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Relationship of ACE1 and ACE2 genetic polymorphisms on SARS-CoV-2 infection and severity of symptoms in different regions of Iraq

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ABSTRACT

This study was conducted in the laboratories of the Biology Department - College of Science - Tikrit University, the laboratories of the Food Science Department - College of Agriculture - University of Anbar, and the Public Health Laboratories - Anbar for the period from December 2021 AD until February 2022 AD, study involved taking samples from hospitalized COVID-19 patients of both sexes, after a confirmed positive PCR test result. As well as those lying in quarantine in three Iraqi governorates (the isolation unit in Karkh, Yarmouk and Kadhimiya hospitals, the Al-Shifa quarry hospital in Anbar, the public health laboratory in Anbar, and the quarantine hospital for thousands in Erbil), in addition to outpatient clinics. They were distributed by 30 patients and 20 healthy subjects from each governorate, with ages ranging from 18-80 years, randomly to men and women in order to determine the role of genetic polymorphisms in the ACE inhibitor gene in the study samples. The results of the genetic polymorphisms of ACE-1rs4646994, ACE-2rs2106809, and ACE-2rs4240157 showed that there were non-significant differences between the frequency of alleles and genotypes in the patient's group compared with the healthy group. The DD mutant genotype of ACE-1rs4646994 appeared in the patient's group in Baghdad and Anbar with a high percentage, while patients in Erbil showed the highest percentage of the heterozygous genotype ID compared to healthy subjects, with an OR= 0.922, OR= 2.750, and OR= 1.118, respectively, for the mutant allele D. Therefore, it is not considered a risk factor for the disease in Baghdad, in contrast to Anbar and Erbil. The polymorphism of ACE-2rs2106809, the homozygous genotype CC was dominant in patients in the aforementioned provinces and may not be considered a risk factor for the disease. The results of ACE-2rs4240157 gene polymorphism, the heterozygous CT genotype showed a high percentage in patients in Baghdad and, while in Anbar patients, the normal genotype TT appeared to be the highest percentage compared to healthy subjects. The C mutant allele in Baghdad, Anbar, and Erbil patients reached OR= 1.306, OR= 4.333, and OR= 9.00, respectively, so this allele is considered a risk factor for the disease. We can conclude from this study the role of each of the polymorphisms rs4646994 ACE-1 and rs4240157 ACE-2 in infection with COVID-19 and the severity of its symptoms according to the geographical region and race, in contrast to rs2106809 ACE-2, as it is possible to conduct clinical studies to demonstrate the role of genetic polymorphisms with Relationship to diseases depending on the region and race.

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علاقة تعدد الأشكال الجينية ACE1 و ACE2 على الإصابة بفيروس SARS-CoV-2 وشدة أعراضه في مناطق مختلفة من العراق

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الملخص

شملت هذه الدراسة أخذ العينات من مرضى COVID-19 المراجعين للمستشفيات من كلا الجنسين، بعد نتيجة اختبار PCR الإيجابية المؤكدة. وكذلك الراقدين في الحجر الصحي في ثلاثة محافظات عراقية (وحدة العزل في مستشفى الكرخ واليرموك والكاظمية، مستشفى الشفاء للحجر الصحي الأنبار ، مختبر الصحة العامة في الأنبار، مستشفى لالاف للحجر الصحي في أربيل) بالإضافة إلى العيادات الخارجية، وزعت عينات الدراسة بواقع ٣٠ مريضاً و٢٠ من الأصحاء من كل محافظة، بأعمار تتراوح بين ١٨-٨٠ سنة، عشوائياً من الرجال والنساء بهدف تحديد دور تعدد الأشكال الجينية في الجين المثبط للإنزيم المحول للأنجيوتنسين.

بينت نتائج تعدد الأشكال الجينية rs4646994 ACE-1 و rs2106809 ACE-2 و rs4240157 ACE-2 بوجود اختلافات غير معنوية بين تكرار الأليلات والتراكيب الوراثية في مجموعة المرضى مقارنة مع مجموعة الأصحاء. ظهر التركيب الوراثي الطافر DD لجين rs4646994 ACE-1 في مجموعتي مرضى بغداد والأنبار بنسبة مرتفعة، بينما مرضى أربيل ظهرت النسبة الأعلى للتركيب الوراثي غير المتماثل ID مقارنة بالأصحاء، وبقيمة OR=0.922 و OR=2.750 و OR=1.118 على التوالي للأليل الطافر D لذلك لا يعد عامل خطورة للمرض في بغداد، على العكس من الأنبار وأربيل.أما بالنسبة لتعدد أشكال جين rs2106809 ACE-2 فكان التركيب الوراثي المتماثل CC هو السائد في مرضى المحافظات المذكورة وقد لا يُعد عامل خطورة للمرض. وبالنسبة لنتائج تعدد أشكال جين rs4240157 ACE-2 فقد ظهر التركيب الوراثي غير المتماثل CT بنسبة مرتفعة في مرضى بغداد وأربيل، بينما مرضى الأنبار ظهر التركيب الوراثي الطبيعي TT هو النسبة الأعلى مقارنة مع الأصحاء. والاليل الطافر C في مرضى بغداد، الأنبار وأربيل، وبلغت OR=1.306 وOR=4.333 وOR=9.00 على التوالي، لذلك هذا الأليل يعد عامل خطورة للمرض.

