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Polymorphism of IL-17A Gene (Rs2275913), In Obese and Smokers of Covid-19 Patients in Wasit Province, Iraq

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Annotation: Severe COVID-19 disease was linked to a severe proinflammatory response and cytokine storm interleukin 17 (IL-17) is one of these cytokines, was associated with severe acute lung injury and multiorgan dysfunction. Single nucleotide polymorphisms (SNPs) in genes coding IL-17 can affect level of IL-17 hence its role in diseases. Various host factors associated with an increased risk of disease such as; older age, obesity and smoking, led to increased risk of getting severe COVID-19. Aim of study to determine SNPs of the IL-17A gene at rs2275913 (G > A) are associated with COVID-19, in groups of smokers and obese covid-19 patients . So this study included 24 obese COVID-19 patients, 25 smokers with COVID-19, 25 non-obese & non-smoking COVID-19 patients (as control positive) and 21 healthy people (as negative control). Genotyping of the IL-17A (rs227591) SNP in studied groups was performed by the ARMS-PCR technique. Results of the analysis shown no statistical difference between IL17A (rs2275913) genotypes or alleles in relation to the different studied

groups. While the genotypic and allelic frequencies observed in all samples studied, the A allele (1.1 %), G allele (16.8%), and AG genotype (82.1 %), test of homogeneity showed that were significantly different in all samples regarding the genotypic and allelic frequencies. The heterozygous AG genotype of IL17A (rs2275913) was have the highest frequency among the different studied groups, also there was noticeable prevalence of the homozygous GG genotype, While the prevalence of the homozygous AA genotype it's almost non-existent. Thus, the different genotypes of IL-17A G/A-rs 2275913 can be proposed as markers of the severity of COVID-19 infections and risk factors (Smoking and Obesity).

Keywords: COVID-19, IL-17A, 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[1]. 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 [1,2] . Coronavirus Disease-19 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome-coronaviruses-2 (SARS-CoV-2) a highly pathogenic and transmissible coronavirus. Most cases of COVID-19 have mild to moderate symptoms, including cough, fever, myalgias, and headache, also coronavirus can lead to severe complications and death in some cases [3]. The first cases were described in late December 2019 in the Chinese city of Wuhan. Since then, the disease has rapidly spread over more than 200 countries and infected millions of cases with high mortality rates. This forced the World Health Organization (WHO) to announce COVID-19 as pandemic[4] .

Various host factors associated with an increased risk of disease include older age, male gender, diabetes, obesity, cardiovascular disease and malignancy[5,6]. Much clinical research suggests a strong relationship between body mass index (BMI) defined obesity and increased risk of testing positive for SARS-CoV-2, as well as increased risk of severe disease among patients with COVID-19[7]. Furthermore, evidence indicates that smokers are more likely to suffer more severe outcomes of COVID-19, such as admission into intensive care units and death, than never smokers[8]. Cytokines are the most important factors of immunity because of their involvement in mediating and controlling immune responses against various infectious agents, as well as inflammation[9]. In COVID-19 patients, the term cytokine storm has been used to describe the uncontrolled excessive production of inflammatory markers in COVID-19 patients [10] .

Interleukin-17 (IL-17) family cytokines are potent drivers of inflammatory responses and its family genes contain six groups (IL-17A to F), Single nucleotide polymorphisms (SNPs) in genes coding IL-17 can affect level of IL-17 hence its role in diseases[11]. Among these SNPs, two widely studied polymorphisms (rs2275913 and rs3748067) have been found to be associated with susceptibility to a wide range of human diseases, studies reported, the G genotypes rs2275913 polymorphism of IL-17A were significantly higher in severe COVID-19 patients[11,12] .

Aim of Study

This study aims to determine whether SNPs of the IL-17A gene at rs2275913 (G > A) are associated with COVID-19, in groups of smokers and obese covid-19 patients in the Iraqi population

Material and Methods

1. The study subjects

This study included One hundred blood samples were collected from individuals for a cross-sectional study of COVID-19 infection, 100 samples were divided into (75 patients infected covid-19 and 25 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.

