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Investigating single nucleotide polymorphisms of ACE2 gene as a genetic factor in the control of COVID-19 infection

Abstract

The severity of the coronavirus illness may be related to host genetic factors in 2019 (COVID-19). In viral cellulitis, angiotensin-converting enzyme 2 (ACE2) is crucial.
The present literature aims to examine the relationship of COVID-19 mortality with rs2285666 and rs2074192 polymorphisms and clinical parameters..
ACE2 rs2285666 and 2074192 polymorphisms were genotyped using RFLP-PCR and Tetra-Arms methods in 556 recovered and 522 deceased patients.

In deceased patients with ACE2 rs2285666 than they were with the TT genotype, both CC frequency and TT frequency were considerably higher, and vice versa for ACE2 rs2074192 than they were with the CC genotype. Higher levels of alanine aminotransferase, alkaline phosphatase, creatinine, erythrocyte sedimentation rate, and C-reactive protein and lower levels of uric acid, cholesterol, low-density lipoprotein, 25-hydroxyvitamin D, real-time PCR Ct values, and the CC genotype were linked to an increased risk of mortality, Based on the results of the multivariate logistic regression analysis..
The prevalence of the CC and TT alleles in ACE2 rs2285666 and ACE2 rs2074192, respectively, was found to be related to the clinical variables, death related to COVID-19, and these findings were supported by our findings. It will take more research to validate these findings.

Keywords: *angiotensin-converting enzyme 2, COVID-19, severe acute respiratory syndrome of coronavirus 2*

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Introduction

A novel coronavirus from the beta-coronavirus family surfaced in December 2019. Each beta-coronavirus is unique and different in some manner (4,5). The SARS-CoV-2 genome, however, is 77% and 50% structurally similar to SARS-CoV and MERS-CoV, respectively, despite having different degrees of structural and genomic similarity. SARS-CoV-2 demonstrated an unprecedented spread of infection in contrast to the comparatively low prevalence of SARS-CoV and MERS-CoV in 2002 and 2012, respectively. The World Health Organization (WHO) consequently designated her COVID-19 infection worldwide pandemic on March 11, 2020. Less than 6 million verified cases and 371,000 fatalities had been reported by the WHO as of June 2020, and as of November, 3.46 million cases and 1.2 million fatalities had been reported [1].

Since the outbreak of the COVID-19 infection outbreak in China, the virus has undergone about 150 changes. Scientists analyzed 103 available genomes in SARS-CoV-2 and found about 149 genetic mutations, most of which have occurred recently [2].

Various factors are involved in spreading or stopping viral infections. These factors can be related to the virus (such as viral load and genotype) and host genetic factors such as patient's age and sex, metabolic diseases (e.g., insulin resistance, obesity, heart and kidney diseases, and lung infection), race, and genetic polymorphisms) [3].

The infected host's cellular components play a major role in SARS-CoV-2 transmission. The heart, respiratory system, kidney, and oral mucosa all contain ACE2, which is crucial for virus entry into host cells [4]. The ACE2 SNPs rs2285666 and rs2074192 have been associated with diabetes, stroke, coronary heart disease, and hypertension in numerous investigations [5]. The A allele is associated with greater serum ACE2 levels in healthy people, diabetics, and people who have both diabetes and stroke [6]. The A allele is not known to be connected to greater circulating levels of ACE2, which would imply that there is more membrane-bound ACE2 in tissues like

the lungs. This polymorphism explains the association between COVID-19 susceptibility and disease progression because the ACE2 gene functions as a cellular receptor for virus entry [7]. Additionally, researchers conclude that there is a direct link between people infected with COVID-19 and their genetics. Some genetic traits can affect host responses to the Coronavirus and drugs. Studies have shown that changes in the ACE2 gene can make people more susceptible to SARS and the Coronavirus. Alterations in this gene have also been linked to heart problems, suggesting that people can become infected with the virus through comorbidities such as diabetes and hypertension, Which means that the virus infection depends on genetic conditions and lifestyle [8].

rs2074192 (ACE2) and rs2285666 may be helpful instruments for forecasting the clinical outcomes of people infected with SARS-CoV-2. Genetic predisposition may play a role in the severe visceral injury and poor prognosis seen in COVID-19 individuals. [9].

The current research aims to examine the role of ACE2 single nucleotide polymorphisms (SNPs) as host genetic factors in the prevention of COVID-19 infection in the Iranian genetic population based on the aforementioned themes.