يمكن أن نستنتج من هذه الدراسة دور كل من الأشكال rs4646994 ACE-1 وrs4240157 ACE-2 في الإصابة بمرض COVID-19 وشدة أعراضه حسب المنطقة الجغرافية والعرق على العكس من rs2106809 ACE-2، إذ بالإمكان أجراء دراسات سريرية في بيان دور الأشكال الجينية ذات العلاقة بالأمراض إعتمادًا على المنطقة والعرق.

Introduction:

Recently, the newest strain of Coronavirus, SARS-CoV2, similar to SARS-CoV, has appeared in China, which has infected large numbers of people with COVID-19 worldwide [1]. Iraq reported its first confirmed cases of SARS-CoV-2 virus on February 22, 2020, in the city of Najaf, and this case was confirmed after laboratory tests were conducted on the same day. The disease outbreak began in all other governorates, especially the capital, Baghdad, where the number reached Alif on April 5 [2]. Renin-Angiotensin-Aldosterone System (RAAS) is the enzyme chain that plays a critical role in the pathogenesis of COVID-19 disease [3].

The angiotensin-converting enzyme (ACE or ACE1) works by converting Ang-1 into Ang-2, and ACE2 works by converting Ang-2 into Ang-(1-7). The interaction between ACE1/ACE2 predicts the risk of body disorders such as high blood pressure and heart disease. Moreover, vascular and pulmonary diseases significantly influence the severity of clinical symptoms and risk factors associated with COVID-19[4]. In addition, SARS-CoV-2 binds to the ACE2 receptor through the spike protein and enters host cells [5]. Angiotensin-converting enzyme1 (ACE1) convert angiotensin I into Angiotensin II that was further metabolized by ACE 2 (ACE2). The binding ACE2 receptor to SARS-CoV-2 facilitate it enter into the host cell [6].

Angiotensin II can induce strong vasoconstriction, proinflammatory effects, and profibrotic effects, while angiotensin 1–7 exhibits antiproliferative, antiapoptotic, and mild vasodilating abilities and protect different cardiovascular effects such as anti–heart failure, anti-thrombosis, anti-myocardial hypertrophy, anti-fibrosis, anti-arrhythmia, anti-atherogenesis, and attenuating vascular dysfunction related to metabolic syndrome [7].

A correlation was found between the ACE2 SNP rs233575 and blood pressure, and ACE2 risk alleles were associated with more severe COVID-19 outcomes in obese, smoking males [8]. Depending on the geographic region, the rs4646994 DD genotype can be considered as a predictive biomarker for determining human susceptibility to SARS-CoV-2 infection and severe outcome to COVID-19, as studies have indicated that individuals with GG and TT genotypes are significantly more susceptible to severe outcome disease, whereas, conversely, individuals with GA, AA, and CC genotypes are less likely to develop severe COVID-19 [9]

Wang and Corpe, studying ACE2 SNPs that included ACE2 SNPs rs2074192, rs6632677, rs4646142, rs2048683, and rs4240157 for the first time in COVID-19 patients using genotyping assays, found that there is a relationship between genetic polymorphisms and allele frequency of ACE2 SNPs and disease severity. [10].

Based on the foregoing and the presence of SARS-CoV-2 infection cases in all Iraqi governorates, this study aimed to determine the effect of ACE1 and ACE2 genetic polymorphisms on SARS-CoV-2 infection and the severity of its symptoms through blood samples of infected and healthy individuals.

Material and method:

This study was conducted in the laboratories of the Biology Department - College of Science - Tikrit University, the laboratories of the Food Science Department - College of Agriculture - University of Anbar, and the Public Health Laboratories - Anbar for the period from December 2021 AD until February 2022 AD,

Study areas and sample collection:

This study included one 150 individuals from different Iraqi governorates in the location and ethnicity (Baghdad, Anbar, and Erbil). It consisted of 30 people confirmed to be infected with Covid-19 and 20 healthy people for each governorate, and the samples were taken randomly from men and women with ages ranging from 18- 80 years old to determine the role of ACE-1 and ACE-2 polymorphism in SARS-CoV-2 infection and the severity of its symptoms.

Collecting blood samples:

Five ml of venous blood was drawn from each patient with Covid-19 and for each individual from the healthy group using a syringe and placed in tubes containing ethyl diamine tetra acetic acid (EDTA) for use in DNA extraction and determination of polymorphisms of the ACE-1 gene rs4646994 and the ACE-2 gene rs2106809 and the gene ACE-2 rs4240157.

DNA Extraction Procedure:

DNA was extracted in several steps according to the method mentioned by[11].

Identification of a polymorphism of the ACE-1:

Tetra-Amplification Refractory Mutation System-PCR (Tetra-ARMS-PCR) was used to detect polymorphisms of the ACE-1 gene, the primer was designed for this study for this study as follows:

Table 1. Primers used to detect ACE-1 gene polymorphisms.

Name	Sequence	Number
ACE F	CTGGAGACCACTCCCATCCTTTCT	24
ACE R	GATGTGGCCATCACATTCGTCAGAT	25

Polymerization reaction method:

1. The rsPrime primers were prepared from the Korean manufacturer, Macrogen, in powder form, at a concentration of 100 picomoles/microliter, after which the primers were dissolved by adding 250 microliters of deionized Nuclease Free Water, according to the manufacturer's instructions.
2. Place the reaction tubes in a thermocycler using the following program:

Table 2. Thermopolymerization software for the ACE-1 gene.

Stage	Temperature	Time	Cycle number
1	95 °C	5 min	1
2	95 °C	45 sec	35
	56 °C	45 sec	
	72 °C	45 sec	
	72 °C	7 min	1
1	4 °C	Infinity	1

3. Take out the samples from the thermopolymer device, conduct the electrophoresis of the PCR product, and record the results.

