

Unveiling the Genetic Symphony: Exploring IL-6 and IL-10 Gene Variations as Pivotal Predictors of COVID-19 Severity in the Babylonian Population

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Abstract— The new coronavirus, which is now many respiratory illnesses in Wuhan, China, under the name SARS-CoV-2, from December 2019. The illness COVID-19 was brought on by this virus. A worldwide pandemic has been caused by the virus spreading from person to person. The investigation tries assess polymorphism effect with some genes like Interleukin-6 and Interleukin- 10 on susceptibility to covid-19 in Babylon province. A total of (113) cases in this study (63) cases have clinical symptoms of covid -19 patient, (50) blood samples were collected from healthy people as a control group in this study during (November 2021 to February 2022) at Al-Morgan Hospital. There were many demographic data included in the present study such as age distribution, sex distribution, and geographic distribution of covid patients. Among (63) samples from suspected covid -19 patients from different age groups (20 to 80 years old). The three genotypes (CC, CG &GG), respectively (5.31, 38.94, 11.50), and the allele frequency of (C, G) were (44.44, 55.56) for patients and genotypes (CC, CG &GG), respectively (15.04, 23.01, 6.19) and the allele frequency of (C, G) was (60.00, 40.00) for controls. three genotypes (GG, GA &AA), (3.54, 51.33, 0.88) respectively, and allele frequency of (G, A) was (52.38, 47.62) respectively for the patients and genotypes (GG, GA &AA), (7.96, 20.35, 15.93) respectively, and allele frequency (G, A) was (41.00, 59.00) respectively for control people.

Index Terms—IL-6, IL-10, genes, single nucleotide polymorphisms, COVID-19 Babylonian, PCR.

I. INTRODUCTION

Millions of people have been impacted by 2019, Coronavirus disease Covid-19, which is brought on by the SARS-CoV-1 (SARS-CoV-2) coronavirus that causes the severe acute respiratory syndrome (MERS-CoV) and four seasonal coronaviruses that cause moderate infections are all members of the Coronaviridae family (Wang *et al.*,2020, Corman *et al.*,2018).

Immune responses in the host and immune-related signs and symptoms highly varied. Patients with SARS-CoV-2 under

effective control, i.e., asymptomatic, and those without effective management, i.e., impacted via COVID-19 severe infection, host immune responses and immunological-related symptoms are tremendously diverse. This shows that in some circumstances, host immunological dysregulation plays a role in pathogenesis. However, it is unclear whether the emergence of a severe variation of the illness governed by immunological either excessive activity or an inability to stop the inflammatory reaction as a result of continued virus reproduction and immunologic mis regulation. In mild cases, the relationship between cytokine levels, viral load in the nasopharynx, and falling viral burden implies that immunological reaction is linked to viral burden (Yang *et al.*,2021, Fajgenbaum and June,2020).

Inflammatory cytokines are crucial in the pathogenesis of disease, as the COVID-19 cytokine storm illustrated (Pedersen and Ho.,2020,Wu and Yang.,2020). which could linked to the progression of COVID-19. The number of nations having information the range of cytokine polymorphisms was 16 to 54. The cytokine polymorphisms utilized, as well as the quantity of nations involved. The allele frequency associate with CFR , DDR (de Meira Leite *et al.*,2021).

II. MATERIALS AND METHODS

Academic Disciplines

The empirical aspect of the current investigation was conducted between November 2021 and March 2022. A total of 113 participants, consisting of 50 healthy individuals and 63 sick, were chosen for the study. A comparative analysis was conducted between Covid-19 diagnosed patients admitted at Mergan Medical City in Iraq and two groups: fifty samples from healthy cases as controls and sixty-three samples' from diagnosed patients with the condition. Using serological and

molecular assays, the current investigation included a group of individuals who were diagnosed with covid-19. The participants ranged in age from 20 to 80 years old and were comprised of 19 males and 44 females. It was determined that the blood and serum samples taken from the patients were quite accurate. Group of individuals who are in good health and serve as a comparison to the experimental group.

