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Original Article

Association between Methylenetetrahydrofolate Reductase (*MTHFR*) and 5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase (*MTRR*) Polymorphisms in Iraqi Patients with COVID-19

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Abstract

Background: The methylenetetrahydrofolate reductase (*MTHFR*) gene is an essential gene in the metabolism of folate-homocysteine. Recently, the level of homocysteine was found to be a significant marker in the follow-up of COVID-19 infection. Thus, this study aimed to detect the effect of genetic polymorphisms for single nucleotide polymorphisms (SNPs) (c.66A>G, c.1298A>C, and c.677CT) on COVID-19 infection.

Methods: Blood samples were collected from 270 patients with COVID-19 in the medical center of Al-Shifa (Baghdad, Iraq) from November 2020 to March 2021. Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique was used for the detection of genotypes of SNPs. The odds ratio (OR) was used to detect the relationship between SNPs and COVID-19 infections. Haplotype analysis was performed by SHEsis software.

Results: There was a significant difference between mild/moderate cases and severe/critical cases for ages (35-45), (46-55), and (56-65) years (P<0.0001, P=0.01, and P=0.006, respectively). The results showed significant differences in the T allele for SNP c.677>C (P<0.0001 and OR=4.58). The C allele for SNP c.1298A>C indicated significant differences (P<0.001 and OR=3.15). Besides, the G allele for SNP c.677C>T showed significant differences (P<0.001 and OR=6.64). Consequently, these SNPs showed a predisposition to the development of COVID-19 infection. With regard to the C-A-A, T-A-A and T-C-G haplotypes indicated significant differences between the control and patient groups. The C-A-A was related to a decreased risk and indicated a protective effect against COVID-19 infection development (P<0.0001 and OR=0.218). The increased risk was associated with T-A-A and T-C-G haplotypes and indicated the risk impact on COVID-19 infection development (P<0.0001, P=0.004, and OR=15.5, OR=6.772, respectively). Furthermore, the linkage disequilibrium (LD) for SNPs was studied, and the complete D' value was 99%.

Conclusion: The genetic polymorphisms of SNPs (c.66A>G, c.1298A>C, and c.677C>T) in the Iraqi population were associated with COVID-19 infection.

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Keywords • COVID-19 • Polymorphism, single nucleotide • *MTHFR* gene • *MTRR* gene

What's Known

- The level of homocysteine can be used as a significant biomarker in the follow-up of COVID-19 infection. The methylenetetrahydrofolate reductase (*MTHFR*) gene is a vital essential gene in the metabolism of folate-homocysteine.
- Homocysteine is a risk agent in thromboembolism, increasing oxidative reactions, endothelial dysregulation, neurotoxicity, and atherosclerotic activities.

What's New

- Single nucleotide polymorphisms (c.66A>G, c.1298A>C, and c.677C>T) in the Iraqi population were associated with susceptibility to COVID-19 infection.
- C-A-A was related to a decreased risk of COVID-19 infection, which indicated a protective effect effect against COVID-19 infection development. T-A-A and T-C-G haplotypes were associated with an increased risk of COVID-19 infection development.

Introduction

The Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic began to spread in China in 2019. The infection of SARS-CoV-2 evolved into coronavirus infection (COVID-19) and was related to 8.8% death rates in the elderly group (60 years and more) compared to 0.46% in the group aged less than 60 years.[1] Moreover, it affected more than 200 states.[2] The coronaviruses give rise to acute syndrome of the respiratory system and Middle East syndrome of the respiratory system, and both can be fatal in several conditions. COVID-19 is among the infectious coronaviruses that were recently detected and distributed over the world.[3] This pandemic increased the rates of death in Italy, the USA, Spain, France, and Iran. The highest mortality rate worldwide occurred in old men (68%) who had at least one concurrent disease with COVID-19.[1] The comorbid diseases were hypertension, cardiovascular diseases, hypercholesterolemia, and diabetes.[4]

While some coronaviruses can cause mild or moderate respiratory system infections, such as the cold, others can create destructive epidemics with an incubation interval of 5-6 days.[5] In contrast, the general population does not need specific therapy during COVID-19 infection. While, individuals suffering from medical troubles such as diabetics, respiratory disorders, and cardiovascular diseases develop critical illness symptoms.[6] The symptoms of COVID-19 were fever, coughing, and dyspnea. In most acute conditions, this infection results in pneumonia or respiratory difficulty.[7]

