




## Association of TNF- $\alpha$ G-308 A Promoter Polymorphism with the Course and Outcome of COVID-19 Patients

Ahmed Saleh <sup>a</sup>, Ahmed Sultan<sup>a</sup>, Mohamed a Elashry<sup>a</sup>, Ahmed Farag<sup>a</sup>,  
Metwaly Ibrahim Mortada<sup>b</sup>, Mayada A Ghannam<sup>b</sup>, Ahmed M Saed<sup>a</sup>,  
and Sayed Ghoneem<sup>a</sup>

<sup>a</sup>Department of Internal Medicine, Hepatology & Gastroenterology Unit, Faculty of Medicine, Mansoura University, Al Mansurah, Egypt; <sup>b</sup>Department of Clinical Pathology, Haematology Unit, Faculty of Medicine, Mansoura University, Al Mansurah, Egypt

### ABSTRACT

**Background:** Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is one of the most important cytokines that manage the host defense mechanism, which may play a role in the pathogenesis of COVID-19 patients. The work aims to study the association of TNF- $\alpha$  G-308 A gene polymorphism with the course and outcome of COVID-19 patients in Mansoura University Hospital.

**Methods:** 900 patients with COVID-19 infection and 184 controls were tested for TNF- $\alpha$  G-308 A promoter polymorphism. Different genotypes of TNF- $\alpha$  G-308 A were compared as regards the severity and prognosis of the disease.

**Results:** No statistically significant difference was found between patients and controls as regards the demographic data. The AA genotype of TNF- $\alpha$  showed a higher incidence of the disease in comparison to the other genotypes. As regards the demographic and laboratory characters, no statistically significant difference was found between the different genotypes except for age, lymphopenia, CRP, and serum ferritin levels. In 336(80.0%) cases of the AA genotype, the disease was severe in comparison to 90(41.7%) cases in the GA genotype and no cases in the GG genotype with  $P = .001$ .

**Conclusion:** People who carry the A allele of TNF- $\alpha$  polymorphism are more prone to COVID-19 infection. The AA genotype of TNF- $\alpha$  is associated with a more aggressive pattern of the disease. In those patients, the use of anti - TNF therapy may be promising.

### KEYWORDS

Tumor necrosis factor;  
COVID-19; Polymorphism

## Background

Cytokines are the key protein regulators of the response of the host to infection and inflammation. They are small proteins that have a molecular weight ranging from 8 to 40 kDa. They initiate and modulate the immune response as well as systemic and local intercellular regulatory factors. According to their function, they can be classified into anti-inflammatory (IL-1ra, IL-4, IL-10, IL-13) and proinflammatory (IL-1, IL-6, IL-8, TNF- $\alpha$ ) molecules, since anti-inflammatory cytokines can suppress the expression of pro-inflammatory cytokines, adhesion molecules, or chemokines (Dinarello 2000).

On exposure to an infectious challenge, proinflammatory cytokines are produced first and very rapidly followed by the production of anti-inflammatory molecules (Dinarello 2000).

In humans, there is growing evidence that the host's cytokine response is determined genetically. In healthy individuals, there are stable and reproducible differences in cytokine production, and these differences are linked with genetic variations in the encoding genes. Most of the cytokine genes are polymorphic, single-nucleotide polymorphisms (SNPs). SNPs are substitutions of a single base at a particular site of the gene. They are stably carried by more than 1% of the population and can potentially affect the protein product when they are located in the promoter region of the gene to which they are related (Bidwell et al. 1999). Inside the TNF- $\alpha$  promoter, several TNF- $\alpha$  polymorphisms have been detected at the positions, relative to the transcription start site, -1031 (T/C), -308 (G/A), -238 (G/A), -851 (C/T), -857 (C/A), -863 (C/A), -419 (G/C), -49 (G/A), -376 (G/A), and -162 (G/A) (Elahi et al. 2009). These polymorphisms may be associated with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis or ankylosing spondylitis. A meta-analysis done by Zhang et al. (2018) showed that TNF- $\alpha$  (-308) polymorphism is associated with increased susceptibility to some infections like dengue fever.

