Original Article

Role of SNPs of IL6 gene in the incidence and progression of COVID19 patients in Wasit province

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Abstract

Background: SARS-coronavirus 2 is the causative of the COVID-19 pandemic. (SARS-COV-2). There is a correlation between illness severity and cytokines of proinflammation such as interleukin-6 (IL-6). The amount of COVID-19 cytokine produced might be affected by polymorphisms in the regulatory areas in genes of cytokine. In this study, an Iraqi population was used to investigate a possible connection between three IL-6 promoter SNPs and COVID-19 susceptibility.

Methods: The goal of this cohort study required the participation of a total of 97 individuals, 52 of whom had been diagnosed with severe COVID-19 and 45 of whom had been diagnosed with intermediate COVID-19. In order to determine the genotypes of three selected SNPs in the promoter region of the IL-6 gene, genomic DNA was extracted from the peripheral blood leukocytes of patients utilizing the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. This was done in order to establish a baseline for future research. These single nucleotide polymorphisms were denoted by the SNP names rs13306435 (T > A), rs2069860 (A > T), and rs2069830 (A > T).

Results: There were no significant changes seen in the genotype or allele distribution of chosen SNPs of IL-6 gene in the promoter region between patients with severe levels of COVID-19 and patients with moderate levels of COVID-19. These SNPs included rs13306435 (-395 T > A), rs2069860 (-632A > T), and rs2069830 (-612A > T).

Conclusion: According to the results of our investigation, these SNPs do not seem to be linked with the severity of COVID-19 in the Arabian community in Iraq.

Keywords: COVID-19, SNPs, interleukin-6, polymorphisms, haplotypes.

INTRODUCTION

It was determined in December 2019 that an outbreak of a novel beta-coronavirus in Wuhan, China, posed a significant risk to the public's health all over the world. On February 11, 2020, the SARS coronavirus was given the classification 2 by the World Health Organization (WHO). (SARS-CoV-2). in order to distinguish it from other coronaviruses that may cause SARS[1]. The coronavirus of 2019 was caused by this virus. (COVID-19). More than 20 percent of SARS-CoV-2 infections produce severe acute respiratory distress syndrome (ARDS), which may lead to fatality in extreme circumstances[2]. Many people infected with COVID-19 show no symptoms or have only mild to severe disease. Infections caused by COVID-19 are caused by the COVID-19 virus. (COVID-19). A common effect of COVID-19 is a state of inflammation that is out of control, a condition known as a cytokine storm. During these episodes, the production of pro-inflammatory cytokines such as IL-6, IFN-, and TNF-alpha might be seen[3]. The immunopathological process known as "cytokine storm" is responsible for the severe clinical course that COVID-19 may cause. It is also the reason why these patients pass away. IL-6 is a substantial inflammatory cytokine that is superior to CRP and other prognostic indicators in predicting and monitoring the development of Covid-19[4]. These other prognosis markers include leukopenia, fibrinogen, ferritin, prothrombin time, and D-dimer. In lung tissue, immune and non-immune cells, such as T lymphocytes, alveolar macrophages, type II epithelial cells, and lung fibroblasts, are responsible for the production of interleukin-6[5]. Cytokine is produced by these cells (IL-6). This cytokine causes lymphocytes to die, which is one factor that contributes to the lymphopenia seen by COVID-19 patients. It is possible that high levels of IL6 might change the expression of lymphocytes (HLA-DR), which can lead to a reduction in the number of natural killer (NK) cells,

