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Role of Genetic Variants AGT rs699 and ACE2 rs2106809 in Increasing the Risk and Severity of COVID-19 Infection in Iraqi Patients

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Abstract

The main aim of our study was to determine if the *ACE2* rs2106809 and *AGT* rs699 polymorphisms increase the risk and severity of Covid-19 infection in Iraqi patients. This case-control study included 102 patients (mean age 52.66±18.82 years) and 92 healthy (mean age 37.88±14.19 years). The patients were grouped based on severity: hospitalized (n = 57) and non-hospitalized (n = 45). Demographic and comorbidity data was also collected. Genotype distribution of two selected SNPs *ACE2* gene rs2106809 and *AGT* rs699 polymorphisms was performed by allele-specific PCR (AS-PCR) and sanger sequencing. The *ACE2* rs2106809 C allele was associated to an increased risk of Covid-19 infection and the severity in males but not in the females (C vs. T OR = 3.04 p=0.01 and C vs. T OR = 4.9, p = 0.03, respectively). While *AGT* rs699 TC genotype was associated with 2.96 folds higher risk of Covid-19 infection (TC vs.TT, CC OR =2.96, 95%CI = 1.12–6.44; p = 0.02). The *AGT* rs699 was not associated with the severity of infection. Among outpatients, benign conditions were associated with a lower risk, however older age males and comorbidities increased the risk. We concluded that the genetic variant of rs2106809 *ACE2* in males was significant with a risk of infection and severe clinical course because it could be located on the X-chromosome, and at the same time *AGT* rs699 polymorphism had an impact on the increased risk of Covid-19 but had no relation with the severity of Covid-19.

Keywords: COVID-19, AS-PCR, ACE2, AGT, Polymorphisms.

دور التغايرات الجينية *AGT* rs699 و *ACE2* rs2106809 في زيادة خطر وشدة الإصابة بفيروس كورونا في المرضى العراقيين

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الخلاصة

كان الهدف الأساسي لدراستنا هو تحديد ما إذا كانت تعدد التغايرات الجينية *ACE2* rs2106809 و *AGT* rs699 تزيدان من خطر وشدة الإصابة بـ Covid−19 في المرضى العراقيين. لذلك تم دراسة عينات الحالات المرضية ومقارنتها بعينات السيطرة للأصحاء، وتضمنت الدراسة 102 شخص مريضا (متوسط أعمارهم كان 52.66±18.82) و 92 كعينات سيطرة (متوسط أعمارهم كان 37.88±14.19)، تم تقسيم المرضى الى فئتين بناءً على شدة المرض الى مرضى راقدين في المستشفيات عددهم (57) ومرضى غير راقدين كانت

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اصابتهم غير شديده عددهم (45)، وتم جمع البيانات الديموغرافية (العمر ،الجنس والتاريخ الطبي) للمرضى المصابين، تم تحري تعدد الاشكال الوراثي بطريقة الاليل المتخصص (AS−PCR) و تقنية Sanger لتحديد تسلسل الحمض النووي . اوضحت النتائج في البحث ان الاليل (C) في الذكور في جين *ACE2* rs2106809 مرتبط في زيادة خطر الإصابة بالفايروس وأيضا في زيادة شدة المرض (95% CI=1.26− 7.28, OR=3.04 و 95% CI=1.13−20.25, OR=4.8) على التوالي، ولكن ليس له دور في الاناث. بينما اظهر تعدد الاشكال لجين *AGT* rs699 بان النمط الجيني TC مقارنة بالنمط السائد TT يلعب دور في زيادة خطر الإصابة ولكن ليس له دور في زيادة شدتها (95% CI=1.12−6.44, OR=2.96). المرضى غير الراقدين والذين تكون اصابتهم متوسطة هذا مؤشر لقلة الخطورة ولكن الاعمار الكبيرة والامراض المصاحبة وجنس الذكور عوامل تزيد الخطورة. نستنتج من خلال النتائج بان تعدد الاشكال الوراثي لجين *ACE2* rs2106809 محدد جزيئي مهم في معرفة خطر الاصابه بالمرض وشدته ويمكن اعتبار تعدد الاشكال لجين *AGT* rs699 محدد جزيئي مهم في التنبؤ بخطر الإصابة بالفيروس.