According to a study conducted in Iraq, the number of COVID-19 cases increased gradually and reached to maximum peak on April 7th, with 684 cases documented. The Ministry of Health in Iraq reported that 2085 individuals were infected overall.[8]

Folate, which is a water-soluble vitamin B, has an essential role in the metabolism of carbon. It is also considered the main cofactor in nucleotide biosynthesis (purines and thymidine). Lack of folate causes anemia and is considered to be a major etiopathological factor in several cardiovascular diseases, neurological tube deficiencies, congenital disorders, mental and neurological disorders, and malignancies.[9] Homocysteine is regarded as a byproduct of the folic acid (folate) cycle. Homocysteine level concentration is utilized as an important biomarker in different neurodegenerative and cardiovascular diseases.[10]

On the other hand, due to its impacts on platelets, recent studies indicated that homocysteine might play a role as a hazard agent in thromboembolism.[11] It also aims to increase oxidative reactions, endothelium dysregulation, neurotoxicity, and atherosclerotic activities.[12] Furthermore, studies indicated that viral infections such as human immunodeficiencies virus (HIV), hepatitis virus, and papillomavirus had higher homocysteine concentrations.[13, 14] A recent study indicated that the level of homocysteine was a key marker in the COVID-19 infection follow-up.[15] The methylenetetrahydrofolate reductase (*MTHFR*) gene is an essential gene in the metabolism pathway of folate-homocysteine. Methylenetetrahydrofolate reductase is generated by *MTHFR*, which in turn stimulates the conversion of the 5, 10-methylene tetra hydrofolate to 5-methyl tetrahydrofolate, which is co-substrate for the homocysteine re-methylation to methionine. Two single nucleotide polymorphisms (SNPs) (c.677C>T and c.1298A>C) are associated with the metabolism of folate homocysteine. SNPs (677C>T and 1298A>C) are located on the exon of the *MTHFR* gene. These SNPs represent the most frequent mutations. Polymorphisms of the *MTHFR* gene change or reduce the methylenetetrahydrofolate reductase activity, causing homocysteine levels to elevate in the blood. Declined folate and elevated levels of homocysteine are related to different conditions, such as thrombosis, cardiovascular diseases, hypertension, and glaucoma.[16]

SNP (c.66A>G) for 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*) gene, which is caused by the substitution of isoleucine with methionine, is located on 5p15.31. This SNP reduces the biological activity of the protein.[17] The genotype GG is associated with lower levels of homocysteine.[18] Besides, the polymorphism of c.66A>G in the *MTRR* gene is associated with lower enzyme efficiency.[19] Thus, this study aimed to detect the effect of these SNPs on COVID-19 infection.

Patients and Methods

Sampling Collection Procedure

The present study included 270 patients with COVID-19, who were admitted to the Al-Shifa Center in the Medical City of Baghdad between November 2020 and March 2021. The patients were of both sexes and aged 35 to 65 years. The patients were divided into 140 mild/moderate cases and 130 severe/critical cases. The severity was classified according to WHO classifications. The clinical symptoms for mild/moderate COVID-19 were no hypoxia, moderate

signs of pneumonia (fever, dyspnea, cough), and oxygen saturation ≥90%. The severe condition of COVID-19 represented pneumonia with one of the following conditions, including a respiratory average of more than 30 bpm, intense difficulty breathing, and oxygen saturation of less than 90%. Acute respiratory distress syndrome, septic shock, intense pulmonary embolism, syndrome of intense coronary, stroke, and rave were among the additional issues that the critical COVID-19 cases represented.[20]

Written informed consent was obtained from all the participants. The study was approved by the Ethics Committee of Department of Biology, College of Science, Baghdad University, Baghdad, Iraq (Reference number: CSEC/0122/0055).

Genetic Detection

DNA was extracted from whole blood in EDTA tubes by using a Geneaid blood kit (Cat Number: GS300/ Korea). Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) technique was used to detect SNPs of *MTHFR* (c.66A>G, c.1298A>C), and *MTRR* (c.677C>T). Each SNP was detected by four primers including two primers for allele-specific, and two general primers in one reaction tube. The primers, listed in table 1, were manufactured by IDT (Integrated DNA Technologies/ USA).[19] Melting temperature was determined by algorithm sequences. The PCR reaction contained 2 µL of DNA sample, master mix 12.5 µL (2×EasyTaq® PCR SuperMix Cat.N: AS112-11/TransGen Biotechnology/China), 1 µL of each primer, and 1.5 µL of free nuclease water, with final volume of 20 µL. Thermal Cycler (Analytik Jena/Germany) was used for amplification. The first step of denaturation in the PCR software was set to 95 °C (5 min). Then, 30 cycles were added for each of the following steps, which included 94 °C (30 sec) for the second step of denaturation, 58 °C (30 sec) for annealing, and 72 °C (40 sec) for extension. In contrast, the final step of the extension was 72 °C (5 min). Agarose gel (1.5%) electrophoresis was used to determine the PCR product through visualization under UV light. The PCR amplicon lengths were 677T allele (146bp), 677C allele (87bp), 1298A allele (281bp), 1298C allele (361bp), 66G allele (458bp), and 66A allele (117bp). The presence of two distinct amplicons for SNP was classified as heterozygote.

