

Research Article

IL-6-174 G/C Polymorphism in COVID-19 Pregnant Women

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A B S T R A C T

Introduction: Cytokines play a role in severely ill patients with COVID-19. Similarly, there are roles of other pro-inflammatory responses in COVID-19 pathogenesis.

Objective: To detect IL-6 G/C +174 polymorphisms in COVID-19 pregnant women.

Method: 77 blood samples were collected from pregnant women with COVID-19 and 25 samples of healthy pregnant women (age range: 20-35 years). The blood samples of pregnant women with COVID-19 were collected (from the 1st of August to the 25th of December, 2020) from Azasi Teaching Hospital and Al-Jumhuri Hospital in Kirkuk, Iraq.

Results: The results showed that C allele in pregnant women with COVID-19 was 31(40.3%) compared to with allele G, which is 46(59.7%), while the allele C was 19(76%), compared to the allele A, which is 6(34%) in healthy pregnant women.

Conclusion: G allele demonstrates significant frequency in pregnant women with COVID-19 and higher rate compared with healthy pregnant women, and G allele may represent a significant risk factor for COVID-19 in the Iraqi population and there is an association between IL-6-174 G/C polymorphism and COVID-19 patients.

Keywords: IL-6 (-174) G/C, Pregnant Women, COVID-19

Introduction

The Coronaviruses (family: Coronaviridae) contain a single-stranded RNA. The subfamily of this virus, according to genetic and serology methods, divided into: α -coronavirus, β -coronavirus, δ -coronavirus and γ -coronavirus.^{1,2} The coronavirus belongs to Beta corona-virus. The phylogenetic analysis results exhibited that COVID-19 entered the similar sub-genus like coronavirus that lead to Severe Acute Respiratory Illness (SARS) prevalence in 2002-2004.^{3,4} The process of replication and duplication of COVID-19 in epithelium called ciliated columnar that causes cell injures and infection.⁵ COVID-19 might pass via mucous

membranes, and then enters to lungs, then COVID-19 would effect and attack targeting organs that express the angiotensin-converting enzyme 2 (ACE2), like the lungs with heart, and digestive tract.⁶⁻⁸ Cytokines are pleiotropic proteins and their molecular weight is usually less than 30 kDa. Cytokines are synthesised and produced by several types of cells such as white blood cells that participate in process of inflammation.^{9,10} It has long been believed that different types of cytokines play a significant role against viral infection. A rapid immune response is the first step and defence line versus viral infection.¹¹⁻¹³ The relevant evidence from severely ill patients with COVID-19 suggests

that responses of pro-inflammatory play a significant role in COVID-19 pathogenesis.¹⁴ So, the aim of the current study was to detect IL-6 G/C +174 polymorphisms in COVID-19 pregnant women.

Material & Method

Blood Samples Collection

Blood samples (77 samples) were collected from pregnant women with COVID-19 and 25 samples of healthy pregnant women (age range: 20-35 years). The blood samples of pregnant women with COVID-19 were collected (from the 1st of August to the 25th of December, 2020) from Azasi Teaching Hospital and Al-Jumhuri Hospital in Kirkuk, Iraq. The blood was put in EDTA tubes as an anticoagulant that was used in process of DNA extraction.

DNA Isolation

The gSTNCTM Kit, which is made by the American company Gene aid, was used for DNA extraction. DNA concentration and purity were determined by utilising a Nanodrop. The DNA samples were stored at -60°C until use.

IL-6 (-174) G/C Gene

Polymorphism of IL-6 (-174) G/C was analysed by using PCR and followed by a step called polymorphism of restriction fragment length. DNA was amplified by utilising the standard conditions: 94°C for 4 min, 35 cycles at 94°C for 30 s, 61°C for 30 s, 72°C for 45 s, and finally 72°C for 10 min. Table 1 shows the primers used for amplification. Amplification of samples was observed on 2% agarose gel by using Gel electrophoresis.

Table 1. IL-6 (-174) G/C Gene Polymorphisms Primers

Primer	Sequence	Reference
Forward	5'-GGAGTCACACACTCCACCT-3'	Gupta et al. ¹⁵
Reverse	GTGGGGCTGATTGGAAACC-3'	

Statistical Analyses

PCR data were analysed by using Minitab-17 program, and the significant differences between the averages were compared by utilising Tukey at a probability level $P < 0.05$. The allele frequencies, genotypes, confidence intervals (CI) and odds ratio (OR) were analysed by utilising the Compare 2 Ver.3.04 program.¹⁶

Results and Discussion

The polymorphism of IL-6 (-174) G/C was studied in two groups (patients and healthy samples) by using the technology of ARMS-PCR. The results of IL-6 (-174) G/C mutant gene amplified by using ARMS-PCR technology demonstrated the presence of two alleles, G and C, and through them, three genotypes were identified: GG, GC and CC. When one package is present in the g domain and absent

in domain c, then the genotype is GG, and in the event that a beam appears in the domain c and is not present in the g domain, the genotype is CC. While, if two packages presence in both domains, so the genotype is GC. The results of the repetitive distribution of the two alleles g and c of the mutant IL-6 (-174) G/C showed different results between pregnant women with COVID-19 and healthy pregnant women, c allele in pregnant women with COVID-19 was 31 (40.3%) compared with G allele, which was 46 (59.7%), while C allele was 19 (76%), compared to the allele a, which is 6 (34%) in healthy pregnant women as shown in Figure 1. Table 2 also demonstrates that the frequency distribution shows a significant difference between pregnant women with COVID-19 and healthy pregnant women. G allele demonstrates significant frequency in pregnant women with COVID-19 and higher rate compared with healthy pregnant women, and G allele appeared as a causative allele associated with infection risk with COVID-19 Etiological Fraction (EF). While C allele appeared as a preventive allele against COVID-19 infection, CC genotype showed a higher rate in healthy pregnant women compared to the pregnant women with COVID-19, the CC showed significant association with the preventive the risk of COVID-19 as shown in Figure 2 and Table 3.