A total of fifty individuals, ranging in age from 20 to 80 years, who are in good health and belong to the Babylon Iraqi community, were selected for the study. These individuals have undergone rigorous laboratory, clinical, and genetic testing to confirm their absence of covid-19 infection.

Collection Of Blood Samples

Within the subjects that took part in this investigation, a total of around five milliliters of blood samples were collected from them. To collect roughly two milliliters of blood for the purpose of genetic analysis, EDTA tubes were utilized as the collection medium. Each individual patient gave two samples of their blood. The initial sample was placed in gel tubes and allowed to sit for a period of thirty minutes. After centrifuging the second sample for fifteen minutes, the serum that was obtained was collected and placed in a freezer at a temperature of around twenty degrees Celsius.

Collection Of Control Samples

There were fifty individuals who were selected to provide venous blood samples for the study. These individuals were in good health and had an age distribution that was equivalent to that of the patients.

Genomic DNA Isolation

The utilization of EDTA tubes was necessary in order to fulfill the requirements of the molecular analysis of human genomic DNA. Proteinase K is widely suggested for use in the process of DNA purification from frozen blood samples. Both the Geneaid and Promega kits, which were manufactured by Geneaid Ltd., were utilized.

Acquiring an understanding of the concentration and purity of DNA:

Following a procedure that was developed expressly for the purpose of separating DNA from whole blood samples of patients and control individuals, genomic DNA was extracted from whole blood samples taken from both patients and controls. RNA and DNA were extracted from fresh blood samples using the gSYNCTm gDNA Extraction kit that was manufactured by Geneaid-Ltd. This kit was used to analyze blood samples.

In order to determine the concentration (ng/L) and purity (OD: 260/280nm) of 2.5 l of DNA that was extracted from the samples, the Nano drop was utilized. The purpose of this measurement was to determine whether or not the samples included any instances of protein. A 260/280 ratio that fell within the range of one to two was obtained as a result of the DNA purification process, as directed by the standard. At the conclusion of the genomic DNA extraction process, agarose gel electrophoresis was utilized in order to validate the proximity and integrity of the DNA that was extracted. A solution of acrylate that had been dissolved in 1x TBE buffer and Safe stain was used to expose the DNA bands with the application of a voltage of 100 Volts for a period of seventy-five minutes.

During the process of manufacturing the primer, the IL-6 (174 Promoter) primer that was provided was utilized for the purpose of identifying diagnostic and pathogenic genes (Table 1). The primer was supplied by Ligo/USA from their end. First, all of the primer pairs were centrifuged before the caps were removed from the tubes that contained the primers. A primer stock solution with a concentration of one hundred picomoles per microliter was produced by adding a predetermined quantity of nuclease-free water to each primer. This activity was carried out in accordance with the instructions provided by the manufacturer. A concentration of 10 Picomole/microliter of free nuclease water was achieved for use in PCR amplification by transferring 10 liters of the primer stock solution into an Eppendorf tube that contained 90 liters of free nuclease water. This was done in order to get the desired result.

TABLE 1. SEQUENCE OF PRIMERS FOR IL-6 AND IL-10 GENE

Gene	Primer		bp.	Reference
IL-6 (174 Promoter)	F	5'-CCC CTA GTT GTG TCT TGC C-3'	288	(Marzieh et al.,2016)
	R	5'-GCC TCA GAG ACA TCA CCA GTC C-3'		
IL-10 1082	F	5-CTACTA AGG CTT CTT TGG GAG-3 5-ACT ACT AAG GCT TCT TTG GGA A-3	258 bp	(Samaneh et al.,2014)
	R	5-CAG TGC CAACTG AGA ATT TGG-3		

Detection of IL-6 (174 Promoter)

Genotype determination for three selected SNPs was performed by (SSP-PCR) method (Gao et al., 2009). PC*R mixture 5- μ l (DNA), 5- μ l master mix, 5 μ l forward, reverse primer. Conditions of *PCR conditions for *IL-6* performed of following table (2).

