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Research Article

Polymorphism of IL-10 gene (rs1800896), in obese and smokers of Covid-19 patients in Wasit Province, Iraq

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Abstract

An inflammatory response and an interleukin 10 (IL-10) cytokine storm were associated with serious acute lung injury and multiorgan dysfunction in patients with severe COVID-19 disease. Genetic variations affecting IL-10 levels and disease are known as single nucleotide polymorphisms (SNPs). Severe COVID-19 was more likely in older adults, those who were overweight, and smokers. Purpose: To determine if COVID-19 is associated with IL-10 gene SNPs at (rs1800896) in individuals who smoke and are overweight. The participants in this study were 24 people with COVID-19 who were overweight, 25 people who smoked, 25 people who were neither overweight nor smokers (control positive), and 21 healthy individuals. The groups that were studied underwent ARMS-PCR genotyping of the IL-10 (rs1800896) SNP. Three samples had the A allele (3.2%), eight samples had the G allele (8.4%), and eighty-four samples had the AG genotype (88.4%). Within the same groups, there was a substantially different prevalence rate of IL-10 (rs1800896) alleles, with low rates of AA and GG genotypes ($P< 0.0001$).

Keywords: COVID-19, IL-10, Polymorphism, Smoking, Obesity

Introduction

In late December 2019, several health facilities in Wuhan, in Hubei province in China, reported clusters of patients with pneumonia of unknown cause (Zhu et al., 2020). Similarly to patients with SARS and MERS, these patients showed symptoms of viral pneumonia, including fever, cough and chest discomfort, and in severe cases dyspnea and bilateral lung infiltration(Zhu et al., 2020, Gralinski and Menachery, 2020). Coronavirus Disease19 (COVID19) is an infectious illness caused by SARSCoronaviruses2 (SARSCoV2), a highly virulent and transmissible coronavirus. The majority of COVID19 infections have mild to moderate symptoms such as cough, fever, myalgias, and headache; nonetheless, coronavirus can cause serious consequences and death in certain situations. (Polatoğlu et al., 2023). The first instances were reported in late December 2019 in Wuhan, China. Since then, the illness has spread fast throughout more than 200 nations, infecting millions of people with significant fatality rates. This compelled the World Health Organization (WHO) to declare COVI-19 a pandemic. (Vannabouathong et al., 2020) .

Older age, male gender, diabetes, obesity, cardiovascular disease, and cancer are all connected with an increased risk of illness. (Zhou et al., 2020, Stefan et al., 2020). Much clinical data reveals a strong link between BMI-defined obesity and an increased likelihood of testing positive for SARS-CoV-2, as well as an increased risk of severe illness in COVID-19 patients.(de Lusignan et al., 2020). Furthermore, data suggests that smokers are more likely than never smokers to experience more severe COVID-19 outcomes, such as hospitalization and mortality. (Alqahtani et al., 2020). Cytokines are the most essential immune factors because they play a role in regulating and modulating immunological responses to numerous infectious pathogens as well as inflammation. (Lin and Leonard, 2019) . In COVİD-19 patients, the term cytokine storm has been used to describe the uncontrolled excessive production of inflammatory markers in COVİD-19 patients (Coperchini et al., 2020) .

A pleiotropic cytokine, interleukin-10 (IL-10) has strong anti-inflammatory and immunosuppressive capabilities. The original idea was that T helper 2 cells were responsible for producing IL-10.. (Saraiva et al., 2019). The severity of viral infections in patients has been associated with polymorphisms in the interleukin-10 (IL10) gene. The IL10 rs1800896 polymorphism was shown to be related with the COVID-19 mortality rate in the Delta and Omicron genotypes, but not in the Alpha variation..(Abbood et al., 2023).

Martial and methods

This study included One hundred blood samples were collected from individuals for a cross-sectional study of COVID-19 infection, 95 samples were divided into (74 patients infected covid-19 and 21 samples of healthy individuals as a negative control group) from Al-Hayat

Center in Al-Zahra Teaching Hospital and External laboratories after fixing their infection, in Wasit provinces/Iraq. This study was carried out during the period between February 2022 to March 2023.

In this study, 74 individuals infected with COVID-19 (41 women and 33 men) The patients were diagnosed as COVID-19 positive cases . For each patient, an information sheet was filled and written consent was obtained. The information included gender, age, body mass index (BMI), and presence and absence of chronic diseases. All patients in this study do not suffer from any chronic diseases.

This 74 patients were divided into three main groups;Group 1: 24 Covid-19 patients suffering from obesity with a body mass index (BMI) higher than 25 kg/m2 (BMI ranging from 29 kg/m2 to 40 kg/m2). Group 2 : 25 smoking patients with Covid-19, with normal body mass index (BMI) less than 25 kg/m2. Group 3: 25 patients with COVID-19 were non-smokers, non-obese, as positive control group. This 74 patients infected with Covid-19 was compared with healthy people(21 samples) who do not have Covid-19, are non-smokers, and non-obese as a negative control group (Group 4) .