1. Introduction

Coronaviruses, member of the Coronaviridae family and are associated with the disease severe acute respiratory syndrome coronavirus2 (SARS-CoV-2), recently appeared in Wuhan, China, and quickly spread throughout the country before spreading world widely [1, 2]. Patients with Covid-19 may develop pneumonia. Clinical outcomes of the Sars-Cov-2 virus are highly variable, ranging from asymptomatic infection to death [3]. The Ministry of Health reported the first Covid-19 case in Iraq on March 24th when an Iranian student visited the city of Najaf. Almost every day following that, the Ministry of Health announced the registration of new cases [4]. The early symptoms that patients observed were fever, cough, myalgia, anorexia, fatigue and sore throat [5]. Elderly adults with comorbid conditions such as diabetes, hypertension, renal disease, lung disease and other medical conditions tend to have higher mortality rates [6]. The virus needs to bind spikes protein to a receptor angiotensin-converting enzyme 2 (ACE2) on the surface of host cells in order to enter host and cause the SARS-CoV-2 infection [7]. In 2003, ACE2 was demonstrated as the SARS-CoV receptor [8]. It was noted that SARS-CoV-2, like SARS-CoV, uses ACE2 as a receptor since their spike proteins have a higher degree of sequence similarity [9]. Following the initiation of Covid-19, ACE2 and the renin-angiotensin-aldosterone system (RAAS) play an important role in regulating the severity of lung damage, fibrosis and failure [10]. A hormonal system known as the renin-angiotensin system (RAS) was located in the liver. The RAAS consists of such substances as angiotensinogen, angiotensin I, angiotensin II, angiotensin converting enzyme (ACE) which converts inactive Ang I to active Ang II, and angiotensin converting enzyme (ACE2) which converts Ang II into angiotensin 1-7[11]. Emerging studies have showed that host-genetic variables may potentially contribute to the variation in Covid-19 phenotypes [12], [13]. Angiotensinogen (AGT) is a prohormone produced by the liver that is encoded by the 1q42.2-mapped AGT gene [14]. All angiotensin peptides in RAS system are mainly produced by angiotensinogen [15]. The rs699, one of the common polymorphisms in AGT (M268T, formerly known as M235T) is a missense polymorphism on exon 2 that encodes a threonine variant correlated with increased angiotensin levels. Angiotensin-I is created when renin cleaves angiotensinogen to produce angiotensin-I which is subsequently converted to angiotensin-II by angiotensin converting enzyme-1 (ACE1) [16]. The X chromosome Xp22.2 region contains the *ACE2* gene which is expressed in a variety of organs [17]. Past studies on SARS-CoV infection which infects host cell by the highly produced ACE2 as receptor protein in pulmonary tissue [18], demonstrated that binding to SARS virus reduces ACE2 activity and expression [19]. ACE2 genetic variants may connect with intracellular susceptibility to SARS-CoV-2 with controversial results [20], [21]. The ACE2 rs2106809 mutation (C-T) in

intron has been found to have a significant impact on Angiotensin-converting enzyme2 and may be a determinant of circulating Ang-(1-7) [22].

This study examined the relationship between *AGT* rs699 C > T and *ACE2* C > T variations and the susceptibility of Covid-19 infection and severity in a sample in the middle Euphrates of Iraq.

2. Materials and Methods

Subjects Selection.

This study included 102 patients (57 hospitalized, 45 non-hospitalized) who had positive results for SARS-COV2 polymerase chain reaction (PCR) tests on nasopharyngeal swabs or lower respiratory tract samples. In addition, 92 healthy controls from similar ethnicities and regions were recruited for the study. All samples, obtained between Feb - Jun 2022, were from the patients that were admitted to Al Diwaniyah and Al-Hussein teaching hospitals, and Marjan medical city in the provinces of the Middle Euphrates of Iraq. Those who tested negative for Covid-19 using both the RT-PCR method and clinical diagnostic criteria, were also included in the control group, clearly ruling out the disease. Whereas the patients were classified and categorized as severe or non-severe based on World Health Organization standards for disease severity [23]. Accordingly, hospitalized cases were defined as patients who required hospital and clinical signs complied with a high degree of pneumonia in addition to having hypoxia (SpO2) on room air of less than 90%, a respiratory rate of more than 30 breaths per minute, or other symptoms of severe respiratory distress. Patients who did not need oxygen and who had no risk factors for developing severe illness, were considered non-hospitalized cases.

