Association of IL-18 Promoter -607 C/A Polymorphism with Severity of Covid-19 in Kurdish Patients

Shayma Khalil Muhammad, Suhaila Nafee Darogha, Ahmed A Al-Naqsbbandi

Department of Biology, College of Education,Scientific Department, University of Salahaddin, Kurdistan Region, Iraq

Abstract— **Background: Coronavirus disease 2019 is a viral infection that throws the immune system out of balance, sets off an inflammatory cytokine storm, and has the potential to drastically alter the makeup of immune cells in circulation.**

Methods: In order to estimate serum IL-18 levels and genotyped SNPs in IL-18 using the ARMS-PCR technique, a case-control research including 210 Kurdish Covid-19 patients visiting three hospitals in Erbil-city and 70 healthy controls was carried out from July to December 2021. Based on their symptoms, the patients who were recruited in this research were classified into three groups: mild, moderate, and severe.

Results: The study results indicated that most Covid-19 patients were 51 years and older (60%). With respect to gender, women were more affected than male (53% vs. 47%). When compared to healthy controls, the median level of IL-18 in the blood of patients in the Covid-19 group was considerably higher. The group with severe symptoms exhibited the greatest level. The primary findings also showed that homozygous for strongly displayed CC was protected against Covid-19 severity, but heterozygous CA showed an elevated risk for Covid-19 severity.

Conclusion: there is a substantial correlation between the severity of Covid-19 and elevated blood levels of IL-18. Additionally, heterozygous CA genotype at the IL-18 -607 position was positively correlated with Covid-19 patients.

Index Terms— **Covid-19, gender, IL-18, Gene polymorphism.**

I. Introduction

Following the virus's quick spread around the world and potential to cause illness, the WHO declared the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic on March 11, 2020 (1). Globally, more than 528 million individuals (2,328,264 in Iraq) had contracted the disease as of the first of June 2022, and about 6.3 million people had died, counting 25,219 in Iraq (2). The virus is spread through respiratory secretions, which infect people when they come into contact with mucosal membranes, either directly or indirectly (3).

There are two stages in the acute phase of SARS infection, which correspond to the disease's intensity and clinical signs. While the second acute stage is marked by a dysregulated immune response that can quickly lead to severe symptoms, the first acute stage is marked by viral replication and relatively moderate symptoms (4).

Natural immune system is the initial line of defense that the body employs in the fight against acute viral infections. It involves stopping infection, quickly identifying as well as eliminating any infected cells, and producing inflammatory cytokines, as well as adaptive immune responses, such as the creation of antibodies that are unique to the infection (SARS-CoV-2) (5). Activation of both the specific and non-specific immune systems in individuals with severe CoV-2 leads to an uncontrolled inflammatory response that generates the cytokine storm (6).

Interleukin-18 is pleiotropic pro-inflammatory cytokine, mainly produced by macrophages, monocytes, as well as dendritic cells, and is involved in both humoral as well as cellular reactions. IL-18 performs a wide range of biological functions, including modifying the immune response by increasing cytokine gene expression, encouraging naive T cells to develop into Th cells, activating NK cells and plays a crucial part in the host's defensive mechanisms versus viral infection (7). IL-18 has played a very important role in ARDS, a feature of severe Covid-19. In certain viral infections marked by cytokine storms, such as "avian influenza, Dengue virus, and Covid-19", serum IL-18 levels have been associated with severe illness and fatality (8).

Variation in the promote area of the L-18 gene plays a significant impact in the regulation of L-18 gene production by affecting the TF binding capability of the gene. The human IL-18 gene contains 6 exons and 5 introns, and it is situated on chromosom 11 at location "11q22.2-q22.3" (9). Research on the exact structure and sequence variations of the human L18 promot has discovered 5 SNPs at the 50-end of the IL18 gene: -656 G/T, -607 C/A, -137 G/C, +113 T/G, and +127 C/T (10). Two common SNPs, −607C/A and −137G/C, associated with levels of IL-18 in people. These SNPs are connected to IL-18 gene transcription and IL-18 protein synthesis (11).

Genetic variations in the IL-18 gene have been associated with a range of inflammatory diseases: as viral infection and atopic asthma (12). According to research by Hirankarn et al. (13), HBV patients had a significantly higher prevalence of the -607A/A genotype than controls. Further research by Li et al.

