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Polymorphism in promotor region of IL6 gene as a predictor for severity in COVID -19 patients

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Abstract

SARS-CoV-2 is the causative agent of coronavirus disease started in 2019 (COVID-19). IL-6 gene is located on chromosome 7. A considerable number of polymorphisms was identified in the IL-6 gene. Polymorphism in IL-6–174C allele is associated with a higher level of IL-6 production and this may lead to severity of in COVID-19 patients. We intended to investigate the role of polymorphism in the promotor region of IL-6 gene as a predictor for disease severity in COVID-19 patients. Fifty patients diagnosed with COVID-19 and classified into moderate and severe groups and twenty apparently healthy controls were enrolled in the study. Genotyping for IL-6 gene (-174G/C) was done by using TaqMan SNP genotyping assay for all studied groups. The distribution of different IL-6-174G/C genotypes among COVID-19 patients was 76% for GG genotype, 22% for GC genotype and 2% for CC genotype. Whereas the distribution of genotypes among the control group was 80% for GG genotype, 20% for GC genotype and 0.0% for CC genotype. The G allele distribution was 87% and 90% in the patients and control groups, respectively, while the C allele was 13% and 10% in the patients and control groups, respectively. There was no significant statistical association between different genotypes, severity and treatment outcome in the patients group. In conclusion, this study showed no relation between -174G/C IL-6 gene polymorphism and disease, in COVID-19 patients.

Keywords: Interleukin-6, Promotor region, Polymorphism, COVID-19, Severity.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that emerged in China at the end of 2019 was a major public health issue and large global outbreak.[1] SARS-CoV-2 is the causative agent of coronavirus disease started in 2019 (COVID-19) where its clinical presentation ranges from mild clinical presentation up to 14% may suffer from severe respiratory distress course and up to 5% of the cases may have a life-threatening respiratory failure and multiorgan failure.[2] Several studies have revealed that COVID-19 associates states of both immunodeficiency and hyperinflammation which is being manifested

by cytokine storm.[3] Among these cytokines is IL6, which was significantly associated with disease severity and has the potential to discriminate between mild and severe disease and possibly may be used as a prognostic marker.[4] Furthermore, host factors such as age, comorbidities and interleukin 6 (IL-6) gene polymorphisms may be involved as a cause of that variability.[5]

IL-6 gene is located on chromosome 7, and a considerable number of polymorphisms was identified in the IL-6 gene, especially inside the non-coding promoter sequence. It has been reported that these polymorphisms exert a powerful influence on gene expression.[6] The most frequently studied polymorphism is the single nucleotide polymorphism (SNP)-174C and -174G in the promoter region, which has been associated with transcription rates of IL-6.[7] Moreover, Ulhaq & Soraya (2020) concluded that polymorphism in IL-6–174C allele is associated with a higher level of IL-6 production and pneumonia severity in COVID-19 patients.[8] Furthermore, some studies had investigated the association between genetic polymorphisms in IL- 6 and different therapeutic agents in rheumatoid arthritis where rheumatoid patients with an IL-6 -174GG genotype respond to treatment better than patients with GC or CC genotype.[6,9] They suggested that -174G/C IL-6 polymorphism may be a genetic marker of responsiveness to treatment in rheumatoid arthritis patients.[9] Consequently, this study aimed to investigate the role of polymorphism in the promotor region of IL-6 gene (-174G/C) as a predictor for disease severity in COVID-19 patients, that might affect clinical implications or therapeutic management.

Patients and Methods

This pilot study included 50 patients diagnosed with COVID 19 with mean age of 61.9 ±16.65 (34 males and 16 females), and 20 controls (12 males and 8 females) with mean age of 43.2±11.08. Patients were stratified into two groups, moderate (12 cases) and severely ill patients (38 cases). These patients were admitted to Ain Shams University hospital during the period from April 2020 to March 2021. All patients were diagnosed as COVID 19, confirmed with SARS-CoV-2 RT-PCR testing on respiratory samples. None of the patients were immune suppressed, on immune suppressive drugs or with autoimmune diseases.

The study protocol was reviewed and approved by Ethical Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 232, 2020). A written informed consent was taken from each participant in the study.

All patients were subjected to full medical history, laboratory investigations including complete blood count, C-reactive protein (CRP), serum ferritin, blood sugar, lactate dehydrogenase, D. dimer, liver and renal function tests.

Genotyping for IL-6 gene was determined by using TaqMan SNP genotyping assay in all studied groups.