DNA Extraction and Genotyping

Blood samples were collected from each participant and placed in an EDTA-containing tube. Genomic DNA was isolated using the Kit (GENEAID, Taiwan) according to the guidelines provided by the manufacturer. NanoDrop spectrophotometer 2000 absorbance measurements were used to assess DNA purity and concentration (Thermo Scientific) [24], and the integrity checked by agarose gel electrophoresis. T Allele specific polymerase chain reaction (AS-PCR) method was applied for genotyping *AGT* rs699 and *ACE2* rs2106809 polymorphisms. Table 1 summarizes primer sequences, optimum conditions, and fragment lengths. The allele-specific reactions must be performed in separate wells, with (wild + common primers) and (mutant + common primers) in first and second well respectively. PCR was carried in a final volume of 25 µL containing 5 µL of genomic DNA, 2 µL of each primer (10 µM) in the first reaction wild and common while in a second mutant and common, and 12.5 µL of GoTaq® G2 Green Master Mix (Promega, USA) and 3.5 µL ddH_2O. PCR conditions included an initial denaturing step at 95°C for 3 min, followed by 34 cycles of 95°C for 30s, annealing at 57°C for *AGT* rs699 and 56°C for *ACE2* rs2106809, in addition to a final extension at 72°C for 5 min. Later on, the product was electrophoretic separately in 2%, agarose gels.

Table 1: The primers sequences for the SNPs in study (rs2106809 *ACE2* and *AGT* rs699) **were designed by online tool.**

Polymorphisms	Primers Sequences (5'-3')	Product Size
***ACE2*/rs2106809**		
Wild Reverse Primer	TGATGTAGAAGTGTGGAGAACT	
Mutant Reverse Primer	TGATGTAGAAGTGTGGAGAACC	**196 bp**
Common Forward Primer	GCTACAACGTGTCCTTGATT	
***AGT*/rs699**		
Wild Reverse Primer	AAGACTGGCTGCTCCCTGAT	
Mutant Reverse Primer	AAGACTGGCTGCTCCCTGAC	**142 bp**
Common Forward Primer	CTGGCTGATCTCAGCTACAC	

Direct DNA Sequencing

PCR product sequencing was done by Macrogen, South Korea. Sanger sequencing was for samples to check the accuracy and validity of the genotyping information obtained using the AS-PCR method. The present study results were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA), version11 software to identify SNPs.

Ethical Consideration

The study was conducted in compliance with ethical guidelines as the patients provided both verbal consent and signed approval before samples were taken. A local ethics committee at College of Science, University of Babylon, reviewed and approved the study protocol and subject information and consent form in accordance with the regulations of the Ministry of Health in Iraq according to the document (B220102 dated Feb 17,2022) to get this approval.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 27.0 software. Categorical data was represented as percentage (%) and continuous variables as a mean and standard deviation (SD), or median and inter-quartile range (IQR). Chi-square or Fisher's exact test was used to compare categorical variables. Minor allele frequency (MAF) and Hardy-Weinberg Equilibrium (HWE) were calculated based on the results of chosen variations. To investigate the relationship between genotypes, COVID-19 infection risk, and disease severity, logistic regression analysis was performed to compute the odds ratios (ORs) and 95% confidence intervals (CIs) under different genetic models. The statistical significance level was set at p 0.05.

3. Results

Baseline Features of the Present Study

There were 102 confirmed Covid-19 patients (48 (47%) males, 54 (53%) females) and 92 apparently healthy in the study group (54 (58.7%) males, 38 (41.3%) females). The mean±SD ages of patients and controls were 52.66±18.82 and 37.88±14.19 respectively. There was a significant difference between them ($p<0.05$), as well as their median (IQR) ages were 53 (36-69) and 31 (24-43) respectively. Table 2 summarizes the demographic and clinical features of Covid-19 patients, according to disease severity. Although there were no changes in sex between hospitalized and non-hospitalized cases ($p=0.31$), a significant difference was found between severe and non-severe cases based on the age of patients in the study (p-value<0.05).