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) was determined using the Chi square test, and Fisher's test was used to compare the observed and expected genotype frequencies. P>0.05 denotes a population compatible with HWE. Moreover, the relationship between SNPs and the incidence of COVID-19 infections was determined using the odds ratio (OR) and confidence interval (CI). The data were analyzed using WINPEPI version 11.65 (Oxford University Press/NewYork).[21] P<0.05 was considered to be statistically significant. Furthermore, SHEsis software (Shanghai Institutes for Biological Sciences, China) was used to analyze haplotype analysis.[22]

Results

Demographic Characteristics of COVID-19 Studied Groups

There was a significant difference between mild/moderate cases and severe/critical cases according to different age classifications (35-45), (46-55), and (56-65) with P<0.0001, P=0.01, and P=0.006, respectively. There was no significant difference between men and women (P=0.999). As shown in table 2, the results indicated no significant differences in chronic diseases (P=0.999).

Tetra-primer ARMS- PCR Analysis

The PCR amplicon lengths were 146bp and 87bp for the 677T allele and 677C allele, respectively.

Table 1: Primers for *MTHFR* (c.66A>G, c.1298A>C) and *MTRR* (c.677C>T) SNPs

Primer	Sequences	Melting temperature (°C)
117F/c.66A>G	CAGTTTCACTGTTACATGCCTTGAAGT	63.8
117R/c.66A>G	CCATGTACCACAGCTTGCTCAGAT	64.8
458F/c.66A>G	CAAAGGCCATCGCAGAAGAAGTG	67.3
458R/c.66A>G	GCCTTTCTTTTGGGGAAAAAAGTG	65
361R/c.1298A>C	GAGGAGCTGACCAGTGATGC	61.1
361F/c.1298A>C	CAGGCAAGTCACCTGGGAGAGA	66.2
281F/c.1298A>C	GGCAAAGAACGAAGACTTCAAAGACACATT	68.9
281R/c.1298A>C	GAAGAAGTTTGCATGCTTGTGGTTG	66.1
87R/c.677C>T	AGCAAAGCTGCGTGATGATGAAATAGG	69
87F/c.677C>T	CCGAAGCAGGGAGCTTTGAGG	67.6
146F/c.677C>T	GAAGGAGAAGGTGTCTGCGGGAAT	67.9
146R/c.677C>T	CCCTCACCTGGATGGGAAAGAT	65.6

Table 2: Demographic characteristics of the clinical severity of COVID-19 infection

Variable		Mild/Moderate n=140 cases	Sever/Critical n=130 cases	P value*
Age(year)	35-45	69 (49.3%)	20 (15.4%)	<0.0001
	46-55	39 (27.9 %)	60 (46.2 %)	0.01
	56-65	32 (22.9%)	50 (38.5%)	0.006
Sex	Male	75 (53.6%)	70 (53.8%)	0.999
	Female	65 (46.4%)	60 (46.2%)	
Chronic diseases	Diabetes mellitus	74 (52.9 %)	64 (49.2%)	0.999
	Hypertension	66 (47.1%)	66 (50.8%)	

The results are presented as frequency and percentages. *Chi square test and the Fisher's test; P<0.01 was considered statistically significant.

Moreover, 1298A allele, 1298C allele, 66G allele, and 66A allele had PCR amplicon lengths of 281 bp, 361 bp, 458 bp, and 117 bp, respectively. Two different amplicons' existence for SNP were indicated as heterozygotes (figures 1-3). Besides, three nonspecific amplicons were formed due to common primer pairing (529, 593, and 183 bp).

Hardy-Weinberg Equilibrium

The results of genotype frequency for all SNPs were in agreement with the Hardy-Weinberg equilibrium. As shown in table 3, there were no significant differences between the observed and expected genotypes for each SNP.