Coronavirus 2019 is a mutational RNA virus named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that causes the clinical syndrome Coronavirus disease 2019 (COVID-19). In the majority of the cases, SARS-CoV-2 infection may be asymptomatic or only cause minor symptoms and is less lethal than MERS-CoV infection. However, in nearly 10 to 20% of the cases, the disease may progress to interstitial pneumonia and acute respiratory distress syndrome (ARDS), especially in those with older age and co-morbidities (Crayne et al. 2019).

As soon as viral antigens are recognized by the immune system, they are presented by the antigen presenting cells both to the CD8-positive cytotoxic T and natural killer cells in the context of major tissue histocompatibility (MHC) antigens as usual. This activates both the innate and adaptive immunity, causing the production of large amounts of chemokines and proinflammatory cytokines. In some patients, this activation becomes so massive that leads to the development of cytokine storm. This results in thrombotic tendency and multi-organ failure and eventually causes death (Li et al. 2020). Previous studies conducted on SARS and the Middle East respiratory syndrome (MERS) induced by different strains of coronavirus, focused on the so-called cytokine storm, showed high release of proinflammatory cytokines such as interleukin-6 (IL-6), TNF- $\alpha$ , IL-8, IL-12 and, IL-1 $\beta$  as well as interferon gamma inducible protein (Lau et al. 2013). In COVID-19-associated ARDS (CARDS), elevated plasma levels of cytokines including IL-6 have been demonstrated and usually correlated with the severity of the clinical course (Huang et al. 2020). IL-6 may play a key role in the inflammatory storm, which underlies the COVID-19 pathogenesis (Huang et al. 2020). Although, higher levels of IL-6 were associated with the need for mechanical ventilation owing to a more severe disease, preliminary data on CARDS patients treated with tocilizumab, favorable outcomes were observed (Liu et al. 2020; Rojas-Martel et al. 2020).

## **Aim of the study**

The work aims to study the association of TNF- $\alpha$  G-308 A gene polymorphism with the course and outcome of Covid-19 patients in Mansoura University Hospital.

## **Subjects and methods**

### ***Study design***

This is a hospital-based case-control study that was conducted on patients admitted to Quarantine department, Mansoura University Hospital.

### ***Subjects***

The study included 900 patients with COVID-19 and 184 controls from health care workers in contact with them during the period from April to July 2020. Patients were selected randomly from both sexes and older than 18 years. The diagnosis of COVID-19 is based on the characteristic suggestive clinical and radiological finding in a computed tomography (CT) scan of the chest (bilateral multiple ground-glass opacities mainly in the periphery) and confirmed by the positive nasopharyngeal swab. Each patient was subjected to:

### ***Complete history with stress on***

- (1) Smoker or not.
- (2) Presence of comorbidities as diabetes, hypertension, cardiac troubles (ischemic or valvular heart diseases), chronic kidney, or liver disease.
- (3) History of contact with patients with COVID-19 or method in which infection was contracted if it is known.
- (4) Prodromal symptoms and severity of the disease.
- (5) Fever, pattern, and level.

### ***Laboratory assessment***

Complete blood count, C-reactive protein (CRP), serum ferritin, D-dimer, and lactate dehydrogenase (LDH) were done to all patients at the time of diagnosis of COVID-19 infection.

### ***Radiology***

CT scan of the chest was done to all patients and controls.

All patients were followed during the period of hospitalization and noticed for improvement of symptoms, the progression of the disease, timing of 1<sup>st</sup>, and 2<sup>nd</sup> negative nasopharyngeal swab, and improvement in CT scan of the chest. According to the severity of the disease, patients were divided into two groups (light and severe). The disease is considered severe if any of the following is present according to the latest guidelines: tachypnea with a respiratory rate more than 30 cycle/min; PaO<sub>2</sub> less than 300 mmHg; oxygen saturation below 93 at rest; shock; respiratory failure or other organ dysfunction. The two groups were compared as regards the distribution of TNF- $\alpha$  polymorphism. After genotyping, the different genotypes of TNF- $\alpha$  were compared as regards the severity, course, and prognosis of the disease.