Table 2: Demographic and clinical features of severe and non-severe patients

Characteristics	Total Patients n (102)	Hospitalized Groups n (57)	Non-hospitalized Groups n (45)	P-value
Age, median (IQR)	53(36-69)	67(60-75)	37 (28-42)	**P=0.001***
Gender	Male/48(47.1%) Female/56(52.9%)	Male/33(68.8%) Female/24(44.4%)	Male/15(31.3%) Female/30(55.6%)	P=0.31
Death	5 (4.9%) 4 /male, 1/female	5 (8.7%)	0	--
Comorbidity	49(48%)	32(68.3%)	17(34.6%)	**P=0.002***
Hypertension	14(13.7%)	11(78.5%)	3(21.42%)	**P=0.04***
Diabetes Meletus	15(30.6%)	9(60%)	6(40%)	**P=0.034***
Cardiovascular disease	9(18.3%)	4(44.4%)	5(55.5%)	P=0.12
Kidney diseases	6(12.2%)	5(83.3%)	1(16%)	**P=0.031***
Cancer	3(6.1%)	1(33.3%)	2(66.6%)	P=0.27
Liver disease	2(4%)	2(100%)	0	--

IQR; Interquartile range, SD; Standard deviation, n; number of samples; *: Significant.

Association Study

We estimated HWE based on genotype frequencies for SNP *AGT* (rs699) which did not match HWE among the control group, however it did match HWE among patients. Females in both patients and control groups had genotype distributions consistent to Hardy-Weinberg equilibrium for *ACE2* rs2016809 (p>0.05) (Table 3).

Table 3: HWE p-values and allele frequencies comparing Covid-19 patients and controls with polymorphisms results

Polymorphism	Gender	Group	Genotype, n (%)			Allele, n (%)		HWE	
								χ^2	p
			TT®	TC	CC	T	C		
***AGT* (rs699)**	**Female and male**	**Control**	18(19.5)	31(33.7)	3(46.8)	67(36.4)	117(63.6)	**6.82**	**0.03***
		Patients	11(10.8)	51(50)	40(39.2)	73(35.8)	131(64.2)	0.78	0.67
***ACE2* (rs2106809)**	**Female**	**Control**	22(57.8)	15(39.4)	1(2.6)	59(64.8)	32(35.1)	0.7	0.7
		Patients	34(62.9)	16(29.6)	4(7.4)	84(77.7)	24(22.2)	1.1	0.57
	Male	**Control**	--			43(62.9)	27(56.8)		
		Patients	--			11(20.3)	21(43.2)		

*: Significant p<0.05, HWE: Hardy–Weinberg equilibrium, $\chi 2$: chi-square, *p*: The p-value.

The *ACE2* rs2106809 and *AGT* rs699 variants are associated with the risk and severity of COVID-19 infection.

In Table 4 allele and genotype distribution of *ACE2* rs2106809 and *AGT* rs699 polymorphisms indicates differences and risk factors of Covid-19 infection between the patients and control. In *ACE2* rs2106809 results classified this variant based on the sex because the *ACE2* gene on X-chromosome, the results demonstrated no significant differences between the *ACE2* rs2106809/female but in males C allele was detected to be significant enough to be risk factor to catch infection compared to T allele (OR = 3.04, 95%CI = 1.26–7.28, p < 0.05, T vs. C allele). The frequencies of the C and T alleles in males were 62.9% vs. 20.3% in control, and 56.8% vs.43.2% in Covid‑19 patients respectively. In addition, the analysis of *ATG* rs699 results revealed that the frequencies of the homozygous variant TT,

heterozygous variant TC and rare homozygous CC of were 19.5%, 33.7%, and 46.8 % healthy controls, and 10.9%, 50%, and 39.2% in Covid‑19 cases respectively. This polymorphism found significant differences that highlight TC genotype as a predictor of Covid-19 infection (OR = 2.69, 95% CI = 1.12–6.44, $p < 0.02$, TC vs. TT®).

As shown in Table 5 and 6, polymorphisms were analyzed in both hospitalized and non-hospitalized cases of Covid-19 to estimate their impact on infection progression. The results presented in Table 5 that *AGT* gene rs699 polymorphism in all genetic models, in spite of the differences in genotype distribution, were not statistically significant and large sample sizes may be required.

Table 6 shows no significant association between Covid-19 severity and *ACE2* gene rs2106809 genotypes and alleles in females, while C allele of *ACE2* rs2106809 was associated with Covid-19 severity in males (OR = 4.8, 95%CI = 1.13-20.25, $p = 0.03$, C vs T). Based on the results of rs2106809 and rs699, two alleles were identified as T and C and three genotypes were identified as TT, TC, and CC **(Figures 1 and 2)**. Analysis of DNA polymorphism was performed by DNA sequencing to confirm the electrophoresis results (**Figures 3 and 4).**