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(14) examined the effect of SNP -607A/C in the IL-18 gene on the likelihood of developing a chronic HBV infection and discovered that patients with the infection had a higher frequency A/A at position - 607 compared to controls.
As ithere is currently a dearth of information on relations between gene polymorphisms of IL-18 - 607 C/A and the severity of Covid-19 disease in Iraq, the current study set out to examine the relationship between serum IL-18 levels and the role of IL-607 A/C with COV-19 severity in Kurdish patients.

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II. Materials and Methods Patients and control groups

A case-control study was conducted on 210 COVID-19 patients, their age groups ranging between (21 to 75) years old, who were attending three hospitals in Erbil-city (Rozhawa Emergency, Emirati, and Lalav ICU) as outpatients and inpatients during the period from July 2021 to December 2021. Reverse real-time PCR was used to detect the virus RNA in nasal-pharyngeal swabs in order to molecularly diagnose Covid-19. Patients classified in to three severity levels depending on clinical manifestation: 1. Mild groups: Thirty-five men and thirty-five women, totaling seventy patients, were found to have COVID-19 signs and symptoms "fever, coughing, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, and loss of taste and smell" but not dyspnea or normal chest imaging. 2. The 70 patients in the moderate groups (of which 39 are female and 31 are male) have changed imaging evidence that is consistent with lower respiratory diseases and pneumonia, which is clinically or radiologically evident, but whose oxygen saturation (SpO2) is ≥94% in room air. 3. Severe groups: 70 patients (38 female and 32 male), shortness of breath, symptoms lower respiratory infection, respiratory tract more than 30 times (breaths)/min, in resting state SpO2 ≤ 90. A control sample of 70 apparently healthy individuals were included, their age ranging between (23 to 70) years.

A. Blood sample collection

Each recruited participant (patient and control) had five milliliters of peripheral blood collected by venipuncture; the blood was split into two tubes: two milliliters were stored at freezer until DNA extraction, and three milliliters were put in a gel tube to extract serum. After centrifuging the tubes for 15 minutes at 3000 rpm, the serum was transferred to a 1.5 ml Eppendorf tube and kept at -20 °C until the IL-18 level was estimated.

B. Determination of serum IL-18

Serum IL-18 levels were quantified using the ELISA technique. The Cloud Clone Corp. kit was employed in accordance with the manufacturer's instructions for accurate measurement.

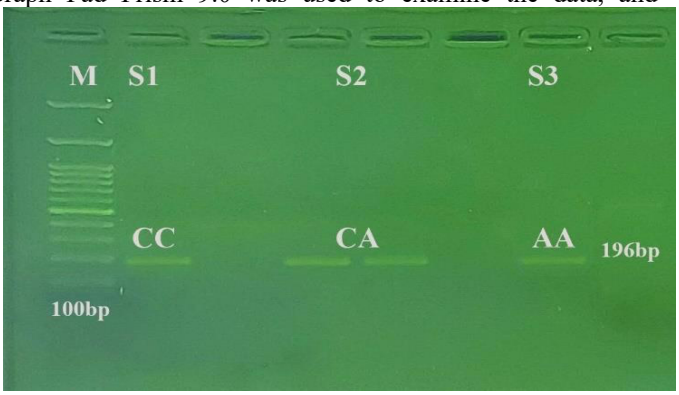
C. Interleukin-18 genotyping

The DNA extraction kit (Bayern GMbH) was used for peripheral blood mononuclear cells in accordance with the manufacturer's instructions to obtain genomic DNA for Covid-19 patients and healthy persons. Utilizing the ARMS-PCR, the IL-18 genotype at (- 607 C/A) (rs 1946518) was determined. 20 µL of reaction buffer containing 1.5 Mm dNTPs, 40 ng genomic DNA, 25 MgCl2, 0.4 units of Tag polymerase (Fermentas), and 1 µL of 10 pmol primers in 1 X Reaction Buffer were used for the tests. For the generic primer of IL-18, targeting the C allele, "the forward primer sequence was 5'-GTTGCAGAAAGTGTAAAAATTA TTAC-3'. On the other hand, for the A allele-specific forward primer, the sequence was 5'- GTTGCAGAAAGTGTAAAAATTA TTAA-3'. The reverse primer sequence for both alleles was 5'-TAACCTCATTCAGGACTTCC-3". Using a thermal cycler, the reaction was carried out under the following conditions: 95 C for two min, followed by 35 cycles of 45 seconds at 95 C, 40 sec. at 58 C, one min at 72 C, and finally a 7 min elongation at 72 C. To analyze the amplified products, a 2% agarose gel was employed.