## **Nasopharyngeal swab**

### **Sample collection and transport**

- (1) Patient nasopharyngeal swabs were collected via Dacron or polyester flocced swabs and transported for testing in viral transport medium (VTM) at 4°C. The samples should be handled in a Biosafety Level 2 facility.

### **RNA extraction**

The purification of viral nucleic acids was done using the QIAamp DSP Virus Spin Kit on a fully automated QIAGEN QIAcube device.

### **Reaction setup**

- (A) A mix of 10 µl of OneStep 2X RT-qPCR Master Mix and 2 µl of COVID-19 primer & probe was added to the wells required for testing for the chosen PCR platform include 1 well for the PCT (positive control Template) and 1 well for the NEC (Negative extraction control).
  
- B) 8 µl of the following was added into the appropriate wells according to the plate setup:
  - i. Samples.
  - ii. PCT.
  - iii. NEC.

### **Data analysis**

Dna- Technology. JSC 2017 Real-Time PCR system V 7.9 using Relative Standard curve and comparative CT Experiments.

## **TNF-α polymorphisms determination**

The DNA was obtained using venous EDTA anticoagulated blood and isolated according to the instructions of the manufacturer using the Wizard Genomic Blood DNA Isolation Kit (Promega, Madison, WI). We stored samples at – 80°C until SNP genotyping by real-time polymerase chain reaction (PCR) was done. All patients were genotyped for the TNF-α G-308A promoter polymorphism using a real-time PCR fluorescence resonance energy transfer assay. We used TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA), according to the instructions of the manufacturer.

### **Statistical analysis**

Data were fed to the computer and analyzed using IBM SPSS Corp. Released in 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Case vs health controls to analyze TNF 308 SNPs in the population. Cases then further analyzed to understand the effect of the varied SNPs on COVID19 illness and outcome. Qualitative data were described using the number and percent. Quantitative data were described using median & inter-quartile range for non-parametric data and mean, standard deviation for parametric data after testing normality using the Kolmogorov-Smirnov test. The significance of the obtained results was judged at the (0.05) level.

Qualitative data were compared using the Chi-Square test, Monte Carlo test & Fischer Exact test as appropriate. Quantitative data were compared using Student t-test and One Way ANOVA test with Post Hoc Tukey test to detect pair-wise comparison (for Parametric data), Kruskal Wallis test & Mann-Whitney U test was used to compare independent groups (for non-parametric data). Binary stepwise logistic regression analysis was used for the prediction of independent variables of the binary outcome. Significant predictors in the univariate analysis were entered into the regression model using the enter method. Adjusted odds ratios and their 95% confidence interval were calculated. Hardy-Weinberg equilibrium was used to compare genotype distribution among studied groups with distribution among the total population.

## Ethics

Written consents from patients and controls who participated in the study or from their families were obtained and approved by the Mansoura medical ethics Committee (MMEC) of the faculty of medicine.

Code: R.20.07.932.

## Results

### *Patient sociodemographic characteristics*

The study has included 900 patients and 184 controls. No significant difference was found between the patients and controls as regards age, sex, presence of diabetes, or hypertension with  $P = .344, 0.521, 0.752,$  and  $0.386$  respectively (Table 1). The mean age of the studied patients was  $51.08 \pm 11.72$  and females represent 44.0% of cases. Diabetes was found in 32.0% of cases and 34.8% of controls while hypertension was found in 21.3% of cases and 28.3% of controls. Chronic liver disease was found in 36 cases, chronic kidney disease in 36 cases, and coronary heart disease in 60 cases in comparison to 5, 6 and, 9 controls respectively. As regards smoking, 48 patients were found to be smokers.