DNA Extraction

Genomic DNA was isolated from whole blood samples, using Thermo Scientific™ Gene JET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, USA), according to the manufacturer's instructions.

SNP typing

The IL-6 gene is located on chromosome 7p21. For the detection of the IL-6 polymorphisms, real time polymerase chain reaction (RT-PCR) for IL-6 (-174G/C) was performed using TaqMan™ SNP Genotyping Assay (Applied Biosystems, USA), using the set provided primers and Taq- Man minor groove binder probes labeled with VIC (VICTORIA) and FAM (FLOURESCEIN AMIDITE) dye.

Context Sequence [VIC/FAM]. The primers sequence was 181 5'GTCTTGCCATGCTAA AGGACG3'-161 for IL-6 −174 C; and -181 5'GTCTTGCGATGCTAAAGGACG3'-16 for IL-6 −174 G.

RT-PCR was conducted according to the manufacturer's protocols using the following amplification program: heating at 50°C for 2 min then 10 min at 95°C, followed by 45 cycles at 95°C for 15 s and a final elongation at 60°C for 60 s.

Type of SNP was obtained automatically from the QIAGEN's real-time PCR cycler machine software (Rotor-Gene Q Software 2.3.3.5 Technician). The subjects' results were analyzed according to IL-6 (-174G/C) SNP into either C or G alleles.

Statistical analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 25). The qualitative variables were described as numbers and percentages. Quantitative variables were presented as medians or mean ± standard deviation. Results were analysed using unpaired student's t-test; Mann-Whitney U test, Fisher's Exact test. $P < 0.05$ was considered significant.

Results

The demographic and clinical data of the enrolled 50 patients are presented in Table (1).

Table 1. Co-morbidities and clinical presentation of the 50 patients, and treatment received by such patients.

Co-morbidities		N (%)	Clinical presentation	N (%)	treatment	N (%)
Diabetes mellitus	Yes	27 (54%)	Fever	4 (8.8%)	Antibiotics, antipyretics and vitamins	8 (16%)
	No	23 (46%)				
Hypertension	Yes	34 (68%)	Fever and dyspnoea	24 (53%)	Antibiotics, antipyretics, vitamins and steroid	21 (42%)
	No	16 (32%)				
Chest diseases	Yes	5 (10%)	Fever and diarrhoea	4 (8.8%)	Antibiotics, antipyretics, vitamins, steroid and anti-IL6	16 (32%)
	No	45 (90%)				
Neurological diseases	Yes	2 (4%)	Disturbed conscious level	8 (17.7%)	Antibiotics, antipyretics, vitamins, steroid and hydroxychloroquine	5 (10%)
	No	48 (96%)				
Renal diseases	Yes	4 (8%)	Vomiting	4 (8.8%)		
	No	46 (92%)				
Cardiac diseases	Yes	8 (16%)	Loss of taste and smell	1 (2.2%)		
	No	42 (84%)				
Hepatic diseases	Yes	4 (8%)				
	No	46 (92%)				
Autoimmune diseases	Yes	2 (4%)				
	No	48 (96%)				
Cancer	Yes	3 (6%)				
	No	47 (94%)				

There was a statistically significant relation between disease treatment outcome and disease severity (*P*=0.005). All the moderately affected patients were improved as well as 18 (47.3%) severely affected patients. The other 20 (52.6%) severely affected patient died (Table 2).

Table 2. Relation between outcome and disease severity in the patient groups.

Outcome	Patient group		*P-Value
	Moderate (12)	Severe (38)	
Improved N (%)	12 (100%)	18 (47.3%)	0.005
Died N (%)	0 (0%)	20 (52.6%)	

*Monte Carlo Fisher's Exact test. *P*<0.05 is significant

The most dominant genotype among COVID-19 patients and control groups was GG genotype, and there was no significant relation between patient sex and different genotypes. Furthermore, G allele distribution was the most dominant in the patients and control groups and there was no significant relation between sex and different alleles in both the patients and control groups (Table 3).

Table 3. Genotypes and allelic distribution and its relation to sex in the two study groups.