SNP c.677C>T Results

Table 4 demonstrates the genetic models (codominant, dominant, recessive, and overdominant models) for the SNP c.677C>T genotypes. The results showed significant differences in a codominant modal (CT, TT) with P<0.0001 and P<0.01, respectively.

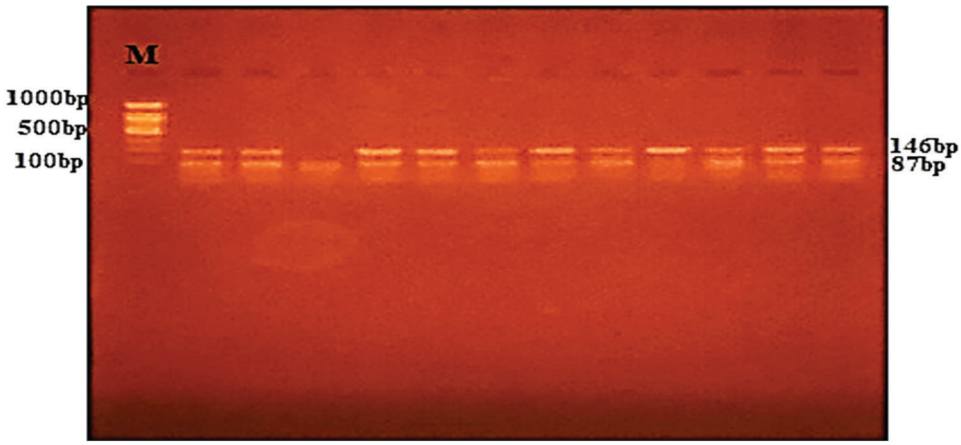

Figure 1: Electrophoresis of 677 T allele (146 bp), and 677C allele (87 bp) was detected by Tetra-primer ARMS-PCR. M lane represents the DNA ladder (1000 bp).

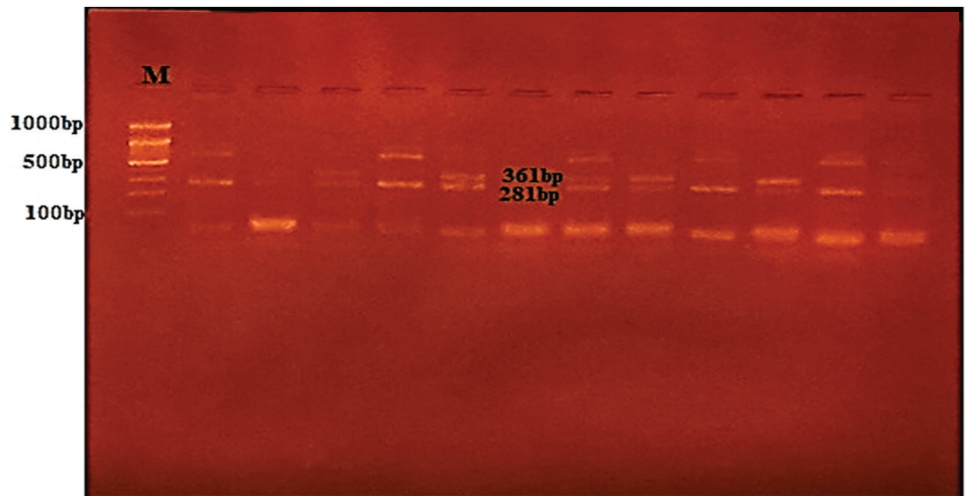

Figure 2: Electrophoresis of 1298A allele (281 bp), and 1298C allele (361 bp) was detected by Tetra-primer ARMS-PCR. M lane represents the DNA ladder (1000 bp).

