

Association of TNF- α -308G/A Gene Polymorphism with Coronavirus Disease-19 Severity

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Abstract

Background: From the time when the first outbreak of coronavirus disease (COVID-19), only a small proportion of infected people developed a severe infection, which is usually a sequel of cytokine overproduction. Genetic variations in the genes of some cytokines can influence the transcription rate of these cytokines. **Objective:** The going research article tried to evaluate the link between tumor necrosis factor (TNF)- α -308 gene polymorphism and COVID-19 severity. **Materials and Methods:** Blood samples were obtained from 60 patients with COVID-19 and verified by reverse transcriptase polymerase chain reaction (PCR) in nasopharyngeal swabs. Patients were categorized into two categories based on the severity of the disease: severe COVID-19 included 30 patients and mild/moderate COVID-19 with 30 patients. The nucleic DNA was obtained from the whole blood, and TNF- α -308G>A polymorphism was genotyped utilizing PCR-restriction fragment length polymorphism. **Results:** Homozygous (GG) and heterozygous (GA) genotypes were more frequent among severe than among mild cases, although the differences were not significant. At the allelic level, the frequency of a mutant allele (A) was higher in severe than in mild cases with a noticeable distinction (odds ratio = 2.49, 95% confidence interval = 1.1–5.64, $P = 0.029$). **Conclusion:** Allele A of TNF- α -308G>A may be deemed a threat for the severity of COVID-19.

Keywords: Coronavirus disease, restriction fragment length polymorphism, tumor necrosis factor-alpha gene polymorphism

INTRODUCTION

In the public health and therapeutic systems around the globe, severe acute respiratory syndrome coronavirus (SARS-CoV-2) infection has had disastrous consequences. The disease associated with this infection is called coronavirus sickness (COVID-19). It was called comprehensive by world health organization and has become a serious issue for people all over the world. According to data that have been collected recently from all patients with COVID-19, about one-fifth of them developed severe or critical disease.^[1] The disease had a wide spectrum of symptoms and signs involving almost all body organs.^[2,3]

In patients with COVID-19, the immunity of the host could be the leading cause of a deadly inflammatory condition identified as cytokine release syndrome.^[4] It is a state of severe inflammation characterized by the quick and extensive secretion of inflammatory cytokines in

response to infectious stimuli. In clinical settings, such a phenomenon is associated with a severe disease that usually has to be conceded to the intensive care unit or even death.^[5]

Tumor necrosis factor- α (TNF- α) is a principal player among proinflammatory cytokines. It is a 17kDa protein comprising 157 amino acids, generated mostly via active macrophages, T lymphocytes, and natural killer cells.^[6] The human TNF gene, positioned within the small arm (p-arm) of chromosome 6, is a 7kb DNA sequence consisting of TNFA and TNFB, encoding TNF- α and TNF- β , successively, everyone carrying four exons and three introns.^[7]

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The promoter zone of the TNF- α gene is a polymorphic region. However, the most common single nucleotide polymorphism (SNP) in this region is TNF- α -308 G/A (rs1800629), which can present on the basis of three distinct genetic phenotypes: the homozygous form G/G as well as the mutated version forms G/A and A/A. The wild-type genetic profile G/G occurs most frequently in a defined sample of a certain group of people, typically linked to people who produce little of TNF- α . The mutated version, G/A, nevertheless, is associated with medium production, whereas the less common kind, A/A, is linked with max-level TNF- α -productive people.^[8] The goal of this going study is to investigate the connection of TNF- α -308 polymorphism with the severity of COVID-19 in local patients in Iraq.

MATERIALS AND METHODS

The study population

This case-control retrospective study was carried out during the period from September to the end of December 2021 and performed on 60 individuals from both genders (35 males and 25 females) with a variety of age groups (mean = 61.8 ± 17.2 , range = 16–90). All patients presented with signs and symptoms suggesting COVID-19 and investigated positive for COVID-19 according to reverse transcriptase polymerase chain reaction (PCR) in nasopharyngeal swabs. Patients' blood samples were collected from two hospitals in Baghdad: Al-Emamain Al-Kadhemain Medical City and Dar AL-Salam, which had frequently served as a quarantine center in Baghdad. Samples were processed in the departments of the Medical Microbiology, College of Medicine, AL-Nahrain University.