TABLE 2. PCR CONDITION FOR *IL-6 (174 PROMOTER)*

Step	Temperature C°	Tim/min.	Cycles
Initial denaturation	*95	4	1
Denaturation	*95	20s	15
Annealing	58	40s	
Extension	72	40s	
Denaturation	95	20s	25
Annealing	54	50s	
Extension	72	50s	
Final extension	72	7	1
Storage	4	∞	

Detection of IL-10 1082

Using polymerase chain reaction, two IL-10 SNPs (IL-10-592 and IL-10-1082) were identified and genotyped (SSP-PCR) (Lu, Y.L et al., 2010). The initial stage was utilizing a gradient temperature to optimize the PCR. This is crucial in figuring out the ideal annealing temperature. 5 l of template DNA, 5 l of master mix, 5 l of each forward and reverse primer, and 20 l of total reaction volume made up the PCR reaction mixture for the gradient. The following table displays the gradient's PCR condition (table 3).

TABLE 3. GRADIENT CONDITION OF *IL-10 1082*

Step	Temperature C°	Tim/min.	Cycles
Initial denaturation	*95	*5	1
Denaturation	*95	*0.5	35
Annealing	55.4-56.2-59.3-61.6-63.4-63.3	0.5	
Extension	72	40s	
Final Extension	*72	7	1
Storge	*4		$-\infty$

Following identification of the clearest band, which was 61.6 C°, as the ideal annealing temperature of IL-101082 genes, a PCR combination of 5 l DNA, 5 l master mix, and 1.5 forward & reverse primers was created. According to the following table, PCR conditions were used (3-21).

TABLE 4. PCR PREREQUISITE TO *IL-10 1082*

Step	Temperature C°	Tim/min.	Cycles
Initial denaturation	*94	1	-1
Denaturation	*94	1	40
Annealing	*61.6	1	
Extension	*72	-1	
Final Extension	*72	10	-1
Storge	4		

III. RESULT AND DISCUSSION

IL-6 Gene Polymorphism

The three genotypes (CC, CG & GG), respectively (5.31, 38.94, 11.50), and the allele frequency of (C, G) were (44.44, 55.56) for patients and genotypes (CC, CG & GG), respectively (15.04, 23.01, 6.19) and the allele frequency of (C, G) was (60.00, 40.00) for controls. Tables (4-5) and

TABLE 5. POLYMORPHISMS OF THE IL-6 GENE THAT ARE CONNECTED WITH PATIENTS WITH COVID-19 AND CONTROL INDIVIDUALS IN TERMS OF THEIR GENOTYPE FREQUENCY

Genotype	Covid Patients		Healthy people		Total	
	No	%	No	%	No	%
CC	6	5.31	17	15.04	23	20.35
CG	44	38.94	26	23.01	70	61.95
GG	13	11.50	7	6.19	20	17.70
Total	63	55.75	50	44.25	113	100.00
Allele Frequency						
C	56	44.44	60	60.00	116	51.33
G	70	55.56	40	40.00	110	48.67
Total	126	100.00	100	100.00	226	100.00

TABLE 6. GENETIC ASSOCIATION OF GENOTYPE *IL-6* GENE WITH DISEASE. POLYMORPHISMS OF (*IL-6*) GENE

ALLELE	OOD RATIO	Significance level	CI 95%
CC*CG	0.2086	0.0034	0.0730 to 0.5956
CC*GG	0.1900	0.0128	0.0514 to 0.7028
GG*CG	1.0974	0.8608	0.3883 to 3.1015

As showed in the above table, there is significant between CC*CG (0.0034), CC*GG (0.0128), GG*CG (0.8608). These results agreed with the findings of the WHO, which indicated Population diversity of the IL-6 gen polymorphisms at the rs1800796/rs1800795 loci revealed that populations of China, Spain, Sweden, Poland, Germany, the UK frequently have the GC genotype while populations from India, Mexico, Turkey, Brazil, Russia, Italy, South Africa, & Greece frequently have the GG genotype. For the rs1800796 polymorphism, only the

Japanese population typically had the CC genotype (WHO, 2020). And these results agreed with the findings of (Falahi *et al.*, 2022) indicated no appreciable variations when it comes to the genotype or allele distribution of a few. There are some differences between patients with severe COVID-19 and those with mild COVID-19 in the promoter region of the IL-6 gene. Another local study by Iman S.H. (2022) explained that G allele from the Iraqi population and there is an association between IL-6-174 G/C polymorphism and COVID-19 patients.