**Table 1.** Demographic characters of the studied groups.

	Control N = 184	Cases N = 900	test of significance
Age/years mean±SD	49.28 ± 6.59	51.08 ± 11.72	t = 0.951 p = .344
Sex	n(%)	n(%)	$\chi^2 = 0.413$
Male	92(50.0)	508(56.0)	p = .521
Female	92(50.0)	392(44.0)	
DM	120(65.2)	616(68.0)	$\chi^2 = 0.100$
-VE	64(34.8)	284(32.0)	p = .752
+VE			
HTN	132(71.7)	712(78.7)	$\chi^2 = 0.751$
-VE	52(28.3)	188(21.3)	p = .386
+VE			
Comorbidities	5(2.7%)	36(4%)	$\chi^2 = 0.100$
CLD	6(3.26%)	36(4%)	p = .852
CKD	9(4.89%)	60(6.6%)	
CHD			

t: Student t test  $\chi^2$ : Chi-Square test DM: Diabetes Melitus HTN: Hypertension  
CLD: chronic liver disease CKD: chronic kidney disease CHD: coronary heart disease

### Genotype testing and distribution

On genotype testing, 420(46.7%) of patients were AA, 288(32.0%) were GA, and 192(21.3%) were GG. As regards the control group, 60(32.6%) of them were AA, 40(21.7%) were GA, and 84(45.7%) were GG (Table 2). From these data, it appears that the AA genotype of TNF- $\alpha$  showed a higher incidence of the disease in comparison to the control group with  $P = .019$ .

### Genotype distribution and disease severity and prognosis

We then studied differences within the affected cases by stratifying the results of labs, clinical course and outcome by promoter genotype. Table 3 shows the demographic and laboratory characters of the studied cases according to the genotype distribution. There was no significant difference between the different genotypes as regards the demographic and laboratory characters except for age, lymphopenia, CRP, and serum ferritin level with  $P = .001, 0.001, 0.03,$  and  $0.001$  respectively. The AA genotype was associated with advanced age, increased degree lymphopenia, high CRP, and serum ferritin in comparison to the other genotypes. Table 4 summarizes the disease severity parameters and outcome in different genotypes of the studied cases. In 336(80.0%) cases of the AA genotype, the disease was severe in comparison to 120(41.7%) cases in the GA genotype and no cases in the GG genotype with  $P = .001$ . The duration until the first and the second swab becomes negative is significantly prolonged in the AA genotype. Mechanical ventilation was needed in 120 cases of the AA genotype in comparison to one case in the GA genotype and no cases in the GG genotype. Table 5 shows risk factors of death among studied cases, where the risk of death was significantly associated with advanced age (above 60 y), degree of lymphopenia, the severity of the disease, need for mechanical ventilation, and lastly the genotype of TNF- $\alpha$ .

## Discussion

In the space of just 3 months, a novel coronavirus – a family of viruses that historically was not viewed as a global health concern – has become daily headline news worldwide. The COVID-19 outbreak represents a global health problem both in developed and developing countries. In developing countries, the large gush of new cases creates a big financial problem. The limited resources in these countries emphasize the importance of identification of high-risk patients who are more liable to develop complications; hence, patients can receive timely and effective treatments.

**Table 2.** Genotype distribution among studied groups.

	Control N = 184(%)	Cases N = 900(%)	test of significance	Odds ratio (95% CI)
Genotypes	60(32.6)	420(46.7)	$\chi^2 = 7.95$	3.06(1.26–7.44)
AA	40(21.7)	288(32.0)	$p = .019^*$	3.15(1.18–8.42)
GA	84(45.7)	192(21.3)		reference group
GG(R)				
Hardy-Weinberg equilibrium	0.001*	0.006*		
Allele	N = 92	N = 150	$\chi^2 = 7.95$	2.18(1.29–3.70)
A	160(43.5)	1128(62.7)	$p = .005^*$	reference group
G (R)	208(56.5)	672(37.3)		