CD4+ lymphocytes, and CD19+ lymphocytes. It's possible that this is caused by reduced lymphocyte expression[6]. There is a possibility that IL-6 is involved in the coagulopathy caused by COVID-19. IL-6 may be responsible for the production of tissue factors and thrombin, as well as the activation of platelets and the promotion of endothelial dysfunction. Previous studies found that people with COVID-19 have greater than normal amounts of IL-6 in their bodies. This is connected to poor clinical outcomes such as admission in an intensive care unit (ICU), acute respiratory distress syndrome (ARDS), and death[7]. This gene has SNPs that contribute to the coding process as well as those that do not. There is a good chance that the IL-6 gene is located on chromosome 7p21–14. SNPs in regulatory areas such as promoters, introns, as well as the 5′-UTR and 3′-UTR, may have an effect on cytokine production. Genetic modifications may affect the parts of the gene that code for proteins, which can either result in a loss of protein function or in a function that is significantly different from the original protein function. Single nucleotide polymorphisms, often known as SNPs, are genetic variants that may be discovered in regulatory areas such as promoters[8]. These variants can cause variations in the production of cytokines[1], [4], [5]. Blood IL-6 levels and the prevalence, incidence, and/or development of a wide variety of illnesses have been associated in a number of studies to the IL-6 gene promoter polymorphisms rs13306435 (T > A), rs2069860 (A > T), and rs2069830 (A > T). These polymorphisms can be found in three different combinations: T > A, A > T, and T > A. Infectious sepsis, chronic obstructive pulmonary disease, and hepatocellular carcinoma are a few examples of this kind of cancer. There is a school of thought that suggests that differences in the genes that code for IL-6 might alter the severity of COVID-19. The purpose of this study is to investigate whether or not there is a connection between COID19 and the three single nucleotide polymorphisms (SNPs) that were found in Iraqi patients. These single nucleotide polymorphisms (SNPs) have been given the following names: rs13306435 (T > A), rs2069860 (A > T), and rs2069830 (A > T).

Method and material

Patients

This study included a total of 97 participants, 54 of whom were discovered to be suffering from a moderate to severe case of COVID-19, and 42 of whom were discovered to be experiencing a mild form of the condition. In order to determine whether or not any of the patients were infected with SARA-COV-2, the researchers performed a nasopharyngeal RT-PCR and looked for a positive response for COVID-19. This allowed them to determine whether or not any of the patients had the virus. Twenty women and thirty-two men made up the entire population of the mild COVID-19 group, which had a mean age of 38.22 years (standard deviation: 7.3 years). These patients were taken to the Medical Clinic in Baghdad because they were experiencing relatively mild symptoms, such as malaise, arthralgia, and anosmia. Additionally, their throats were painful and they had lost their sense of smell. Their ages ranged anywhere from 38.22 to 7.3 years old, with an average of 38.22. The severe COVID-19 group consisted of 18 women and 30 men and had a mean age of 45.32 years with a standard deviation of 13.4 years. Each of the patients in this group had been admitted to the critical care unit of the Alsader hospital in order to receive treatment for one of the following conditions: respiratory distress or respiratory failure, mechanical ventilation, low oxygen concentration in arterial blood, or low oxygen partial pressure. Patients were all members of Iraq's Arab population, and they all hailed from the same region in the center of the country, which also happens to be the site of the nation's capital city. After being provided with sufficient information, each participant gave their assent to take part in the investigation, and the Ethics Committee of Bagdad University, which had already granted its approval to the inquiry, gave the study its final approval.

DNA extraction and genotyping

The peripheral circulation of the body's blood supply was used to obtain blood samples, which were then treated with EDTA to stop the formation of blood clots, and then frozen at a temperature of -20 degrees Celsius until they were needed again. These blood samples were later used to isolate genomic DNA from the blood supply. When it was first invented, this particular type of extraction was given the name of the salting-out operation. This term has stuck ever since. Using a method that is known as polymerase chain reaction-restriction fragment length polymorphism, the researchers were able to identify the genotyping of three SNPs that are located in the promoter region of the IL-6 gene (PCR-RFLP). Single nucleotide polymorphisms, or SNPs for short, are variations in the sequence of a single nucleotide. These variations may be passed down across generations. These variations are able to be seen, for instance, in the genetic markers rs13306435 (-395T > A), rs2069860 (-623A > T), and rs2069830 (-612A > T). The table that can be found at the beginning of this article provides an explanation of the PCR-RFLP primers that were used in the process of determining the genotype of the IL-6 polymorphisms (Table 1).