Identification of a polymorphism of the ACE-2 gene at rs2106809:

Tetra-Amplification Refractory Mutation System-PCR (Tetra-ARMS-PCR) was used to detect polymorphisms of the ACE-2 gene at rs2106809 for this study as follows:

Table 3. Primers were used to detect ACE-2 gene polymorphisms at locus rs2106809.

Name	Sequence
OF09	GCTCTCTCTTCACAGCTTCTGGTA
OR09	GGAATTTCTTCTCTCACAGATCCC
IF09	TTTTTTTTCCATATCTCTATCTGATTGG
IR09	GCTGATGTAGAAGTGTGGAGACGT

Polymerization reaction method:

1. The rsPrime primers were prepared from the Korean manufacturer, Macrogen, in powder form, at a concentration of 100 picomoles/microliter, after which the primers were dissolved by adding 250 microliters of deionized Nuclease Free Water, according to the manufacturer's instructions.
2. Place the reaction tubes in a thermocycler using the following program:

Table 4. Thermopolymerization program of the ACE-2 gene at site rs2106809.

Stage	Temperature	Time	Cycle number
1	95 °C	5 min	1
2	95 °C	45 sec	35
	59 °C	45 sec	
	72 °C	45 sec	
	72 °C	7 min	1
1	4 °C	Infinity	1

3. Take out the samples from the thermopolymer device, conduct the electrophoresis of the PCR product, and record the results.

Identification of a polymorphism of the ACE-2 gene at rs4240157:

Tetra-Amplification Refractory Mutation System-PCR (Tetra-ARMS-PCR) was used to detect polymorphisms of the ACE-2 gene at rs4240157 for this study as follows:

Table 5. Primers were used to detect ACE-2 gene polymorphisms at locus rs4240157.

Name	Sequence	Number
ACE2 OF	GCTGAGTTCTCAAAATAATGCCATAGAT	28
ACE2 OR	GCATTTCTTTCCAATCATTAAGAGTTCA	28
ACE2 IF-T	GCCTCAGAACATTACAGAATCAACCT	26
ACE2 IR-C	GAGGGTTGGTAAATAGTGTTCAGTGG	26

Polymerization reaction method:

1. The rsPrime primers were prepared from the Korean manufacturer, Macrogen, in powder form, at a concentration of 100 picomoles/microliter, after which the primers were dissolved by adding 250 microliters of deionized Nuclease Free Water, according to the manufacturer's instructions.
2. Place the reaction tubes in a thermocycler using the following program:

Table 6. Thermopolymerization program of the ACE-2 gene at site rs4240157.

Stage	Temperature	Time	Cycle number
1	95 °C	5 min	1
2	95 °C	45 sec	35
	56 °C	45 sec	
	72 °C	45 sec	
	72 °C	7 min	1
1	4 °C	Infinity	1

3. Take out the samples from the thermopolymer device, conduct the electrophoresis of the PCR product, and record the results.

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Results & Discussion

DNA Extraction:

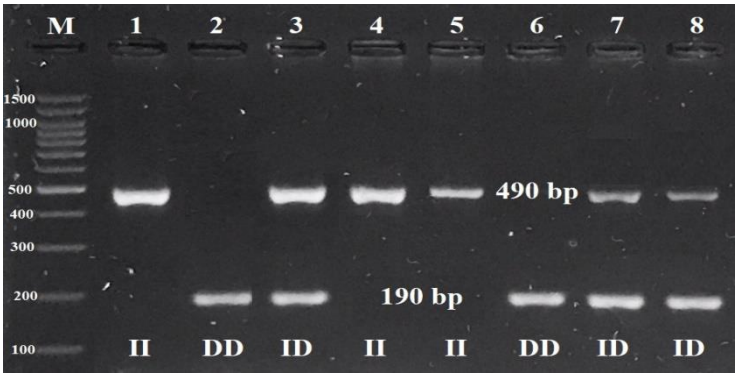
A DNA extraction process was carried out from blood samples of patients with COVID-19 disease and healthy persons taken from the aforementioned governorates, DNA was obtained with high concentration and purity using the electrophoresis method. Noting that the severity of clinical symptoms was evaluated in patients in each governorate, which included severe and moderate symptoms. Moreover, mild, as shown in Table (7).

Table 7. Percentage of severity of clinical symptoms among patients in the three governorates.

Governorates	The severity of clinical symptoms(%)		
	Severe	Moderate	Mild
Baghdad	١٣,٧	٤٩,٨	٣٦,٤
Anbar	١٠	٦٢,٢	٢٧,٧
Erbil	٢٨,٥	٢٨,٥	42.8

Polymorphism of the ACE-1 gene at rs4646994:

The results of the analysis using the Tetra-ARMS-PCR technique for the ACE-1 gene at rs4646994 showed three genotypes (II, ID, DD) as shown in the Figure (1):



(I) is the natural allele, and (D) is the mutant allele.

Figure 1. Electrophoresis of an amplified segment of the ACE-1 gene at locus rs4646994 by Tetra-ARMS-PCR on a 2% agarose gel.

The results are shown in Figure (1) of three genotypes, including the natural Wild II genotype, represented by the band (490 bp). The mutant genotype DD, represented by the band (190 bp), and the heterozygous genotype ID, represented by the two bands (190 bp, 490 bp).