Materials and methods

In the current cross-sectional study, the population was sampled from 1078 COVID-19 patients' records, and their information was entered into a questionnaire. After obtaining written consent forms, 10 ccs of whole blood was sampled from the patients. ACE2 gene polymorphisms were analyzed using the Ficoll PBMC solution. To examine the polymorphisms, the genome was extracted using the salting out protocol for 2 consecutive days, and then frozen at −70 °C for the tests. The absorbance of samples and the analysis of genomic DNA extraction were investigated by spectrophotometry. The bands were then examined on the electrophoresis gel.

Extracted DNA was certified using a NanoDrop spectrophotometer. In this study, the concentrations of the extracted DNA samples were in the range of 50-100 ng/µl and their OD 260/280 was between 1.7 and 1.9, indicating good concentration and purity.

ACE2 rs2285666 and rs2074192 polymorphisms were determined by PCR-RFLP and Tetra Arms PCR techniques, respectively, using the sequencing methods. For the ACE2 gene polymorphism test, we first extracted the base sequence of the desired SNPs was first extracted from PubMed.SNP.database. After the BLAST process, a primer for this region was designed from different software according to the genotyping method. All the obtained data were analyzed by the SPSS 22 statistical software.

Results

Cognitive characteristics in the population of COVID-19 patients

The clinical and demographic details of the subjects are presented in Table 1. This research involved 1078 COVID-19 patients, who were split into two groups with average ages of 51.5 ± 12.7 and 57.8 ± 11.3 years, respectively: recovered (n = 556) and deceased (n = 522). After COVID-19, higher mortality was linked to high levels of ALT, AST, ALP, FBS, Cr, CRP, ESR, uric acid, 25(OH)D, real-time PCR Ct, TG, cholesterol, HDL, and LDL.

Table 1. Comparison of laboratory parameters between deceased and recovered COVID-19 patients

Variables	**Dead patients (n = 522)**	**Recovered patients (n = 556)**	***P*-value**
Mean age ± SD	57.8 ± 11.3	51.5 ± 12.7	<0.001
Gender (male/female)	280/242 53.6/ 46.4%	289/267 52.8/48.0%	0.585
ALT, IU/L (mean ± SD) (Reference range: 5-40)	44.3 ± 23.6	33.0 ± 24.5	<0.001
AST, IU/L (mean ± SD) (Reference range: 5-40)	37.0 ± 12.9	30.7 ± 15.3	<0.001
ALP, IU/L (mean ± SD) (Reference range: up to306)	201.1 ± 66.0	169.2 ± 89.6	<0.001
Cholesterol, mg/dL (mean ± SD) (Reference range: 50-200	116.5 ± 38.4	123.2 ± 36.9	<0.001
TG, mg/dL (mean ± SD) (Reference range:60-165)	117.6 ± 42.2	132.2 ± 36.9	0.009
LDL, mg/dL (mean ± SD) (Reference range: up to 150)	71.4 ± 36.7	111.4 ± 48.7	<0.001
HDL, mg/dL (mean ± SD) (Reference range: >40)	30.5 ± 11.0	34.5 ± 11.8	<0.001
WBC, 10^9/L (mean ± SD) (Reference range: 4000-10000)	7600.4± 2712.8	7738.8 ± 2901.1	0.589
CRP, mg/L (mean ± SD) (Reference range: <10 mg/L Negative)	66.6 ± 21.5	56.4 ± 20.8	<0.001
ESR, mm/1st h (mean ± SD) (Reference range:0-15)	55.1 ± 15.8	46.4 ± 15.5	<0.001

FBS, mg/dL (mean ± SD) (Reference range: 70-100)	110.2 ± 42.6	106.1 ± 41.5	0.002
Platelets × 1000/cumm (mean ± SD) (Reference range:140000-400000)	185 ± 77	184 ± 67	0.317
Hemoglobin, g/dL (mean ± SD) (Reference range:12-18	14.4 ± 2.1	12.4 ± 1.8	0.363
Uric acid, mg/dL (mean ± SD) (Reference range: 3.6-6.8)	3.4 ± 1.2	5.8 ± 1.6	<0.001
Creatinine, mg/dL (mean ± SD) (Reference range: 0.6-1.4)	1.1 ± 0.3	0.8 ± 0.3	<0.001
T3, ng/dL (mean ± SD) (Reference range: 2.3-4.2)	3.0 ± 1.1	2.6 ± 0.9	0.051
T4, mcg/dL (mean ± SD) (Reference range: 5.6-13.7)	9.0 ± 4.7	8.4 ± 5.8	0.352
TSH, mu/L (mean ± SD) (Reference range: 0.4-4.5)	3.5 ± 1.8	3.3 ± 1.6	0.626
25-hydroxy vitamin D, ng/mL (mean ± SD) (Sufficiency:21-150)	27.0 ± 10.2	36.2 ± 13.5	<0.001
Real-time PCR Ct values	12.5 ± 6.9	26.1 ± 8.1	0.021

