

The Role of ACE2 rs41303171 Gene Polymorphism in Susceptibility for Infection with SARS-CoV-2

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Abstract

Background: Angiotensin-converting enzyme 2 (ACE2) is a human receptor for Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2), and successful interactions between the spike protein of SARS-CoV-2 and ACE2 are responsible for the initial and complete infection. The aim of the current study included: the determination of Single Nucleotide Polymorphisms A/G of the ACE2 rs41303171 gene and their association with the susceptibility to infection with SARS-CoV-2.

Material and Methods: Allele Specific-polymerase Chain reaction (AS-PCR) method was used for investigating Single Nucleotide polymorphisms A/G of ACE2 rs41303171 in different states of patients with COVID-19,

Results: The wild genotype of (GG) for the ACE2 rs41303171 gene may be more susceptible to the infection with SARS-CoV-2. In contrast, the heterozygote genotype (AG) and mutated genotype (AA) for ACE2 rs41303171 gene may be associated with more protection from the infection with SARS-CoV-2.

Conclusion: the main findings of our study show an apparent positive correlation between susceptibility to infection and severity of COVID-19 with the qualitative expression of the ACE2 gene in Iraqi people.

Keywords: COVID-19, Single Nucleotide Polymorphism of ACE2 rs41303171, ACE2 gene polymorphisms, Genetic Susceptibility to Infection with SARS-CoV-2.

1. Introduction

The angiotensin-converting enzyme 2 (ACE2) is the host receptor for SARS CoV, HCoV-NL63, and the novel COVs; SARS CoV-2. While the particular function of ACE2 is regulated blood pressure and preserved blood pressure homeostasis through the renin-angiotensin system [1, 2]. The susceptibility to SARS CoV was associated with the level expression of ACE2 in the lung cells particularly the alveolar epithelial type II cells which are the first expression site of ACE2 in healthy human lungs and, play an important role in the gas exchange [3]. However, the expression rate of ACE2 in the various organs, tissues, and cells may be crucial to the susceptibility and symptoms of the covid-19 disease. Thus, the genetic variation in the ACE2 could be critical to the outcome of SARS CoV-2 infection [4, 5]. The single-nucleated polymorphism (SNP) of the ACE2 gene may affect the entry of the SARS CoV-2 into the host cell, either by shifting its expression levels or increasing the binding affinity for the virus [3]. Thus the genetic polymorphisms that control the expression of ACE2 or the strength of these interactions can impact the severity of COVID-19. Recent studies aimed to compare the ACE2 functional coding variants in populations with endemic COVID-19 [6]. Single-nucleotide polymorphism studies have been done in different populations to find the correlation with various diseases. SNPs have been found to affect gene expression resulting in disease outcomes [7]. On the other hand, single nucleotide polymorphisms (SNPs) in ACE2 may result in modulation of the RAS pathway and associated cardiovascular diseases [8]. Different populations may exhibit a different

response to SARS-CoV-2 because of such genetic differences [1]. SNPs of ACE2 have also been studied in different populations to find the correlation with diseases, including essential hypertension, dyslipidemia, hypertrophic cardiomyopathy, ventricular hypertrophy, and cerebral malaria. Many of the studied polymorphisms were found to affect ACE2 activity resulting in the downregulation of circulating angiotensin [7].

ACE2 I/D polymorphism greatly varies from population to population. SNPs rs200180615 and rs140473595 were found in the Han Chinese population with the allele frequency, AF < 0.01. Polymorphism rs2285666 occurred at higher AF in South Han Chinese populations compared to Mixed American, African, and European populations. The reported frequency of homozygous genotype was almost double in males (0.550), than that of females (0.310) in the Chinese population [8]. Understanding the natural ACE2 polymorphism spectrum not only provides information on the SARS-CoV-2 susceptibility but can also be used to create high affinity, rationally designed soluble ACE2 receptor molecules. Such agents that carry naturally occurring polymorphism(s) will lead to no or low immunogenicity in a drug setting and can be utilized as a decoy-receptor for treating patients [5]. Furthermore, the database analysis had been revealed the presence of 1700 variants in the ACE2 region on the X chromosome, and they identified 14 SNPs and 1 insertion/deletion with higher minor allele frequencies in the Asian population than in the European population [9]. Furthermore, various bioinformatics systems including Protein analysis through evolutionary relationships (PANTHER), Functional analysis of SNPs through Hidden Markov Models (FTHMM), and Analysis of protein stability and alteration by (iPTREE software) predicted that rs41303171, rs4646116, and