The percentage, genotypes, and allele frequency of the ACE-1 gene at rs4646994 for the infected and healthy group in the three governorates:

The results showed in Table (8) the percentage of genotypes of the ACE-1 gene at the rs4646994 site for three genotypes (II, ID, DD) for the two groups of COVID-19 patients and healthy ones in Baghdad Governorate, and the highest frequency was for individuals carrying the mutated genotype DD and heterozygous ID, which is the same value (46.67%) for the sick group versus (66.66%) and (10%) for the healthy group, respectively, and for individuals carrying homozygous genotype II, the recurrence rate was (6.66%) and (43.23%) for patients and healthy people, respectively.

Table 8. Percentage, genotypes, and frequency of alleles of the ACE-1 gene at rs4646994 for the infected and healthy group in Baghdad governorate.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
II	2	6.66	5	23.34	0.004*	1 Ref.	-
ID	14	46.67	3	10		16.333	2.197 - 121.429
DD	14	46.67	12	66.66		2.450	0.441 - 13.593
Alleles	No.	%	No.	%	P value	OR	(95% CI)
I	18	30	14	28.3	0.840	1 Ref.	-
D	32	70	36	71.67		0.922	0.4197 - 2.027

The value of the risk factor was OR = 16.333 for individuals carrying the heterozygous genotype ID at the level ($p < 0.05$) and in a non-significant way compared between patients and healthy people. In contrast, its value was OR = 2.450 for individuals carrying the mutated genotype DD at the same probability level and in an insignificant manner. Moral. The frequency of the natural allele I was (30%) for the patient's group compared to (28.3%) for the healthy group, and the frequency of the mutant allele D was (70%) and (71.67%) for the patients and healthy groups, respectively, and the value of the risk factor OR = 0.922 At a confidence interval of 0.4197 - 2.027 = 95% CI in the frequency ratio of the mutant allele D to the normal allele I when comparing the two groups of patients and healthy subjects as shown in Table (8). This indicates that the mutant allele D does not represent a risk factor for the disease at the probability level ($p<0.05$).

The results showed in Table (9) the percentage of genotypes of the ACE-1 gene at the rs4646994 site for three genotypes (II, ID, DD) for the two groups of COVID-19 patients and healthy people in Anbar Province, the highest frequency of individuals carrying the mutant genotype DD was (66.66 %) for the patient's group compared to (27.27 %) for the healthy group. For individuals carrying the homozygous genotype II, the frequency rate was (20%) and (27.27 %) for patients and healthy people, respectively, and the lowest frequency rate for individuals carrying the heterozygous genotype ID was (13.34%)) and (45.45%) for the sick and healthy group, respectively.

Table 9. Percentage, genotypes, and frequency of alleles of the ACE-1 gene at rs4646994 for the infected and healthy group in Anbar Province.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
II	6	20	6	27.27	0.010*	1 Ref.	-
ID	4	13.34	8	45.45		0.40	0.0791 - 2.021
DD	20	66.66	6	27.27		3.333	0.7791 to 14.261
Alleles	No.	%	No.	%	P value	OR	(95% CI)
I	16	26.67	25	50	0.014*	1 Ref.	-
D	34	73.33	25	50		2.750	1.2081 - 6.260

Moreover, the value of the risk factor was OR = 0.40 for individuals carrying the heterozygous genotype ID at the level ($p < 0.05$) and in an insignificant way compared between patients and healthy people, while its value was OR = 3.333 for individuals carrying the mutated genotype DD at the same level of probability and in an insignificant way. Moral. The frequency of the natural allele I, which was (26.67%) for the patient's group, compared to (73.33%) for the healthy group, and the frequency of the mutant allele D was the same in the patients and healthy groups, amounting to (50%). As the value of the risk factor OR = 2.750 at the confidence interval 1.2081 - 6.260 = 95% CI in the ratio of the frequency of the mutant allele D to the normal allele I when compared between the two groups of patients and healthy people as shown in Table (9), and this indicates that the mutant allele D represents a Risk factor for the disease at the level ($p<0.05$).

The results showed in Table (10) the percentage of genotypes of the ACE-1 gene at the rs4646994 site for three genotypes (II, ID, DD) for the two groups of COVID-19 patients and healthy people in Erbil Governorate, and the highest frequency was for individuals carrying the heterozygous genotype ID by (55%) for the patient's group compared to (60%) for the healthy group, and for individuals carrying the DD mutant genotype, the percentage was (40%) and (35%) for the patients and healthy groups, respectively, while for individuals carrying the homozygous genotype II, the recurrence rate was The same in both groups and amounted to (5%).

Table 10. Percentage, genotypes, and frequency of alleles of the ACE-1 gene at rs4646994 for the infected and healthy group in Erbil Province.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
II	2	5	1	5	0.946	1 Ref.	-
ID	17	55	12	60		0.923	0.0518 - 16.456
DD	11	40	7	35		1.1429	0.0597 - 21.871
Alleles	No.	%	No.	%	P value	OR	(95% CI)
I	13	32.5	18	35	0.935	1 Ref.	-
D	37	67.5	32	65		1.118	0.4424 - 2.826

Moreover, the value of the risk factor was OR = 0.923 for individuals carrying the heterozygous genotype ID at the level ($p < 0.05$) and in an insignificant way compared between patients and healthy people, while its value was OR = 1.1429 for individuals carrying the mutated genotype DD at the same level of probability and in an insignificant way. Moral. The frequency of the natural allele I was (32.5%) for the sick group compared to (35%) for the healthy group, and the frequency of the mutant allele D was (67.5%) and (65%) for the patients and healthy groups, respectively, and the value of the risk factor OR = 1.118 At a confidence interval of 0.4424 - 2.826 = 95% CI in the frequency ratio of the mutant allele D to the normal allele I when comparing the two groups of patients and healthy subjects as shown in Table (10). This indicates that the mutant allele D represents a risk factor for the disease at the probability level ($p <0.05$).