Associations between ACE2 rs2285666, ACE2 rs2074192, and COVID-19 mortality

The effect of ACE2 rs2285666 on COVID-19 mortality is shown in Figure 1-A; patients with the CC genotype of ACE2 rs2285666 had significantly greater mortality rates than patients with the different genotypes, while improved individuals had the TT genotype. The majority of deaths belonged to the TT genotype, and the majority of recoveries belonged to the CC genotype. Figure 1-B shows the relationship between genotypes and mortality; in this category, 522 people perished and 556 people recovered.

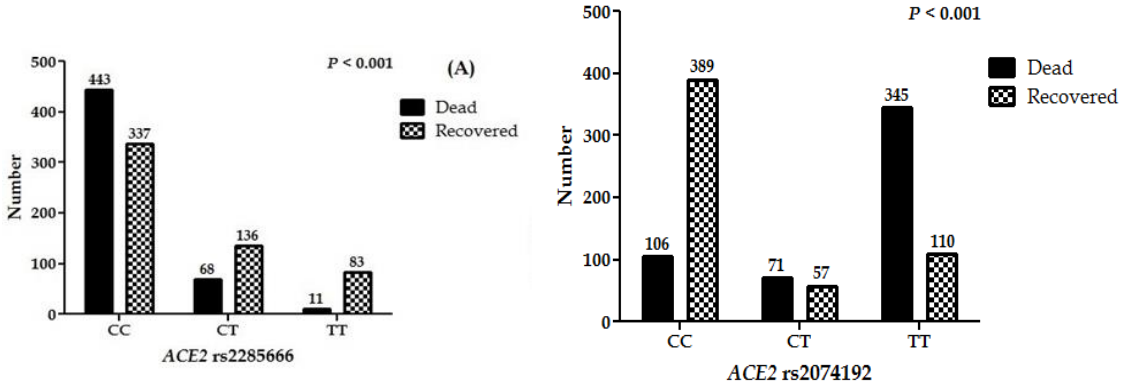

Figure 1. Frequencies of ACE2 rs2285666 (A) and ACE2 rs2074192 (B) in COVID-19 patients

COVID-19 infection mortality rate was correlated with the patients' average age.

Factors associated with COVID-19 mortality

Multivariate logistic regression analysis was used to examine several factors associated with COVID-19 mortality. The

Table 2. Factors associated with deceased patients infected with COVID-19

Factors		
Baseline Predictors	**OR (95 % CI)**	***P*-value**
Mean age ± SD	0.959 (0.942–0.977)	<0.001
Cholesterol, mg/dL	1.039 (1.020–1.059)	<0.001
LDL, mg/dL	1.016 (1.011–1.021)	<0.001
Uric acid, mg/dL	2.169 (1.882–2.498)	<0.001
Creatinine, mg/dL	0.049 (0.025–0.097)	<0.001
ESR, (mm/1st h)	0.971 (0.957–0.984)	<0.001
CRP, mg/L	0.985 (0.976–0.995)	<0.004
25-hydroxyvitamin D, (ng/Ml)	1.062 (1.042–1.082)	<0.001
ALT, IU/L	0.983 (0.974–0.993)	<0.001

ALP, IU/L	0.997 (0.995–1.000)	<0.029
Real-time PCR Ct values	0.912 (0.901-0.965)	<0.003
ACE2 rs2285666 (TT)	7.452 (4.867-11.410)	<0.001
ACE2 rs2274192 (TT)	0.212 (0.131-0.345)	<0.001

ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; Ct, cycle threshold; *ACE2*, Angiotensin-converting enzyme2; SD, standard deviation; OR: Odds ratios; CI: confidence intervals; *Statistically significant ($p < 0.05$).

The results of determining ACE2 rs2285666 and ACE2 2074192 polymorphisms were obtained by the RFLP PCR and Tetra-Arms PCR techniques using the designed primers with lengths of 673 and 777 base pairs, respectively (Fig. 2 a & b).