rs149039346 have affected function and protein stability through deleterious, and disabilities of ACE2 [10]. On the other hand, Wang et al. [11] constructed a complex model of human ACE2 binding to spike protein of ACE2 by molecular dynamic simulation and noted that the human ACE2 is a polymorphous gene and defined 317 missense Single nucleotide variants (SNVs) from the dbSNP database. They addressed three SNVs K26R (rs4646116), E329G (rs143936283), and M821 (rs267606406) which have a significant decrease in binding free energy, which marked higher binding affinity than wild-type ACE2 and increased susceptibility to infection with SARS-CoV-2 for people with them [11].

Up to our Knowledge, there is the first local study for detecting the ACE2 rs41303171 gene polymorphisms and it is associated with susceptibility to infection with SARS-CoV-2 by using the Allele Specific-PCR method.

Patients and Controls

The case-control study included one hundred sixty patients who were diagnosed (clinically and radiology) with COVID-19 (50 Severe, 40 Moderates, and, 70 Mild COVID-19) and sixty healthy volunteers as a control group who had no history or clinical evidence of COVID-19, their range age was over 18years. The patients were charged to AL-Amal Hospital for Communicable diseases, Najaf/Iraq during the period from October 2021 to July 2022.

Collection of samples

All patients' specimens were collected under biosafety precautions and handled in a BSL2 biosafety hood with BSL3 practice. Samples were collected from patients with COVID-19 who tested positive for SARS-CoV-2 RT-PCR and were admitted to AL-Amal for communicable diseases. As well as 60 healthy volunteers in a control group who tested negative for SARS-CoV-2 RT-PCR with no clinical evidence of COVID-19. Five ml of whole blood had collected after cleaning the skin with 70% alcohol by venipuncture, then the venous blood samples were collected into EDTA tubes for the Allele specific-PCR method. On the other hand, the samples were taken after getting the consent of all participants in the study.

2. Material and Methods

The allele Specific-polymerase Chain reaction (AS-PCR) method was used to analysis of ACE2 polymorphism in patients with COVID-19. AS-PCR is a technique effectively used to analyze single nucleotide polymorphism (SNP) that includes the insertion/deletion, transition, and transversion polymorphism and has been mostly used in the study of disease and forensic biological evidence [12]. Furthermore, AS-PCR is an extension of the allele-specific hybridization principle, this method is accomplished by allele-specific primer annealing followed by PCR amplification [13]. The kits used in the Allele-specific PCR method for detection of ACE2 SNP polymorphism included: ReliaPrep Blood gDNA MiniPrep System Promega from the USA for DNA extraction and PCR Master Mix {Taq DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), Tris. HCl pH 9.0, KCl and MgCl₂}. In addition to Specific six primers of ACE2 gene polymorphism which included: three primers of rs41303171, which were designed by Fast PCR, those primers were provided by IDT

(Canada) as shown in table (1).

3. Statistical Analysis

The stational analysis of results was performed by using a multiples computer program that includes the Chi-square test to assess the genotypes and allele frequencies between patients and controls, in addition to the Hardy-Weinberg equilibrium (HWE) which is used to the representativeness of alleles and genotypes by comparing the observed and expected frequencies of genetic variants. However, the genotype and allele distributions were determined in each group, and the odds ratios (OR) with 95% confidence intervals (95% CI) have been calculated. As well, a p-value of <0.05 was considered statistically significant as a confidence interval (CI) of 95%.

Primers	Sequence	Allele	Amplicon	Source
ACE2 rs41303171 A/G	F1 5- TGATGCTTCCGTCTGAATGA CA-3	A	728bp	NCBI
	F2 5- TGATGCTTCCGTCTGAATGA CG-3	G		
	R 5- GAAATCGAGGTTGCAGTGAA CT-3			

4. The Results

Molecular Assessment of ACE2 rs41303171 Polymorphism that Associated with Different Severity of COVID-19.