Table 1: primers of SNPs used in this research.

rs- SNPs	Primers	Size	Temperature	Enzyme	NM
rs13306435	F: TCCTTCTCCACAAACATGTAACAA R: AGCTGCGCAGAATGAGATGA	395bp	58	Taq (1)	NM_0013180 95.2
rs2069860	F: GGAGATGTCTGAGGCTCATTCTG R: CAGGCTGGCATTTGTGGTTG	623bp	59	Bsrb1	NM_0013180 95
rs2069830	F: GTCCAGTTGCCTTCTCCCTGG R: CCCATGCTACATTTGCCGAAG	619bp	59	Bsrb1	NM_000600. 5

The total volume of the reaction mix was 16 microliters, and it was comprised of the following: distilled water in the amount of 6 microliters, the master mix in the amount of 8 microliters, each primer in the amount of 1.5 microliters, and extracted DNA in the amount of 0.7 microliters. It took up a total of 16 microliters of space during the reaction. The conditions for performing PCR on the three SNPs were as follows: an initial denaturation step at a temperature of 95 degrees Celsius for three minutes, followed by 32 cycles of denaturation at a temperature of 95 degrees Celsius for forty seconds, an annealing temperature of 35 seconds at 59 degrees Celsius for rs13306435, and 20 seconds at 65 degrees Celsius for rs2069860 and rs2069830, and a final extension step at a temperature of 72 degrees CelsiusRotrogene 6000 was used for the PCR procedure (USA). The specificity of the PCR fragments for the rs13306435, rs2069860, and rs2069830 polymorphisms was examined by electrophoresis on a 3 percent agarose gel stained with 1.8 L Green Viewer. The length of each of these fragments was 395 base pairs (bp), 623 base pairs (bp), and 612 base pairs (bp), respectively. The RFLP digestion was carried out with particular enzyme restriction for each genetic variation in accordance with the instructions that were supplied by the manufacturer, and the digested products were observed by electrophoresis on an agarose gel that had a concentration of two percent. In the instance of rs13306435, the 395 bp PCR product was digested with Taq1 restriction enzyme at 37 degrees Celsius for twenty hours. This process took place at the same temperature. The A allele is resistant to restriction enzymes and may be cut, resulting in pieces of 395 and 35 base pairs. On the other hand, the A allele cannot be cut, and the fragment that is produced is still 395 base pairs. samples that showed both 395 bp and 216 bp bands were assigned the homozygote AA genotype; samples that showed both 395 bp and 35 bp and 216 bp bands were assigned the AT heterozygote genotype; and samples that showed only one fragment of 395 bp were assigned the homozygous TT genotype (Figure 1).

