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Genetic Susceptibility of ACE1 Gene Polymorphism with Comorbid Diseases among Sudanese COVID-19 Patients

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Abstract: Introduction: *The variation in symptoms and severity of COVID-19 among patients could be linked to genetic issues, including defects in the renin-angiotensin system (RAS) pathway. The ACE1 Insertion/Deletion (I/D) polymorphism gene, which regulates the RAS pathway, may also explain the genetic susceptibility to COVID-19 and its association with other underlying health conditions, as well as the different ways the disease presents in individuals.* ***Methodology:*** *In this research, a total of 161 Sudanese individuals who were hospitalized due to COVID-19 were included. The objective of the study was to investigate the relationship between ACE (I/D) gene polymorphism and the presence of comorbid diseases in this population. Blood samples were collected from the participants to detect the ACE1 gene Insertion/Deletion (I/D) polymorphism. The data on socio-demographic characteristics and risk factors were then analyzed in correlation with the detected polymorphism.* ***Results:*** *Among the 161 patients included in the study, males accounted for 68.3% (110/161) while females accounted for 31.7% (51/161). The most common comorbidities observed were hypertension and diabetes mellitus, which accounted for 52.8% (85/161) of the cases. The ACE (I/D) gene polymorphism showed significant differences when comparing asthmatic and non-asthmatic COVID-19 patients, with a p-value of 0.027. The mutant (D) allele was found to be present in 100% of asthmatic, cardiac, renal disease, diabetic, and hypertensive patients, with odds ratios (OR) of 9.08, 1.83, 1.35, 2.72, and 1.88 respectively.* ***Conclusion:*** *The presence of ACE gene polymorphism, specifically the mutant (D) allele, was found to be associated with various comorbid diseases, particularly asthma and cardiovascular diseases, in the studied COVID-19 patients. Additionally, the I/I genotype was not observed to be a risk factor for comorbid diseases in this population.*

Keywords: COVID-19, ACE (I/D) polymorphism, comorbid diseases.

Introduction

An unprecedented pandemic has been caused by the rapid global spread of coronavirus disease-19 (COVID-19) (1). As of 18 December 2020, 222 countries and territories had received reports of more than 72 million cases and 1.6 million fatalities (2). On February 26, 2020, Sudan reported its first verified COVID-19 case; as of March 30, 2021, there were 29825 confirmed cases (of which 2587 were reported in Gezira State) (3). Despite reports of increased infection rates among African Americans, the information regarding verified COVID-19 infections by race is mainly uncomplete (4). Therefore, it is possible that the gap in COVID-19 mortality rates in black communities is being influenced by genetic polymorphisms in numerous genes and an underlying genetic susceptibility to SARS-CoV-2 infection. The fact that thrombotic issues are markers of severe COVID-19 and are associated with multi-organ failure and greater mortality is relevant (5). In COVID-19 patients receiving care in intensive care units (ICUs), deep vein thrombosis and pulmonary embolism were observed to occur in 20.6% to 49.0% of cases (6). It is currently unknown what causes the elevated thromboembolic risk in pneumonia caused by COVID-19. The direct cytotoxic effect of the virus, endothelial cell inflammation, and the dysregulated immune response are just a few of the mechanisms at work in this process. These factors all help to activate the complement and coagulation cascades, platelet aggregation, and the recruitment of inflammatory cells (7). The cooperation of ACE1 and ACE2 in the local vasoconstrictor/proliferative (ACE1/Ang-II axis) and vasodilator/anti-proliferative (ACE2/Ang1-7 axis) activities is balanced by (RAS). As a result, the anticoagulant, anti-inflammatory, anti-fibrotic, anti-alveolar epithelial cell apoptosis, and anti-oxidative stress actions become antagonistic, protecting the organs and blood vessels (8). Given