2. COVID-19 patients Groups

In this study, 74individuals 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. Note\\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 75 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**) .

3. Methods

A- 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.

B- 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

C- Determination of IL17 Gene Polymorphism

Ninety five, 74 patients and 21 controls were genotyped for the IL-17A gene (rs2275913) polymorphism using a polymerase chain reaction with sequence specific primers (Arms-PCR). The primer sequences used are Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for the rs2275913 SNP genotyping. The ARMS-PCR is based on allele specific amplification of desired fragment using primers corresponding to each allelic variant. The

primers used are: Allele A specific forward, Allele G specific forward & Common reverse. As an internal control, Human Growth Hormone (HGH) gene primers Forward & Reverse were included in every PCR mix to verify successful amplification. The sequences of the primers used are shown in Table 1:

Table (1): Primers sequence and design for IL17A & HGH gene.

Primers	Primer sequence (5' - 3')	Product Size (bp)
IL17A Allele A Forward	5′-ATGGTGTTAATCTCATCTGGTGGG-3′	312 bp
IL17A Allele G Forward	5′-ATGGTGTTAATCTCATCTGGTGGC-3′	
IL-17A _ C-R Common reverse	5′-ATGCCCACGGTCCAGAAATAC-3′	
HGH Forward HGH_F	5′-GCCTTCCCAACCATTCCCTTA-3′	148 bp
HGH Reverse HGH_R	5′-TCACGGATTTCTGTTGTGTTTC-3′	

For the detection of IL-17A gene polymorphism, DNA was amplified in a 20 ul final volume of reaction mixture, containing of 1µl of DNA template, 1µl of each IL-17A 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 performed in a Multigene Gradient thermal cycler (LabNet International, NJ, USA). The conditions included initial denaturation (94 °C for 5 min) following a 35 time cycles of denaturation at 94 °C for 30 s, annealing at 58°C for 50 s and extension at 72°C for 45 s each cycle; and final extension at 72°C for 5 min. PCR products were visualized in a 2% agarose gel stained with SYBR™ Safe DNA Gel Stain dye using the UV transluminator gel system.

D- 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 & Discussion:

Genotyping of the IL-17A (rs227591) SNP in groups of smokers and obese covid-19 patients was performed by the ARMS-PCR technique as described in Material and Methods section. **Table(2)** shows the genotypic and allelic frequencies distribution observed in all samples studied. When we compared the A allele (1.1 %) (No.1 samples) against G allele (16.8%) (No.16 samples) , and AG genotype (82.1 %) (No.78 samples) , Chi-Square test of homogeneity showed that were significantly different in all samples regarding the genotypic and allelic frequencies (P< 0.0001) (Figure 1).

Table (1): SNP of IL-17A (rs2275913) In all samples studied

Alleles Of IL-17A	Total frequency	Percentage (%)	Chi square (χ^2)	P value
A	1	1.1 %	**110.42**	**<0.0001****
G	16	16.8%		
A\G	78	82.1 %		
Total	95	100 %		