In Mild cases of COVID-19, the genotypes (GG, GA) of ACE2 rs41303171 gene polymorphism displayed a significant association (p-value=0.002) in comparison with the control group. While the Genotypes of ACE2 (AA) showed no significant association between Mild cases of COVID-19 and the control group. In addition to that, allele ACE2 G rs41303171 had a high ratio in Mild cases of COVID-19 (71.42%) in comparison with the control group. In contrast, allele ACE2 A rs41303171 had a high ratio in the control group (58.33%). The genotypes (GG, GA, AA) and alleles (G, A) of the ACE2 rs41303171 gene were significantly associated with the Moderate cases of COVID-19 and the control group. In detail, the wild genotype ACE2 (GG) of rs41303171 genes has a high ratio in the Moderate cases (60%) of COVID-19 disease. While genotype ACE2 (GA) of rs41303171 genes had a high ratio in the control group. Allele G was highly significant in Moderate cases of COVID-19 disease, while Allele A was highly significant in the control group. On the other hand, in Severe cases of COVID-19, wild genotypes (GG) and heterozygotes genotypes (GA) and the homozygote genotypes (AA) of ACE2 rs41303171 gene polymorphism displayed significant association (p-value= 0.0001) in comparison with the control group. However, the genotype of ACE2 (GG) had a high ratio (92%) in Severe cases of COVID-19 while the genotypes (GA) of ACE2 showed a high ratio in control groups (70%). In addition to that, allele G of ACE2 rs41303171 had a high ratio in

Severe cases of COVID-19 (96%), while, allele A of ACE2 rs41303171 had a high ratio in the control group (58%).

Table (2) Distribution of the genotypes (GG, GA, AA) and alleles (G, A) of ACE2 rs41303171 gene in the Mild, Moderate, and Severe COVID-19 and the control group							
Genotype / allele	Mild COVID N=70		Control N=60		P-value	OR	95% CI
	No.	%	No.	%			
Genotype:							
GG	38	54.28	4	6.66	0.0001(S)	16.6250	5.4347 to 50.8564
GA	24	34.28	42	70	0.0001(S)	0.2236	0.1066 to 0.4689
AA	8	11.42	14	23.33	0.0763(NS)	0.4240	0.1642 to 1.0948
Alleles							
G	100	71.42	50	41.66	0.0001(S)	3.500	2.0893 to 5.8631
A	40	28.57	70	58.33	0.0001(S)	0.2857	0.1706 to 0.4786
Genotype / allele	Moderate COVID-19 N=40		Control N=60		P value	OR	95% CI
	No.	%	No.	%			
Genotypes							
GG	24	60	4	6.66	0.0001(S)	21	6.3537 to 69.4079
GA	14	35	42	70	0.0008(S)	0.2308	0.0984 to 0.5414
AA	2	5	14	23.33	0.0258(S)	0.1729	0.0370 to 0.8088
Alleles							
G	62	77.5	50	41.66	0.0001(S)	4.8222	2.5477 to 9.1273
A	18	22.5	70	58.33	0.0001(S)	0.2074	0.1096 to 0.3925
Genotype / allele	Severe COVID-19 N=50		Control N=60		P value	OR	95% CI
	No	%	No	%			
GG	46	92	4	6.66	0.0001(S)	161	38.1545 to 679.36
GA	4	8	42	70	0.0001(S)	0.0373	0.01117 to 0.1190
AA	0	0	14	23.33	0.0176(S)	0.0614	0.0018 to 0.5474
Alleles							
G	96	96	50	41.66	0.0001(S)	33.6	11.5943 to 97.37
A	4	4	70	58.33	0.0001(S)	0.0298	0.0103 to 0.0862

S: Significant association at P <0.05; NS: No significant association at P>0.05.

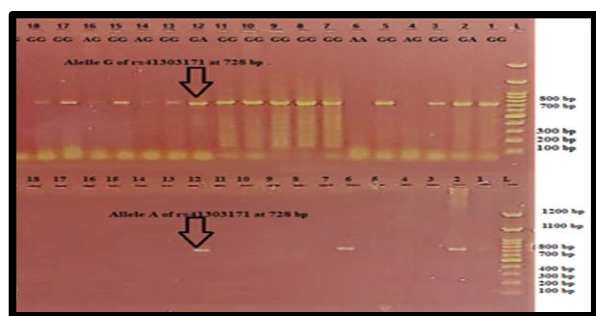


Figure (1): Agarose gel electrophoresis (1.5%, 1x TBE) showing 728 bp PCR product of ACE2 rs41303171 for alleles A and, G by an allele-specific PCR assay. Line 4, 6, 12, 14, 16, appeared the genotype AG, while the line 1, 3, 5, 7, 8, 9, 10, 11, 13, 15, 17,18, showed the genotype GG. Agarose gel at 80% volts for one hour, Line L: 100-1500 bp ladder.