Table 2. Frequency of Gand Gof IL-6 (-174) G/C Mutant Gene

Gene	Allele	Patients (77)	Control (25)	(95%CI) OR	P value
IL-6 (-174) G/C	C	31 (40.3%)	19 (76%)	0.38 (0.52-0.16)	*0.000
	PF	52.3%			
	G	46 (59.7%)	6 (34%)	3.44 (1.74-6.73)	
	EF	40.7%			

PF = Preventive Fraction, OR = Odds Ratio, EF = Etiological Fraction, CI = Confidence Intervals

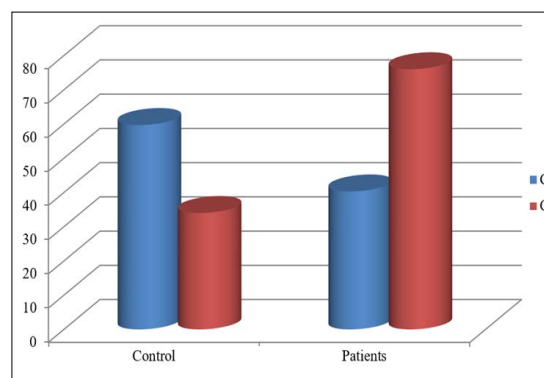


Figure 1. Frequency of Gand GAlleles for the IL-6 (-174) G/C

Table 3. Frequencies of the Genotypes for IL-6 (-174) G/C

Gene	Genotype	Patients (77)	Control (25)	(95%CI) OR	P value
IL-6 (-174) G/C	GG	37(48%)	3(12%)	3.18(9.32-1.25)	*0.000
	E.F		24%		
	GC	29(37.7%)	6(23.33%)	1.72(0.64-5.13)	0.152
	E.F		14.2%		
	CC	11(14.3%)	16(16.67%)	0.22(0.07-0.55)	*0.021
	P.F		49.5%		

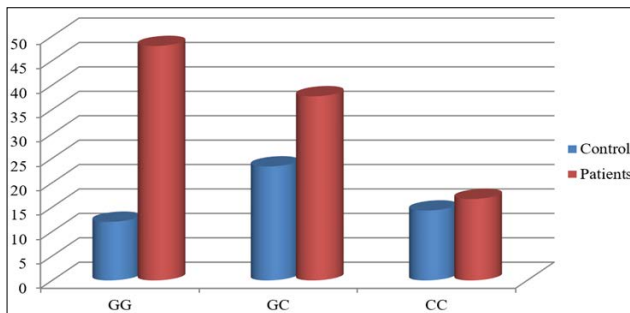


Figure 2. Genotypes for IL-6 (-174) G/C

The current study demonstrates that G allele is a significant frequency in pregnant women with COVID-19 and higher rate compared with healthy pregnant women. IL-6 is defined as a cytokine that plays an important role in the immune regulatory process with various functions in the angiogenesis process, pro-inflammation process, and anti-inflammatory response. These types of responses are triggered by activation of the family of JAK tyrosine kinases and then stimulated by signalling pathways of MAPK and PI3K.¹⁷⁻¹⁹ IL-6 coded by a gene called IL-6 in man, play as pro-inflammatory cytokine. Jin and Wang briefed the rate of single nucleotide polymorphisms (SNPs) associated with the immune responses.²⁰ Also, various lung disorders and diseases such as chronic obstructive pulmonary (COP) and asthma disease, the severity of disease were differentially seen through analysis of gene polymorphism.^{21,22} For example, polymorphisms on the promoter region of IL-6 are linked to pneumonia risk.²³ Also, the polymorphism in the IL-6 was connected to several viral infections such as hepatitis C and B (HCV & HBV).^{24,25} An experimental research study with bleomycin for inducing pulmonary fibrosis suggested that the inhibiting release of IL-6 in the early phase of lung damage assists fibrosis lesion and that inhibition in the damage stages at the onset of the fibrotic phase might improve fibrosis.²⁶

These results are in agreement with Kadhim MM and Intesar AS²⁷, who referred that the IL-6 (-174G/C) genotype was associated with an elevated risk of obesity ($p=0.004$), where, levels of IL-6 were higher in samples with GG genotype compared with patients with CG or CC genotype.

Conclusion

G allele demonstrates significant frequency in pregnant women with COVID-19 and higher rate compared to healthy pregnant women. It may represent a significant risk factor for COVID-19 in the Iraqi population and there is an association between IL-6-174 G/C polymorphism and COVID-19 patients.

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Conflict of Interest: None

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