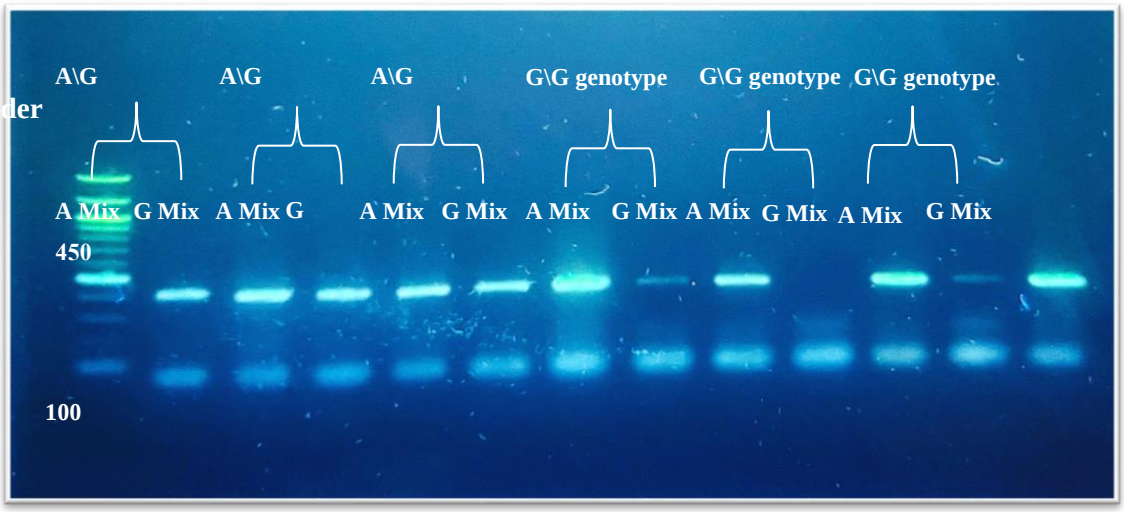

Figure (1): SNP of IL-17A (rs2275913) In groups of smokers and obese covid-19 patients

Results of the analysis of the association between IL17A (rs2275913) polymorphism with the different studied groups are shown in Table(3). There was no statistical difference between IL17A (rs2275913) genotypes or alleles in relation to the different studied groups.

This study showed a frequency rates of the A allele 4% (No. 1 samples) in the obese covid-19 patients group , and no frequencies of the A allele appeared in the other groups. In contrast, the frequency of the G allele was 29.2% (No. 7 samples) in the obese covid-19 patients group, While the frequency of the G allele was 20%, (No. 5 samples) in smokers covid-19 patients group, the frequency of the G allele was 16% (No.4 samples) in patients with Covid-19 only (Positive control group). While in the negative control group (healthy people) no frequency ratio was shown for the G allele. On the other hand, frequency rates were 100% (No.21 samples) in the negative control group (healthy people), 66.6% (No.16 samples) in the obese covid-19 patients group, 80% (No.20 samples) in smokers covid-19 patients group and 84% (No. 21samples) in patients with Covid-19 only (Positive control group).As shown in Table(3):

Table (3): IL17A (rs2275913) genotypes or alleles in relation to the different studied groups.

IL-17 Allele	Control		Obese Covid-19 Patients		Smokers Covid-19 Patients		Covid-19 patients	
	No.	%	No.	%	No.	%	No.	%
A	0	0	1	4.2	0	0	0	0
Chi square (χ^2)	NON		NON					
P value	-		-					
G	0	0	7	29.2	5	20	4	16
Chi square (χ^2)	-		4.09					
P value	-		0.13 NS					
A & G	21	100	16	66.6	20	80	21	84
Chi square (χ^2)	6.70		2.05					
P value	0.082 NS		0.36 NS					

While the prevalence rate of IL17A (rs2275913) alleles within same groups, the result of current study showed there was a high significantly difference (P< 0.0001) of IL17A (rs2275913) alleles prevalence within each patient COVID-19 groups, as shown in table (4) & figure (2).

Table (4): Prevalence Rate Of IL17A (rs2275913) Alleles Within Each COVID-19 Patient Groups

Groups	IL-17 Alleles	Total frequency	Percentage (%)	Chi sequare (χ^2)	P value
Obese COVID-19 Patients	**A**	1	4.2	**60.83**	**<0.0001****
	G	7	29.2		
	A\G	16	66.6		
Smokers COVID-19 Patients	**A**	0	0	**36**	**<0.0001****
	G	5	20		
	A\G	20	80		
COVID-19 patients	**A**	0	0	**46.24**	**<0.0001****
	G	4	16		
	A\G	21	84		