Figure 1: Agarose gel electrophoresis (75 volts/cm2; 45 minutes) of PCR products of IL-18 196 bp C/A.

Statistical Analysis

Graph Pad Prism 9.0 was used to examine the data, and



variables with a regularly distributed set of values were suitably expressed as mean ± SD. The area under the COVID-19 curve (AUC), cut-off value, specificity, sensitivity, and other metrics were used to illustrate the predictive significance of the study's determination of severity using receiver operating characteristic (ROC) curve studies. The 2-tailed Fisher's exact probability was utilized to assess if the genotype distributions of COVID-19 patients and controls differed significantly from one another. The IL-18 genotypes were displayed as percentage frequencies. Direct gene counting techniques were used to determine the allele frequencies of the cytokine gens; the Hardy-Weinberg (H-W) calculator was utilized to assess the genetic equilibrium of two alleles. P <0.05 was used to determine the variables' statistical significance.

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Results and discussion

The result of the current study revealed that females were more affected than men; the were 112 (53%) and males were 98 (47%) as shown in Figure 2, and in terms of age group (≤30, 31-50 and ≥51) years. The majority of COVID-19 patients (5%), were older than 30 (5%), 31-50 years (35%) and ≥51 years (60%), the older age (60%) was more risk of covid-19 patients than the other ages (5% and 35%) (Figure 3).

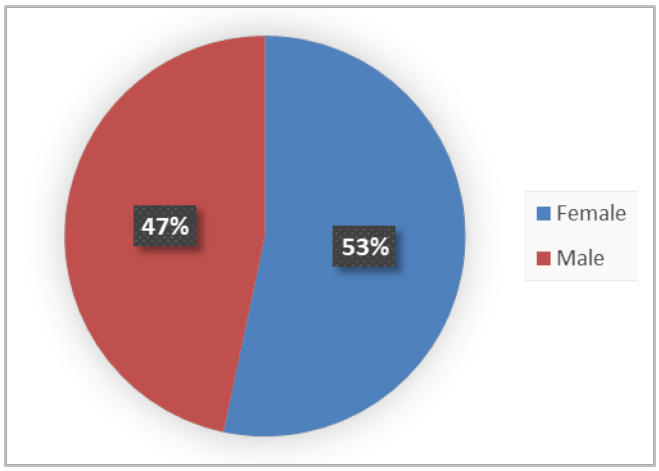

Figure 2: Percentage male and female of covid-19 patients.

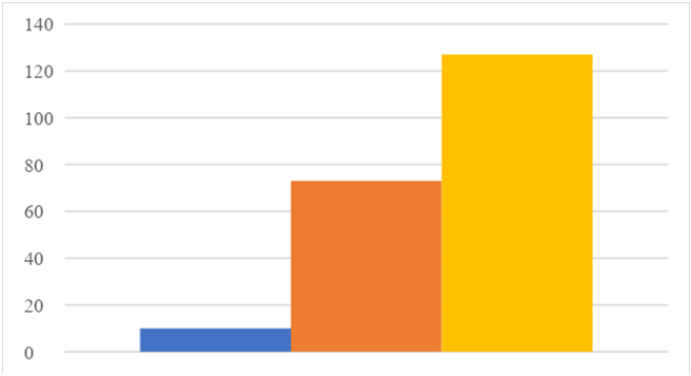

Figure 3: Percentage age categories of covid-19 patients.

The majority of COVID-19 cases that have been reported have involved adults; a notable fraction of these patients exhibit severe pneumonia, which frequently progresses to acute respiratory distress syndrome (15). All age groups have been found to be susceptible to SARSin this investigation; however, older individuals have a higher chance of developing a severe illness than middle-aged adults, and as such, they remain at risk of contracting the virus as long as the prerequisites for transmission are fulfilled (16). According to the data that was released, there was a substantial relationship between the severity of an illness and age, with older patients being more likely to have severe diseases and even have higher death rates (17, 18).