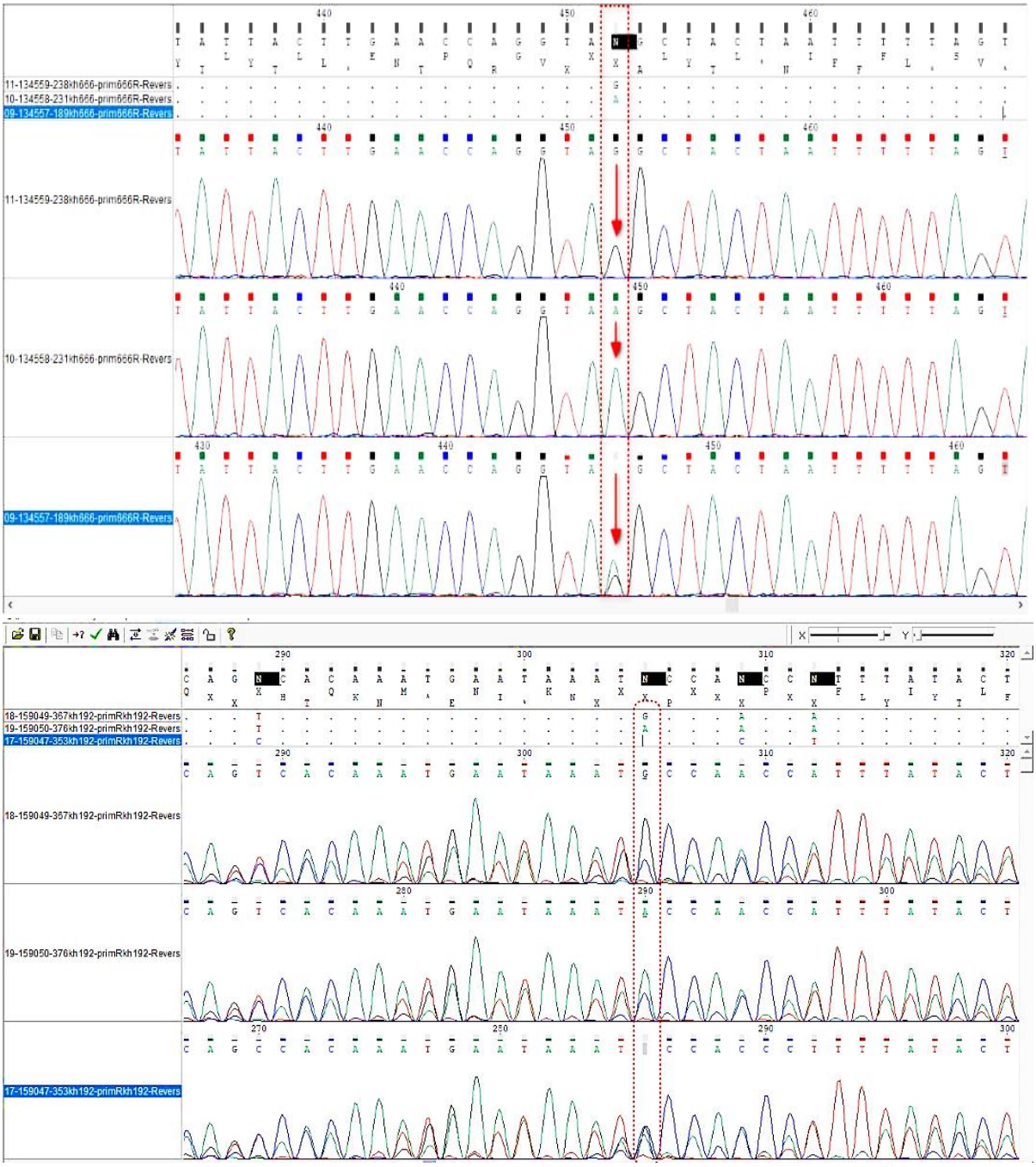

Figure 2. Confirmation of the results of rs2285666 (A) and rs2074192 (B) polymorphisms by the sequencing method

Discussion

This thorough study aimed to determine whether the severity and death rate of SARS-CoV-2 infection in Persian patients are linked to the ACE2 rs2285666 and rs2074192 polymorphisms in COVID-19 patients. Our findings indicate a connection among COVID-19 death rate and the ACE2 alleles rs2285666 and rs2074192. Numerous ongoing studies are currently accessible regarding the consequence of host genetic factors on the probability of SARS-CoV-2 infection or the severity of COVID-19. According to the above scientists' theory, the polymorphisms of the gene ACE or ACE2 may affect the likelihood of infection [5].

A comparison of the two patient groups showed that the CC genotype and ACE2 rs2285666 had a greater mortality rate than the TT genotype. According to other research [10, 5], a higher mortality rate was seen in TT than in CC in the ACE2 rs2074192 study. Numerous studies have connected the genotypes CC and TT of these two SNPs to the frequency and risk of SARS-CoV-2 infections. However, it was impossible to find any studies on how this SNP affects the COVID-19 mortality rate or the severity of the illness [11].

