Association Between Covid-19 Risk and The Genotypes (Gg, Gc and Cc) And Its G and C Alleles for Interlukin-6 (Il-6) Gene Polymorphisms

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Abstract

COVID-19 disease is a highly contagious respiratory inflammation occur by viral infection (SARS-CoV-2). Cytokines are proteins that mediators of dysregulated immunity, and stimulated of immune response such as interlukin-6 (IL-6). Our study included two hundred and twenty samples of COVID 19 patients of Iraqi population, their ages ranged from 21 to 79 year (mean of ages 37.8 ± 1.4) and one hundred and twenty of the controls (outwardly healthy), their ages ranged from 19 to 54 year (mean of ages 27.9 ± 1.9). We confined the frequency of IL-6 Gene polymorphism by using Tetra-amplification refractory mutation system-polymerase chain reaction technique (TARMS PCR). Also, we determined the association of IL-6 Gene polymorphism in position (-174 G/C) with COVID 19 for Iraqi community. Data showed significant difference in genotypes frequency of IL-6 Gene polymorphism with COVID-19 patients and control.

The genotype of GG (homozygotes) show high frequency in COVID 19 patients was ratio 63.64%, comparison with control was 47.50% and showed related with etiological fraction risk of SARS-CoV-2 ratio was 30.7%, and OR (CI 95%) was 1.63(1.23 to 3.03). The genotypes of GC (Heterozygotes) show high frequency in control ratio was 50%, while in COVID-19 patients show low frequency was 32.72%, also GC genotypes showed relationship with preventive fraction risk of SARS-CoV-2 ratio was 25.7% and OR (CI 95%) was 0.49 (0.31 to 0.77). The CC genotype show non-significant between COVID-19 patients and control and ratio was 3.64% and 2.50% respectively, and was OR (CI 95%) 1.47 (0.38 TO 5.63).

The G allele show high in frequency of COVID-19 patients comparison with control ratio was 80% and 72.50% respectively, and present with etiological fraction risk of SARS-CoV-2, while C allele show low frequency in COVID-19 patients comparison with control ratio was 27.50% and 20% respectively, and present related with preventive fraction for SARS-CoV-2. Our findings demonstrate that the IL-6 gene in position -174 G/C may represent of SARS-CoV-2 risk development of COVID-19 patient's in Iraqi population

Keywords: lL-6 Gene Polymorphisms, COVID19, Tetra ARMS PCR, Genotypes

1. Introducion

Coronaviruses (SARS) are a family of single strain RNA viruses that infect several hosts, including the human, causing respiratory infected [1], and it's has the ability to spread quickly in population [2]. SARS-CoV-2, also referred to COVID-19, emerged from Wuhan, China in December 2019. So far, infect humans and cause disease, with powerful infectivity [3]. the viral genome of the virus which is RNA has a molecular weight of approximately 26,000 to 32,000 base pairs [4]. COVID-19 associated infections have been well described and include cytokine storm, inflammation, pathologic coagulation, endothelial dysfunction, kidney and heart problems [5].

However, there is no treatment or vaccine currently available for COVID-19, but some treatments and antiviral drugs have been adopted, such as plasma from recovered patients, and the use of vitamins that increase the immunity of the infected patients, as well as cytokines [2]. Cytokine storm is an interesting point in COVID-19 patients. High levels of inflammatory cytokines have been observed in more severely ill COVID-19 patients and have been associated with inflammation and lung damage as well as failure of multiple organs such as the kidneys, heart, and lungs [6]. Stimulation the production of cytokines by active Th1 cells and cytotoxic T, and can also be produced by natural kill cells, and it can stimulate its production by macrophage [7]. Interleukin-6 is protein encoded by IL-6 gene and plays a role in response for many diseases and is a cytokine which has both pro- and anti-inflammatory properties. IL-6 is secreted from several type of cells such as T cells, macrophages, endothelial cells, fibroblasts and monocytes [8]. The targets of the IL-6 are B cells, Th cells, and leucocyte which include basophils, eosinophils and neutrophils. The functions of IL-6 are differentiation of the B cells as well as IgA, IgM and IgE production by plasma cells [9]. IL-6 is a cytokine which controls the immune response in addition to cell proliferation and differentiation. Human IL-6 is a gene mapped in chromosome 7p21 and is comprised of 212 amino acids, including a 28-amino-acid signal peptide [10]. The study indicates that the G allele and GG genotype of the IL-6 gene has been significantly associated with Covid-19 infection as it indicates a genetic risk factor associated with COVID-19, also shows that decreasing IL-6 production during infection can increase

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the recurrence of the Coronavirus and increase the risk of infection [11]. The aim of this study is to detect the phenotypic polymorphism of the IL-6 gene in position -174G/C by the replication impedance system with ARMS-PCR technology for the studied samples to find out the relationship and linkage of the IL-6 genotypes with COVID-19 Patients in Iraqi Population.