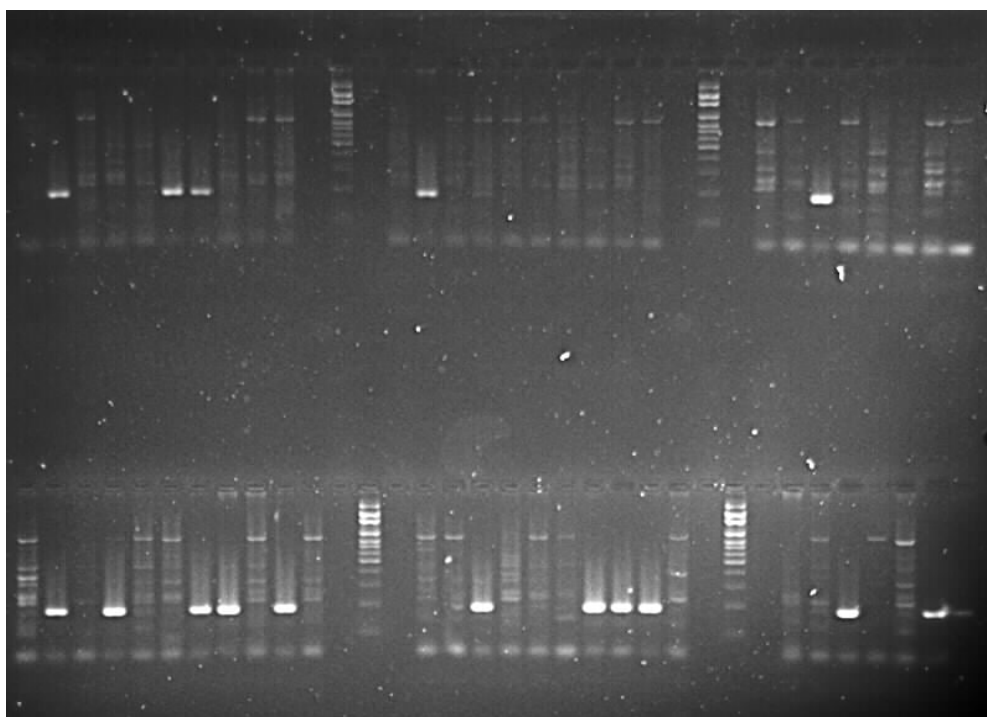


Figure (1): The Electrophoresis Pattern of IL6 gene Polymorphisms. At 100 V. The 100 bp DNA Ladder is in the L lane along with a 1X TBE buffer with 5% NuSieve® 3:1 agarose gel with 5 l of Safe red dye for an hour.

A reliable predictive test that can reasonably anticipate a patient's progression to a severe stage of disease is urgently needed for effective diagnosis and care given the continued prevalence of COVID-19 in most societies. Early studies on COVID-19 suggested that the respiratory tract submucosa of activated mast cells has a role in escalating the inflammatory state and pathogenesis by releasing pro-inflammatory cytokines like IL-6 and TNF- (Conti *et al.*, 2020, Kritas *et al.*, 2020, Ross and Conti 2020). When SARS-CoV2 activates the innate and adaptive immune systems, a high number of cytokines, including IL-6, are released. Many individuals with severe COVID-19 experience a systemic inflammatory reaction known as cytokine release syndrome (CRS), which is a significant cause of death.) Zhang and Li 2020(. Increased levels of IL-6, a pro-inflammatory molecule, are known to

