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Association between ACE1 Gene polymorphism and COVID-19 Disease Severity among Sudanese Hospitalized Patients

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Abstract: Introduction: *The diversity of symptoms, signs, and severity in COVID-19 patients has been attributed to various factors, including hereditary issues such as the renin-angiotensin system (RAS). The ACE1 Insertion/Deletion (I/D) polymorphism has been considered a genetic risk factor for COVID-19 and its associated disorders. This case-control hospital-based study included 140 normal individuals and 161 COVID-19 hospitalized patients of different age groups and genders.* ***Objectives:*** *The study aimed to investigate the association between ACE (I/D) polymorphism and disease severity in COVID-19 hospitalized patients.* ***Methods:*** *Venous blood samples were collected for DNA extraction and conventional PCR was performed to detect the ACE (I/D) polymorphism. Data were analyzed using statistical software.* ***Results:*** *The study found that the ACE D/D, ACE D/I, and ACE I/I genotypes were present in 56.5%, 35.4%, and 8.1% of the cases, respectively. The mutant and wild-type alleles were found in 91.9% and 8.1% of the cases, respectively. In the control group, the ACE D/D, ACE D/I, and ACE I/I genotypes were present in 86.4%, 5.7%, and 7.9% respectively, with mutant and wild-type alleles found in 91.9% and 8.1% respectively. The ACE I/D genotype was associated with a higher risk of the disease compared to the control group (OR = 9.04). Furthermore, ACE1 DD genotype was significantly associated with disease severity (P value = 0.007).* ***Conclusion:*** *ACE gene polymorphism, particularly the mutant (D/D) genotype, was associated with disease severity, while the (I/D) genotype was associated with an increased risk of infection.*

Keywords: COVID-19, ACE (I/D) polymorphism, severe disease.

Introduction

In December 2019, a novel coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing COVID-19, emerged. COVID-19 death rates in the black population were found to be six times higher than in the white population, according to United States demographic data (1). Non-Hispanic Blacks have experienced the highest mortality rates among all racial groups in the USA, suggesting that genetic polymorphisms in certain genes and underlying genetic factors may be influencing COVID-19 death rates in black communities (2). Coagulopathy, particularly micro-clots in the lungs, has been associated with COVID-19 and linked to disease severity (3, 4). Evidence suggests that the severe thrombotic symptoms of COVID-19 may be the result of SARS-CoV-2's ability to enter endothelial cells via ACE-2 (angiotensin-converting enzyme 2) when the virus's spike-protein domains bind to ACE2, acting as an anchor for viral homing and replication in cells. ACE2 is expressed on cell membranes in target pulmonary and intestinal host cells (5, 6). The Renin Angiotensin System (RAS), which plays a crucial role in controlling vascular physiology, is negatively regulated by ACE2. ACE1 (angiotensin-converting enzyme 1) is responsible for transforming angiotensin-1 (Ang-I) into angiotensin-2 (Ang-II) in the RAS system, while ACE2 breaks down Ang II into Ang (1, 7). Ang II is known to have pro-inflammatory, pro-fibrotic, and vasoconstrictive effects, while Ang (1, 7) acts as a vasodilator peptide with anti-apoptotic, anti-heart failure, anti-thrombotic, and anti-myocardial hypertrophy properties (8-9). The ACE1 gene is located on chromosome 17 (locus 17q23.3) and displays varying levels of expression due to different gene variations in various populations (10). The ACE1 gene also has an insertion/deletion polymorphism that results in three distinct genotypes: DD and II homozygotes, and ID heterozygotes, with the ID genotype exhibiting intermediate levels of ACE1 activity and the II genotype showing the lowest levels. In contrast, the DD genotype exhibits the highest ACE1 activity, likely due to maintaining two active sites that favor Ang-I to Ang-II formation (8). Several studies have aimed to establish a relationship between the geographical variation of the I/D polymorphism of the ACE1 gene with COVID-19 mortality and disease severity (8). The severity of the disease in hospitalized patients is categorized as moderate or

severe, with moderate cases requiring hospitalization and severe cases requiring ICU treatment and mechanical ventilator support due to acute respiratory distress syndrome and/or non-pulmonary involvement (11).

Methodology:

Study setting and population:
A case-controlhospital-based study carry out to find the relationship between ACE gene polymorphism and the severity of COVID-19 infection. This study included 161 patients who tested positive for SARS-CoV-2 as cases and 140 normal healthy individuals as control. Patients with SARS-CoV2 infection who presented with the COVID-19 characteristic symptoms of fever, cough, and were admitted to the hospital were enrolled.