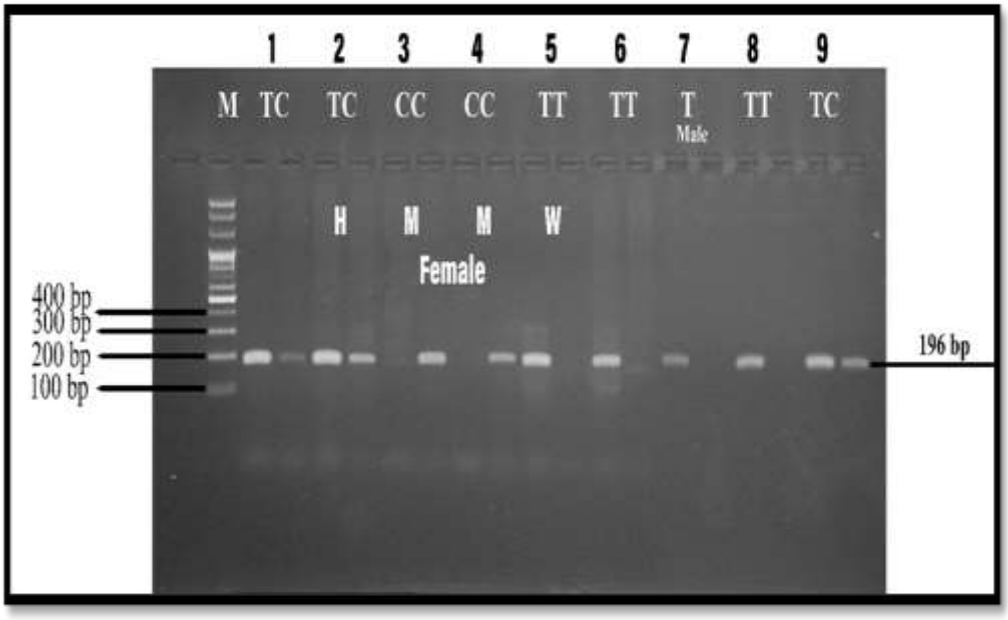

Figure 1: Allele-specific PCR for *ACE2* T>C (rs2106809) was performed on 2% agarose gel electrophoresis. M: 100 bp DNA ladder, lanes 1, 2 and 9: PCR products TC heterozygous (H), Lanes 3 and 4 PCR products obtained CC mutant(M) homozygous, lanes 5, 6 and 8 TT wild(W) homozygous and Lane 7 primer specific to the T allele for male.

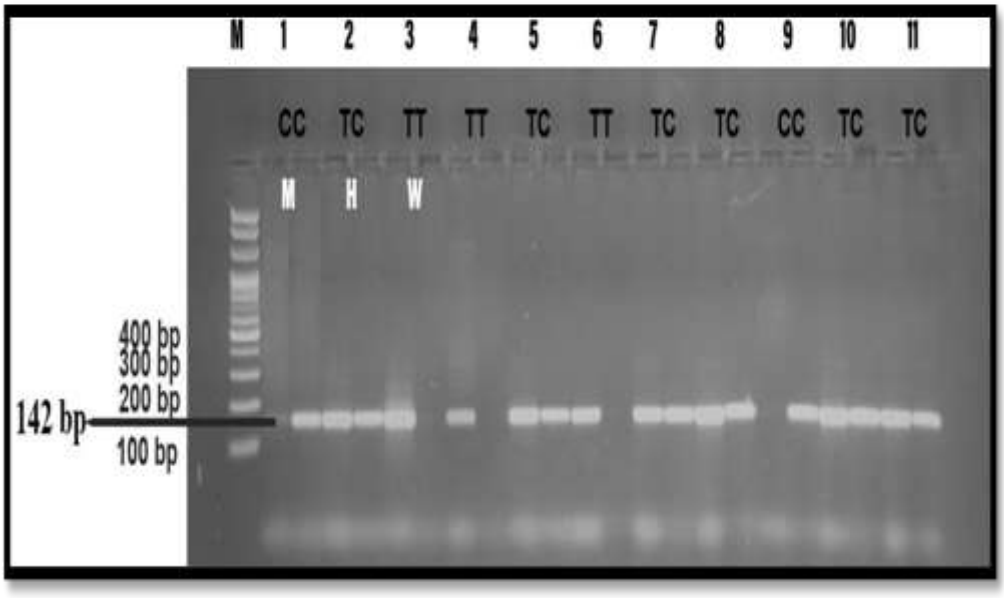

Figure 2: Electrophoresis on 2% agarose gel for allele-specific PCR for *AGT* T>C (rs699). M: DNA ladder of 100 bp. PCR products on lane 1 and 9 using C allele primer (mutant-M homozygous); PCR products on lanes 2, 5, 7, 8, 10, and 11 using T primer and C primer (heterozygous-H), and PCR products on lanes 3, 4, and 6 using allele-specific T primer (wild-W homozygous).