that ACE and ACE2 have the opposite effects, decreased ACE2 receptor gene expression is closely associated with increased ACE expression (9). Because the viral intracellular translocation and replication result in the depletion of membraneal ACE2 through degradation and shedding, the balance between ACE and ACE2 activities occurring in COVID-19 may be critical in the thrombo-inflammatory process (7, 10). Therefore, the absence of ACE2 allows Angiotensin II to act unchecked, which causes vasoconstriction, endothelial damage, endovascular thrombosis, and increased blood volume (7). Angiotensin II is a powerful vasoconstrictor as well as a pro-atherogenic substance that raises plasminogen activator inhibitor-1 (PAI-1) levels and reduces fibrinolytic activity (11). The mutual levels are largely regulated by common genetic variants of the ACE1 and ACE2 genes. There have been reports of significant racial and gender disparities in the frequency of ACE1 and ACE2 gene polymorphisms in subgro ups of individuals with a high risk of a bad outcome from COVID-19 (such as male sex, Black ethnicity, and cardiovascular disease). Additionally, these variations seem to be associated to COVID-19 morbidity and mortality rates (8). The findings of a biological predisposition for high-risk comorbid conditions, on the other hand, may be crucial for comprehending and solving COVID-19 deaths among blacks (12-13). Therefore, the purpose of this study is to ascertain how the ACE gene polymorphism and the severity of COVID-19 infection are related.

Methodology

Study setting and population:

It was intended to conduct a cross-sectional prospective hospitalized study to examine the relationship between the ACE gene polymorphism and the severity of COVID-19 infection. This study included 161 of the patients who tested positive for SARS-CoV-2. Patients who had already been diagnosed with SARS-CoV2 infection and were admitted to the hospital with the COVID-19 typical symptoms of fever and cough were included in the study.

Sample Collection:

Each patient's 2.5 ml of venous blood was drawn into a K3EDTA container for DNA extraction. Anticoagulant was combined with the blood sample by repeatedly inverting the container. The blood sample was transported to the lab on crushed ice.

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 was separated, and pellets frozen at −20°C. Genomic DNA was extracted from peripheral blood leucocytes using the G-spinTM total DNA Extraction Kit.

Molecular analysis of ACE gen 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:

- The PCR was performed in 15 µL volume containing 10 pmol of each primer, 1× PCR master mix, 3 mM MgCl2, and 2 U Taq polymerase.
- PCR sequence amplification was performed under the conditions: Initial denaturation at 95 ◦C for 5 min, followed by 35 cycles of denaturation at 95 ◦C for 45 s, annealing at 60 ◦C for 1.15 min, extension at 72 ◦C for 2.30 min and final extension at 72 ◦C for 10 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:
    - ✓ Insertion (I allele): The major allele indicates the 490 bp 481 bp fragment that embraces the 287bp sequence.
    - ✓ Deletion (D allele):The 190 bp 194bp fragment represents the minor allele with the deletion of the sequence
    - ✓ Heterozygosity specifies the combination of both major and minor fragments, that is, 490/190 bp.

Genotypes: I/I = Insertion in homozygosis, I/D = Insertion/Deletion, and D/D = Deletion in homozygosis.
Alleles: mutant (D) D/D and D/I alleles.

Statistical Analysis:

Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, New York, United States). Significance was observed with P value < 0.05.The genotyping and alleles were analyzed using SNPSTAT online program.

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

There were 161 participants in this study, representing a variety of ages and genders. According to (Table 3) results from conventional PCR used to determine the frequencies of ACE I/D gene polymorphism in patient peripheral blood samples, 91 (56.5%)

of SARS-CoV-2-infected patients had ACE D/D polymorphism, 57 (35.4%) had ACE D/I polymorphism, and 13 (8.1%) had ACE I/I polymorphism. More specifically, the mutant and broad type alleles account for 148 (91.9%) and 13 (8.1%) of the total. 37 (100%) patients with asthma, 9 (100%) patients with cardiac disease, 15 (93.8%) patients with renal illness, 81 (95.3%) patients with diabetes, and 80 (94.1%) patients with hypertension all have mutant (D) alleles, according to research (Table 4). The COVID-19 patients with various ACE genotypes demonstrated that there were no differences in all variants across age groups and gender. On the other hand, there are no differences in community and home contact across all ACE1 genetic variations. When comparing asthmatic and non-asthmatic COVID-19 individuals, the ACE (I/D) polymorphism reveals substantial differences. (p- value 0.027). There are no appreciable variations among COVID-19 patients for the other concomitant disease (Table 5).