5. Discussion

The current study aimed to find the possible relation between ACE2 polymorphisms and infection with Severe Acute Respiratory Disease Syndrome Coronavirus-2 (SARS-CoV-2) in Iraqi people. The results addressed variants of the ACE2 gene rs41303171 A/G as the following:

Only the genotype (GG) of the ACE2 rs41303171 gene was highly significantly associated with Mild, Moderate and, Severe infection with COVID-19 that reported (OR= 161,

21 and, 16.63) respectively in comparison with the control group as it is shown in tables (2). This genotype (GG) revealed a common wild type in the ACE2 rs41303171 gene in Iraqi people while the heterozygous genotypes (GA) reported a high ratio in the control group (n=42, 70%), in comparison with Mild (OR=0.2236), Moderate (OR=0.2308), and Severe (OR=0.0373) cases of COVID-19. The homozygous mutated genotype (AA) of the same gene had a high ratio in the control group (n= 14, 23.33%) and in comparison, to Mild (n=8, 11.42%), OR (0.4240), Moderate (n=2, 5%), OR (0.1729) and Severe (0.0%), OR (0.0614) cases of COVID-19. These results suggested that the genotype (GG) of ACE2 rs41303171 is probably related to infection with COVID-19 in contrast to the genotypes (AG) and (AA) of ACE2 rs41303171 may be associated with resistance to the infection with SARS-CoV-2. In addition to that, allele G displays a highly significant association with different statuses of COVID-19 Mild, Moderate, and Severe, with odds ratios of (3.500), (4.822), and (33.6), respectively. While allele A highly presented in control groups (58.33%, n=70) with an odds ratio (0.2857), (0.2074), and (0.0298) in Mild, Moderate, and Severe cases of COVID-19, respectively.

These results were in agreement with Benetti et al. [14] who suggested that ACE2 rs41303171 A (p.Asn720Asp) and other SNPs maybe interfere with the stability and protein structure. Also, they found in the Italian population a higher significant allelic variability at p-

value < 0.029, between the patients and control when they compared 131 patients with 258 control cases in ACE2 WES data [15]. Additionally, Möhlendick et al. [15] suggested that the ACE2 polymorphism influences the risk for infection with SARS-CoV-2 and the severity of COVID-19 independently of other known risk factors [15]. This indicates the clinical variability of individuals that were observed in patients with COVID-19 can return to genetic backgrounds.

Furthermore, Various Bioinformatics Systems including Protein Analysis Through Evolutionary Relationships (PANTHER), Functional Analysis of SNPs Through Hidden Markov Models (FTHMM), and Analysis of Protein Stability and Alteration by (iPTREE software) predicted that rs41303171 and rs4646116 have affected function and protein stability through deleterious, and disabilities of ACE2 [10]. However, the current results were in line with Hou et al. [16], who proposed that the ACE2 polymorphisms associated with susceptibility to SARS-CoV-2 infection and human genetics may be the first step in fighting COVID-19 [12].

Overall, many researchers have indicated that ACE2 protein played an important role in the entry of SARS-CoV-2 into the cell and it is considered a primary host cell receptor of the viral spike protein and responsible for the final infection [14, 16].

Many researchers have studied Single-nucleotide polymorphism in various populations to discover the relationship with different diseases, due to the affecting of SNPs on gene expression and finally on the prognosis of the disease. However, SNPs of ACE2 had previously been studied to find a relation with many diseases like hypertension, ventricular hypertrophy, and hypertrophic cardiomyopathy [7]. Furthermore, the database analysis had been completed by [4] revealed the presence of 1700 variants in the ACE2 region on the X chromosome, and they identified 14 SNPs and 1 insertion/deletion with higher minor allele frequencies in the Asian population than in the European population [9]. The current study is the first study for SNPs of the ACE2 rs41303171 gene in Iraq by the Allele Specific-PCR method, and further studied needed to confirm the association of this polymorphism with susceptibility to infection with SARS-CoV-2.

6. Conclusion

The infection with SARS-CoV-2 has been affected by the single nucleotide polymorphisms in the ACE2 gene. The wild genotype of (GG) for the ACE2 rs41303171 gene may be more susceptible to the infection with SARS-CoV-2. While the heterozygote genotype (AG) and mutated genotype (AA) for ACE2 rs41303171 gene may be associated with more protection from the infection with SARS-CoV-2.

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