suppress NK cell activity and have also been linked to decreased levels of granzyme and perforin, which degrade lytic activities (Cifaldi *et al.*, 2015). Exacerbation symptoms in COVID-19 patients included elevated body temperature, elevated inflammatory markers such as CRP and serum ferritin, and advanced chest computed tomography imaging. These symptoms were linked to elevated IL-6 levels, which decreased as the condition improved (Liu *et al.*, 2020). IL-6 has been linked to pulmonary diseases before, namely in individuals with pneumonia potentially severe pneumonitis brought on by radiation treatment (Chen *et al.*, 2001). Since the coronavirus disease of 2019 (COVID-19) epidemic in Wuhan, China, it has spread quickly to numerous other nations. While the majority of individuals were thought to be mildly unwell, deaths from critically ill patients who suffer from respiratory failure and

multiple organ dysfunction syndrome are not unusual. It has been hypothesized that cytokine storm is connected to negative results (Han *et.al.*,2020). looked at 113 people; 50 healthy controls, 63 people with Covid, and a total of 113 people. Results for the *IL-6* polymorphism were obtained. Logistic regression use evaluates a distribution frequency of variables across the study groups. The *IL-6* GG*GA genotype ($p = 0.049$) & male gender ($p 0.001$) relate with severe COVID-19. COVID-19 severity was found to be associated with the *IL-6* genotype through In the context of the co-dominant inheritance paradigm, multiple logistic regression GG*CG, CC*GG, and CC*GG alleles all have 95% confidence intervals of 0.0730 to 0.5956, 0.0034 for significance and 0.2086 for odds ratio, respectively (1.0974). assuming that severity off Covid-19 associated with a *IL6-CC*CG* genotype. this study agreed with (Han *et.al.*,2020) 102 -COVID-19 patients who had been admitted to Renmin Hospital were enrolled (Wuhan, China). In accordance with their symptoms, all patients were divided into three groups: moderate, severe, and critical. Additionally, 45 healthy volunteer control samples were used. This study agreed with (Michot *et.al.*,2020) COVID-19 with hyperinflammatory pulmonary symptoms is associated with a

cytokine storm involving interleukins and chemokine dysregulation. This study agreed with (Batur and Hekim 2020). This study agreed with (Ulhaq and Soraya 2020) Excessive cytokine production and a higher mortality rate are characteristics of these severe COVID-19 cases. shows the course of COVID-19 is closely correlated with an increased level of interleukin-6 (IL-6) and C-reactive protein (CRP). This study agreed with (Szulc-Kielbik, Kielbik, Nowak, and Klink, 2021) Patients with ovarian cancer had high IL-6 levels in their serum and ascites. In light of this, its level is addressed in the literature as a potential biomarker that may aid in differentiating between malignant and benign ovarian tumors and enable the prediction of the chemotherapy response.

IL-10 Gene Polymorphism

Figures 2 show the three genotypes (GG, GA & AA), (3.54, 51.33, 0.88) respectively, and allele frequency of (G,A) was (52.38, 47.62) respectively for the patients and genotypes (GG, GA & AA), (7.96, 20.35, 15.93) respectively, and allele frequency (G,A) was (41.00, 59.00) respectively for control people. Table 4-7 displays the genotyping results for patients with Covid-19 and control people.

TABLE 7. POLYMORPHISMS OF THE *IL-10* GENE THAT ARE CONNECTED WITH PATIENTS WITH COVID-19 AND CONTROL INDIVIDUALS IN TERMS OF THEIR GENOTYPE FREQUENCY

Genotype	Covid Patients		Healthy people		Total	
	No	%	No	%	No	%
GG	4	3.54	9	7.96	13	11.50
GA	58	51.33	23	20.35	81	71.68
AA	1	0.88	18	15.93	19	16.81
Total	63	55.75	50	44.25	113	100.00
Allele Frequency						
G	66	52.38	41	41.00	107	47.35
A	60	47.62	59	59.00	119	52.65
Total	126	100.00	100	100.00	226	100.00

