The severity of COVID-19 infection correlation with IL-17 polymorphism

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Abstract

Single nucleotide polymorphisms (SNPs) in many studied genes have been related to the severity of COVID-19. This study was carried out to investigate whether the polymorphisms of two SNPs (rs763780 and rs2275913) of the gene polymorphisms for IL-17 are connected to the COVID-19 severity. The effect of these polymorphisms on the levels of IL-17 and the relationship between the level of IL-17 and the severity of COVID-19 were also investigated. RT-PCR was performed to detect SARS-COV-2. Blood samples were taken for analyses of IL-17 levels by ELISA test, and Genomic DNA was extracted for genotyping. Genotyping was performed using Real-Time –Polymerase Chain Reaction. The results indicated that the IL-17 level significantly increases in patients compared with control (healthy people), and there is a relationship between the severity of COVID-19 and IL-17 levels ($p<0.01$) between severity groups. Mean ± SE of IL-17 was 67.99 ±2.05 ng/L for the control group, 147.60 ±3.34 for mild or moderate, (218.15 ±6.27) for the severe group, and (283.97 ±5.59) for the critical group. Furthermore, there is no-significant Relationship between SNP80 and IL-17 level (ng/L) with different types of severity, and there is no-significant Relationship between SNP13 and IL-17 level (ng/L) with different types of severity ($p \geq 0.05$) both of them. The severity of COVID-19 and the prevalence of the AA genotype were shown to be significantly correlated. Nevertheless, rs2275913 A-allele carriers in the patients were shown to be at risk ($P = 0.021$) comparison with the control group, show increase risk in the severe group (p-value= 0.005, $p<0.05$) and critical group (p-value = 0.023, $p<0.05$) but no compact between the mild group, There is no relationship ($p> 0.05$) between the

prevalence of the GA genotype and the severity of COVID-19. Among C-allele carriers of rs 763780 at IL-17, a strong association between the frequency of the TC, CC genotype at and the severity of COVID-19 risk was discovered, (P value = 0.0001, p< 0.05) both of them show increase risk in all groups, mild or moderate group (p-value= 0.0001, p< 0.05) for both TC and CC genotype, severe group (P value = 0.0001, p< 0.05) TC genotype and (P value = 0.04, p< 0.05) CC genotype, critical group (P value = 0.0001, p< 0.05) for both TC and CC genotype. According to the current research, COVID-19 prognosis and severity were substantially correlated with IL-17 level and two IL-17 SNPs, rs2275913 and rs763780. It demonstrated that the two SNPs might be potential markers for predicting COVID-19 risk and development. There is an insignificant relationship between (SNP13 and SNP80) and IL-17 levels (ng/L) with different types of severity.

Keywords: Polymorphism, COVID-19, RT-PCR, SARS-CoV-2, Interleukin-17, HRM RT-PCR, SNP

1. Introduction

The 2019 Coronavirus Disease (COVID-19) is a coronavirus 2 (SARS-CoV-2) caused infectious disease pandemic that produces severe acute respiratory distress syndrome (ARDS), which originally surfaced in December 2019 in Wuhan, China; since then, it has expanded the globe (1). This 2019-nCoV is the third and most dangerous human pathogen following a zoonotic transmission epidemic of CoV, SARS-CoV (in 2003), and MERS-CoV (in 2012). Positive sense RNA was found, which affects birds and a variety of other creatures, including humans (2). Viral pneumonia and host inflammation characterize each of these diseases; as a result, pulmonary edema develops and a state resembling ARDS acute respiratory distress syndrome (3).

Coronaviruses are members of the Coronavirinae subfamily of the Coronaviridae family in the order Nidovirales. In humans and many other animals, it can induce respiratory, digestive, and neurological system problems. The Coronavirus particles are spherical, varying from 80 to 160 mm. The spike (S) protein is coated on the envelope's surface. The S proteins are membrane (M) and envelope (E). A helical nucleocapsid comprises genomic RNA and phosphorylated