In addition to fading immunity, there are several additional age-related variables that may contribute to increased mortality and morbidity in the elderly. As people aged, the average number of comorbid conditions rose gradually. Compared to younger and middle-aged patients, Covid-19 patients had a considerably higher score (19). Chronic, subclinical systemic inflammation is another well-known aspect of aging immunity (20). Chronic respiratory conditions, cancer, diabetes mellitus, hypertension, and cardiovascular disease were all linked to an increased risk of mortality as people aged (21). This may explain why individuals over 60 are more likely to develop chronic illnesses, engage in less physical activity, lose muscle mass, and gain weight as they age. Random studies have also shown that infections often result in death (22).

Males and females react differently to the same viruses, such as SARS CoV2. These disparities between the two genders are mostly due to variances in illness incidence and severity. Multiple factors contribute to the variation in the progression of sex-specific illnesses following viral infections. Both X-linked gene activity and steroid activity specific to a particular sex affect the immune response, altering both the non- specific and specific immune responses to viral infection. Moreover, variations in the expression of the cellular serine protease TMPRSS2 and the ACE- 2 receptor, which are essential for the binding and priming of SARS CoV2, might potentially have a significant impact (23).

Similar to our findings, 60% of the SARS CoV2-infected patients were female, and 40% were male, in this investigation, which was done in Baghdad City. The female patients had greater effects than the male patients (24). Furthermore, it was discovered by Gebhard et al. (25) that the percentage of female infections was greater than that of male infections, reaching (52% in Switzerland and Belgium, 54% in Portugal, 53% in France, and 60% in South Korea).

Additional studies revealed that a greater percentage of patients were men (26). Males are thought to be more likely than females to smoke currently, and men also have less CD4 cells than women, which implies that fewer B cells are created to manufacture antibodies, which accounts for the distributional disparity. Additionally, the X chromosome aids in the synthesis of antivirals such as type 1 interferon. Because men only have one X chromosome, they generate less antiviral therapy than women to combat the SARS-CoV-2 infection (27).

Serum level of IL-18

The results of the statistical analysis revealed a significant increase in serum IL-18 levels in Covid-19 patients compared to healthy controls (P = 0.000). Additionally, there was a significant difference in IL-18 levels between the Covid-19 patient group and the healthy control group. The mean ± SD of L-18 in the control group was 33.99 ± 16.76 pg/ml, while in the mild, moderate, and severe Covid-19 groups, the IL-18 levels were 62.88 ± 22.03 pg/ml, 86.63 ± 26.87 pg/ml, and 141.5 ±

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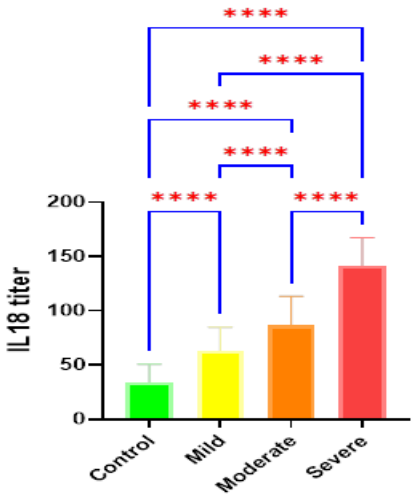
25.96 pg/ml, respectively. These findings are shown in Table (1).

Table 1: Serum IL-18 mean ± SD levels in Covid-19 patients and healthy controls .

Category	Mean ± SD (pg/ml)	P – value
Mild	62.88±22.03	0.000
Moderate	86.63±26.87	0.000
Severe	141.5±25.96	0.000
Healthy Control	33.99±16.76	0.000

Receiver Operating Characteristic analysis

The diagnostic significance of elevated blood levels of IL18 in Covd-19 patients was assessed using ROC analysis. According to ROC curve analysis results, patients with severe conditions had an AUC of 0.993 and a P-value of 0.000. Therefore, 70.97 pg/ml was the cutoff value of IL-18 to predict severity, with a sensitivity of 95.71% and a specificity of 91.43% reported. With a sensitivity of 92.86% and specificity of 91.43%, the cut-off value for the serum IL-18 level in the moderate group was 62.35 pg/ml and the AUC was 0.946 with a significant value of P = 0.000. The mild group had a significant AUC of 0.897 (P = 0.000); the cut-off value for serum IL-18 level was 44.60 pg/ml. The corresponding sensitivity and specificity were 90% and 85.71%, as shown in Table 2 and Figure 4.