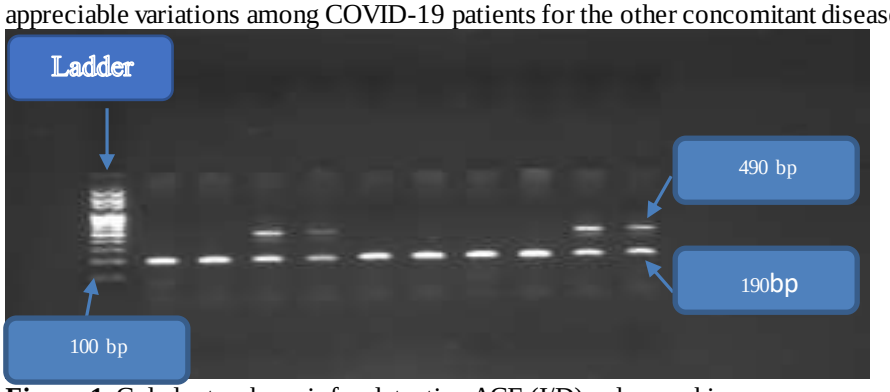

Figure 1. Gel electrophoresis for detection ACE (I/D) polymorphism:
PCR on blood samples of SARS-CoV-2 patients for detection of ACE I/D polymorphism. I allele is demonstrated as a 490 bp band and the D allele as a 190 bp band.

Table 1. Demographic characteristics of study population.

Factors	Cases (N = 161)
Age group (years)	
65 years and Less	78 (48.4%)
More than 65 year	83 (51.6%)
Gender	
Male	110 (68.3%)
Female	51 (31.7%)
House hold contact	
Yes	57 (35.4%)
No	104 (64.6%)
Community contact	
Yes	63 (39.1%)
No	98 (60.9%)

Table 2. Frequency of comorbidities among study population.

Comorbidities	Frequency (%)
Hypertension	85 (52.8%)
D.M	85 (52.8%)
Asthma	37 (23%)
Renal disorders	16 (9.9%)
Cardiac diseases	9 (5.6%)

Table 3. Frequency of ACE (I/D) gene polymorphism among study population.

Factors	Cases (N = 161)
Gene polymorphism	
D/D	91 (56.5%)
I/D	57 (35.4%)
I/I	13 (8.1%)
Mutant	
Present	148 (91.9%)
Absent	13 (8.1%)

Table 4. Odd ratio of ACE (I/D) gene polymorphism with comorbidity.

Polymorphism / variables		D/D	I/D	I/I	Total	P value
Age groups	≤ 65 years	44 (56.4%)	28 (35.9%)	6 (7.7%)	78 (100%)	0.981
	>65 years	47 (56.6%)	29 (34.9%)	7 (8.4%)	83 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
. Gender	Male	63 (57.3%)	37 (33.6%)	10 (9.1%)	110 (100%)	0.675
	Female	28 (54.9%)	20 (39.2%)	3 (5.9%)	51 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Hypertension	No	39 (51.3%)	29 (38.2%)	8 (10.5%)	76 (100%)	0.335
	Yes	52 (61.2%)	28 (32.9%)	5 (5.9%)	85 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Diabetes	No	42 (53.3%)	25 (32.9%)	9(11.8%)	76 (100%)	0.243
	Yes	49 (57.6%)	32 (37.6%)	4 (4.7%)	85 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Asthma	No	69 (55.6%)	42 (33.9%)	13 (10.5%)	124 (100%)	**0.027**
	Yes	22 (59.5 %)	15 (40.5%)	0 (0.0%)	37 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Renal disorders	No	80 (55.2%)	53 (36.6%)	12 (8.3%)	145 (100%)	0.581
	Yes	11 (68.8%)	4 (20.5%)	1 (6.3%)	16 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Cardiac disorders	No	84 (55.3%)	55 (36.2%)	13 (8.6%)	152 (100%)	0.257
	Yes	7 (77.8%)	2 (22.2%)	0 (0.0%)	9 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
House hold contact	No	56 (53.8%)	38 (36.5%)	10 (9%)	104 (100%)	0.496
	Yes	35 (61.4 %)	19 (33.3%)	3 (5.3%)	57 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	
Community exposure	No	54 (55.1%)	34 (34.7%)	10 (10.2)	98 (100%)	0.441
	Yes	37 58.7%)(	23 (36.5%)	3 (4.8%)	63 (100%)	
	Total	91 (56.5%)	57 (35.4%)	13 (8.1%)	161 (100%)	

