections, Clinica Chimica Acta. 505 (2020) 192–193.
[31] M. Saadat, No significant correlation between ACE Ins/Del genetic polymorphism and COVID-19 infection, Clinical Chemistry and Laboratory Medicine (CCLM). 58 (7) (2020) 1127–1128.
[32] J.D. Mosley, C.M. Shaffer, S.L. Van Driest, P.E. Weeke, Q.S. Wells, J.H. Karnes, et al., A genome-wide association study identifies variants in KCNIP4 associated with ACE inhibitor-induced cough, Pharmacogenomics J. 16 (3) (2016) 231–237.

[33] B.E. Himes, K. Sheppard, A. Berndt, A.S. Leme, R.A. Myers, C.R. Gignoux, et al., Integration of mouse and human genome-wide association data identifies KCNIP4 as an asthma gene, PloS One 8 (2) (2013), e56179.
[34] D. Gemmati, V. Tisato, Genetic Hypothesis and Pharmacogenetics Side of Renin-Angiotensin-System in COVID-19, Genes. 11 (9) (2020).
[35] H.T. Nguyen, S. Zhang, Q. Wang, S. Anang, J. Wang, H. Ding, et al., Spike glycoprotein and host cell determinants of SARS-CoV-2 entry and cytopathic effects, J. Virol. 95 (5) (2021) e02304–e2320.
[36] K.A. Chan, N.L. Tang, D.S. Hui, G.T. Chung, A.K. Wu, S.S. Chim, et al., Absence of association between angiotensin converting enzyme polymorphism and development of adult respiratory distress syndrome in patients with severe acute respiratory syndrome: a case control study, BMC Infect. Dis. 5 (1) (2005) 1–6.
[37] M. Akbari, M. Taheri, G. Mehrpoor, S. Eslami, B.M. Hussen, S. Ghafouri-Fard, et al., Assessment of ACE1 variants and ACE1/ACE2 expression in COVID-19 patients, Vasc.Pharmacol. 142 (2021), 106934.
[38] N. Yamamoto, Y. Ariumi, N. Nishida, R. Yamamoto, G. Bauer, T. Gojobori, et al., SARS-CoV-2 infections and COVID-19 mortalities strongly correlate with ACE1 I/D genotype, Gene 758 (2020), 144944.