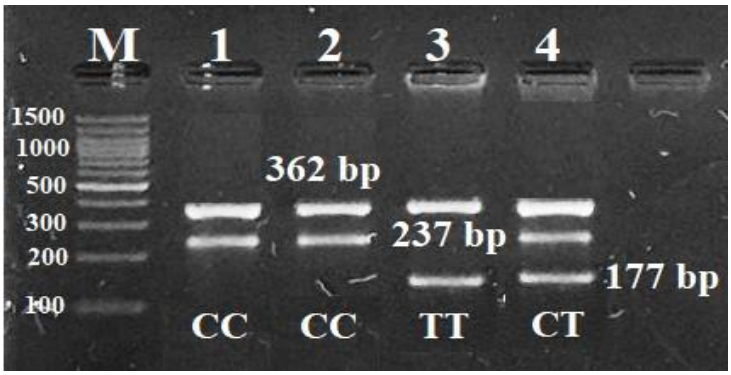
The results of the study in Baghdad and Anbar governorates agreed with [12], who found that the elevation of the DD mutant genotype at rs4646994 based on geographic region and ethnicity is a predictive biomarker for determining human susceptibility to SARS-CoV-2 infection and the severity of its symptoms, as it is This pattern could significantly increase the risk of SARS-CoV-2 infection by 1.7-fold in the Asian population, in contrast to the results for Erbil Governorate in which the heterozygous ID genotype had the highest frequency. While he indicated that the normal genotype II was more resistant to the severity of COVID-19, and the results of the frequency ratio of the mutant allele D to the normal allele I agreed with Anbar and Erbil governorates that it constitutes a risk factor for the disease with what the same researcher found for patients infected with SARS-CoV -2.

Found that the ACE1-DD genotype is associated with COVID-19 severity, while the ACE1-II genotype plays a protective role against the development of severe COVID-19 infection. As indicated by Verma, the severity of symptoms in COVID-19 patients depends on the DD mutant genotype at locus rs4646994 in addition to age, diabetes, and hypertension [13].

The study also agreed [14] presence of the D allele is associated with ethnic differences and that the higher frequency of the D allele was in African American and European countries infected with SARS-CoV-2 and is considered to have a higher mortality rate compared to Indians, Asians, and white people. The results of this study are in agreement [15], who found a significant association between the ACE1 rs4646994 polymorphism and an increased risk of severe infection with COVID-19 in D versus I alleles, that is, DD is dominant versus II+ID, and that the DD genotype is higher. At locus ACE1 rs4646994 leads to increased levels of ACE in the blood, causing an increased risk of clinical outcomes of COVID-19 and diseases such as hypertension and cardiovascular disease, and infected people with this pattern have a 2.06-fold increased risk of developing severe symptoms.

Polymorphism of the ACE-2 gene at rs2106809:

The results of the analysis using the Tetra-ARMS-PCR technique for the ACE-2 gene at rs2106809 showed three genotypes (CC, CT, TT), as shown in the Figure (2):



(C) is the natural allele, and (T) is the mutant allele.

Figure 2. Electrophoresis of an amplified segment of the ACE-2 gene at locus rs2106809 by Tetra-ARMS-PCR, on a 2% agarose gel.

The results showed in Figure (2) three genotypes, which included the normal wild genotype CC, represented by the two bundles (237 bp and 362 bp), the mutant genotype TT, which was represented by the two bundles (177 bp, 362 bp), and the heterozygous genotype CT represented by Three packages (177 bp, 237 bp, and 362 bp).

The percentage, genotypes, and allele frequency of the ACE-2 gene at rs2106809 for the infected and healthy group in the three governorates:

The results showed in Table (11) the percentage of genotypes of the ACE-2 gene at the site rs2106809 for three genotypes (CC, CT, TT) for the two groups of COVID-19 patients and healthy people in Baghdad Governorate, and the highest frequency was for individuals carrying the homozygous CC genotype by (83.34% for the sick group compared to (90%) for the healthy group. For individuals carrying the heterozygous genotype CT, the percentage was (10%) and (6.66%) for the patients and healthy groups, respectively, while for individuals carrying the mutated genotype TT, the recurrence rate was (6.66 %) and (3.33 %) for the patients and healthy groups, respectively.

Table 11. Percentage, genotypes, and frequency of alleles of the ACE-2 gene at rs2106809 for the infected and healthy group in Baghdad governorate.

	Patients No. (30)		Control No. (20)				
Genotypes	No.	%	No.	%	P value	OR	(95% CI)
CC	25	83.34	17	90	0.737	1 Ref.	-
CT	3	10	2	6.66		1.620	0.249 - 10.512
TT	2	6.66	1	3.33		2.160	0.184 - 25.317
Alleles	No.	%	No.	%	P value	OR	(95% CI)
C	43	88.34	47	93.34	0.348	1 Ref.	-
T	7	11.66	3	6.66		1.849	0.511 - 6.681

The value of the risk factor was OR = 1.620 for individuals carrying the heterozygous genotype CT at the level ($p < 0.05$) and in an insignificant way compared between patients and healthy people, while its value was OR = 2.160 for individuals carrying the mutated genotype TT at the same level of probability and in an insignificant way. Moral. The frequency of the normal C allele was (88.34%) for the sick group, compared to (93.34%) for the healthy group. The frequency of the mutant allele T was (11.66%), and (6.66%) for the sick and healthy groups, respectively, and the value of the risk factor OR = 1.849 At a confidence interval of 0.511 - 6.681 = 95% CI in the frequency ratio of the mutant allele T to the standard allele C when comparing the two groups of patients and healthy subjects as shown in Table (11), this indicates that the mutant allele T represents a risk factor for disease at the level of probability ($p <0.05$).