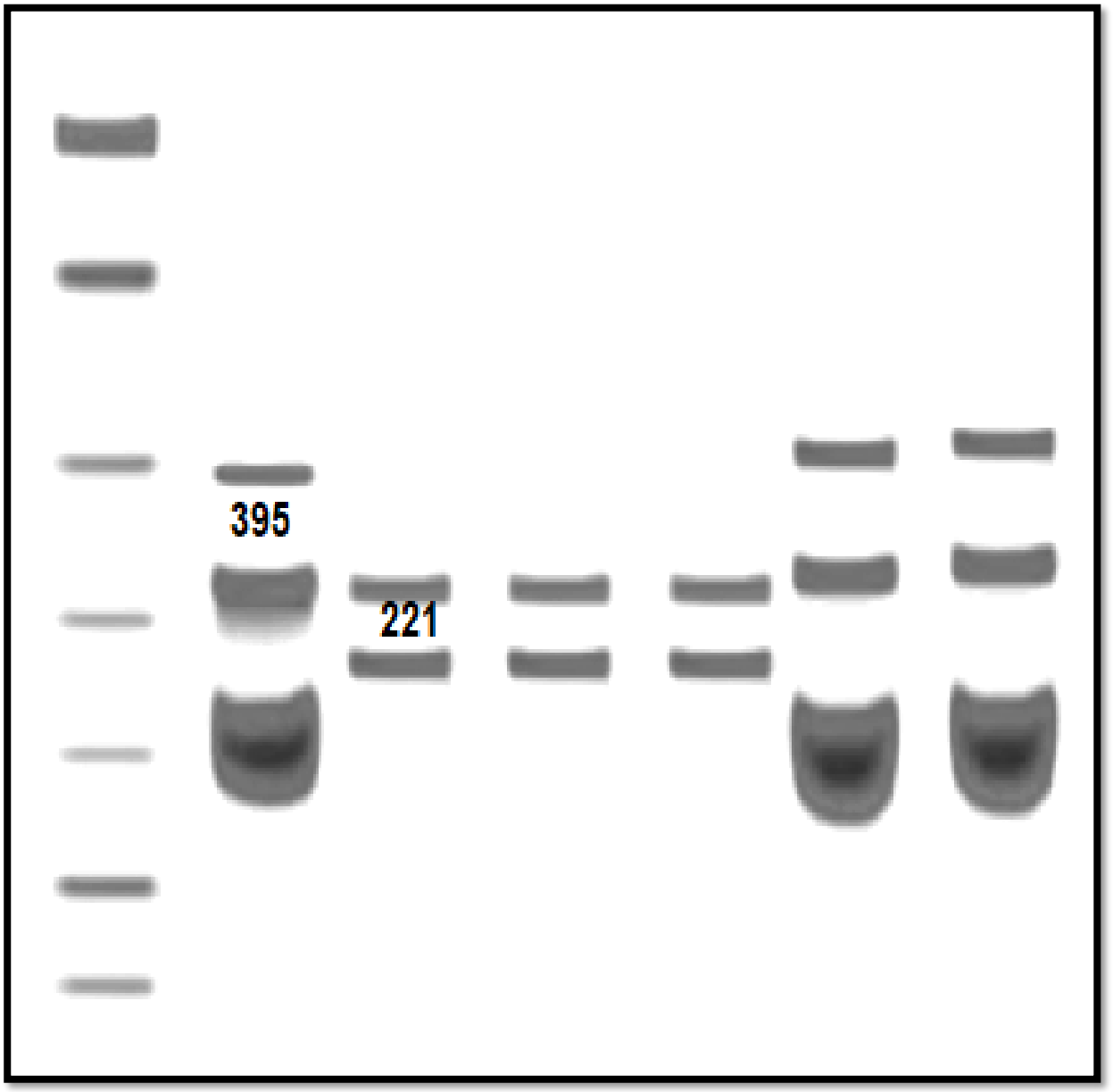

Figure 1: On an agarose gel containing 1 percent restriction enzyme, the products of restriction digestion (Taq1) were detected when the IL-6 rs13306435 A > T polymorphism in the promoter region was subjected to restriction digestion. Homozygous genotype of the wild-type allele AA (395 bp + 35 bp), heterozygous genotype of the allele AT (221 bp + 395 bp + 35 bp), and homozygous genotype of the mutant allele TT (395 bp + 35 bp) are the three possible outcomes of this experiment (221 bp).

The reaction mix includes distilled water, master mix, primers, and extracted DNA. 16 microliters were needed. Initial denaturation at 95°C for 3 minutes, followed by 32 cycles of denaturation at 95°C for 40 seconds, annealing at 65°C for rs2069860 and rs2069830, and a final extension at 72°C. PCR used Rotrogene (USA).

In the instance of rs2069860 (-623A > T), the 623 bp PCR product was digested with BsrbI restriction enzyme at 37 degrees Celsius for 16 hours. This process took place at the same temperature. The T allele has retained its resistance to restriction enzymes, and the length of the resultant fragment has remained unchanged at 512 base pairs. On the other hand, the A allele may be cut, which leads to the creation of fragments of 623 and 512 base pairs respectively. samples that exhibited 612 bp and 45 bp bands were typed as homozygote AA; samples that exhibited 612 bp and 612 bp and 623 bp bands were typed as AT heterozygotes; and samples that had one fragment of 623 bp were reported as homozygous TT. There are three potential alleles for this locus, and they are AA, AT, and TT (Figure. 2).

The PCR result for the rs2069830 mutation, which had a length of 623 base pairs and had 619 transitions from the G to the A, was digested with the BtscI restriction enzyme for 20 hours at a temperature of 45 degrees Celsius. The result was successful. Allele A may be cut, which produces a fragment with either 619 or 623 base pairs as a consequence; however, allele G is resistant to restriction enzymes and always produces a fragment that is still 619 base pairs in length. Those samples were considered to be AA homozygotes if they showed bands of 487 bp, 619 bp, and 606 bp. On the other hand, those samples that had bands of 487 bp, 619 bp, and 606 bp were considered to be AT heterozygotes. In order to determine whether or not the samples were homozygous for the TT status, each sample needed to have exactly one fragment of 606 base pairs (Figure 3).