Samples Collection

About 1-2 ml of whole human blood were collected from each subject (cases and controls) involved in the present study samples, using plastic disposable syringes, then there was put in tube containing ethylene diamine tetra acetic acid (EDTA) for genomic DNA extraction and then labeled and stored in -20C.

DNA Extraction

Genomic DNA was extracted from blood samples using the extraction kit (FAVORGEN-BIOTECH CORP- Taiwan), according to the manufacturer’s recommendations. The extracted DNA was checked by using Quantus™ Fluorometer (Promega. USA), using QuantiFluor® dsDNA Dye that check DNA concentration

Determination of IL-10 Gene Polymorphism

We genotyped 95 individuals for the IL-10 gene (rs1800896) polymorphism using a polymerase chain reaction with sequence specific primers (Arms-PCR). This included 74 patients and 21 controls. The ARMS-PCR, or primer sequence amplification refractory mutation system, was used for the genotyping of the rs1800896 SNP. Primers unique to each allelic variant are used to amplify the target segment in the ARMS-PCR procedure. Primers that were specific to allele A, allele G, and common reverse were used. To double-check that the amplification was effective, each PCR mix also included Forward and Reverse primers for the Human Growth Hormone (HGH) gene. A look at Table 1 reveals the primer sequences that were utilized:

Table (1). Primers sequence for IL-10 & HGH genes

Primers	**Primer sequence (5' - 3')**	**Product Size (bp)**
IL10_ C-R Common Revers	5′-CAGCCCTTCCTTTTACTTTC-3′	550 bp
IL-10_ G-F Allele G Forward	5′-TACTAAGGCTTCTTTGGGAG-3′	
IL-10 _ A-F Allele A Forward	5′-CTACTAAGGCTTCTTTGGGAA-3′	
HGH Forward HGH_F	5′-TGCCTTCCCAACCATTCCCTTA-3′	431 bp
HGH Reverse HGH_R	5′-CAACTCACGGATTTCTGTTGTGTTTC-3′	

For the detection of IL-10 gene polymorphism, DNA was amplified in a 20 ul final volume of reaction mixture, containing of 1µl of DNA template, 1µl of each IL-10 primers, 1µl of each Human growth hormone (HGH) for internal control, 12.5 µ of PCR Premix and then completed to 20 µl with Nuclease free Water (Promega). The amplification was carried out using a Multigene Gradient thermal cycler from LabNet International in New Jersey, USA. First, the DNA was denaturated at 94 °C for 5 minutes. Then, it was subjected to 35 time cycles of denaturation, annealing, and extension at different temperatures. The final extension was performed at 72 °C for 7 minutes. On a 2% agarose gel dyed with SYBRTM Safe DNA Gel Stain dye, the PCR results were seen using the UV transluminator gel equipment.

Statistical Analysis

The Results of the collected data were analyzed using SPSS (v.20) program by independent - test and one way ANOVA as appropriate and obtaing least significant difference (LSD). Pearson chi-square (correlation G-efficient) test was performed, to detect a Correlation between the studied parameters - The data were presented as mean ± S.E, while the significant differences was Considered at $P \leq 0.05$.

Results and Discussion

The ARMS-PCR methodology was used to genotype the IL-10 (rs1800896) SNP in groups of obese and smoking COVID-19 patients, as detailed in the material and methods section. The genotypic and allelic frequency distributions observed in all samples investigated are shown in Table(2). When we compared the A allele (3.2%) (No.3 samples) to the G allele (8.4%) (No.8 samples) and the AG genotype (88.4%) (No.84 samples), the Chi-Square test of homogeneity revealed that the genotypic and allelic frequencies were significantly different in all samples (P 0.0001), as shown in figure (1).

Table 2. Polymorphism of IL-10 (rs1800896) In all studied samples

Alleles	**Total frequency**	**Percentage (%)**	**Chi square $(\varkappa^2)$**	**P value**
A	3	3.2 %	**137.87**	**<0.0001****
G	8	8.4%		
A\G	84	88.4 %		
Total	95	100 %		