TABLE 8. GENETIC ASSOCIATION OF GENOTYPE *IL-10* GENE WITH DISEASE

Polymorphisms of (<i>IL-10</i>) gene			
ALLELE	OOD RATIO	Significance level	CI 95%
GG*GA	0.1762	0.0075	0.0493 to 0.6295
GG*AA	8.0000	0.0806	0.7761 to 82.4596
GA*AA	45.3913	0.0003	5.7231 to 360.0120

As showed in the above table, there is significant between GG*GA (0.0075), GG*AA (0.0806), GA*AA (0.0003). These results agreed with (Neumann *et.al.*,2020) A

characteristic of activated regulatory T cells, which are found in organs like the lung, is the production of IL-10. This population, which is typically uncommon in healthy people,

increased to around 10% of the regulatory T-cell pool in patients with severe COVID-19. The murine counterpart of this population has a strong anti-inflammatory effect and environmental interactions. (Bedoya *et.al.*,2013) When it comes to lung viral infections, *IL-10* restrains a development of *IL-17*-producing cells that damage the tissue, (Chaudhry *et.al.*,2011,McKinstry *et.al.*,2009) reduces the generation of cytokines including IL-6, which have been linked to COVID-19 morbidity, and suppresses the innate inflammatory response to particles.(Chang, Kunkel and Chang (2009). (Rojas, Avia, Martín, and Sevilla (2017) Potentially, A blood-based biomarker for cases that proceed to more severe lung injury could be provided by increased IL-10. A more intriguing

possibility is that individuals with higher *IL-10*-Regulatory T cells with impaired adaptive immunity produce. Many persistent viral infections are characterized by the presence of IL-10+ regulatory T cells, which are also linked to long-term persistence. IL-10 effectively inhibits antiviral responses in respiratory infections. (Sun, Torres, and Metzger (2010), reduces the immunological response superinfection with bacteria.(Chaudhry *et.al.*,2011,van der Sluijs *et.al.*,2004) Since secondary infection leading to pneumonia ,a leading reason for mortality of influenza, and perhaps for some patients with COVID-19 is well,(Cox, Loman, Bogaert and O'Grady (2020), Zhou *et.al.*,2020) excessive *IL-10* production