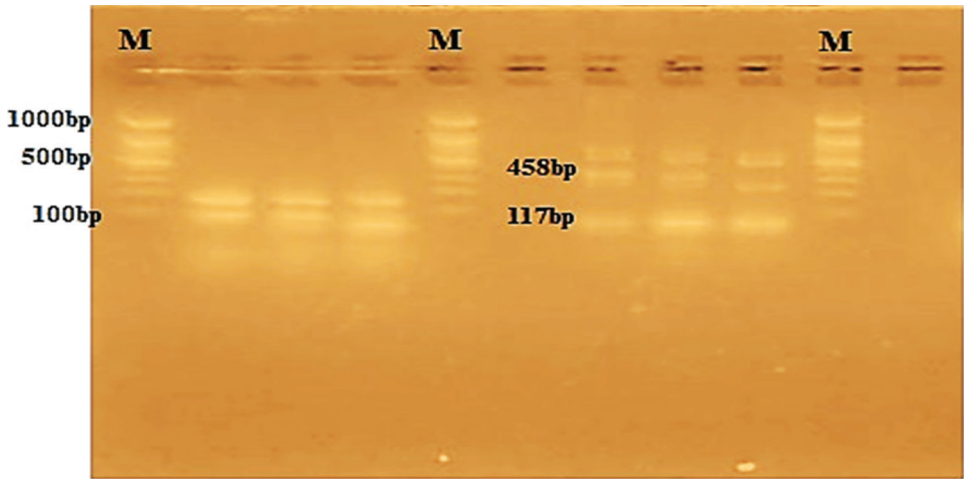

Figure 3: Electrophoresis of 66G allele (458 bp), and 66A allele (117 bp) was detected by Tetra-primer ARMS-PCR. M lane represents the DNA ladder (1000 bp).

Table 3: Genotypes frequency and Hardy-Weinberg Equilibrium (HWE) of SNPs (c.677C>T, c.1298A>C, c.66A>G)

SNPs	Groups		Genotypes frequency			
			CC (%)	CT (%)	TT (%)	HWE P≥0.05*
c.677C>T (n=270)	Mild/Moderate (n=140)	Observed	124 (88.5)	13 (87)	3 (2)	0.054
		Expect	122 (87.1)	17.7 (12.6)	0.6 (0.4)	
	Severe/Critical (n=130)	Observed	75 (57.6)	45 (34.6)	10 (7.6)	0.4
		Expect	73.1 (56.2)	48.8 (37.5)	8.1 (6.2)	
			AA (%)	AC (%)	CC (%)	
c.1298A>C (n=270)	Mild/Moderate (n=140)	Observed	120 (85.7)	18 (12.9)	2 (2.1)	0.2
		Expect	118.9 (84.9)	20.3 (14.3)	0.9 (0.6)	
	Severe/Critical (n=130)	Observed	84 (64.6)	37 (28.5)	9 (6.9)	0.09
		Expect	80.8 (62.3)	43.4 (33.3)	5.8 (44.6)	
			AA (%)	AG (%)	GG (%)	
c.66A>G (n=270)	Mild/Moderate (n=140)	Observed	132 (94.3)	7 (5)	1 (0.7)	0.34
		Expect	131.1 (93.6)	8.7 (6.2)	0.1 (0.07)	
	Severe/Critical (n=130)	Observed	90 (69.2)	33 (25.4)	7 (5.4)	0.1
		Expect	87.2 (67.1)	38.5 (29.6)	4.2 (3.2)	

*Chi square test

Table 4: The genetic modal of c.677C>T of the clinical severity of COVID-19 infection

Genetic model	Genotype and allele	Mild/Moderate n (%) n=140	Severe/Critical n (%) n=130	OR (95% CI)	P value*
Codominant	CC ref	124 (88.6)	75 (57.7)	---	---
	CT	13 (9.3)	45 (34.6)	5.72 (2.91-11.27)	<0.0001
	TT	3 (2.1)	10 (7.7)	5.51 (1.48-20.54)	0.01
Dominant	CC ref	124 (88.6)	75 (57.7)	---	
	CT/TT	16 (11.4)	55 (42.3)	5.68 (3.05-10.61)	<0.0001
Recessive	CT/CC ref	137 (97.9)	120 (92.3)		
	TT	3 (2.1)	10 (7.7)	3.81 (1.03-14.08)	0.04
Overdominant	CC/TT ref	127 (90.7)	85 (65.4)		
	CT	13 (9.3)	45 (34.6)	5.17 (2.64-10.14)	<0.0001
Allele	C ref	261 (93.2)	195 (75)	---	
	T	19 (6.8)	65 (15.9)	4.58 (2.66-7.88)	<0.0001

*Chi square test; ref: reference genotype; OR: Odds ratio; CI: Confidence interval; P<0.05 was considered statistically significant.

The OR for CT and TT was 5.72 and 5.51, respectively. Besides, the dominant model (CT/TT) revealed a significant association between the studied groups (P<0.0001 and OR=5.68). Besides, the recessive (TT) model and the overdominant model (CT) had significant associations (P<0.0001 and P=0.04; OR=3.81 and OR=5.17, respectively). Moreover, the

T allele showed significant variation (P<0.0001 and OR=4.58). This indicated the susceptibility of SNP in the development of infection.