Sample collection:
A 2.5 ml venous blood sample was collected from each patient into K3EDTA container. The blood sample was mixed with anticoagulant by gently inverting of the container several times. The blood sample was be kept on crushed ice until delivery to the laboratory.

DNA extraction:
DNA extraction and whole genome amplification was done upon admission, venous blood samples was collected in EDTA-containing test tubes.

- Within two hours after collection, plasma will be separated, and pellets frozen at −20°C.
- Genomic DNA was extracted from peripheral blood leucocytes using the QIAamp Blood Mini Kit.

PCR Detection of ACE Insertion/Deletion (I/D) Polymorphism(rs1799752):

- The D/I polymorphism of the ACE gene presence of a 287-base pair (bp) Alu repeat sequence in intron 16. The polymerase chain reaction well be done using specific primers:

Forward primer	5′-CTGGAGACCACTCCCATCCTTTCT- 3′
Reverse primer	5′-GATGTGGCCATCACATTCGTCAGAT-3′

PCR protocol:

Components	Volume
ACEForward primer	0.5 µL
ACE Reverse primer	0.5 µL
Master Mix	4 µL
DNA	5 µL
D.W	10 µL
Total volume	20µL

PCR program:

	Temperature	Time	No. of cycles
Initial denaturation	94^0C	1 min	30 CYCLE
denaturation	94^0 C	45 sec	
Annealing	62^0 C	1 min	
Extension	72^0 C	1 min	
Final extension	72^0C	5 min	

- PCR products was checked on 2 % agarose gel and was visualized by gel documentation system.
- Allele (I/D) types and genotype for each sample well be determined based on the PCR product sizes:

Allele	Size
Insertion (I allele)	(481 - 490) bp
Deletion (D allele)	(190 - 194) bp
Heterozygosity	490/190 bp

Genotypes: I/I = Insertion in homozygosis, I/D = Insertion/Deletion, and D/D = Deletion in homozygosis.

Statistical Analysis:
SPSS version 25.0 and SNPSTAT were used to conduct the statistical analyses. P value 0.05 indicated significance.

Ethical statement:

Ethical clearance was obtained from the Scientific and Research Ethics Committee, Ministry of Health, Gezira State, Sudan. Informed consent was obtained from patients.

Results

The study included a total of 161 cases and 140 controls, representing different age groups and genders. The ACE (I/D) polymorphism was genotyped using conventional PCR. Among the cases, the prevalence of ACE D/D polymorphism was found to be 91 (56.5%), ACE D/I polymorphism to be 57 (35.4%), and ACE I/I polymorphism to be 13 (8.1%), respectively. In contrast, the mutant and wild types allele represented 148 (91.9%) and 13 (8.1%), respectively (Table 1). Among the controls, the ACE D/D polymorphism was determined to be 121 (86.4%), ACE D/I polymorphism to be 8 (5.7%), and ACE I/I polymorphism to be 11 (7.9%) (Table 1). The moderate cases represented 109 (67.7%) while the severe cases represented 52 (32.3%) (Table 1). A significant difference (P-value = 0.0001) was observed when comparing the recessive D/D genotype against the I/D and I/I genotypes, but it was not associated with the risk of the disease (OR = 0.2). There was no significant difference (P-value = 0.944) and no association with the risk of the disease (OR = 0.97) when comparing the dominant D/D and I/D genotypes against the I/I genotype (Table 2). However, a highly significant association was found between COVID-19 infection and the over-dominant I/D genotype (OR = 9.04) (Table 2). There was also a highly significant difference between ACE (I/D) polymorphism and disease severity (P-value = 0.007) (Table 3).

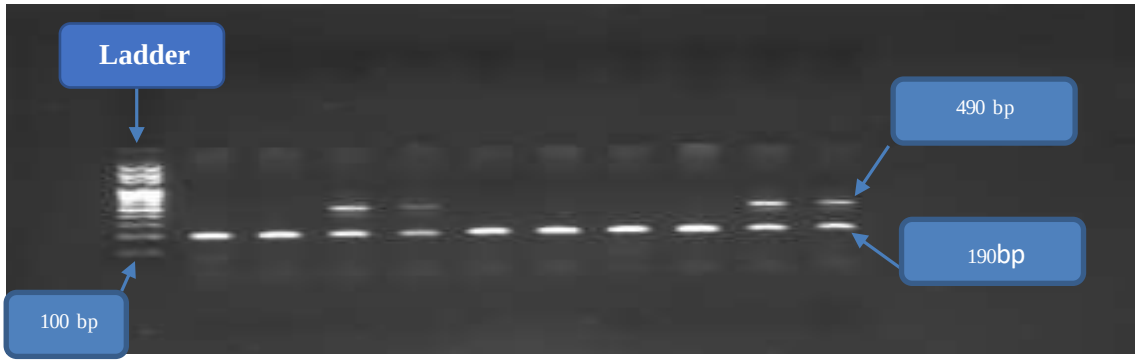

Figure (1): gel electrophoresis for detection ACE (I/D) polymorphism:
PCR on blood samples of SARS-CoV-2 patients for detection of ACE I/D polymorphism. I allele is demonstrated as a 490 bp band and the D allele as a 190 bp band.