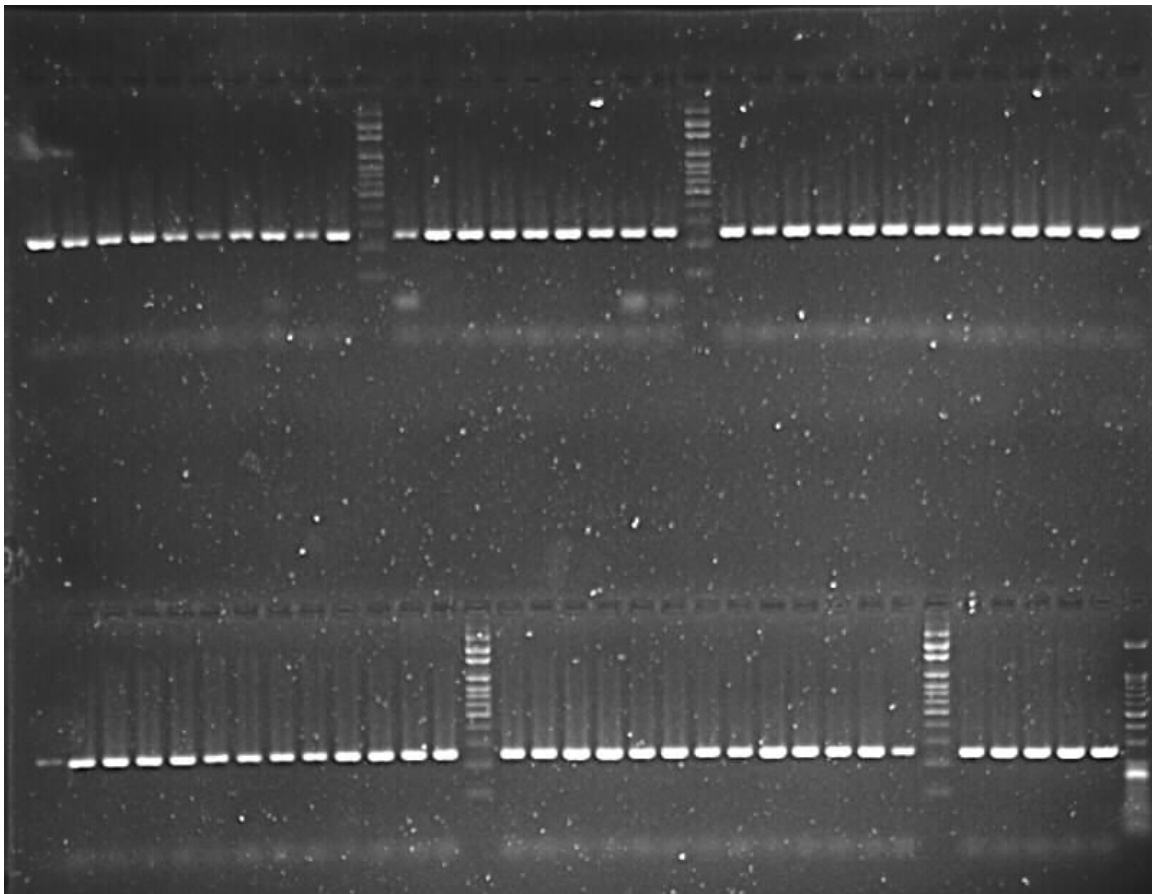


Figure 2. The Electrophoresis Pattern of *IL10* gene Polymorphisms. At 100 V. The 100 bp DNA ladder is in the L lane along with a 1X TBE buffer with 5% NuSieve® 3:1 agarose gel and 5 l of Safe red dye for an hour.

IL-10 is an anti-inflammatory cytokine that was discovered to be increased with COVID-19 (Huang *et al.*, 2020, Chen *et al.*, 2019). The main purpose of the multipurpose cytokine *IL-10* is to suppress the inflammatory response. *IL-10* is also known to cause T-cells to become anergic or unresponsive during the anti-tumor cell response (Moore, *et al.*, 2001) as well as in viral infection (Maris, Chappell, and Jacob, 2007). One of the several mediators involved in the pathophysiology of COVID-19 is *IL-10*. *IL-10* is present in high concentrations during influenza infection, particularly during the adaptive immune response. 58 Critical COVID-19 patients had considerably greater serum *IL-10* and *IL-6* levels than moderate. Viral infection was eradicated by blocking *IL-10* with an antibody against *IL-10* or its receptor or by genetically removing *IL-10*. (Brooks *et al.*, 2006, Ejrnaes *et al.*, 2006) or bacterial pathogen (Biswas *et al.*, 2007). our looked at 113 people; 50 healthy controls, 63 people with Covid, and a total of 113 people. Results for the *IL-10* polymorphism were obtained. Regression using logit was used to assess distribution frequencies of variables across the study groups. COVID-19 severity was found to be associated with the *ACE2-CT* genotype through. The co-dominant inheritance model and multivariate logistic regression GG*GA Allele, 95% CI (0.0493 to 0.6295), Significance level, 0.0075, Odd Ratio, 0.1762, GG*AA Allele, 95% CI (0.7761 to 82.4596), Significance level, 0.0806, Odd Ratio, 8.0000, and GA*AA Allele (45.3913). assuming that the severity of covid-19 was related to the genotype *IL10*-GG*GA. This study agreed with (Chen *et al.*, 2020) increased cytokine levels of *IL-10* to correlate with COVID-19 disease severity. This study differed with (Zhu *et al.*, 2021) the levels of *IL-10* of male were noticeably higher than those of female.

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