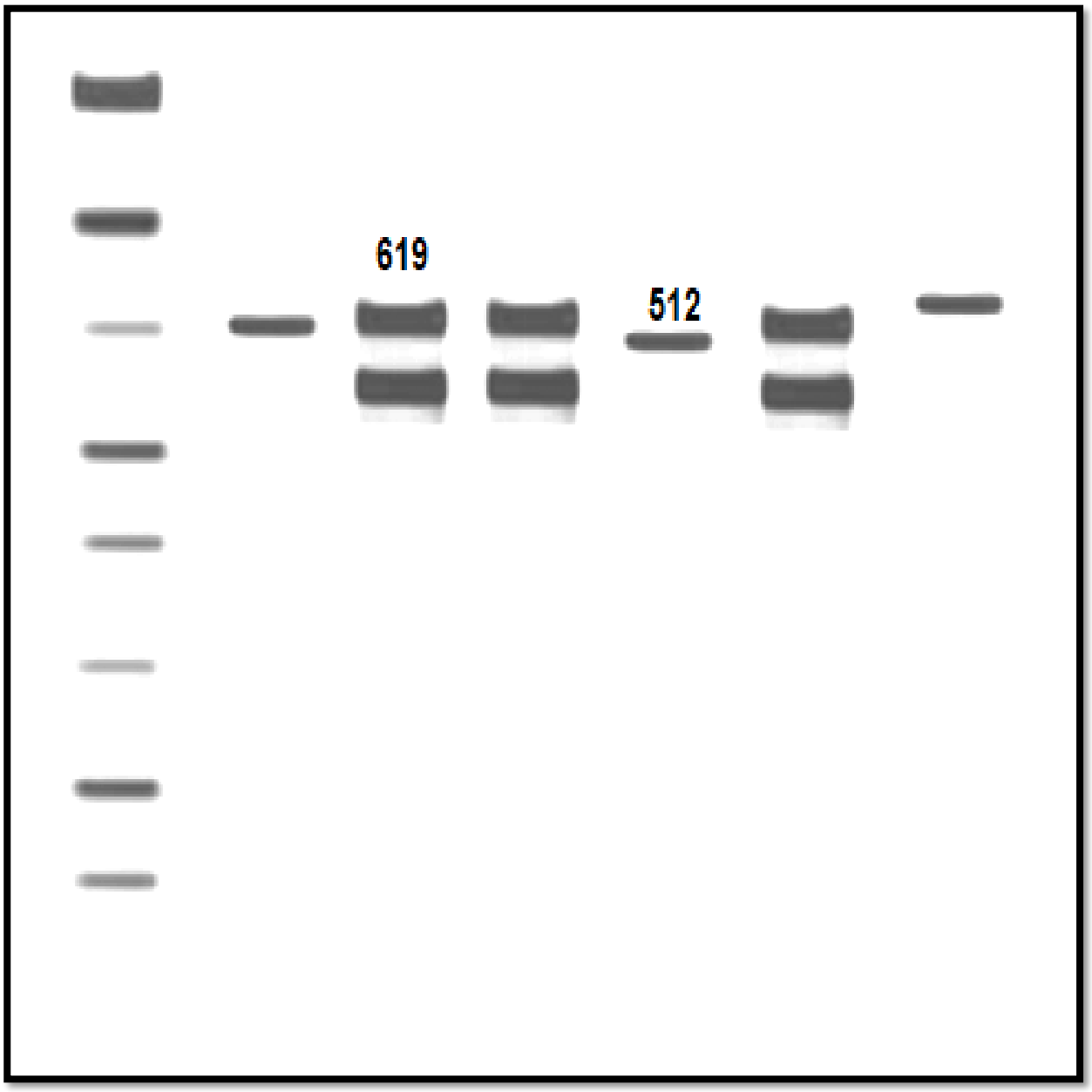

Figure 2: On an agarose gel containing 1 percent restriction enzyme, the products of restriction digestion (Taq1) were detected when the IL-6 rs2069860 A > T polymorphism in the promoter region was subjected to restriction digestion. Homozygous genotype of the wild-type allele AA (623 bp + 45 bp), heterozygous genotype of the allele AT (512 bp + 623 bp + 45 bp), and homozygous genotype of the mutant allele TT (623 bp + 45 bp) are the three possible outcomes of this experiment (512bp).