The results showed in Table (12) the percentage of genotypes of the ACE-2 gene at the site rs2106809 for three genotypes (CC, CT, TT) for the two groups of COVID-19 patients and healthy people in Anbar Province, and the highest frequency was for individuals carrying the homozygous CC genotype by (66.66 %) for the patient's group compared to (81.82 %) for the healthy group, and for individuals carrying the heterozygous genotype CT the percentage was (30 %) and (13.64 %) for the patients and healthy groups, respectively, while for individuals carrying the mutated genotype TT the recurrence rate was (3.34% and (4.54%) for the patients and healthy groups, respectively.

Table 12. Percentage, genotypes, and allele frequency of the ACE-2 gene at rs2106809 for the infected and healthy group in Anbar Province.

	Patients No. (30)		Control No. (20)				
Genotypes	No.	%	No.	%	P value	OR	(95% CI)
CC	20	66.66	16	81.82	0.382	1 Ref.	-
CT	9	30	3	13.64		2.700	0.631 - 11.551
TT	1	3.34	1	4.54		0.900	0.052 - 15.466
Alleles	No.	%	No.	%	P value	OR	(95% CI)
C	39	81.66	40	65	0.079	1 Ref.	-
T	11	18.34	10	35		2.764	0.8860 - 8.623

The value of the risk factor was OR = 2.700 for individuals carrying the heterozygous genotype CT at the level ($p < 0.05$) in an insignificant manner compared to healthy and sick patients. In contrast, its value was OR = 0.900 for individuals carrying the mutated genotype TT at the same probability level and in an insignificant manner. Moral. The frequency of the natural allele C, which was (81.66%) for the sick group compared to (65%) for the healthy group, and that the frequency of the mutant allele T was (18.34%) and (35%) for the sick and healthy groups, respectively, and the value of the risk factor OR = 2.764 At a confidence interval of 0.8860 - 8.623 = 95% CI in the frequency ratio of the mutant allele T to the standard allele C when comparing the two groups of patients and healthy subjects as shown in Table (12). This indicates that the mutant allele T represents a risk factor for the disease at the probability level (p). <0.05).

The results showed in Table (13) the percentage of genotypes of the ACE-2 gene at the site rs2106809 for three genotypes (CC, CT, TT) for the two groups of COVID-19 patients and healthy people in Erbil Governorate, and the highest frequency was for individuals carrying the homozygous CC genotype by (75% for the patient's group compared to (45%) for the healthy group, and for individuals carrying the heterozygous genotype CT, the percentage was (20%) and (55%) for the patients and healthy groups, respectively, while for individuals carrying the mutated genotype TT, the recurrence rate was (5% and (0%) for the patients and healthy groups, respectively.

Table 13. Percentage, genotypes, and frequency of alleles of the ACE-2 gene at locus rs2106809 for the infected and healthy group in Erbil Province.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
CC	20	75	9	45	0.0559	1 Ref.	-
CT	6	20	11	55		0.218	0.053 - 0.895
TT	4	5	0	0		1.838	0.067 - 49.901
Alleles	No.	%	No.	%	P value	OR	(95% CI)
C	34	85	37	72.5	0.177	1 Ref.	-
T	16	15	13	27.5		0.465	0.1531 - 1.413

The value of the risk factor was OR = 0.218 for individuals carrying the heterozygous genotype CT at the level ($p < 0.05$) and in an insignificant way compared between patients and healthy people, while its value was OR = 1.838 for individuals carrying the mutated genotype TT at the same level of probability and in an insignificant way. Moral. The frequency of the natural allele C was (85%) for the patient's group, compared to (72.5%) for the healthy group. The frequency of the mutant allele T was (15%), and (27.5%) for the patients and healthy groups, respectively, and the value of the risk factor OR = 0.465 At a confidence interval of 0.1531 - 1.413 = 95% CI in the frequency ratio of the mutant allele T to the standard allele C when comparing the two groups of patients and healthy subjects as shown in Table (13), this indicates that the mutant allele T does not represent a risk factor for the disease at the probability level ($p<0.05$).

Studies have shown contradictory results regarding the association of ACE2 rs2106809 with COVID-19 disease [16], in their study of 155 patients with COVID-19 in Turkey, found that there was no relationship between the severity of clinical symptoms of COVID-19 patients and the genotypes of the ACE-2 gene. rs2106809, while [17] found that ACE2 rs2106809 was associated with an increased risk of hospitalization and increased disease severity with heterozygote genotypes, and in a 2020 study by [18], the ACE2 genotype was found to be rs2106809 is an important SNP in susceptibility to COVID-19.

Mohammadi-Berenjestanaki investigated the possible association between ACE2 rs2106809 and the severity of clinical symptoms to relieve disease severity in Iranian patients with COVID-19, as the frequency of the mutant allele was found to be higher than normal, noting that more is needed. Of the studies in this aspect, his study did not agree with the studies that found that the frequency of the mutant allele in this gene is higher than normal in members of the European population only, in contrast to the level of the natural gene is higher in Asians, and that the recent studies agreed with the results of our study [19].