Patients were categorized into two categories based on the severity of COVID-19 according to guidelines issued by the Iraqi Ministry of Health. The mild/moderate group was composed of 30 individuals hospitalized in the hospital's medical suite, whereas the severe group included also 30 patients who required intensive care unit admission because of the complications of the disease.

DNA extraction and genotyping

Quick protocol SYNCTM DNA extraction Kit (Geneaid) has been applied to obtain highly pure genomic DNA using venous ethylene diamine tetra-acetic acid anticoagulated blood samples. A specific pair of primers was used in conventional PCR to detect a partial DNA sequence of the TNF- α gene, where -308G>A SNP is situated. The forward and reverse primers were: F: GAGGC AATAGGTTTTGAGGGCCAT and R: GGGACACAC AAGCATCAAG as previously described.^[9] The following PCR criteria were used: 94°C for 4min, then 36 cycles of 94°C for 20s, 65°C for 20s, and 72°C for 40s, cutoff at 72°C for 5min. Five μ L of PCR product was subjected to 3% agarose gel electrophoresis with ethidium bromide (0.5 μ g/mL; Sigma). Amplicon can be seen by using an Ultraviolet, after that a photograph was taken via a camera.

Restriction fragment length polymorphisms were used for genotyping of TNF- α -308 G/A. The reaction was completed in a 50 μ L reaction that contains 20 μ L of PCR yield, 1 μ L of NCOI restriction enzyme (New England Biolabs LTD, Beijing, China) mixed with 5 μ L of 1 X NE buffer. The volume was brought to 50 μ L by adding deionized water. Eppendorf tubes were incubated for 37°C/1 h in a PCR thermocycler. Then, the reaction was stopped by adding 10 μ L of 6 X gel loading dye. Five μ L of the digested PCR product was subjected to 3% agarose gel electrophoresis.^[10] Amplicon can be visualized via Ultra Violate after that a photograph was taken using a camera. Allele amplicon size G: 126 bp A: 147 bp.

Statistical analysis

All of the statistical analyses were conducted via SPSS software (Chicago, IL, USA) version 25. A $P < 0.05$ was deemed to be the level of significance. Numerical variables were cited as mean as well as standard deviation and examined with a student t-test. Categorical data were cited as numbers, and to evaluate the relationship between the TNF-308 gene polymorphism and the likelihood of developing severe COVID-19, logistic regression was employed to generate the odds ratio (OR) and the related 95% confidence intervals (CI).

Ethical endorsement

The study was carried out in conformity with the moral standards set forth in the Helsinki Declaration. Before the sample was taken, it was done with the patients' verbal and analytical consent. A local ethics committee examined and approved the study protocol, subject information, and permission form on December 28, 2020 in accordance with document 202011129.

RESULTS

Patients' demographic characteristics

Patients were divided into severe and mild/moderate cases according to guidelines issued by the Iraqi Ministry of Health. Patients' mean ages with severe infection were 62.1 ± 15.28 years that did not differ noticeably from that of mild/moderate infection (59.0 ± 19.71 years). Likewise, the two groups had an almost similar distribution of gender with no significant difference. Diabetes, hypertension, and renal failure were slightly more frequent among severe cases (20%, 36.67%, and 16.67%, respectively) than among mild/moderate cases (16.67%, 23.33%, and 6.67%, respectively); however, the differences were not significant [Table 1].