2. Materials and methods

a. Study samples

The total of Study samples was of 340 samples, and clouded 220 of COVID-19 patients, there ages range from 21 to 79 year, and 120 controls (outwardly healthy), there ages range 19 to 54 year of Iraqi population. All the samples of patients were collected from Hospitals for COVID-19 patients in Baghdad/Iraq. They had an established diagnosis of SARS-CoV-2 viral by the laboratory and clinical examination.

b. Genotyping of IL-6 Gene in position -174 G/C with COVID-19

Samples were collected by size two ml of blood from each COVID-19 samples and control group by venipuncture, later, 2 ml was added into EDTA tubes (anticoagulant) then DNA was extracted by DNA isolation kit from ProMega, the according to manufacture instructions manual. Take into account DNA purity with concentration was about 1.5 ±1.9. All Samples were kept under temperature for until study. IL-6 gene (-174 G/C) polymorphisms were examined by Tetra-amplification refractory mutation system-polymerase chain reaction technique (TARMS-PCR). The primers are designed according to Heydari-Mehrabadi et al. [12] as shown in table 1. The twenty µl were the total volume of reaction mix (Pio-Neer: Korea), included 5 µl premix master mix and including Taq, dNTP, Buffer, and Mg2+, and 1 µl of each primer, and 5 µl from the DNA, and 6 µl for RNase-free, and the molecular marker size is 100-2000 base pair prepared from Pro: Mega (USA). The program for Tetra ARMS-PCR technology for the IL-6(-174 G/C) gene polymorphism is summarized in table 2 according to Heydari-Mehrabadi et al. [12]. The genotypes were established by analyzing electrophoresed 2% gel of agarose stained with diamond dye (Pro-Mega), and under voltage 75.

Table (1): primer sequences of IL-6 gene (-174 G/C) polymorphism by TARMS-PCR technique.

The studied Gene	primer	Sequences of primer (5`→ 3`)	Size (bp)
IL-6 gene (-174 G/C)	Outer (F)	GACTTC AGCTTT ACTCTTTGTCAAGACA	326 bp
	Outer (R)	GAATGAGCCTCAGACATCTCCAGTCCTA	
	F inner (G allele)	GCACTT TTCCCC CTAGTTGTGTCTTCCG	205 bp
	R inner (C allele)	ATTGTGCAATGTGACGTCCTTTAGCTTG	184 bp

Table (2): The program IL-6 (-174 G/C) Gene polymorphism by using TARMS PCR technique for COVID 19 patients and control group.

Target gene	Steps	Temperature (co)	Number of cycles	Time (second)
IL-6 -174 G/C gene	Pre-denaturation	96		600
	Initial denaturation	95	40	30
	Annealing	55		30
	Extension	72		30
	Final Extension	4		600

3. Statistics

Differences in the frequencies of IL-6 (-174 G/C) gene for COVID 19 patients in this study with control were analyzed by Fisher's test (value P<0.05). The OR (Odds Ratios) and CI (Confidence Intervals) were calculated by Compare 2 Ver.3.04 software Abramson, J. (2003-2013). The Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www. had2know.com.

4. Results

The study of genetic polymorphisms for IL-6 gene in position -174 G/C for 220 COVID 19 patients was ages mean 37.8 ± 1.4 year, and 120 control group was ages mean 27.9 ± 1.9 year. Notably, the two alleles G/C are more present for IL-6 gene polymorphism in position -174 G/C with GG, GC and CC genotypes in bladder cancer patients and control (figure 1), by Tetra-ARMS PCR technique. The frequency distribution of genotypes polymorphism showed significance in COVID 19 patients comparison with control group by Fisher's test and value P>0.05. The frequency of IL-6 -174 G/C genotyping was significant with COVID 19 patients, so GG genotype show significant and high frequency in COVID 19 patients compared with control group, and it ratio was 63.64% and 47.50% respectively as shown in figure 1, also the OR and CI 95% of GG genotype was 1.63 (1.23 to 3.03) as shown in table 3. and present association with etiological fraction for SARS-CoV-2 risk ratio was 30.7, while GC genotype present high frequency in COVID 19 control compared with patients and ratio was 32.72% and 50% respectively (Figure 2), also the OR and CI 95% was 0.49 (0.31 to 0.77), and show related with protective fraction of SARS-CoV-2 risk and ratio was 25.7% as shown in table 3, As well CC genotype show non-significant between COVID-19 patients and control group, was of frequency in COVID-19 patients high from control group and ratio was 3.64% and 2.50% respectively, also the OR and CI 95% was 1.47 (0.38 to 5.63), and present relationship with etiological fraction of SARS-CoV-2 risk and ratio was 1.2%. Briefly, the results showed that GG and CC homozygotes genotypes was

correlated etiological fraction with the risk SARS-CoV-2 risk, while GC heterozygote genotype were correlated with the protective fraction of SARS-CoV-2 risk, and the results correspond to Ferhan and Bugra, 2021 [11].