Statistical analyses

All investigations utilized IBM SPSS 22. Chi-square test compared genotype and allele frequencies of rs13306435 (-395T > A), rs2069860 (-623A > T), and rs2069830 (-612A > T). Chi-square test used to investigate Hardy–Weinberg equilibrium for

three COVID-19 SNPs. ORs and 95% CIs were calculated using logistic regression (CIs). 0.05 or less was significant.

Results

Correlation between demographics of patients and COVID19

54 individuals had a severe case of COVID-19 and 42 had a moderate form. A nasopharyngeal RT-PCR was done to test for SARA-COV-2 infection using COVID-19. The mild COVID-19 group included 20 women and 32 men with a mean age of 38.22 7.3. These people presented with malaise, sore throat, arthralgia, and anosmia to the Medical Clinic in Baghdad. Age range: 38.22± 7.3 years. The severe COVID-19 group included 18 women and 30 men aged 45.32±13.4. Mild COVID-19 patients were younger than severe COVID-19 patients (p=0.00).

Correlation of IL-6 gene polymorphisms with COVID-19 degree

Research was carried out on three different polymorphic sites in order to ascertain whether or not there is a connection between IL-6 promoter polymorphisms and the severity of COVID-19. These three polymorphic sites were designated as rs13306435 (-395T > A), rs2069860 (-623A > T), and rs2069830 (-612A > T). People who have severe and moderate COVID-19 have their distributions of genotypes, allele frequencies, and multiple genetic models (dominant, additive, and recessive) displayed here. This information is included in the article, which you can see here (Table 2). There was not the slightest hint of a significant departure from the Hardy–Weinberg equilibrium (HWE; P > 0.05) in any of the examples at all. In fact, there was not even the faintest suggestion.

Table 2: The following chart shows the distribution of the allele and genotype frequencies of chemR23 gene polymorphisms in patients with AR and controls.

Rs SNPs	**High degree of severity**	**Low degree of severity**	**P value**
rs13306435			
T	313 (76%)	316 (76%)	**0.787**
A	**77 (24 %)**	**78 (24%)**	**0,453**
TT	**188 (59%)**	**210 (62%)**	**0.234**
AT	**84 (41%)**	**67 (38%)**	**0.423**
AA	**11 (5.3%)**	**20 (10.5%)**	**0.132**
rs2069860			
T	222 (92%)	214 (90%)	**0.456**
A	**77 (8%)**	**78 (10%)**	**0.243**
TT	**188 (59%)**	**210 (62%)**	**0.654**
AT	**99 (41%)**	**96 (38%)**	**0.871**
AA	**14 (5.3%)**	**21 (10.5%)**	**0.432**
rs2069830			
T	413 (33%)	316 24%)	**0.186**
A	**83 (67%)**	**86 (76%)**	**0.679**

TT	**212 (44%)**	**213 (62%)**	**0.564**
AT	**87 (33%)**	**83 (38%)**	**0.345**
AA	**13 (5.3%)**	**19 (10.5%)**	**0.243**