$\chi^2$ :Chi-Square test \*statistically significant

**Table 3.** Demographic and laboratory characters of the studied cases according to genotype distribution.

	Genotypes			test of significance	within group significant
	AA n = 420	GA n = 288	GG n = 192		
Age/years Mean±SD	55.43 ± 11.08	50.13 ± 11.94	43.0 ± 8.09	F = 7.37 P = .001*	P1 = 0.069 P2 < 0.001* P3 = 0.045*
Sex	192(45.7)	216(75.0)	96(50.0)	$\chi^2 = 5.25$ P = .072	P1 = 0.03* P2 = 0.776 P3 = 0.104
Male	228(54.3)	72(25.0)	96(50.0)		
Female					
DM	312(74.3)	144(50.0)	156(81.2)	$\chi^2 = 5.50$ P = .064	P1 = 0.056 P2 = 0.586 P3 = 0.046*
-VE	108(25.7)	144(50.0)	36(18.8)		
+VE					
HTN	324(77.1)	252(87.5)	132(68.8)	$\chi^2 = 2.10$ P = .350	P1 = 0.316 P2 = 0.523 P3 = 0.146
-VE	96(22.9)	36(12.5)	60(31.2)		
+VE					
Smoking	312(74.3)	276(95.8)	168(87.5)	$\chi^2 = 5.10$ P = .078	P1 = 0.03* P2 = 0.287 P3 = 0.327
-VE	108(25.7)	12(4.2)	24(12.5)		
+VE					
White blood cell count	4.62 ± 1.53 4.5(3.3–5.5)	5.16 ± 2.18 4.4(3.92–7.07)	4.58 ± 2.05 4.4(2.6–5.7)	KW $\chi^2 = 0.461$ P = .794	P1 = 0.740 P2 = 0.655 P3 = 0.507
Haemoglobin (gm/dl)	11.98 ± 1.13	11.84 ± 2.80	12.65 ± 1.29	F = 1.01 P = .369	P1 = 0.778 P2 = 0.234 P3 = 0.180
Lymphocytes	1105.43 ± 650.77 900(600–1400)	1655.83 ± 695.84 1850 (942.5–2192.5)	1900.81 ± 697.29 1950(1575–2275)	KW $\chi^2 = 45.37$ P = .001*	P1 = 0.005* P2 < 0.001* P3 = 0.287
C-reactive protein (mg/L)	42.29 ± 28.98 32(24–48)	34.95 ± 31.28 24(12–48)	21.56 ± 14.85 12(12–32)	KW $\chi^2 = 6.84$ P = .03*	P1 = 0.150 P2 = 0.009* P3 = 0.304
Ferritin(ng/ml)	433.40 ± 218.68 423(300–543)	287.50 ± 166.94 278.5 (148.5–366.5)	212.69 ± 89.48 199 (123.25–288.75)	KW $\chi^2 = 18.07$ P = .001*	P1 = 0.004* P2 < 0.001* P3 = 0.119
D-dimer(mg/L)	2.063 ± 3.89 1.23(0.80–1.68)	1.26 ± 0.57 1.05(0.825–1.70)	1.33 ± 0.55 1.20(0.90–1.7)	KW $\chi^2 = 0.55$ P = .760	P1 = 0.567 P2 = 0.919 P3 = 0.462

P1: difference between GG & GA, P2: difference between GG & AA, P3: difference between GA & AA  
KW $\chi^2$ : Kruskal Wallis test, F: One Way ANOVA test,  $\chi^2$ : Chi-Square test, Parameters described as mean ± SD, Median (inter-quartile range).

The key aspect of the study was the selection of our patients and controls. Patients were selected randomly from both the Quarantine department and admitted patients to the intensive care unit (ICU) with varying degrees of severity. We selected the controls from the health care workers who are in contact with the patients to maximize the degree of exposure to infection. Controls were included to study differences in the TNF promoter SNPs in the whole population but the additional thrust of the study is to compare patients with COVID and the different SNPs. In our study, there is no statistically significant difference between the patients and controls as regards the demographic data and this indicates that the two groups are cross-matched.