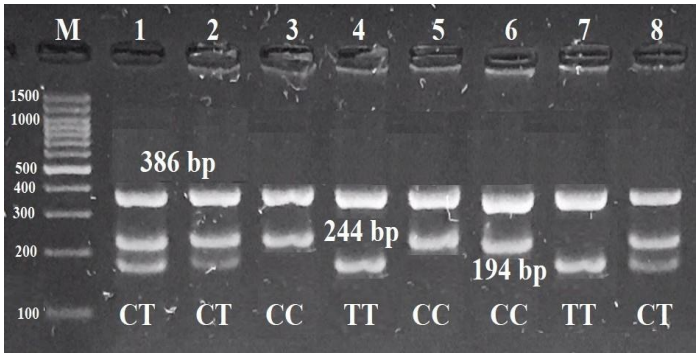
Suleiman indicated that the high degree of ACE-2 genetic polymorphism among different populations around the world, especially at loci rs2106809 and rs2074192, explains the genetic differences between males and females towards COVID-19, as the TT genotype is higher among males. It could contribute to their severity of infection compared to females, but the absence of such variants in females explains their lower infection and mortality rates [20].

ACE2 polymorphisms can affect susceptibility to COVID-19, but it is not yet clear whether polymorphisms of ACE2 can influence the severity of COVID-19, as [21] found no association between rs2106809

and outcome. Disease in COVID-19 patients and the differences in the results of research on rs2106809 may be the result of epigenetic effects or that other genetic changes play an essential role in the increase in the disease of COVID-19 and the severity of its symptoms, such as inflammatory cytokines, clotting markers, and others that play a role in personal differences in patients [17]. Several studies indicated that rs2106809 is one of the most important SNPs associated with ACE2, which is associated with diseases such as hypertension, left ventricular hypertrophy, atrial fibrillation, and dyslipidemia that can increase susceptibility to COVID-19 [22].

Polymorphism of the ACE-2 gene at rs4240157:

The results of the analysis using the Tetra-ARMS-PCR technique for the ACE-2 gene at rs4240157 showed three genotypes (TT, CT, CC), as shown in the Figure (3):



(T) is the natural allele, and (C) is the mutant allele.

Figure 3. Electrophoresis of an amplified segment of the ACE-2 gene at locus rs4240157 by Tetra-ARMS-PCR on a 2% agarose gel.

The results shown in the Figure (3) three genotypes, which included the natural wild genotype TT represented by two bundles (194 bp and 386 bp), the mutant CC genotype represented by the two bundles (244 bp and 386 bp), and the heterozygous genotype CT represented by Three packages (194 bp, 244 bp, and 386 bp).

The percentage, genotypes, and allele frequency of the ACE-2 gene at rs4240157 for the infected and healthy group in the three governorates:

The results showed in Table (14) the percentage of genotypes of the ACE-2 gene at the site rs4240157 for three genotypes (TT, CT, CC) for the two groups of COVID-19 patients and healthy people in Baghdad Governorate, and the highest frequency was for individuals carrying the heterozygous genotype CT with a percentage (76.66%) for the patient's group compared to (83.33%) for the healthy group, and for individuals carrying the CC mutant genotype, the percentage was (13.34%) and (3.33%) for the patients and healthy groups, respectively. As for individuals carrying the normal TT genotype, the recurrence rate was (10% and (13.34%) for the patients and healthy groups, respectively.

Table 14. Percentage, genotypes, and frequency of alleles of the ACE-2 gene at rs4240157 for the infected and healthy group in Baghdad governorate.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
TT	3	10	4	13.34	0.363	1 Ref.	-
CT	23	76.66	15	83.33		1.226	0.247 - 6.078
CC	4	13.34	1	3.33		5.333	0.375 - 75.779
Alleles	No.	%	No.	%	P value	OR	(95% CI)
T	24	48.34	30	55	0.4653	1 Ref.	-
C	26	51.66	20	45		1.306	0.637 - 2.678

The value of the risk factor was OR = 1.226 for individuals carrying the heterozygous genotype CT at the level ($p < 0.05$) and in an insignificant way compared between patients and healthy people, while its value was OR = 5.333 for individuals carrying the mutated genotype CC at the same level of probability and in an insignificant way. Moral. The frequency of the normal T allele, which was (48.34%) for the sick group compared to (55%) for the healthy group, and that the frequency of the mutant allele C was (51.66%) and (45%) for the sick and healthy groups, respectively, and the value of the risk factor OR = 1.306 At a confidence interval of 0.637 -

2.678 = 95% CI in the frequency ratio of the mutant allele C to the standard allele T when comparing the two groups of patients and healthy subjects as shown in Table (14). This indicates that the mutant allele C represents a risk factor for the disease at the probability level (p <0.05).

The results showed in Figure (14) the percentage of genotypes of the ACE-2 gene at the site rs4240157 for three genotypes (TT, CT, CC) for the two groups of COVID-19 patients and healthy people in Anbar Province, and the highest frequency was for individuals carrying the homozygous genotype TT by (46.67% for the patient's group compared to (70%) for the healthy group. For individuals carrying the mutant genotype CC, the percentage was (33.33%) and (0%) for the patients and healthy groups, respectively, while for individuals carrying the heterozygous genotype CT, the recurrence rate was (20% and (30%) for the patients and healthy groups, respectively.