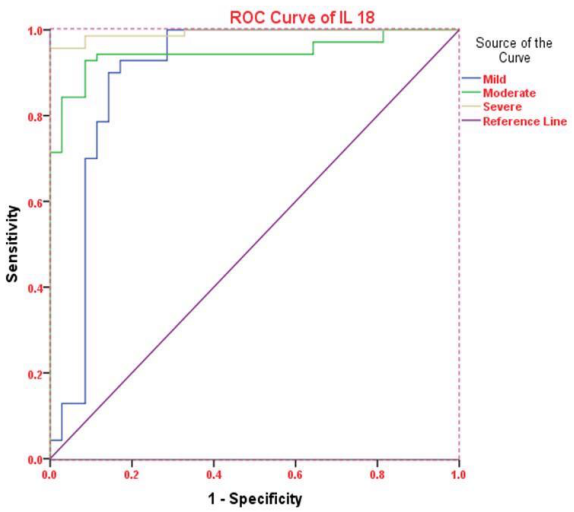


Figure 4: (A) Serum IL-18 levels in both the Covid19 patient group and the healthy control group, and (B) ROC curve analysis depicting the IL-18 levels specifically for the Covid-19 patient groups.

Table 2: The ROC curve evaluation of IL-18 in individuals in the Covid-19 group

Category groups	Area under curve (AUC)	Optimal cut-off value	Sensitivity %	Specificity %	P-value
Mild	0.897	44.60	90	85.71	0.000
Moderate	0.946	62.35	92.86	91.43	0.000
Severe	0.993	70.97	95.71	91.43	0.000

Host immune cells react differently to different infectious agents in response to infections. A variety of immune mediators are produced in response to viral-cell interactions to combat the invasive virus (28). While a successful immune response is necessary to manage and eradicate viral infection, an overly strong or protracted response may lead to immunopathogenesis. Overproduction of inflammatory mediators plays in the causing of organ failure and immunopathology (29).

Host cells release a wide family of cytokines during SARS-CoV-2 infection to mediate innate immune responses and start inflammatory responses (30). A hyper-inflammatory state known as a "cytokine storm" may occur in severe COVID19 patients, even though the majority of patients show no symptoms or mild signs. During this time, the immune system produces an excessive amount of inflammatory cytokines and chemokines, such as IL-6, IL-10, IL-18, TNF-α, CCL2, CCL20, and CXCL10 which can lead to "acute respiratory distress, pulmonary edema, and multiorgan failure" (31, 32, 33).

The current investigation examined IL-18 serum concentration in three cohorts of Covd19 patients to forecast the role of this cytokine in the illness. We discovered that serum IL18 levels are significantly elevated and positively correlated with the severity of COVID-19 in patients. The Erbil research is the first to look into IL-18 in patients with COVD19. Our results are in

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line with earlier research showing elevated levels of inflammatory cytokines in COVD19 patients with more severe illness, including IL-16, IL-1α, IL-10, IL-18, and TNF-α (34, 35).

Our study's results on Covid-19 patients' higher levels of IL-18 were in line with other research that had been published. In their investigation, Chen et al. discovered that "IL-1β, IL-1Ra, IL-10, IL-6, IL-18, and TNF-α" were much more in severe COVID-19 patients than in healthy controls. Among the measured factors, IL-18 levels demonstrated the highest discriminative ability for severe COVD19, with an area under the curve (AUC) of 0.948. At a cut-off value of 190.5 pg/mL, IL-18 levels exhibited a sensitivity of 91.3%, specificity of95.8%, and accuracy of91.5%. This finding was statistically significant ($P < 0.005$) and suggests that IL-18 levels can effectively distinguish severe COVID-19 cases (36, 37).