After genotype testing, a statistically significant difference between the patients and controls was found as regards the genotype distribution, where the A allele is more expressed in patients Vs controls with  $P = .005$ . 420(46.7%) of patients were AA, 288 (32.0%) were GA, and 192(21.3%) were GG Vs 60(32.6%), 40(21.7%) and 84(45.7%) in the control group respectively. This indicates that persons carrying the A allele (AA and GA) are more susceptible to the disease.

**Table 4.** Disease severity and prognosis in different genotypes of the studied cases.

	Genotypes			test of significance	within group significant
	AA n = 420	GA n = 288	GG n = 192		
Severity of disease	84(20.0)	168(58.3)	192(100.0)	$\chi^2 = 29.26$	P1 = 0.003*
Mild	336(80.0)	120(41.7)	0(0.00)	$P = .001^*$	P2 < 0.001*
severe					P3 = 0.003*
Duration till first swab negative (days)	13.91 ± 5.23 13(11–18)	10.67 ± 5.39 9(6.25–15.75)	6.31 ± 2.63 6(4–7.75)	KW $\chi^2 = 21.45$ $P = .001^*$	P1 = 0.026* P2 < 0.001* P3 = 0.007*
Duration till second swab negative(days)	21.74 ± 6.79 21(17–27)	17.71 ± 7.47 14(12–25.5)	11.94 ± 2.72 11(11–1.75)	KW $\chi^2 = 22.69$ $P = .001^*$	P1 = 0.029* P2 < 0.001* P3 = 0.012*
Mechanical Ventilation	300(71.4)	276(95.8)	192(100.0)	MC	P1 = 0.018*
-VE	120(28.6)	12(4.2)	0(0.0)	$P = .006^*$	P2 = 0.017*
+VE					P3 = 0.408
Survival	360(85.7)	288(100.0)	192(100.0)	MC	P1 = 0.053
Survived	60(14.3)	0(0.0)	0(0.0)	$P = .047^*$	P2 = 0.111
Died					P3 = .....

P1: difference between GG & GA, P2: difference between GG & AA, P3: difference between GA & AA

KW $\chi^2$ : Kruskal Wallis test MC: Monte Carlo test  $\chi^2$ : Chi-Square test, Parameters described as mean ± SD, Median (interquartile range), number (percentage)

The response of TNF- $\alpha$  to infection is regulated partly at the transcriptional level, the role of genetic polymorphisms of the TNF- $\alpha$  promoter in the determination of susceptibility to inflammatory disease, or as a marker of the disease severity has been the subject of our research. TNF- $\alpha$  is a central element in the host defense response. To preserve the cellular homeostasis, the production of TNF- $\alpha$  has to be tightly regulated. Interestingly, Molvig et al. (1991) reported that even in healthy subjects, a marked inter-individual variability of TNF- $\alpha$  production in response to different stimuli exists. Family studies indicate that this variability is genetically determined in up to 60% of cases (Westendorp et al. 1997).

In our study, all patients showed elevated levels of CRP, D-dimer, serum ferritin, LDH, and varying degrees of lymphopenia. This is not new and consistent with a recent study by Zhu et al. (2020) who showed elevated levels of D-dimer, CRP, serum ferritin, and LDH in patients with COVID-19 infection. When we compared these parameters in different genotypes of TNF- $\alpha$ , The AA genotype was associated with advanced age (above 60 years), increased degree lymphopenia, high CRP, and serum ferritin in comparison to the other genotypes (Table 3). This indicates that this genotype may be associated with a different entity of the disease. Huang et al. (2020) and Ding et al. (2004) showed that patients with SARS-CoV-2 infection who progress to interstitial pneumonia, and ARDS are noticed to have very high levels of D-dimer and serum ferritin levels, hepatic dysfunction, thrombotic tendency, and disseminated intravascular coagulation (DIC) indicating the occurrence of macrophage activation syndrome (MAS). Paules et al. (2020) reported similar clinical and laboratory findings in patients with SARS-CoV and MERS-CoV infections.