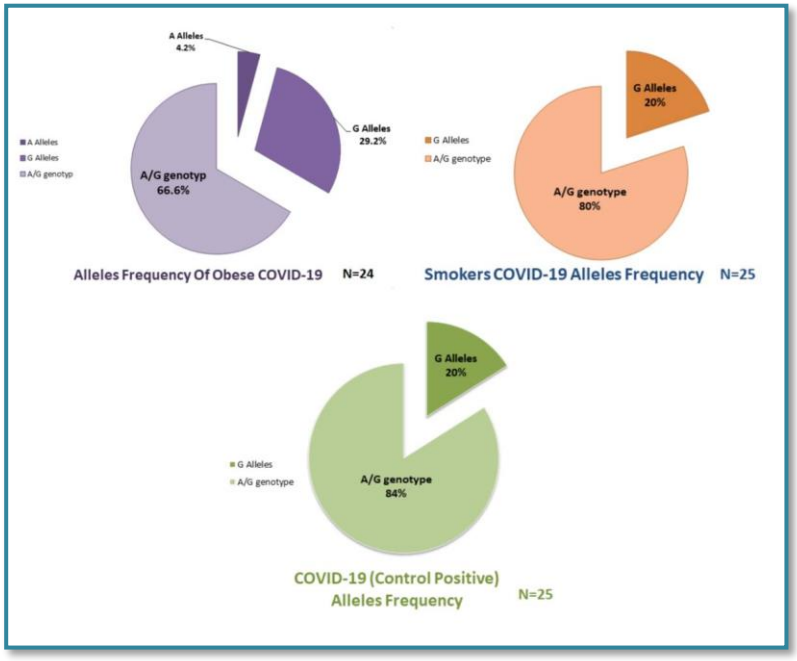

Figure (2): Prevalence Rate Of IL17A (rs2275913) Alleles Within Each COVID-19 Patient Groups

Several studies have demonstrated similar findings to ours. The results of Azevedo *et al.*, 2021 in study to evaluate the involvement of SNPs and tissue expression of IL-17A patients who died of severe forms of COVID-19 comparing to those who died by the pandemic Influenza A virus H1N1. This study showed that genotype GG of IL-17A (rs2275913) could be associated with the higher IL-17A tissue expression than GA in the COVID-19 group . Also the result showed that the IL-17A tissue expression was slightly lower in rs2275913 than rs3819025 for the COVID-19 group and significantly higher in rs2275913 than rs3819025 for the H1N1 group[13]. However, Rushdy *et al.*, 2022 reported no relation between the IL-17A rs2275913 gene polymorphism and the severity of COVID-19 disease in a sample of Egyptian population[12] .

Also In contrast with our findings the result of Goda *et al.*, 2023 showed that GG genotypes of IL-17A were significantly higher in severe COVID-19 patients (p=0.004) and by using multivariate logistic regression analysis revealed AG, GG genotypes of IL-17 and IL-17A were independent predictors of COVID-19 disease severity ($p < 0.0001$, p=0.06 and p=0.04, respectively)[11].

On the other hand, the result of Mazurek-Mochol *et al.*, 2021 showed no statistically significant differences in the distribution of the IL-17F rs763780 and IL-17A rs2275913 genotypes and alleles

between smoking patients with Periodontitis (PD) and smoking control subjects, and between non-smoking patients with PD and non-smoking control.

In the present study, there was no difference in genotypes and allele frequencies of IL-17A (rs2275913) and between COVID-19 groups and the controls. Interestingly, it has been demonstrated that rs2275913 (G > A) is a functional polymorphism that allows stronger binding of NFAT (nuclear factor of activated T-cells), a transcriptional factor, to the IL17A promoter, leading to higher transcription and synthesis of the IL-17A protein[15]. IL-17A promotes chemotaxis to the inflammation zone (especially attracting neutrophils). This effect leads to lung tissue damage and the progression of the disease[16]. So The IL-17 was shown as an important proinflammatory cytokine that involved in several chronic inflammatory and autoimmune diseases[17] .

Conclusion

Findings of the current study showed that were significantly different in all samples regarding the genotypic and allelic frequencies (P< 0.0001). The heterozygous AG genotype of IL17A (rs2275913) was have the highest frequency among the different studied groups, also there was noticeable prevalence of the homozygous GG genotype, While the prevalence of the homozygous AA genotype it's almost non-existent. So this study showed that there was a high significantly difference (P< 0.0001) of IL17A (rs2275913) alleles within same groups. Thus, the different genotypes of IL-17A A/G-rs 2275913 can be proposed as markers of the severity of COVID-19 infections and risk factors (Smoking and Obesity).

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