	Patient group, N (%)	**Sex**		***P* value**	**Control group, N (%)**	**Sex**		***P* value**
		male	female			male	Female	
Wild (GG)	38 (76%)	26 (68.4%)	12 (31.6%)	NS	16 (80%)	10 (62.5%)	6 (37.5%)	NS
Mutant (GC)	11 (22%)	8 (72.7%)	3 (27.3%)		4 (20%)	2 (50.0%)	2 (50.0%)	
Mutant (CC)	1 (2%)	0 (0.0%)	1 (100%)		0 (0%)	0 (0.0%)	0 (0.0%)	
G Allele	87 (87%)	60 (69%)	27 (31%)	NS	36 (90%)	11 (61.1%)	1 (50%)	NS
C Allele	13 (13%)	8 (61.5%)	5 (38.5%)		4 (10%)	7 (38.9%)	1 (50%)	

P>0.05 is not significant (NS)

In the patients' group, there was no significant relation between different genotypes and allelic distribution and different laboratory parameters. However, there was a significant relation between lymphocytic count, different alleles and different genotypes (*P*=0.005) (Table 4).

Table 4. Relation between different laboratory parameters with genotypes and allelic distribution.

laboratory parameters	Genotypes			Alleles	
	GG	GC	CC	G	C
	P value			*P* value	
D-Dimer μg /mIF (N=22)	NS			NS	
CRP mg/l (N=37)	NS			NS	
S. ferritin ng/ml (N=32)	NS			NS	
Blood sugar mg/dl (N=21)	NS			NS	
Sodium mmol/l (N=42)	NS			NS	
K mmol/l (N=43)	NS			NS	
Total bilirubin mg/dl (N=33)	NS			NS	
AST IU/L (N=40)	NS			NS	
ALT IU/L (N=41)	NS			NS	
LDH IU/L (N=31)	NS			NS	
BUN mg/dl (N=40)	NS			NS	
Lymphocytes (*103) /μl (N=45)	NS			NS	

P>0.05 is not significant (NS)

Of the 38 patients with severe COVID-19 presentation, 30 patients were of GG genotype, and 8 of GC genotype. While of the 12 patients with moderate

COVID-19 presentation, 8 cases were of GG, 3 of GC and one of CC. There was no statistically significant association between disease severity and different genotypes.

The 22 patients with GG genotype were clinically improved and 16 died, while 8 patients with GC genotype were improved and 3 died, the only patient with CC genotype was clinically improved. There was no significant statistical association between different genotypes and patient's outcome after treatment.

Meanwhile, 16 patients received anti IL-6 treatment, 10 of them were GG genotype, and 6 were GC genotype. Six patients were responded to treatment, three with GG genotype and three with GC genotype and there was no significant statistical association between genotype distribution and response to anti IL6 treatment (Table 5).

As regards laboratory investigations, there was no significant statistical relation between the severity of the disease and all laboratory parameters. Except for the ALT level which was significantly higher in the moderately affected group than in patients with severe disease (*P*=0.0045, Table 6).

Table 5. Relation between IL-6–174GC genotype with disease severity, outcome and response to anti-IL-6 therapy.

Patient's parameters	Genotype			*P*-value
Disease severity	GG	Moderate	8 (21.1%)	NS
		severe	30 (78.9%)	
	GC	Moderate	3 (27.3%)	
		severe	8 (72.7%)	
	CC	Moderate	1 (100%)	
		severe	0 (0.0%)	
Treatment outcome	GG	Improved	22	NS
		Died	16	
	GC	Improved	8	
		Died	3	
	CC	Improved	1	
		Died	0	
Response to anti-IL-6 therapy	GG	Responder	3	NS
		Non-Responder	7	
	GC	Responder	3	
		Non-Responder	3	
	CC	Responder	0	
		Non-Responder	0	

P>0.05 is not significant (NS)

Table 6. laboratory parameters in the patient group and their relationship with disease severity.

Laboratory parameters	Patient group		*P*-Value
	Moderate Median	Severe Median	
D-Dimer μg /mIF (N=22)	1.2	1.7	NS
CRP mg/l (N=37)	23	89	NS
S. ferritin ng/ml (N=32)	367	665	NS
blood sugar mg/dl(N=21)	80	354	NS
Sodium mmol/l (N=42)	135.5	137	NS
K mmol/l(N=43)	4.1	3.9	NS
Total bilirubin mg/dl(N=33)	0.6	0.7	NS
AST IU/L (N=40)	40	38.5	NS
ALT IU/L (N=41)	41	29	0.0045
LDH IU/L (N=31)	377	398	NS
BUN mg/dl (N=40)	38.5	31	NS
Lymphocytes (*103) /μl (N=45)	1.05	0.7	NS

P>0.05 is not significant (NS)

Discussion

Single nucleotide polymorphisms are now being linked to immune responses, in attempt to find an association between genetics and immune systems "immunogenetic profiling" which may provide new diagnostic and therapeutic ways for some disease.[10] Therefore, the aim of this study was to investigate the role of polymorphism in the promotor region of IL-6 gene (-174G/C) as a predictor for disease severity in COVID-19 patients. Such finding

might affect clinical implications or the disease therapeutic management.