The fact that our study's group size was larger than that of earlier studies may be the cause of this discrepancy. Studies carried out all over the globe have produced conflicting results regarding the association between the ACE2 rs2285666 and rs2074192 polymorphisms and COVID-19 predisposition. This argument might be the outcome of racial disparities among the categories dues to the presentation of variants some population-based differences variants [12].

Despite the possibility that ACE2 receptor gene expression can have effect on COVID-19 susceptibility, no evidence exist which shows ACE2 gene polymorphisms are directly linked to the severity of COVID-19 [13].

The T allele for SNP rs2074192 in intron 16 of the ACE2 gene was found to be significantly more common in this study's symptomatic group than in the asymptomatic group in both males and women. The rs2074192 polymorphism's T allele is presently recognized for its links to cardiovascular risk, type 2 diabetes-related retinopathy, hypertension, and hypertensive left ventricular hypertrophy. The severe effects of SARS-CoV-2 infection are therefore exacerbated by the low amount of ACE2 [14].

Despite the potential for conflicting findings, this should not cause anyone to ignore how the ACE2 gene polymorphisms affect COVID-19 susceptibility or the severity of the disease. The ACE2 enzyme is associated with substantial lung damage and organ deterioration in those who suffer from COVID-19 infection. These receptors are also necessary for the entry of SARS-CoV-2 into cells, Salt and water retention in high blood pressure and induction of fibrotic and inflammatory diseases leading to cytokine storm [15].

Recent studies suggest that age, liver enzymes, a lipid profile, uric acid, and 25(OH)D are all crucial risk factors for chronic diseases in COVID-19 individuals, this can assist in determining the risk of a serious condition.

The finding of fatty degeneration and centrilobular necrosis in postmortem liver tissue of SARS patients is evidence that SARS-CoV-2 can infect living individuals.

Since her hepatocytes contain ACE2 receptors, a direct viral cytotoxic influence is not possible to be ruled out completely. An indicator of a strong inflammatory reaction is the protein interleukin-6, which is linked to both inflammatory and repair reactions in liver disease [16].

Through the host cell receptor ACE2, SARS-CoV-2 regulates the ACE2/renin-angiotensin pathway [17]. Therefore, acute lung injury and acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 can be mitigated as a result. It has been demonstrated that 25(OH)D modulates the SARS-CoV-2 receptor ACE2. The reduction of ARDS and acute lung injury induced as a result of SARS-CoV-2 may be possible in principle due to the modulation of ACE2 by 25(OH)D [18].

High serum uric acid levels may harm different body tissues, which would increase ACE2. Low urinary urate may hinder ACE2 synthesis in the kidneys as a result of high serum uric acid levels, which are primarily brought on by reduced urinary urate excretion. Importantly, it has recently been discovered that uric acid levels are a key indicator of COVID-19 deterioration. The worsening of COVID-19 may be related to a potential link between uric acid and ACE2. A future study regarding connection among ACE2 and uric acid is required [19].

Elevated amounts of ACE2 in the heart may make it more susceptible to the SARS-CoV-2 infection. Autopsies of SARS-infected patients showed that 35% of patients were positive for the SARS-CoV genome in cardiac tissue, and these patients reported a more aggressive disease than disease-free individuals. Myocardial stromal edema, inflammatory cell infiltration, and myocardial fiber atrophy were all seen in individuals who have SARS and myocardial injury . Heart damage is very common in COVID-19 patients, and this study demonstrates a relationship between an early acute myocardial injury and an increased chance of death rate.

it remains appparent that the ACE2 axis have a positive impact in the heart by reducing cardiac pathological remodeling, relaxing coronary arteries, suppressing oxidative stress, and enhancing post-ischemic cardiac performance. ACE2 expression is normally increased during the early stages of cardiac injury but decreases as the disease progresses. Based on recent studies and our data, hypertension as a comorbidity exists in a significant number of critically ill patients. It is possible that in these individuals RAS may have been overactivated prior to infection. In COVID-19, severe ACE2

downregulation and Ang-II upregulation led to an overactivation of RAS, and the loss of angiotensin 1-7's protective effects may exacerbate and extend cardiac injury. ACE2 is primarily found in pericytes, though it is only weakly expressed in cardiomyocytes [20].

Conclusion

The present data showed a significant association between the COVID-19 mortality rate and the clinical ACE2 gene-related parameters. Furthermore, compared to individuals with the TT genotype, those with the CC genotype in ACE2 rs2285666 had higher chances to die from COVID-19-induced complications. Patients with the TT allele were more likely than those with the CC genotype to die from COVID-19 in ACE2rs2074192.

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