SNP c.1298 A>C Results

Table 5 demonstrates the genetic models (codominant, dominant, recessive, and overdominant models) for SNP c.1298 A>C. There was a significant variation in the codominant modal (AC, CC) at P=0.001 and P=0.01, respectively, and OR=2.94 and 6.43, respectively. Furthermore, the dominant model (AC/CC) showed a significant association between the studied groups (P<0.0001 and OR=3.29). Besides, the recessive (CC) model and overdominant model (AC) showed significant associations (P=0.03 and P=0.002, respectively; OR=5.31 and OR=2.7, respectively). Moreover, the C allele indicated a significant difference at P<0.001 and OR=3.15. These findings indicated the susceptibility of these SNPs in the development of infection.

SNP c.66A>G Results

Table 6 shows the genetic models (codominant, dominant, recessive, and overdominant models) for SNP c.1298 A>C. There was a significant difference in a codominant model (AG, GG) with P<0.001 and P=0.01, respectively, and OR=6.9 and 10.3, respectively. The dominant model (AG/GG) also indicated a significant association between the studied groups (P<0.001 and OR=7.3). In addition, the recessive model (GG) and overdominant model (AG) showed significant associations (P=0.03 and P<0.001, respectively). The OR was 7.9 and 6.46, respectively. Moreover, the G allele showed a significant difference (P<0.001 and OR=6.64).

Haplotype Results

Haplotypes were determined for SNPs c.677C>T,c.1298A>C, and c.66A>G. There were significant differences between the control and patient groups for haplotypes C-A-A, T-A-A, and T-C-G. The C-A-A was related to decline risk significantly (OR=0.218 and P<0.0001). Thus, it indicated the protective effect of haplotype C-A-A on the development of COVID-19 infection.

Table 5: The genetic model of c.1298A>C of the clinical severity of COVID19- infection

Genetic model	Genotype and allele	Mild/Moderate n (%) n=140	Severe/Critical n (%) n=130	OR (95% CI)	P value*
Codominant	AA ref	120 (85.7)	84 (64.6)	-----	
	AC	18 (12.9)	37 (28.5)	2.94 (1.57-5.49)	0.001
	CC	2 (1.2)	9 (6.9)	6.43 (1.36-30.3)	0.01
Dominant	AA ref	120 (85.7)	84 (64.6)	----	
	AC/CC	20 (14.3)	46 (35.4)	3.29 (1.82-5.94)	<0.0001
Recessive	AC/AA ref	138 (98.6)	121 (93.1)	----	
	CC	2 (1.2)	9 (6.9)	5.31 (1.09-24.08)	0.03
Overdominant	AA/CC ref	122 (87.1)	93 (71.5)	---	
	AC	18 (12.8)	37 (28.5)	2.7 (1.45-5.02)	0.002
Allele	A ref	258 (92.1)	205 (78.8)	---	
	C	22 (7.9)	55 (21.2)	3.15 (1.86-5.33)	<0.001

*Chi square test; ref: reference genotype; OR: Odds ratio; CI: Confidence interval; P<0.05 was considered statistically significant.

Table 6: The genetic model of c.66A>G of the clinical severity of COVID-19 infection

Genetic model	Genotype and allele	Mild/Moderate n (%) n=140	Severe/Critical n (%) n=130	OR (95% CI)	P value*
Codominant	AA ref	132 (94.3)	90 (69.2)	----	
	AG	7 (5%)	33 (25.4)	6.9 (2.94-16.26)	<0.001
	GG	1 (0.71)	7 (5.4)	10.3 (1.25-84.18)	0.01
Dominant	AA ref	132 (94.3)	90 (69.2)	-----	
	AG/GG	8 (5.7)	40 (30.8)	7.3 (3.29-16.35)	<0.001
Recessive	AG/AA ref	139 (99.3)	123 (94.6)	----	
	GG	1 (0.71)	7 (5.4)	7.9 (0.97-64.71)	0.03
Overdominant	AA/GG ref	133 (95)	97 (74.6)	-----	
	AG	7 (5)	33 (25.4)	6.46 (2.75-15.18)	<0.001
Allele	A ref	271 (96.8)	213 (81.9)	----	
	G	9 (3.2)	47 (18.1)	6.64 (3.19-13.84)	<0.001

*Chi square test; ref: reference genotype; OR: Odds ratio; CI: Confidence interval; P<0.05 was considered statistically significant.