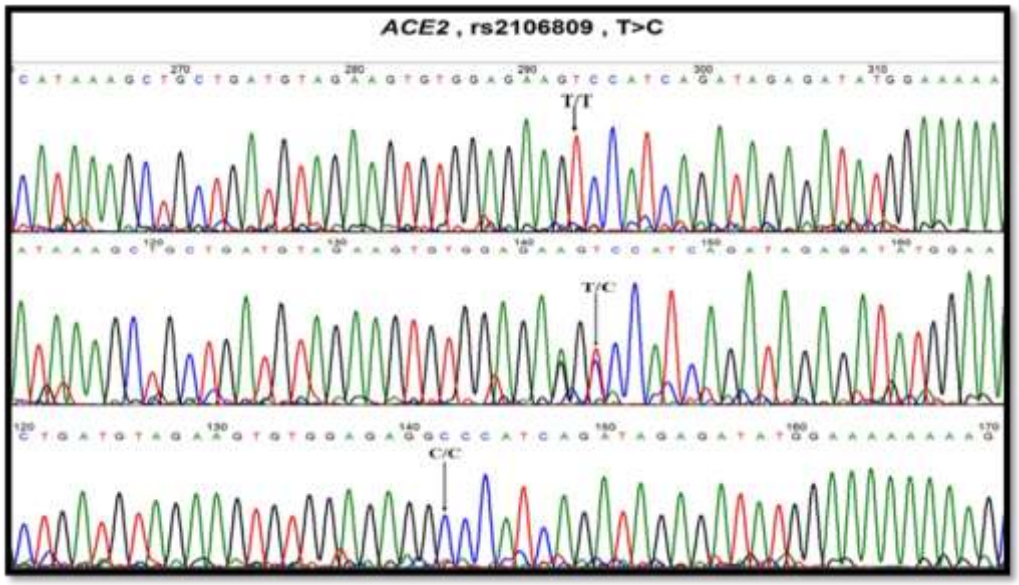

Figure 3: Direct DNA sequencing results shows three genotypes TT, TC, and CC of *ACE2* rs2106809.

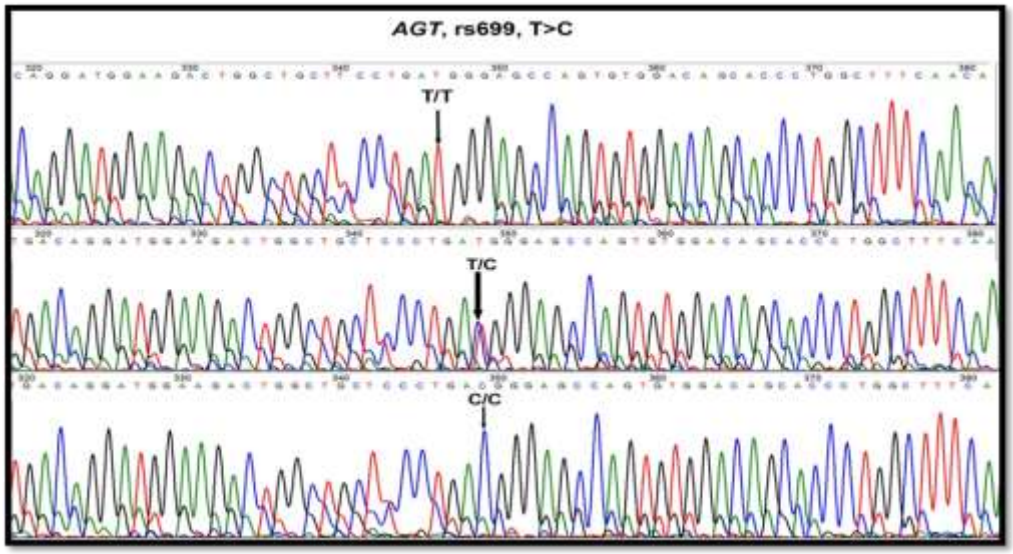

Figure 4: Sanger sequencing results of patients' and control extracted DNA to confirm TT, TC, and CC in *AGT* rs699.