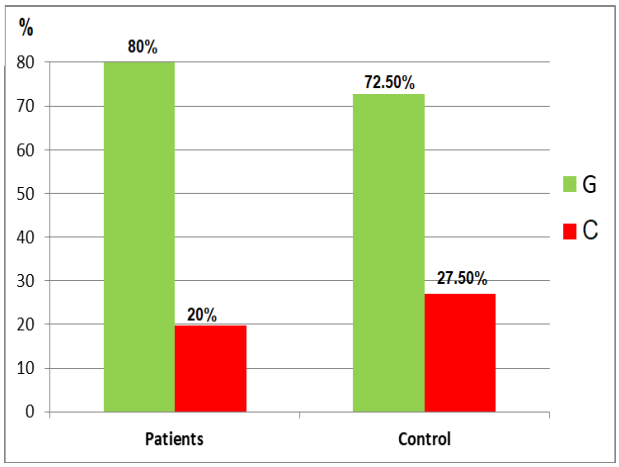

Figure (1): The frequencies of IL-6-174 G/C genotypes in COVID 19 patients and control group.

The G and C allelic show significant between COVID-19 patients and control group (P<0.05 by Fisher's test). The G allele show frequency of bladder cancer patient compared with control (healthy), so G allele show high frequency in COVID-19 patients comparison with control and ratio was 80% and 72.50% respectively (Figure 2), while OR and CI 95% was 1.52 (1.05 to 2.19), and related with etiological fraction for SARS-CoV-2 risk, presented in table 3, while C allele show low frequency with COVID-19 patients comparison with control group and ratio was 20% and 27.50% respectively (figure 2), and was OR and CI 95% 0.66 (0.46 to 0.95), and related with protective fraction for SARS-CoV-2 (presented in table 3). Briefly, polymorphism of IL-6 -174 G/C gene show that G allele and GG genotype are an etiological fraction and it's described that the C allele and GC genotype be a preventive fraction that correlated with COVID-19 patients risk in Iraqi population.

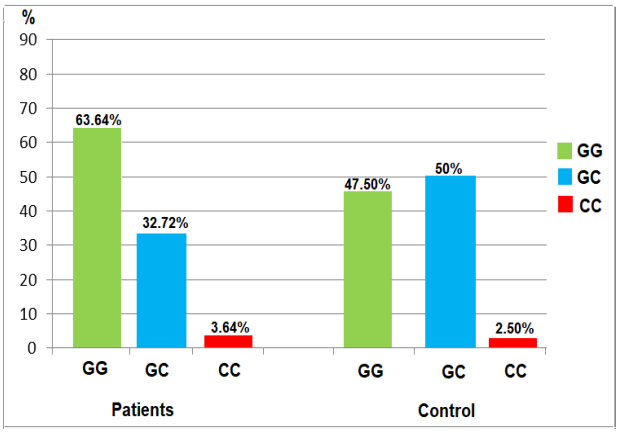

Figure (2): The frequencies of IL-6 -174 G/C allelic in COVID 19 patients and control group.

Table (3): The frequency of genotypes and allelic of COVID 19 patients and control for IL-6 Gene (-174 G/C).

Gene	Genotype & Allele	Bladder cancer (%)Number	Healthy(%) Number	OR (CI 95%)	P. value
IL-6 (-174 G/C) gene	GG	140 (63.64%)	57 (47.50%)	1.63 (1.23 to 3.03)	* 0.006
	E. F	30.7%			
	GC	72 (32.72%)	60 (50%)	0.49 (0.31 to 0.77)	*0.002
	P. F	25.7%			
	CC	8 (3.64%)	3 (2.50%)	1.47 (0.38 to 5.63)	0.753
	P. F	1.2%			
	G allele	352 (80%)	174 (72.50%)	1.52 (1.05 to 2.19)	*0.028
	E. F	27.3%			
	C allele	88 (20%)	66 (27.50%)	0.66 (0.46 to 0.95)	
	P. F	9.4%			

Notes: OR (Odds ratio), CI (Confidence Interval), E.F (Etiological fraction), P.F (Preventive fraction), P<0.05 by Fisher's

5. Discussion

Interleukin-6 is a pleiotropy functions cytokine that plays important roles in inflammatory responses [13] and its related with development of some virus's infection and increase risk of SARS-CoV-2 [14]. Among the cytokines produced by active macrophages, IL-6 is the main cytokine contributing to the development of COVID-19. The most important evidence confirming this is the correlation between IL-6 high levels and disease severity observed in studies of COVID-19 patients [15]. IL-6 cytokine in gene expression (increased or decreased) is an important factor with SARS-CoV-2 in the immune response of COVID-19 patients. The increasing IL-6 expression can contribute to disease exacerbation [16]. In a single nucleotide polymorphisms study conducted in patients with some diseases, which is another condition in which IL-6 levels play an important role in immune response, also IL-6 gene polymorphism show individuals with the position rs1800795 (-174G/C) GG genotype were found to have more severe COVID-19 disease [17]. Most SNP (signal nucleotide polymorphism) of IL-6 gene polymorphism in position -174 G/C influence on related of the receptor and thus repress transcriptional activation of inflammatory proteins [18]. The IL-6 gene polymorphism of G allele and GG genotypes is significantly correlated with increased risk of COVID-19 [11].

6. Conclusion

The data of study demonstrate the polymorphisms association of IL-6 gene in position -174 G/C with risk of SARS-COV-2, and indicate that the IL-6 gene (-174 G/C) may represent a significant risk factor in Iraqi population patients for COVID-19.

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