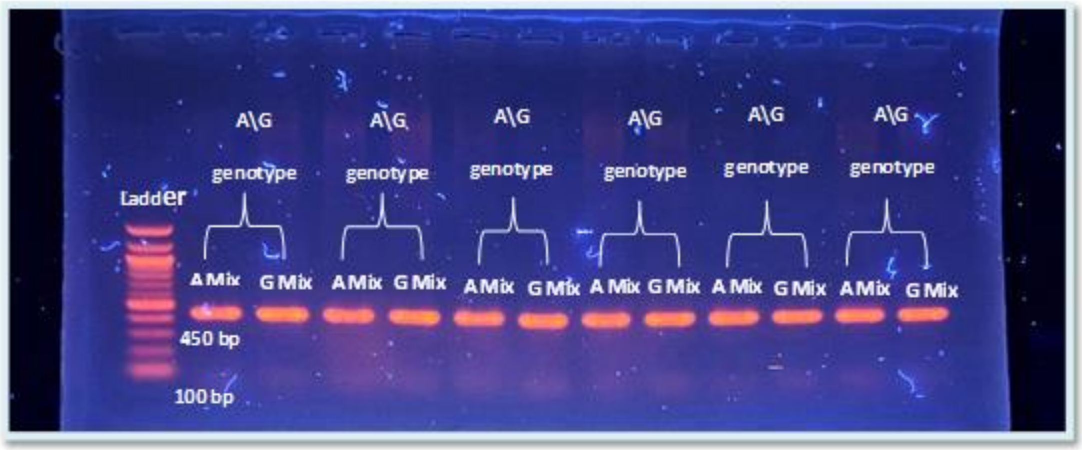

Figure (1): Polymorphism of IL-10 (rs1800896) In COVID-19 Patients Samples

The prevalence rate of IL-10 (rs1800896) alleles within same groups, the result of current study showed there was a high significantly difference (P< 0.0001) of IL-10 (rs1800896) alleles prevalence within each patient COVID-19 groups, as shown in table (3) and Fig. (2).

Table 3. Prevalence Rate Of IL-10 (rs1800896) Alleles Within Each COVID-19 Patient Groups

Groups	IL-10 Alleles	Total frequency	Percentage (%)	Chi sequare ($\varkappa^2$)	P value
Obese COVID-19 Patients	**A**	1	4.2	**84.64**	**<0.0001****
	G	0	0		
	A\G	23	95.8		
Smokers COVID-19 Patients	**A**	1	4	**116.84**	**<0.0001****
	G	3	12		
	A\G	21	84		
COVID-19 patients	**A**	0	0	**46.24**	**<0.0001****
	G	4	16		
	A\G	21	84		

The result of this study showed that the prevalence rate of IL-10 (rs1800896) alleles in obese COVID-19 groups as followings: A allele (4.2 %) (No.1 samples), G allele (0 %) and AG genotype (95.8 %) (No.23 samples), and in smokers COVID-19 patients theIL-10 alleles prevalence rate: A allele (4 %) (No.1 samples), G allele (3 %) (No.3 samples) and AG genotype (84 %) (No.21 samples). While in COVID-19 patients (control positive) the IL-10 alleles prevalence rate : A allele (0 %), G allele (16 %) (No.4 samples) and AG genotype (84 %) (No.21 samples) . In other words, the frequency of AA and GG genotypes was low among all patients groups, while thefrequency of AG genotype was higher among all patients groups, that showed there was no association of obese and smoking risk on COVID-19 severity with homozygous and heterozygous of IL-10 (rs1800896) genotypes.

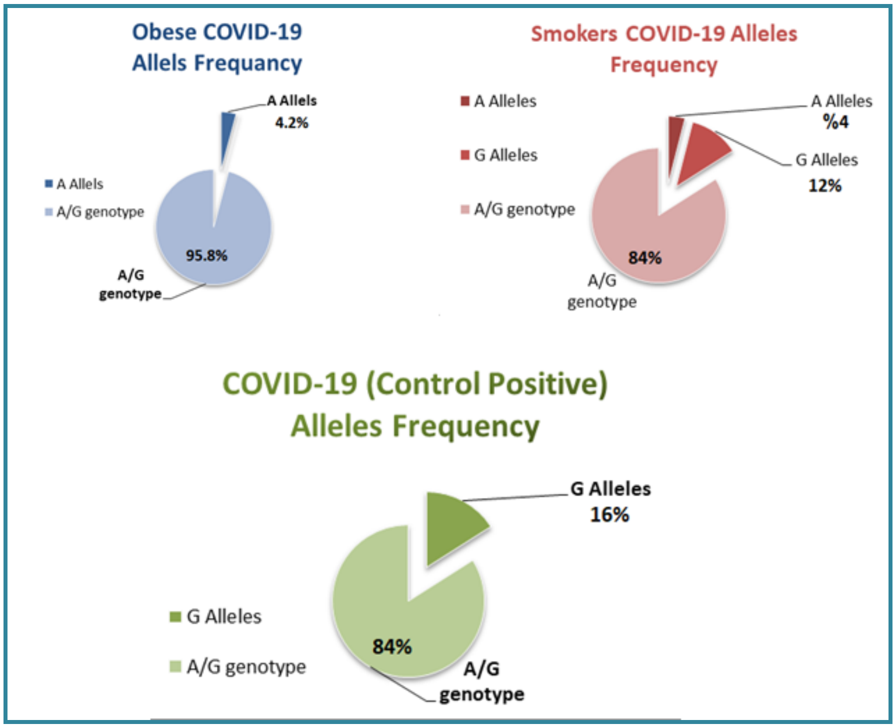

Figure 2. Prevalence Rate of IL-10 (rs1800896) alleles within Different Patients Groups