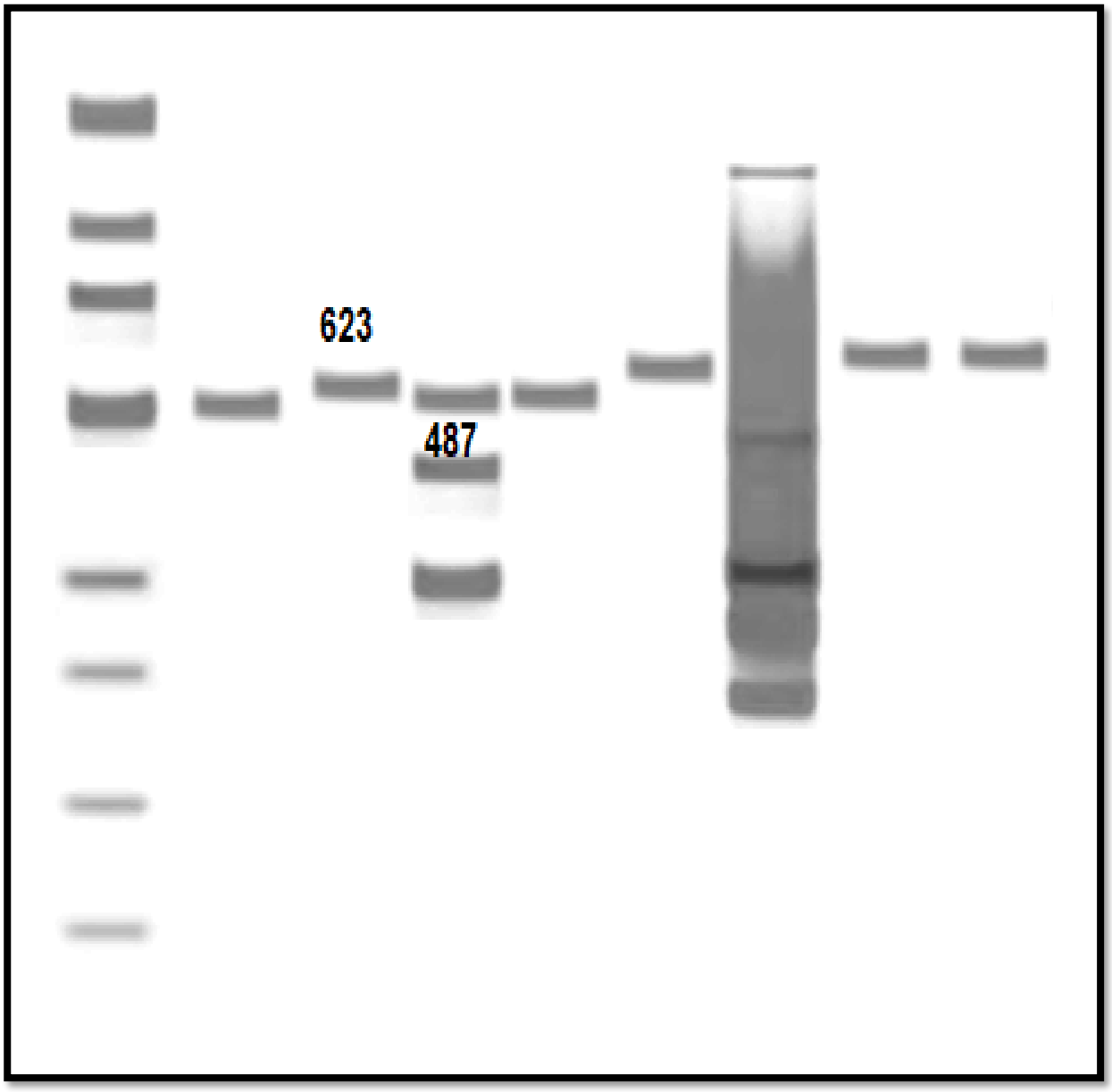

Figure 3: On an agarose gel containing 1 percent restriction enzyme, the products of restriction digestion (Taq1) were detected when the IL-6 the rs2069830 A > T polymorphism in the promoter region was subjected to restriction digestion. Homozygous genotype of the wild-type allele AA (619 bp + 35 bp), heterozygous genotype of the allele AT (487 bp + 619 bp + 35 bp), and homozygous genotype of the mutant allele TT (619 bp + 35 bp) are the three possible outcomes of this experiment (487 bp).