Table 7: Frequencies of haplotype among SNPs c.677C>T, c.1298A>C, and c.66A>G of the clinical severity for COVID-19 infection

Haplotype	Mild/Moderate	Severe/Critical	OR (95% CI)	P value*
C-A-A	0.750	0.918	0.218 (0.126-0.377)	<0.0001
C-C-A	0.00	0.014	---	--
T-A-A	0.04	0.003	15.5 (2.339-102.111)	0.004
T-A-G	0.00	0.001	--	--
T-C-A	0.031	0.033	0.910 (0.348-2.380)	0.8
T-C-G	0.181	0.031	6.772 (3.213-14.272)	<0.0001

*Chi square test; OR: Odds ratio; CI: Confidence interval; P<0.05 was considered statistically significant.

T-A-A and T-C-G haplotypes were associated with an increased risk of COVID-19 infection (OR=15.5 and P<0.0001, and OR=6.772 and P<0.0001, respectively). The risk impact of T-A-A and T-C-G haplotypes with the development of COVID-19 infection is shown in table 7. Moreover, the linkage disequilibrium (LD) for SNPs was investigated, and the D' value was 99% between the control and patient groups (figure 4).

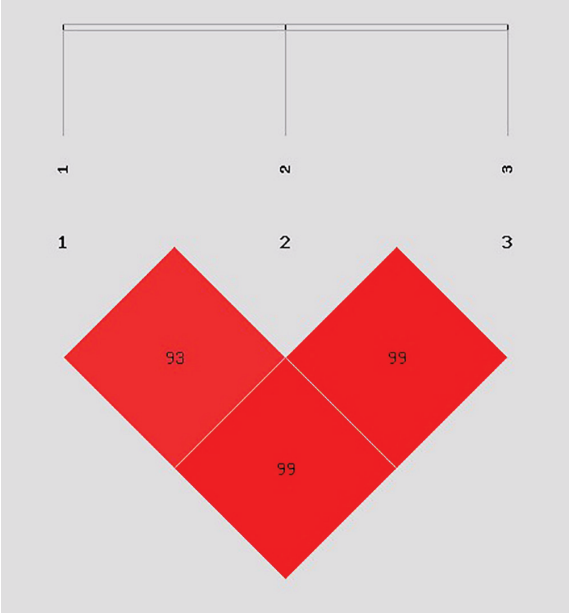

Figure 4: Analysis pairwise of linkage disequilibrium among SNPs c.677C>T; c.1298A>C; c.66A>G with the clinical severity for COVID-19 infection. 1 represents c.677C>T. 2 represents c.1298A>C. 3 represents c.66A>G.

Discussion

The results showed significant differences between mild/moderate cases and severe/critical cases in age (35-65) years. The results showed significant differences between the T allele for SNP c.677C, C allele for SNP c.1298A>C, and G allele for SNP c.677C>T. Therefore, these SNPs may indicate a predisposition to the development of COVID-19 infection. The haplotype C-A-A was related to a lower risk, which indicated a protective effect against COVID-19 infection development. The increased risk was associated with T-A-A and T-C-G haplotypes, which showed a risk impact on COVID-19 infection development.

COVID-19 mortality is more dependent on age than death from other diseases. Men have a higher risk than women.[23] The present study showed significant differences in age categories based on the clinical severity of COVID-19 infection groups. Whereas some Iraqi studies reported that age influenced the severity of COVID-19 infection.[24, 25] In addition, another study found that significant differences were higher in older people, with a positive relationship between COVID-19 infection and age.[26]

Individuals with age more than 65 years had a higher risk of infection and death from COVID-19 than other age categories, making immunization against COVID-19 a top priority.[27] COVID-19 infection is common in elderly patients and can be caused as a result of lower immunity, chronic disease, malnourishment, increased ACE-2 expression, and organ failure.[28] Moreover, previous research indicated that chronic diseases could cause an increase in virus load during COVID-19 infection, indicating that hypertension and diabetes were risk factors in patients.[26] Besides, another study reported that patients with chronic disease in Wuhan (China), died at a higher rate (7.3%, 6.3%, 6%, and 5.6%) due to diabetes, chronic respiratory diseases, hypertension, and cancer, respectively.[29]

It represents glucose and glutamine, which are energy sources for viruses,[30] could affect immunity function, and conversely, weakens the immune system and causes macrovascular problems.[31] Individuals with diabetes might have high glucose levels, which could create an environment conducive to excess.[32] Moreover, SARS CoV-2 infection could cause hyperglycemia even in people without previously existing diabetes.[33] It could worsen diabetes, if not controlled properly, could increase the risk of COVID-19 complications and even death.[32]