People with severe COVID-19 were thought to have greater amounts of IL-10 because of its anti-inflammatory characteristics. (Zhao et al., 2020). People who had the IL-10 (rs1800896) polymorphism and were found to be heterozygous AG had a much decreased chance of illness severity, according to Elhabyan et al. (2020). Additional evidence that the A allele of rs1800896 in the IL-10 gene is associated with an increased risk of pneumonia-induced sepsis suggests that this variant is also associated with an increased risk of pulmonary infections.. (Elhabyan et al., 2020). Also, the frequency of the AG genotype of rs1800896 was positively correlated with the incidence of COVID-19 in a research that included 23 nations. An analysis of IL10 gene polymorphisms at the rs1800896 locus in various populations has shown that whereas the AA genotype is common in China, Mexico, Tunisia, and Japan, it is less common in India, Iran, Spain, the Netherlands, Finland, Brazil, the Czech Republic, Poland, Germany, and Norway. The GG genotype of the rs1800896 polymorphism is more common in the Italian population.. (Karcioglu Batur and Hekim, 2021).

Furthermore, Genotyping analysis of Rizvi *et al.,* (2022) A research found no link between the IL-10 (rs1800896) gene polymorphism and the severity of COVID-19 in the population investigated. (Rizvi et al., 2022) . Also, (Fernández-de-Las-Peñas et al., 2022) A research published recently found no link between the IL-10 rs1800896 polymorphism and post-COVID symptoms in COVID-19 hospitalized patients who were followed up on for 18 months.

In a recent study by Sheikhi et al. (2023), the association between IL-10 gene polymorphisms and COVID-19 severity in pediatric patients was examined. The findings revealed that having the GA genotype at the IL10-1082 locus protected against severe symptoms. Additionally, the study found that all severe cases were males with the AA/GA genotype. The graphs showed that having the GA genotype was protective, but none of the individuals with severe symptoms had it. The COVID-19 mortality rate was shown to be associated with IL10 rs1800896 GG and AG genotypes in the Delta and Omicron, according to Abbood et al. (2023), but no correlation was found with the rs1800896 polymorphism..(Sheikhi et al., 2023).Consistent with earlier research, COVID-19 risk. Among the groups analyzed, the heterozygous AG genotype was most common, occurring in 88.4% of cases.

On the other hand, Maculewicz et al. (2022) discovered that IL10 polymorphisms play a role in controlling body weight in physically active people. They analyzed the correlation between the IL10 rs3024505 genotype and BMI and found that this SNP is significantly associated with a BMI worth more than 25, while rs1518111, rs1878672, rs3024496, and rs3024498 IL10 (Maculewicz et al., 2022). On the flip side, research conducted by Kuo et al. (2014) demonstrated that individuals in Taiwan who smoked cigarretes had an increased risk of gastric cancer, and that the combination of smoking and the AG and GG genotype at IL10 A-1082G further increased this risk. (Kuo et al., 2014) . Butov et al. (2016) found that smoking increases the risk of developing a multidrug-resistant TB genotype by increasing the likelihood of a substantial activation polymorphism in the G1082A gene, which is associated with the IL-10 gene. (Butov et al., 2016) . There was no statistically significant difference between the healthy controls and lung cancer patients in terms of the genotype and allelotype distribution of IL-10-1082 polymorphisms, according to Chen et al. (2017). Smokers with the A/A genotype had a substantially higher risk of lung cancer as compared to those with the G/G genotype when stratified by smoking status. (Chen et al., 2017).

In addition, many studies have shown a substantial association between the IL-10 (rs1800872) gene polymorphism and the severity of COVID-19. (Yessenbayeva et al., 2023) . To determine the association between the investigated IL-10 gene variants and the severity of COVID-19, more in-depth investigations with bigger samples of genetic variations are needed, as we have proposed..

Conclusion

The current investigation found that the genotypic and allelic frequencies were substantially different in all samples (P 0.0001). The IL-10 polymorphism (rs1800896) had no influence on the risk of obesity or smoking with COVID-19 severity. The prevalence of the AG genotype of the IL-10 (rs1800896) polymorphism was also greater in all patient groups.

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