Table 4: Comparing the genotype distributions of *ACE2* rs2106809, and *AGT* rs699 in Covid-19 patients with healthy controls and based on the risk of Covid-19.

Polymorphism		Genotype	Control, n (%)	Patients, n (%)	OR (95% CI)	*p*-value
***AGT* (rs699) Female and male**		**TT®**	18(19.5)	11(10.8)	1.00 (Ref.)	--
		TC	31(33.7)	51(50)	2.69(1.12-6.44)	**0.02***
		CC	43(46.8)	40(39.2)	1.52(0.64-3.61)	0.34
***ACE2* (rs2106809)**	**Female**	**TT®**	22(57.9)	34(62.9)	1.00 (Ref.)	--
		CT	15(39.5)	16(29.7)	0.69 (0.28 - 1.67)	0.41
		CC	1(2.6)	4(7.4)	2.58 (0.27 - 24.7)	0.4
	Male alleles	**T®**	43(62.9)	27(56.8)	1.00 (Ref.)	--
		C	11(20.3)	21(43.2)	3.04 (1.26 - 7.28)	**0.01***

OR: odds ratio; CI: confidence interval, *P < 0.05 was considered statistically significant.

Table 5: *AGT* rs699 polymorphisms in patients with Covid-19 (hospitalized and non-hospitalized) in different genetic models were examined.

Polymorphism	Genetic Models	Genotypes and Alleles	Hospitalized, n= (%)	Non-hospitalized, n= (%)	Od (95% CI)	P-value
***AGT* (rs699) Female and male**	**Codominant**	**TT®** **TC** **CC**	6(10.5) 28(49.1) 23(40.4)	5(11.1) 23(51.1) 17(37.8)	1.00(Ref.) 1.01(0.27-3.75) 1.12(0.29-4.31)	-- 0.98 0.86
	Dominant	**TT®** **TC+CC**	6(10.5) 51(89.5)	5(11.1) 40(88.9)	1.00(Ref.) 1.06(0.3-3.73)	-- 0.92
	Recessive	**TT+CT®** **CC**	34(59.6) 23(40.4)	28(62.2) 17(37.8)	1.00(Ref.) 0.89(0.4-2)	-- 0.79
	Over-dominant	**CC+TT®** **TC**	29(50.9) 28(49.1)	22(48.9) 23(51.1)	1.00(Ref.) 1.08(0.49-2.36)	-- 0.84
	Allele frequency	**T®** **C**	40(35) 74(65)	33(36.7) 57(63.3)	1.00 1.07(0.6-1.9)	-- 0.81

OR: odds ratio; CI: confidence interval, *P < 0.05 was considered statistically significant.

Table 6: ACE2 *rs2106809 polymorphisms were analyzed in hospitalized and non-hospitalized patients with COVID-19.*

Polymorphism	Sex	Genetic Models	Genotypes and Alleles	Hospitalized, n= (%)	Non-hospitalized, n= (%)	Od (95% CI)	P-value
***ACE2* (rs2016809)**	**Female**	**Codominant**	**TT®** **CT** **CC**	13 (54.1) 10 (41.6) 1 (4.1)	21 (70) 6 (20) 3 (10)	1.00 (Ref.) 2.69 (0.79-9.17 0.53 (0.05-5.74)	-- 0.11 0.23
		Dominant	**TT®** **CT+CC**	13 (54.2) 11 (45.8)	21 (70) 9 (30)	1.00 (Ref.) 1.97 (0.64-6.05)	-- 0.23
		Recessive	**TT+CT®** **CC**	23 (87.7) 1 (12.2)	27 (57.7) 3 (42.2)	1.00(Ref.) 0.39 (0.03-4.02)	-- 0.43
		Over-dominant	**CC+TT®** **CT**	14(58.4) 10(41.6)	24(80) 6(20)	1.00(Ref.) 2.85 (0.85-9.56)	-- 0.08
		Allele frequency	**T®** **C**	84(77.7) 24(22.3)	59(64.8) 32(35.2)	1.00 (Ref.) 0.62 (0.35-1.09)	-- 0.1
	Male	**Genotype allele**	**T®** **C**	15 (45.5) 18 (54.5)	12 (80) 3 (20)	100 (Ref.) **4.8 (1.13-20.25)**	-- **0.03***

OR: odds ratio; CI: confidence interval, *P < 0.05 was considered statistically significant.