According to the findings of the present study, patients with COVID-19 were at a higher risk of developing thrombosis and coagulopathy. It is

hypothesized that homocysteine is an amino acid that plays a critical role in coagulation. Currently, several genes, such as SNPs in ABO and *MTHFR*, which contributed to the development of those disorders, regulate different levels of homocysteine.[34]

It is worth mentioning that no previous Iraqi study was comparable to the present study. The present study found that patients with COVID infections were sensitive to the SNPs c.677C>T, c.1298A>C, and c.66A>G. Other studies, such as Karst and others indicated that estimating homocysteine levels as well as studying *MTHFR* gene polymorphisms could be useful in evaluating susceptibility to COVID-19 infection.[35] Sezer and others showed that the SNP *MTHFR* C677T distribution was analogous in COVID-19 groups with critical, mild, and asymptomatic symptoms.[36] Moreover, the frequencies of SNP *MTHFR* C677T were similar across patients who died and survived. These findings suggested that the presence of SNP *MTHFR* C677T and *MTHFR* A1298C had no effect on the severity of COVID-19 symptoms. Ponti and others also found a correlation between *MTHFR* C677T polymorphism and COVID infection, with Latinos having a higher prevalence of T alleles and a higher risk of death from COVID-19 infection than other populations.[37]

Methylenetetrahydrofolate reductase is an enzyme that contributes to folate metabolism. *MTHFR* is involved in the synthesis of purine-pyrimidine in the synthesis of RNA and DNA, the transformation of homocysteine to methionine, and the methylation. There is a significant polymorphism in the *MTHFR* gene, including *MTHFR* 677C/T, which causes the enzyme to be thermolabelile and increases homocysteine. This increase leads to a decrease in endothelial activity and increase the potential of clot formation. Therefore, this condition causes an elevated risk of venous thrombosis.[38, 39]

The role of homocysteine in different metabolism and inflammation actions were confirmed, and several populations with diverse ethnical dominances exhibited varying distributions to *MTHFR* mutations gene variants and *MTHFR* activity.[40]

Some of the adverse biochemical effects of thermolabile-enzyme encoded by the T allele, such as an increase in homocysteine levels, could be reversed by increasing folic acid intake and vitamin B consumption.[37]

MTRR (Methionine synthase reductase) is a catalyzed enzyme that re-methylates homocysteine to methionine via a process involving cobalamin and folate. Cobalamin serves as a methyl transporter between *MTHFR* and homocysteine. *MTRR* has an important role in preserving cobalamin in an effective form, making it a relevant marker of total homocysteine concentration in plasma.[41] The 66A>G polymorphism in the *MTRR* gene converts isoleucine to a methionine residue, which results in reduced enzyme activity.[42]

Remarkably, the findings of the present study revealed that the analysis of haplotypes for SNPs C677T, A1298C, and A66G was significantly associated with an increasing risk, indicating that haplotype C-A-A has a protective effect on the development of COVID infection. Although the rising risk was associated with T-A-A and T-C-G haplotypes, the risk impact of T-A-A and T-C-G haplotypes with the development of COVID-19 infection was shown. Besides, the linkage disequilibrium (LD) for the studied SNPs was complete. There were no studies demonstrating the association of haplotypes for SNPs analyzed in this study, except for a study on the Syrian population that reported there were linked haplotypes (CA, TA, CC, TC) between C677 T and A1298C, in addition to the linkage disequilibrium was complete, D' value=100%.[19]

One of the limitations of this study was that due to a lack of funding, we were unable to examine the effect of biomarkers on COVID-19 patients and determine their association with SNPs. It is recommended that additional studies be conducted in the future to determine the effect of gene expression for *MTHFR* and *MTRR* genes in COVID-19 patients.

Conclusion

T allele for SNP c.677C, C allele for SNP c.1298A>C, and G allele for SNP c.677C>T indicated significant differences. These SNPs indicated a predisposition to the development of COVID-19 infection. However, the C-A-A haplotype was associated with a decrease in risk. In contrast, the T-A-A and T-C-G haplotypes were associated with increased risk.

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Authors' Contribution

Both authors contributed to the study conceptualization; data curation; investigation; methodology; project administration; resources; and software. N.N.B: wrote the original draft;

reviewing and editing; S.F.A.: Reviewing and editing. Both authors have read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

Conflict of Interest: None declared.

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