Table 1. Demographic characteristics and ACE (I/D) gene polymorphism of study cases.

Factors	Cases (N = 161)	Controls (N = 140)
Age group (years)		
65 years and Less	78 (48.4%)	-
More than 65 year	83 (51.6%)	
Gender		
Male	110 (68.3%)	101 (72.1)
Female	51 (31.7%)	39 (27.9%)
Gene polymorphism		
D/D	91 (56.5%)	121 (86.4%)
I/D	57 (35.4%)	8 (5.7%)
I/I	13 (8.1%)	11 (7.9%)
Mutant		
Present	148 (91.9%)	129 (92.1%)
Absent	13 (8.1%)	11 (7.9%)
Disease severity		
Moderate	109 (67.7%)	-
Severe	52 (32.3%)	-

Table 2. Comparison of ACE(I/D) gene polymorphism between cases and controls.

Model	Genotype	STATUS=Cases	STATUS=Control	OR (95% CI)	P-value
Recessive	**D/D**	91 (56.5%)	121 (86.4%)	0.20 (0.11-0.36)	**0.0001**
	I/D-I/I	70 (43.5%)	19 (13.6%)		
Dominant	**D/D-I/D**	148 (91.9%)	129 (92.1%)	0.97 (0.42-2.24)	0.944
	I/I	13 (8.1%)	11 (7.9%)		
Over dominant	**I/D**	57 (35.4%)	8 (5.7%)	9.04 (4.13-19.79)	NA
	D/D-I/I	104 (64.6%)	132 (94.3%)		

Table 3. Association between ACE (I/D) gene polymorphism and disease severity.

Severity	I\D Polymorphism			Total	P-value
	D/D	I/D	I/I		
Moderate	53 (48.6%)	47 (43.1%)	9 (8.3%)	109 (100%)	**0.007**
Severe	38 (73.1%)	10 (19.2%)	4 (7.7%)	52 (100%)	
Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	

Discussion

This study is a hospital-based case-control study conducted during the second wave of COVID-19 in 2021 at a Mycetoma isolation center in Gezira state, Sudan. The study included 161 cases and 140 controls. The majority of participants were males, accounting for 68.3% of cases and 72.1% of controls, which was a significant majority. The majority of participants were also older than 65 years old, representing 51.6% of the total population.

Among the cases, the ACE (D/D) genotype was the most common, accounting for 56.5% of cases, followed by ACE (I/D) and ACE (I/I) genotypes, each representing 35.4% of cases. The wild type and mutant alleles were represented by 8.1% and 91.9% of the population, respectively. These findings are consistent with previous studies conducted in Sudan (12-14).

In terms of disease severity, moderate cases were more common than severe cases, accounting for 67.7% and 32.3% of cases, respectively. The results showed that the ACE (I/D) genotype was associated with a higher risk of disease when compared to controls under an overdominant pattern of inheritance, with an odds ratio (OR) of 9.04. This is supported by the overdominance model, which suggests that heterozygotes have higher expression compared to homozygotes in the Sudanese population. On the other hand, the ACE (I/I) and ACE (D/D) genotypes were considered as protective genotypes under a recessive and dominant mode of inheritance, with ORs of 0.20 and 0.97 respectively (15).

Furthermore, the ACE (D/D) genotype was significantly associated with disease severity, with a p-value of 0.007, which is in agreement with previous studies (8, 16-17) but disagrees with some other studies, possibly due to differences in ethnic origin of the study population (18). The ethnic origin of the study population may contribute with this finding.

Overall, this study suggests an association between ACE (I/D) polymorphism and COVID-19 severity among hospitalized patients in Sudan, with the ACE (I/D) genotype being associated with a higher risk of disease and the ACE (I/I) and ACE (D/D) genotypes potentially providing a protective effect. However, further research with larger sample sizes and consideration of confounding factors is needed to confirm these findings and better understand the role of ACE (I/D) polymorphism in COVID-19 severity in different populations.

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