nucleocapsid (N) protein and can be found inside the envelope (4). COVID-19 is a pleomorphic or spherical encased particle; single-stranded (positive-sense) RNA is associated with a nucleoprotein within a matrix protein-based capsid. The envelope is adorned with club-shaped glycoprotein projections. Several coronaviruses have the hem agglutinin-esterase protein (HE) (5). Coronaviruses are the RNA viruses with the largest genomes (26.4–31.7 kb) with levels of G+ C ranging (from 32 – 43%). A variety of small ORFs (spike, membrane, envelope, nucleocapsid, and ORF1ab) reside downstream of the nucleocapsid gene between many conserved genes and in different coronavirus lineages. The spike protein's unique N-terminal region is a defining feature of the viral genome. S, E, M, and N are the genes that code for the key structural proteins in all coronaviruses, and they exist in the 5′–3′ order (6). COVID-19 patients might present with a variety of symptoms. At least 26 of these symptoms were found even though far from a complete list of symptoms. Given that general terminology like neurological and dermatological symptoms indicate a spectrum of individual reactions, the necessity of stressing the vast number of presentations cannot be emphasized. This might lead to a rise in the number of symptoms and signs. Dyspnea, fever, cough, and headache are non-specific common SARS-CoV-2 symptoms. From asymptomatic patients to those dying of severe pneumonia. The infection's severity may differ; shortness of breath, fever, and cough were the initial symptoms of the condition. The list was expanded to include chills, headache, muscle discomfort, sore throat, and loss of taste or smell (neurological manifestations) by the US Centers for Disease Control and Prevention (7). There are three severity degrees in the 2019 Coronavirus Disease levels. Flu, for example, may occur in the first stage due to viral pneumonia and viral infection; patients may be admitted to the hospital or placed on a ventilator for a lengthy period. The second stage also distinguishes between pulmonary inflammation and coagulopathy, which can happen sequentially but overlap. The disease's last stage is fibrosis. In individuals who require mechanical ventilation, two respiratory phenotypes can be distinguished low and high elastance. The H-type has greater lung edema, resulting in larger lung weight and worse lung compliance (8). Due to the respiratory system's prominent involvement in suspected COVID-19 cases, a chest CT scan is strongly advised for initial screening and follow-up. X-rays of the chest have a limited diagnostic value in the early stages, even though CT scans can be obtained before the beginning of symptoms. Furthermore, when an initial false-negative result utilizing real-time reverse-transcriptase polymerase chain reaction (RT-PCR) was obtained, CT

results were diagnostic in a few cases (9). One of the key challenges for facilitating public health initiatives is the reliability of biomarker testing. Real-time PCR is commonly utilized to identify causal viruses in respiratory secretions in acute respiratory infections (10). Even though coordinated cytokine stimulation is important for host immune response, impairment of production regulation is linked to immunopathology, which can cause the onset of cytokine storm (11). Cytokines associated with COVID-19 include pro- and anti-inflammatory interleukins (ILs), chemokines, and interferons (12). IL-17 is produced by T-helper 17 (Th17) cells; its signaling is associated with immune functions of barrier epithelial tissues and host defense against extracellular bacterial and fungal infections (13). IL-17 includes unique structures composed of IL-17A and IL-17F (17); it is an inducer of antimicrobial proteins, various chemokines, acute-phase response mediators, and inflammatory functions (14). Recent studies have found increased inflammatory responses in COVID-19 patients because of IL-17 overproduction (Amatya *et al*., 2017; Orlov *et al*., 2020). Genetic polymorphisms implicated in disease-origin research also contribute to preventing infection transmission and developing potentially effective therapies. Single nucleotide polymorphisms (SNPs) are a common type of such polymorphisms that are recognized to play a crucial role in the pathogenicity of a microbial agent, disease immunity, susceptibility, and severity of diseases (AL-Suhail and Ali, 2021). A growing body of research suggests that COVID-19's severe symptoms might be caused by genetic differences in genes linked to immunological diseases, infections, and/or cytokine storms (15).

Additionally, ARDS patients with genetic variants that resulted in decreased IL-17 production had a higher 30-day survival rate, according to research on IL-17 gene polymorphisms in these patients; however, The lower survival rate was linked to a polymorphism that increased the production of IL-17 (16). In order to explain the clinical course of COVID-19 infection and the survival and/or death due to COVID-19 infection, we postulated that SNPs in the genes encoding for IL-17A and IL-17F might be involved. On chromosome 6p12, the rs2275913 is located 2 KB upstream of the IL17A gene. The IL-17A gene's promoter region contains the rs2275913 gene, the A allele of this gene is linked to the gene's promoter activity, and the rs763780 is located in exon 1 of the IL17f gene, and the C allele of this gene is linked with increasing severity of the disease.