Table 5. Association between ACE (I/D) genotypes and risk factors.

Comorbidity		D/D-I/D	I/I	OR (95% CI)	P-value
Asthma	Yes	37 (100%)	0 (0%)	9.08 (0.53-156.49)	0.130
	No	111 (89.5%)	13 (10.5%)		
Cardiac Disease	Yes	9 (100%)	0 (0%)	1.83 (0.10-33.35)	0.680
	No	139 (91.5%)	13 (8.6%)		
Renal disorder	Yes	15 (93.8%)	1 (6.2%)	1.35 (0.16-11.15)	0.770
	No	133 (91.7%)	12 (8.3%)		
Hypertension	Yes	80 (94.1%)	5 (5.9%)	1.88(0.59-6.02)	0.280
	No	68 (89.5%)	8 (10.5%)		
Diabetic	Yes	81 (95.3%)	4 (4.7%)	2.72 (0.80-9.23)	0.100
	No	67 (88.2%)	9 (11.8%)		

Discussion

With regard to COVID-19 hospitalized patients who visit the Mycetoma isolation Centre in Gezira state, Sudan, during the second wave of 2021, the goal of this hospital-based cross-sectional study is to ascertain the relationship between ACE (I/D polymorphism and concomitant disorders. There were 161 participants in this study. Males made up 68.3% of the population, which is a significant majority. The majority of participants (51.6%) were older than 65 years old based on the age distribution. After 1.5% gel electrophoresis and DNA extraction, the results are amplified and then seen under a UV lamp. SPSS version 25 and the online SNPSTAT programme were used to analyze the data. There are no statistically significant differences in the frequency of these genetic polymorphisms when compared to other age groups or genders (P values 0.981 and 0.675, respectively). This result differs

from that reported by (14-15), and it is possible that this is because the sample size and racial makeup were different. When comparing the asthmatic and non-asthmatic COVID-19 patients with different genotypes, there was a significant difference with regard to comorbid diseases in 37 (100%) asthmatic patients with mutant D allele (OR = 9.08); this was explained as the asthmatic patients associated with ACE (I/D) and (D/D) genotypes; this result is consistent with (15-17). 15 Patients with renal illness who carry the mutant D allele are 93.8% (OR = 1.83). A mutant D allele was found in 9 (100%) cardiac patients (OR = 1.35).Nine (100%) cardiac patients (OR = 1.83), 15 (93.8%) renal disease patients (OR = 1.35), 81 (95.3%) diabetic patients (OR = 2.72), and 80 (94.1%) hypertension patients (OR = 1.88) all have the mutant D allele. This outcome agreed with (14, 18-19). Between ACE (I/D) polymorphism and other concomitant diseases (hypertension, diabetes mellitus, renal disorders, and cardiac disorders), there were no appreciable changes (P value = 0.335, 0.243, 0.581 and 0.257 respectively). Other confounding factors, such as the presence or absence of acquired or genetic problems, may have contributed to these findings, as well as the presence or absence of multiple or a single comorbid disease in some patients. Additionally, this study discovered that there were no statistically significant differences between the groups in the prevalence of cardiac illnesses linked to the ACE (D/D) and (I/D) genotypes.

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