In order to better understand the differing presentations and progression of COVID-19 infection, we compared the different genotypes as regards the disease severity and prognosis; we found that the AA genotype is associated with more aggressive disease and less favorable course in comparison to the other genotypes (Table 4). In the AA genotype, the disease was severe in 336(80.0%) cases in comparison to 120(41.7%) cases in the GA genotype and no cases in the GG genotype with  $P = .001$ . The duration until the first and



**Table 5.** Risk factors of death among studied cases.

	Survived	Died	test of significance
Age/years	50.31 ± 11.76	61.8 ± 1.79	t = 2.17
Mean±SD			P = .03*
Sex	492(58.6)	12(20.0)	FET
Male	348(41.4)	48(80.0)	P = .163
Female			
DM	600(71.4)	12(20.0)	FET
-VE	240(28.6)	48(80.0)	P = .03*
+VE			
HTN	684(81.4)	24(40.0)	FET, P = .06
-VE	156(18.6)	36(60.0)	
+VE			
D-dimer(mg/L)	1.67 ± 2.78	1.306 ± 0.46	Z = 0.458
	1.2(0.80–1.7)	1.2(1.0–1.66)	P = .647
Haemoglobin (gm/dl)	12.16 ± 1.88	10.90 ± 0.96	t = 1.48
			p = .144
Lymphocytes	1508.47 ± 736.26	650.0 ± 320.16	Z = 2.722
	1500(890–2102.5)	500(450–925)	P = .006*
C-reactive protein (mg/L)	33.09 ± 26.10	69.6 ± 38.32	Z = 2.07
	24(12–48)	96(30–96)	P = .038*
Ferritin(ng/ml)	312.11 ± 173.23	724.8 ± 195.18	Z = 3.34
	300(177.5–421.25)	664(565–915)	P = .001*
Severity of disease	444(52.9)	0(0.0)	χ <sup>2</sup> = 5.22
Mild	396(47.1)	60(100.0)	P = .02*
Severe			
Duration till First swab negative (days)	N = 70	N = 2	Z = 1.01
	11.04 ± 5.67	14.5 ± 2.12	P = .31
	9.5(6–16)	14.5(13–16)	
Duration till second swab negative(days)	N = 70	N = 1	Z = 0.832
	18.1 ± 7.4	23.0 ± 0.0	P = .406
	17(12–23.25)	23(23–23)	
Ventilation	768(91.4)	0(0.0)	χ <sup>2</sup> = 31.17
-VE	72(8.6)	60(100.0)	P < .001*
+VE			
Genotype	360(42.9)	0(0.0)	MC
GG	288(34.2)	0(0.0)	P = .047*
GA	192(22.9)	60(100.0)	
AA			

Z: Mann-Whitney U test, FET: Fischer exact test t: Student t test MC: Monte Carlo test χ<sup>2</sup>: Chi-Square test, Parameters described as mean ± SD, Median (interquartile range), number (percentage)

DM: Diabetes Mellitus HTN: Hypertension

the second swab becomes negative is significantly prolonged in the AA genotype. Mechanical ventilation was needed in 120 cases of the AA genotype in comparison to 12 cases in the GA genotype and no cases in the GG genotype.

These data suggest that the A allele is associated with more aggressive disease and this may be related to variations in TNF-α levels in serum. Similarly, Elahi et al. (2009) suggested that the role of TNF-α appears to be contradictory and related to the genetic polymorphisms in the genes regulating its production and effect and the polymorphisms in TNF locus itself. Tsukamoto et al. (1998) reported that the genetic alterations in the TNF-α locus are involved in the production of high TNF-α level (12). TNF-α has two biallelic polymorphisms that are responsible for increased production of TNF-α and have been associated with severe infectious conditions. Located at the position \_308 upstream of the transcriptional start, the first one consists of a G (TNF1) to A (TNF2) substitution responsible for a 6- to 9-fold increase in the transcription of TNF-α in vitro (Wilson et al. 1997) and higher plasma levels of TNF-α in vivo (Warzocha et al. 1998).