Discussion

The purpose of this study was to investigate whether or not an Iranian population is more susceptible to COVID-19 as a result of three possible functional SNPs located in the promoter region of the IL-6 gene. The genotype and allele frequency distributions of these SNPs were stable across all COVID-19 patient groups[1]–[3]. Depending on the pathway it takes to send its signals, IL-6 can either alter inflammatory or anti-inflammatory responses[9]–[11]. It has an effect on the survival and proliferation of leukocytes, as well as their differentiation and trafficking. Following an injury to the tissue or an infection, IL-6 can be released from macrophages, fibroblasts, keratinocytes, mast cells, dendritic cells (DCs), monocytes, mesangial cells, T and B lymphocytes, and endothelial cells (ECs). Cytokine storms may be triggered by IL-6, which then causes harm to tissue and disrupts immune response regulation[3]–[5]. IL-6 modifies the immune response to viral infections as well as cancer by increasing[12], [13] the expression of PD-1 and PD-L1 (PDL-1). Cytokine storms and lymphopenia are symptoms caused by the SARS-CoV, MERS-CoV, and SARS-CoV-2 viruses. Extreme cases of COVID-19 are associated with elevated levels of IL-6, which leads to lung infection and damage[5]–[7]. There is some evidence that IL-6 can predict the progression of diseases from mild to severe. Polymorphisms in the regulatory regions of cytokine genes may have an effect on production. IL-6 gene promoter SNPs, serum IL-6 levels, and inflammatory disease risk have been related[1], [4]. IL-6 polymorphism rs13306435 (-395T > A), which was investigated in a recent study, was found to be associated with an increased risk of COVID-19 in the Turkish population. In the MAV group, having the A allele was a risk factor for having an elevated serum IL-6 level and progressing to MAV, but this was not the case in the MAS group. In the course of our research, we found that the A allele and the AA genotype were more common than the T allele and the TT genotype[2], [5], [7], [8], [14]. There was no significant difference between those with severe and mild COVID-19 symptoms ($p > 0.05$). When compared to the population frequency, the researchers Fishchuk et al. found a substantial rise in the frequency of the TT genotype as well as the T allele of the rs2069860 (-623A > T) SNP in 31 COVID-19 pneumonia patients[3], [6], [8], [15]. There was a correlation between the 395 A/T rs2069830) SNP and COPD in Caucasians but not in North Indians. One research found an association between the IL-6 rs2069830 (-612A > T) CC gene variant and the risk of dying from septic shock in Caucasian Europeans who had heart or abdominal surgery[16], [17]. Researchers from China discovered that those with the IL-6–395 TT genotype had a significantly increased likelihood of developing pneumonia-induced sepsis and mRNA levels[18]–[20]. A meta-analysis found that having the 395 A/T (rs2069830) T allele affects the severity of pneumonia as well as the amount of IL-6 that is produced. The TT genotype is connected with poor IL-6 production and an attenuated immune response to chronic HCV infection. High blood IL-6 levels and SVR in HCV and HIV co-infected patients are related with the AA genotype of rs13306435174 A/T rs2069830 395 A/T[6], [14], [21]. There was no association found between the IL-6 rs13306435 (-572 A > T) polymorphism in an Egyptian population with either HCV infection or HCC. HBV has been linked to the genetic variant rs1800796. The role of rs13306435 in HCC has been confirmed by meta-analysis (HCC)[1], [7], [21]. SNPs rs13306435 (-395T > A), rs2069860 (-623A > T), and rs2069830 (-612A > T) were shown to have a substantial correlation with cervical cancer susceptibility in the Lithuanian population in a separate research that evaluated two IL-6 genetic polymorphisms (rs13306435 and rs2069830)[6], [15], [16]. The sample size, patient inclusion and exclusion criteria, disease pathogens, geographical location, ethnicity, and racial background may all play a role in producing inconsistent research findings. These three SNPs do not have any connection to the COVID-19 strain in Iraq. Our study is restricted in several waysTo begin, in order to determine the impact of the SNP[22]–[25], we did not look at the levels of RNA expression or IL-6 protein. Second, the sample size was quite small, and every single patient was from Iran. Additional IL-6 SNPs were not investigated in this research[7], [8]. We suggest doing research on IL-6 genetic variants and COVID-19 pathogenesis in populations of various ethnicities so that these results may be verified[1], [7], [14]–[17], [21].

Conclusion

In this study, we were unable to demonstrate any significant association between three potential SNPs in the promoter region of the IL-6 gene—rs13306435 (-395T > A), rs2069860 (-623A > T), and rs2069830 (-612A > T)—and susceptibility to COVID-19 severity in the Arab population from Iraq. These limitations included ethnicity, sample size, and the selection of genetic variants. For the purpose of elucidating and validating the relationship between genetic variants in IL-6 and SARS-COV-2 infection, more genetic research incorporating a greater number of SNPs and a more extensive sample size is necessary.

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