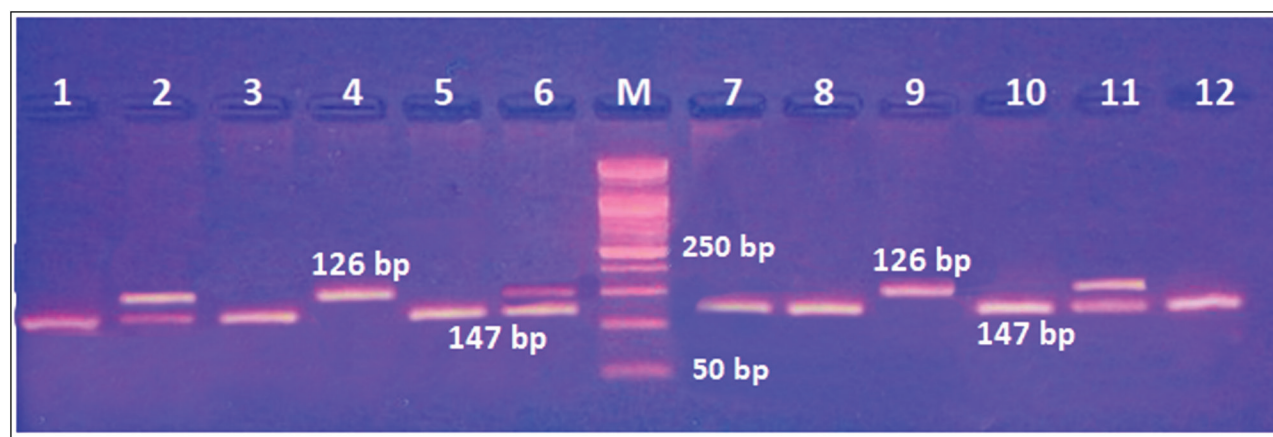
Tumor necrosis factor- α -308 gene polymorphisms

Based on the enzymatic digestion pattern visualized in gel electrophoresis [Figure 1], TNF- α -308 gene polymorphism appeared in three genotypes, which were GG, AG, and AA.

Table 1: Characteristics of studied population's demographics

Variables	Severe cases (30)	Mild/moderate cases (30)	P value
Age (years)			0.499
Mean $\pm$ SD	62.1 $\pm$ 15.28	59.0 $\pm$ 19.71	
Range	16.0–90.0	17.0–89.0	
Gender			0.791
Males	17 (56.67%)	18 (60%)	
Females	13 (43.33%)	12 (40%)	
Comorbidities			
T2DM	6 (20%)	5 (16.67%)	0.739
Hypertension	11 (36.67%)	7 (23.33%)	0.260
Renal failure	5 (16.67%)	2 (6.67%)	0.424
IHD	2 (6.67%)	3 (10%)	1.00

IHD: ischemic heart disease, SD: standard deviation, T2DM: type 2 diabetes mellitus

**Figure 1** : Gel electrophoresis for TNF- α -308 PCR yields after being stained with ethidium bromide and observed by ultraviolet light. M: 50–500 bp ladder; lanes 1, 3, 5, 7, 8, 10, and 12: wild-type (GG), lanes 2, 6, and 11: heterozygous genotype (GA), lanes 4 and 9: mutant genotype (AA)

The distribution of different genotypes, whether in severe or mild/moderate cases, was in good accordance with Hardy Weinberg equilibrium. Comparison between genotypes and allele frequencies between mild/moderate and severe cases revealed that both homozygous (AA) and heterozygous (GA) genotypes were more common among severe cases (36.67% and 20%, respectively) than among mild/moderate cases (26.67% and 6.67%, respectively). However, the differences were not significant. In the recessive inheritance model, the frequency of the GA + AA genotype was far more common in severe cases (56.67%) than in mild/moderate cases (33.33%) with a difference very close to the considerable level. At the allelic level, the mutant allele (A allele) was more common in severe than in mild/moderate cases (38.33% vs. 20%) with a significant difference (OR = 2.49, 95% CI = 1.1–5.64, P = 0.029) as illustrated in Table 2.