Comparing the results of this study to those of a previous study by Kalinina et al., which involved 73 patients with COVID-19 (41 moderate and 32 severe cases) and 11 as the control group, it was found that 22 out of 47 cytokines had height levels of proinflammatory cytokines, in the COVID-19 group when compared to controls who were healthy (38).

A variety of inflammatory events can cause the release of IL-18, a cytokine that is part of the pro-inflammatory IL-1 family. It contributes to both adaptive and innate immune responses (39). Pro-IL-18, its inactive precursor, must be processed by caspase-1 to become an active cytokine. Monocytes and macrophages primarily generate IL-1β and IL-18 in response to pathogenic stimuli, such as viruses (40). Their stimulation requires the activation of inflammasomes, specifically the NOD and NLRP3inflammasomes. Viral components and cytosolic danger signals can trigger the inflammasome. As a result, pro-caspase-1 auto-cleaves into pro-IL-18, 1β, and the pyroptotic component gasderm in D (41).

Prior research has demonstrated that SARS-CoV, through its structural components ORF8b and E protein, which form an ion channel, may activate the NLRP3 inflammasome and cause human macrophages to produce IL-18 (42). Numerous investigations examined viral infections, metabolic disorders, and inflammatory conditions in which IL18 plays a significant impact in the host response (43).

Interleukin 18gene at position 607 C/A (IL-18-607)

The genotype and allele frequencies of IL-18 at the -607C/A location (rs 1946518) in Covid-19 patients and controls were examined in this study. Genotype CC, CA, and AA were represented by the two alleles (C and A) found at IL-18-607. Hardy-Weinberg equilibrium (HWE) showed that the genotype frequencies in the moderate and severe Covid-19 group patients differed significantly ($P = 0.000$) from the expected frequencies, whereas the genotype frequencies in the control and mild groups did not agree with HWE ($P = 0.222$). While the predicted rates were 42.25%, 27.18%, and 37.72%, respectively, heterozygous CA was found in the mild, moderate, and severe groups at a frequency of 58.57%, 78.57%, and 71.42%, respectively (Table 3).

Table 3: Observed numbers and Hardy-Weinberg (H-W) equilibrium of IL-18 genotypes and alleles in COVID-19 patients and control.

Categories		IL-18 -607C/A (re 194518)					
		Genotypes			HWE p.value	Alleles	
		CC	CA	AA		C	A
Mild	Observed	25(31.75%)	41(58.57%)	4(5.71%)	0.055	91(65%)	49((35%)
	Expected	29.58(42.25%)	31.85(45.5%)	8.57(12.24%)		Not estimated	
Moderate	Observed	9(12.85%)	55(78.57%)	6(8.57%)	0.000	73(52.14%)	67(47.85%)
	Expected	19.03(27.18%)	34.94(49.91%)	16.03(22.9%)		Not estimated	
Severe	Observed	18(25.71%)	50(71.42%)	2(2.85%)	0.000	86(61.42%)	54(38.57%)
	Expected	26.41(37.72%)	33.17(47.38%)	19.41(27.72%)		Not estimated	
Healthy control	Observed	32(45.71%)	35(50%)	3(4.28%)	0.222	99(70.71%)	41(29.28%)
	Expected	35(50%)	28.99(41.41%)	6(8.57%)		Not estimated	

Comparing Covid-19 group patients with controls revealed that the IL-18 -607 CA genotype was significantly increased in moderate and severe group patients (78.57% and 71.42% vs. 50% in control; $P = 0.01$ in moderate and $P = 0.015$ in severe group). Such a difference in the mild group was associated with an RR of 3.67 and EF value of 0.57, while in the moderate group it was associated with RR of 2.50 and EF value of 0.42.

In the moderate and severe groups, the CC was significantly less common than in HC (12.85% and 25.71% vs. 45.71% in HCP = 0.000 in the moderate group and $P = 0.021$ in the severe group). However, the AA genotype frequency was high, non-significantly, in mild and moderate Covid-19 group patients than in HC. The C allele frequency was lower in Covid-19 patients than in HC, while the frequency of A allele was higher in mild, moderate, and severe group patients than in HC (35%, 47.85%, and 38.57%, respectively) vs. 29.88% in HC (Table 4). A strong protective effect gains Covid-19 was observed with the CCI genotype in the moderate group (RR = 0.18, 95% CI 0.08–0.40; $P = 0.000$) and the severe group (RR = 0.41, 95% CI 0.20–0.83; $P = 0.021$).