This study revealed that hypertension was the most dominant comorbidity in the patient group 68%, followed by diabetes mellitus 54%. Furthermore, fever and dyspnea were observed in more than half of the patients (53%). These results came in agreement with findings of a study by Gold et al., 2020.[11]

This study showed a statistically significant relation between disease outcome and severity among studied patient groups. All moderately affected patients were clinically improved, while 47.3% of severe COVID-19 patients improved.

This study showed that the distribution of different IL-6-174G/C genotypes among COVID-19 patients were 76% for GG genotype, 22% for GC genotype and 2% for CC genotype. Furthermore, the distribution of such genotypes among the control group were 80% for GG genotype, 20% for GC genotype and 0.0% for CC genotype. These findings go in accordance with those of a study conducted by Batur & Hekim (2019), who showed that in Indian population the most detected genotype was GG genotype (68.6%) followed by the other genotypes GC (26.4%) and CC (5 %).[12]

Moreover, these results come in agreement with findings of another study conducted on Egyptian population by Gaber et al., (2013), who showed that 64.86% of patients with rheumatoid arthritis were of GG, 29.73% with GC and 5.41% with CC. While 90% of the control group were of GG genotype and 10% with GC genotype.[13]

However, this study showed no significant association between IL-6-174G/C and COVID-19 severity. This result was consistent with a study done by Batur & Hekim (2019) as they found no correlations between IL-6 gene polymorphisms of COVID-2019 patients and the mortality rate.[12] On the other hand, a meta-analysis conducted by Ulhaq and Soraya (2020), concluded a relation between the IL6 gene polymorphism and COVID-19 severity especially in the Caucasian population.[8] Kirtipal & Bharadwaj (2020) concluded that immunogenetic impact of IL6 polymorphisms as reported in viral diseases and lung diseases could consider IL6 polymorphism as a major factor that affect COVID 19 severity and could help in understanding the therapeutic response against the COVID-19.[14]

Furthermore, a study by Feng et al., (2015), conducted on patients suffering from pneumonia, concluded that IL-6 –174G/C had a 2.42-fold higher risk for septic shock following pneumonia. Such finding indicates that individuals with IL-6 –174C allele have the tendency to develop severe pneumonia due to higher production of IL-6,[15] as another study showed that CC genotype was significantly correlated with higher IL-6 levels.[16]

Another study conducted by Kerget & Kerget (2021) investigated the relationship between the frequency of IL-6 polymorphisms and the progressive course among COVID-19 in Turkish patients. They reported that the most prevalent genotype among the studied patents was the GG genotype, and they also found a significant positive correlation between the frequency of G allele and IL-6 levels and concluded that the IL-6 synthesis is increased by presence of G allele which may affect disease severity.[17] On the other hand, a study conducted on Asian population by Kirtipal & Bharadwaj (2020), found that GC polymorphism was not significantly associated with pneumonia.[10]

In this study, the frequency of G allele of IL-6-174G/C was higher in both the patients group (87%) and the control group (90%) than the C allele. This comes in agreement with a study done by Batur & Hekim (2019), who showed that the most detected allele was G allele (68.6%).[12]

Moreover, this study showed no significant statistical relation between the severity of the disease and all laboratory parameters, except ALT level which was significantly higher in the moderately affected patients than in patients with severe disease. On the other hand, Kazmi et al., (2021) showed a significant difference between COVID-19 cases regarding D-dimer however, no difference was detected regarding ALT.[18]

In conclusion, our work is one of few studies to investigate the role of polymorphism in the promotor region of IL-6 gene as a predictor for disease severity in COVID-19 patients. The distribution of different IL-6-174G/C genotypes

among COVID-19 patients indicated that GG genotype was the highest (76%) followed by GC (22%) and CC (2%). We did not find a relation between -174G/C IL-6 gene polymorphism and COVID-19 disease severity.

Author Contributions

MAK, SAA and LAA; constructing an idea for the manuscript. SAA, MAK and SAA; planning research methodology. SAA, MAK and SAA; providing financial support for the project. RHR; referring patients. SA, MAK, SAA and LAA; presenting the results. SAA, MAK, SAA and LAA; writing and revising the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by Ethical Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 232, 2020).

Informed consent

A written informed consent was taken from each participant in the study.

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