We believe that any theory explaining the pathogenesis of COVID-19 should give reasons for the very high levels of both D-dimer and ferritin in the serum out of proportion with the severity of infection, as well as a tendency for monocytosis, rather than lymphocytosis. As regards the prognosis of the disease and survival, the AA genotype showed poor prognosis where 60(14.3%) cases died in comparison to no deaths in the other genotypes (Table 4). After adjustment of other variables, the risk of death from COVID-19 infection is correlated with the genotype of TNF- $\alpha$  (Table 5). Our data showed increased risk of death from COVID-19 in patients with age above 60 years. Parallel to our data, Wang et al. (2020) demonstrated that elderly males especially those with comorbidities showed poor prognosis and increased risk of severe condition or even fatality from COVID-19. Immunosenescence or decline in the immune function occurs with aging where a series of changes occur, a decrease in the generation of CD3 + T cells, a decrease in B lymphocytes, and an increase in regulatory T cells (Li et al. 2019; Weng et al. 2009). Wong and Pamer (2003) showed that the poor outcome in elderly patients with COVID-19 induced cytokine storm was related to immunosenescence. Stebbing et al. (2020) observed that patients with COVID-19 infection have much higher serum levels of TNF- $\alpha$  and they are positively correlated with the severity of the disease. In this study, they found that patients admitted to the ICU have higher levels of TNF- $\alpha$ . This is in agreement with our findings because it is well known that the A allele is associated with high TNF- $\alpha$  production. In those patients with cytokine storm, trials of new treatment modalities like anti-TNF may be promising. Although anti-TNF therapies were suggested as a potential treatment for COVID-19, no sufficient data is available concerning this issue (Ferro et al. 2020). However, in elderly patients, immunomodulatory therapies targeting cytokine storm showed potential for such approaches in improving outcomes and reducing mortality due to COVID-19 (Panigrahy et al. 2020). Feldmann et al. (2020) described the rationale for the use of anti-TNF therapies in COVID-19. These therapies neutralise TNF which is a major component of the cytokine response that is part of the damaging excess inflammatory phase of COVID-19, which is termed hyperinflammation or cytokine storm. In addition, the outcome of patients with inflammatory bowel disease who develop COVID-19 and on anti-TNF therapies is better than those on alternative therapies (Brenner et al., 2020). Similarly, patients with rheumatic disease using anti-TNF therapies showed lower rates of hospital admission for COVID-19 (Gianfrancesco et al. 2020).

Owing to the association between cytokine storm and severe COVID-19 complications and the association of A allele with high TNF- $\alpha$  production, we suggest large multicenter studies to assess the validity of TNF- $\alpha$  G-308 A promoter polymorphism in COVID-19 patients.

The study may be limited by some factors, the small number of patients; it is a single-center study which may affect the gene distribution, lack of assessment of serum levels of TNF- $\alpha$  and lastly, we looked at a single site of TNF- $\alpha$  but this was due to financial support. To summarize, it appears that the gene distribution of TNF- $\alpha$  is an independent factor in the control of the severity and prognosis of COVID-19 infection.

## Conclusion

We concluded that the A allele of TNF- $\alpha$  is significantly expressed in patients with COVID-19 in comparison to controls. This means that the AA genotype of TNF- $\alpha$  is more prone to the disease. The AA genotype of TNF- $\alpha$  is associated with a more aggressive pattern of the disease. In those patients, the use of anti – TNF therapy may be promising.

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## Competing interests

The authors declare that there are no conflicts of interest.

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## ORCID

Ahmed Saleh  <http://orcid.org/0000-0001-8028-1997>

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