Table 4: Presents a statistical analysis of the correlation between Covid19 infection severity and IL-18 genotypes and alleles

Case Categories	IL-18-607C/A		Patients (n=70)	Healthy controls (n=70)	Relative Risk	Etiology or Preventive Fraction	Exact Fishers Probability	95% Confidence Intervals
Mild	Genotypes	CC	25(35.71%)	32(45.71%)	0.66	0.15	0.302	0.34 – 1.29
		CA	41(58.57%)	35(50%)	1.41	0.17	0.396	0.73 – 2.74
		AA	4(5.71%)	3(4.28%)	1.35	0.015	1.000	0.29 – 6.21
	Alleles	C	91(65%)	99(70.71%)	0.77	0.16	0.370	0.47 – 1.27
		A	49(35%)	41(29.28%)	1.30	0.081	0.370	0.79 – 2.15
Moderate	Genotypes	CC	9(12.85%)	32(45.71%)	0.18	0.37	0.000	0.08 – 0.40
		CA	55(78.57%)	35(50%)	3.67	0.57	0.001	1.67 – 7.63
		AA	6(8.57%)	3(4.28%)	2.09	0.045	0.493	0.51 – 8.64
	Alleles	C	73(52.14%)	99(70.71%)	0.45	0.38	0.002	1.36 – 3.62
		A	67(47.85%)	41(29.28%)	2.22	0.26	0.002	1.36 – 3.62
Severe	Genotypes	CC	18(25.71%)	32(45.71%)	0.41	0.26	0.021	0.20 – 0.83
		CA	50(71.42%)	35(50%)	2.50	0.42	0.015	1.25 – 5.00
		AA	2(2.25%)	3(4.28%)	0.66	0.015	1.000	0.11 – 4.00
	Alleles	C	86(61.42%)	99(70.71%)	0.66	0.24	0.130	0.40 – 1.08
		A	54(38.57%)	41(29.28%)	1.52	0.13	0.130	0.92 – 2.49

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Impact of Genotypes on IL-18 Serum Levels

The CC, CA, and AA genotypes are recorded approximated means of IL-18 levels in the Covid-19 group patients or healthy control; the genotype CC recorded the highest level of IL-18 in severe Covid-19 patients, then moderate and mild (152.97 ± 20.19 pg/ml, 85.47 ± 2.43 pg/ml and 47.33 ± 18.7 pg/ml, respectively), while such a genotype was observed with the lowest (33.27 ± 12.2 pg/ml) in healthy control, and the deference was significant (P = 0.000). The genotypes AA and CA were observed to have the highest IL-18 levels in severe patients (147.11 ± 2.71 pg/ml and 137.15 ± 27.17 pg/ml, respectively) (Figure 5).

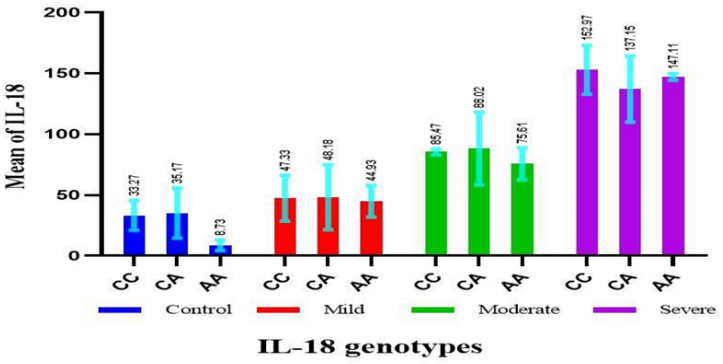

Figure 5: Serum level of IL-18 in IL-18-607 genotypes of Covid-19 group patients and healthy controls.

Understanding the processes behind the pathophysiology of illnesses can be gained through genetic investigations. It has been demonstrated that some genetic variants directly affect cytokine expression, which in turn controls the host's immunological response and may have an impact on the course of the illness (10,44).