4. Discussion

Angiotensin system is associated with the cause of Covid-19, so this study was conducted to investigate the association between candidate gene polymorphisms and the presence and severity of Covid-19 in Iraq. This study found a borderline association between males carrying the *ACE2* rs2106809 C allele and an increased risk and severity of Covid-19. Moreover, we found an association between polymorphism in *AGT* rs699 and Covid-19 risk without relation to severity. In this study, significant differences in age to increase the severity of COVID-19 were detected. According to studies in Iraq, the age factor contributed to increased risk and severity of Covid-19 [25, 26].

All angiotensin peptides originate from the same precursor molecule, AGT, which acts as the sole substrate of RAS [15]. RAS may have a crucial role in Covid-19 pathogenesis and severity. The ACE2, a major RAS component and the principal binding site for SARS-CoV-2, links RAS and COVID-19 [27]. Cafiero *et al.* [28] found *AGT* rs699 genotype and allele frequency distribution and that the T/C genotype is less frequent in asymptomatic patients than in symptomatic individuals with Covid-19. The *AGT* rs699 SNP showed potential as a method for predicting clinical outcomes associated with risk of Covid-19. In male C57BL/6J mice, TMPRSS2 mRNA expression in the lungs significantly lowered after 14 days of treatment with AGT antisense nucleotides to limit AGT expression [29]. It was suggested that preventing AGT might make it more difficult for virus to enter cells and may lead to reduce the severity of infection. Susceptibility to Covid-19 pathogenesis may vary depending on a number of factors, including but not limited to genetic variants that affect the expression and function of RAS components. It has been shown before that the *AGT* rs699 C allele (threonine variation) is linked to elevated plasma angiotensinogen and hypertension[16], [30], [31]. Therefore, it is possible that this variant is associated with the increased risk of COVID-19 infection. The TC genotype of rs699 was shown in our study to be associated with a 2.69-fold higher risk of Covid-19 than the TT genotype. Kouhpayeh *et al.* found similar results that TC genotype is associated with elevation of risk of Covid-19 and non-association with severity [32]. *ACE2* SNP rs2106809 is located in consensus intronic splicing nucleotides, suggesting that it might affect the conversion of total *ACE2* RNA to mRNA, ultimately affecting the level of protein. The ACE2 is a receptor for the virus to enter cells and converts angiotensin II into angiotensin 1-7. The virus uses ACE2 to affect Ang 1-7 levels. According to a study by Liu *et al*., level of angiotensin 1-7 circulating in the body depends on the *ACE2* gene and the polymorphism in the gene rs2106809. Plasma ACE2 levels were greater in men than in women which could means that this receptor for SARS coronavirus infections was expressed more in men's tissues [22]. This could explain why men might be more likely to get SARS-CoV-2 or suffer more from its effects [33]. Similar to our findings, Sienko et *al.* in his study *and* depending on the severity of their symptoms, a total of 155 patients were classified into three groups mild, moderate and severe Covid-19. In females Covid-19 infection severity was not related to the ACE2 receptor gene rs2106809 polymorphism [34]. The second study investigated 155 Covid-19 patients based on disease severity and found no association between the *ACE2* rs2106809 variation with the outcome of Covid-19 disease [35]. We found that the C allele was associated with 4.5-fold increase in the severity in our population of study. These results were consistent with another study that found that rs2106809 SNP in male Covid-19 patients was shown to be associated with an increased risk of hospitalization and death (OR = 11.41, 95%IC: 1.12-115.91, p = 0.012)[36]. A recent Italian study found that polymorphisms in the RAS pathway genes are associated with infection susceptibility and clinical outcome of SARS-CoV-2 infection[28].

In conclusion, our findings indicated that males with the *ACE2* rs2106809 variant had increased chances of acquiring the Covid-19 infection and Covid-19 disease severity. The variant rs699 of *AGT* contributes to Covid-19 disease risk but is not associated with severity of the disease. *AGT* rs699 and rs2106809 (C in males) may be useful as predictive biomarkers, for example, in the diagnosis of patients at greater risk of developing Covid-19 infection. Severe cases of Covid-19 infection are also associated with advanced age and chronic health conditions.

Limitations of study: a larger sample size should be used in future studies across many Iraqi populations to confirm our findings.

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Authors Contributions

All authors conducted experimental design and data analysis. All authors read and approved the final version of the manuscript.

Disclosure and Conflict of Interest

There are no conflicts of interest.

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