DISCUSSION

In the present study, there was not a considerable difference between severe and mild/moderate groups of COVID-19 patients in terms of age, gender, and comorbidities. That is because the patients in the mild/moderate group

were carefully selected to match those in the severe group in these characteristics. Otherwise, advanced age and comorbidities were frequently associated with severe COVID-19 infection. For example, Ambrosino *et al.*^[11] highlighted the existence of chronic diseases such as type 2 diabetes mellitus, hypertension, and cardiovascular diseases, and obesity disorders can exacerbate the mortality risk associated with age above 60 years. Furthermore, 31% of adult patients had associated diseases, including heart failure (10.5%), type 2 diabetes mellitus (10.4%), and coronary heart disease (8.5%), according to meta-analyses designed to investigate the influence of comorbidities on the progression and clinical consequence of COVID-19. It was discovered that certain preexisting associated diseases are related to the severity of COVID-19 (relative risk = 2.11, P = 0.046).^[12]

The most interesting finding in the present study was that allele A of TNF- α -308A>G polymorphism was significantly related to severe COVID-19 (OR = 2.49, 95% CI = 1.1–5.64, P = 0.029). This implies that patients carrying allele A of this polymorphism have about 2.5-time chance of developing severe infection compared with those carrying allele G, regardless of other characteristics.

Table 2: Genotype and allele frequencies of TNF- α -308G>A gene polymorphism in severe and mild/moderate cases

TNF- α -308	Severe cases (30)	Mild cases (30)	P value	OR (95%CI)
Genotypes				
GG	13 (43.33%)	20 (66.67%)	0.154	1.0
GA	11 (36.67%)	8 (26.67%)	0.086	4.62 (0.81–26.45)
AA	6 (20%)	2 (6.67%)	0.406	2.18 (0.35–13.75)
HWE	0.109	0.361		
Dominant				
GG + GA	24 (80%)	28 (93.33%)	0.146	1.0
AA	6 (20%)	2 (6.67%)		3.5 (0.65–18.98)
Recessive				
GG	13 (43.33%)	20 (66.67%)	0.072	2.62 (0.92–7.46)
GA + AA	17 (56.67%)	10 (33.33%)		
Alleles				
G	37 (61.67%)	48 (80%)	0.029	1.0
A	23 (38.33%)	12 (20%)		2.49 (1.1–5.64)

HWE: Hardy Weinberg equilibrium

Significance of bold P value= $P < 0.005$

This result is consistent with an Egyptian study done by Saleh *et al.* who disclosed that AA genotype of this polymorphism was associated with a more aggressive pattern of the disease.^[13] Likewise, Sotomayor-Lugo *et al.* mentioned higher propensity of being asymptomatic in persons carrying the GG genotype.^[14]

Several previous studies on other diseases indicated the association -308A/G polymorphism with serum level of TNF- α . Nasser and Ezzat^[15] showed that the existence of a G>A SNP at stance -308 in the promoter of the TNF- α gene might boost levels of TNF- α 6–7-fold in plasma. Ruan and Lv^[16] showed asthmatic patients carrying with at least one A allele (genotype AA or AG) demonstrated significantly greater TNF- α serum levels than those carrying GG genotype. Likewise, Yang *et al.*^[17] indicated that people carrying the GA genotype possess higher quantities of TNF- α mRNA, and serum levels of this cytokine, than those with the GG genotype.

Increased serum level of proinflammatory cytokines including TNF- α is considered a prominent risk element for the progression of severe COVID-19 and its complications. A meta-analysis including 23 relevant studies stated that TNF- α level in the serum was significantly greater in severe COVID-19 cases compared with nonsevere cases regardless of other factors.^[18] Some other investigations also indicated that serum levels of TNF- α in severe COVID-19 patients were markedly greater than in nonsevere patients.^[19–21]

CONCLUSIONS

These data indicate the role of the A allele of TNF- α -308A/G polymorphism in the development of severe disease in COVID-19 regardless of age and comorbidities, that is, because this allele is associated with increased TNF- α gene transcription. Thus, it seems reasonable to

genotype this polymorphism in COVID-19 at admission. Anti-TNF- α agents could be used for patients with AA genotype to reduce the risk of development of severe infection.

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Conflicts of interest

There are no conflicts of interest.

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