It has been shown that variationsin the IL-18 gene are significant in the genesis of various disorders. However, the correlation between IL-18 variation and Covid-19 pathogenesis has only been the subject of a very small number of studies (of which we are aware of just one). This study is the first to examine the relationship between the susceptibility and severity of Covid-19 infection and the-607 C/A SNP of the IL18 gene promotor regionin the Iraqi-Kurdish population. The primary findings of this investigation showed that homozygous for strongly displayed CC was protected against Covid-19 severity, but heterozygous CA showed an elevated risk for Covid-19 severity.

We discovered substantial difference in the allelic and genotypic distributions between intermediate Covid-19 patients and HC in our investigation of the IL18-607 C/A SNP, but no differences between mild and HC in these distributions. Only the CC and CA genotypes differed significantly from HC in individuals in the severe category. Numerous infectious illnesses, including hepatitis B and C virus, pulmonary TB, type 1 diabetes, rheumatoid arthritis, and numerous malignancies, have been linked to an increased risk associated with the A-allele/AA-genotype (45, 46). Additionally, in line with our findings, the mutant allele/genotype of IL18-607 CA is linked to more dire consequences in various infectious illnesses, such as increased TB, HIV, SARS-CoV, and bacterial infections following liver transplantation (47, 48).

It has been revealed that SNP in the promoter region of IL-18, which affect the transcriptional factor binding capacity of the gene, are essential in regulating the expression of the IL-18 gene. The IL-18 -607C/A polymorphisms are common SNP in the promoter region that alter transcription activity of the IL-18 gene, change its expression, and correlate with the production of IL-18 protein, leading to increased levels of IL-18 through disruption of the nuclear factor binding sites for histone H4 transcription factor and Camp-responsive element-binding protein, respectively (49, 50).

Our findings somewhat corroborate other research that found a substantial correlation between certain infectious disorders and the SNP located at the IL-18-607 location. The relationship between illnesses and this genetic variation has not been thoroughly studied. Among them, Sobti et al. (51) used sequence-specific PCR to examine the relationship between IL-18 promoter polymorphism −607C/A and HIV. They found that the AA genotype had a significantly lower risk of HIV-1 infection than the CA genotype, indicating that variation in IL-18 gene may be protective in the spread of HIV infection in the Indian population, according to a 2016 study by Tavares et al. (52). They also discovered that this polymorphism causes a decrease in IL-18 production as a result of a weak immune response against HPV, which raises the risk of infection.

The correlation between serum IL-18 and its SNPs (-607 C/A and -137 G/C) and chronic hepatitis C (CHC) was demonstrated in research conducted by Said et al. (53). Furthermore, compared to HC patients, CHC patients had considerably greater plasma IL-18 levels, according to the research. The cytomegalovirus (CMV) replication incidence may be greater in individuals with the IL-18 (607C/A and -137G/C) genotypes, according to Pérez-Flores et al. (54) results.

Our findings contradict those of earlier research; Hassuna et al. (55) discovered no discernible risk for tuberculosis (TB) related to either of the homozygous genotypes, AA or CC; on the other hand, the AC genotype was linked to a somewhat decreased risk for tuberculosis infection. In their investigation, Oliveira et al. (56) discovered that there are notable protective benefits of the IL-18 (-105G/A and -137C/G) genetic variations on the severity of Covid-19. Furthermore, they discovered that IL18-105 GA-associated age, BMI, heart conditions, type 2 diabetes, hypertension, and inflammation index may be utilized to forecast the onset of mild or severe illness in individuals.

Compared to other genotypes, the C-allele (-607 C) and CC genotype carriage has been linked to greater blood levels of IL-18 (Sakai et al., 2008; Shi et al., 2017; Hasan and Naif, 2017; Alves et al., 2018).

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Conclusion

The presented results promoted that most of the infected patients were female and old ages and revealed that elevated serum L-18levels are significantly correlated with the severity of Covid-19 and can be used as excellent biomarkers for predicting Covid-19 progression. Also, the results indicated that the heterozygous CA genotype at the607 position ofthe IL18 gene had a positive association in Covid-19 patients and may render susceptibility, while the homozygous CC genotype reduced significantly in Covid-19 patients indicating a negative association and offering resistance to Covid-19 infection.

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