Table 15. Percentage, genotypes, and frequency of alleles of the ACE-2 gene at rs4240157 for the infected and healthy group in Anbar Province.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
TT	14	46.67	14	70	0.015*	1 Ref.	-
CT	6	20	6	30		1.000	0.258 - 3.867
CC	10	33.33	0	0		21.000	1.122 - 392.88
Alleles	No.	%	No.	%	P value	OR	(95% CI)
T	29	56.67	42	85	0.0043**	1 Ref.	-
C	21	43.33	8	15		4.333	1.583 - 11.861

The value of the risk factor was OR = 1,000 for individuals carrying the heterozygous genotype CT at the level (p < 0.05) and in a non-significant way compared between patients and healthy people, while its value was OR = 21,000 for individuals carrying the mutated genotype CC at the same level of probability and in an insignificant manner. Moral. The frequency of the normal T allele, which was (56.67%) for the sick group compared to (85%) for the healthy group, and that the frequency of the mutant allele C was (43.33%) and (15%) for the sick and healthy groups, respectively, and the value of the risk factor OR = 4.333 At a confidence interval of 1.583 - 11.861 = 95% CI in the frequency ratio of the mutant allele C to the standard allele T when comparing the two groups of patients and healthy subjects as shown in Table (15). This indicates that the mutant allele C represents a risk factor for the disease at the probability level (p). <0.05).

The results showed in Table (16) the percentage of genotypes of the ACE-2 gene at the site rs4240157 for three genotypes (TT, CT, CC) for the two groups of COVID-19 patients and healthy people in Erbil Governorate, and the highest frequency was for individuals carrying the heterozygous genotype CT with a percentage (90%) for the sick group compared to (10%) for the healthy group. As for individuals carrying the normal genotype TT, the percentage was (5%) and (85%) for the sick and healthy groups, respectively. As for individuals carrying the mutant genotype CC, it amounted to the same Recurrence rate (5%) for the patients and healthy groups.

Table 16. Percentage, genotypes, and frequency of alleles of the ACE-2 gene at rs4240157 for the infected and healthy group in Erbil Province.

Genotypes	Patients No. (30)		Control No. (20)		P value	OR	(95% CI)
	No.	%	No.	%			
TT	4	5	17	85	0.001**	1 Ref.	-
CT	22	90	2	10		15.000	12.680 - 146.004
CC	4	5	1	5		17.00	0.551 - 523.823
Alleles	No.	%	No.	%	P value	OR	(95% CI)
T	25	50	45	90	0.0004**	1 Ref.	-
C	25	50	5	10		9.00	2.6981 to 30.021

The value of the risk factor was OR = 15,000 for individuals carrying the heterozygous genotype CT at the level (p < 0.05) and in an insignificant way compared between patients and healthy people. In contrast, its value was OR = 17.00 for individuals carrying the mutated genotype CC at the same probability level and in an insignificant way. Moral. The frequency of the normal T allele was (50%) for the sick group compared to (90%) for the healthy group. The frequency of the mutant allele C was (50%) and (10%) for the patients and healthy groups, respectively, and the value of the risk factor was OR = 9.00 At a confidence interval of 2.6981 - 30.021 = 95% CI in the frequency ratio of the mutant allele C to the standard allele T when comparing the two groups of

patients and healthy subjects as shown in Table (16), this indicates that the mutant allele C represents a risk factor for disease at the level of probability ($p < 0.05$).

The polymorphism of ACE2 rs4240157 and its variation correlate with the severity of COVID-19 disease through the increased specific expression of ACE2 in certain tissues of the body that requires hospitalization of patients [23], in addition to the variations of the genetic polymorphisms of each of the ACE2 2 rs2074192 and rs4240157 and rs4646188 showed higher risk in patients with COVID-19 with co-hypertension in patients of Australian descent, particularly genetic polymorphisms of rs4240157 associated with hypertension and cardiovascular disease that increase the risk of infection [18].

The results of Baghdad and Erbil governorates agreed with [24], who studied the association between TT and CC ACE2 rs4240157 genotypes and the C and T alleles with the risk of COVID-19 in cancer patients with ACE polymorphisms in Babil Province assuming that the ACE2-CC genotype is CT was associated with the severity of COVID-19 disease, as it was found that the disease was associated with the ACE2-CT genotype and that the ACE2 polymorphism does not affect the development of their cancer. The researcher indicated that the genetic polymorphism ACE2 rs4240157 differs significantly between patients with COVID-19 and that the difference in the polymorphism of this gene increases gene expression in tissues and its relationship with increased blood pressure.

The results of the study in Baghdad and Erbil governorates are also in agreement with [13], who found a high rate of heterozygous CT genotypes of the ACE-2 gene at rs4240157 in infected people, followed by TT and CC genotypes (40.17%), (35%), and (35%). (24.78%), respectively, and indicated that the frequency ratio of the mutant allele C to the normal allele T constitutes a risk factor for the disease, and the genotypes CT and CC are associated with the severity of SARS-CoV-2 infection compared to healthy subjects and are not consistent with the results of Anbar Governorate.

ACE2 plays a complex role in acute lung infection caused by COVID-19. On the other hand, high levels of its receptor on the cell surface may accelerate SARS-CoV-2 invasion during the very early stage of infection. Low levels of the disease can exacerbate the course of pulmonary complications due to insufficient conversion of Ang II in severe cases of COVID-19, so studies should be applied to broader cohorts and note the risk of genetic variation and its association with the disease [25].

Conclusions

We can conclude that gene polymorphisms have a role in susceptibility to SARS-CoV-2 infection, disease progression and treatment of patients by geographic region and ethnicity in the studied Iraqi governorates, especially ACE-1 rs4646994 and ACE-2 rs4240157.

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