2. Materials and Methods

2.1. Samples collection

This study has been achieved at Dar al-salam Hospital, Alyarmok Teaching Hospital, and Alshefaa hospital from (November 2021 to January 2022). The Ethics Committee approved this study, Department of Biology, College of Science, University of Baghdad (ref no. CSEC/0122/0020). The study includes 100 Iraqi patients who tested positive for SARS-CoV nasopharyngeal swab samples through real-time reverse transcriptase PCR and were admitted to various isolation wards. Patients with vomiting, diarrhea, weakness, systemic diseases, and lower oxygen saturation were hospitalized. Blood Specimens were collected on the second day of admission to the hospital by taking venous blood from each patient 5 ml divided into 2 ml of whole blood were placed into EDTA heparinized tube to test Genomic DNA isolation Kits used in the study included: EasyPure® Genomic DNA Kit TransGen, TransStart® Top Green qPCR Super Mix (biotech, China) and Eva Green® for HRM (Wizbio, Korea) and 3 ml of whole blood in the non-heparinized tube (gel tube) to test human IL-17 level by ELISA KIT.

2.2. Detection of SARS- COV- 2 infections by RT-PCR

SARS-COV-2 RNA was detected in the nasopharyngeal swabs using Real-time Polymerase Chain Reaction (PCR) with the PCR Rotor-Gene, Zybio SARS-COV-2 Assay kit (Zybio company, China).

2.3. Real-time PCR detection protocol

This product qualitatively detected the RNA of SARS-CoV-2 in the specimen by measuring the change of fluorescence signal intensity during RT-PCR amplification with specific primers and probes against the conserved sequences of N, RdRP, and S genes).

2.4. RNA extraction

The RNA was extracted using the Viral Nucleic Acid Extraction Kit (Zybio company, China) and performed according to the manufacturer's instructions.

2.5. Sample preparing

Respectively ten microliters of nucleic acid sample SARS-CoV-2 Negative Control and SARS-CoV-2 Positive Control were added into each PCR reaction tube with filter tips and were

covered, then transferred to the amplification detection zone after quick centrifugation to avoid producing bubbles in tubes.

2.6. PCR amplification

PCR reaction tubes were put into a fluorescent PCR instrument (ABI 7500, USA) following the protocol listed in table 1.

Table 1. RT-PCR protocol used to detect SARS-CoV-2

Steps	Temperature	Time	cycle
UNG reaction	37°C	1 min	1
Reverse transcription	50°C	5 min	1
Initial denaturation	95°C	2 min	1
Denaturation	95°C	5 sec	45 with amplification and fluorescence detection step
Amplification and fluorescence detection	60°C	30 sec	

2.7. Human genomic DNA isolation

The protocol supplied by Transgenbiotech Company was used for DNA isolation (***EasyPure***® **Genomic DNA Kit)** was followed to extract the genomic DNA. The purified DNA was stored at

-20 °C. After that, the extracted DNA was run in agarose gel electrophoresis to confirm the presence and integrity (Russell and Sambrook, 2001).

2.8. HRM Real-Time PCR

To detect genetic variation in the IL-17 gene. Two SNPs (rs763780, rs2275913) were selected to investigate their association with the positive SARS-CoV 2 patient. SNPs detection was achieved by using HRM real-time PCR.

2.9. Primers

Primers used in this study were designed according to their reference sequence in the National Centre for Biotechnology Information (NCBI) database. The Primer 3 plus, V4, and University Code of Student Conduct (UCSC) programs were used to design the primers and synthesized by Alpha DNA, SENC (Montreal)) and stored lyophilized. The sequences of primers used in the experiments in this study are shown in table 2.

Table 2. Designed primers used in the study

Primer	Sequence (5′→3′ direction)
IL-17A (HRM)	
Forward	TCTTTAGGAACATGAATTTCTGC
Reverse	CTCCTTCTGTGGTCACTTACG
IL-17F (HRM)	
Forward	GCATTTCTACAGCTTCTTCAGC
Reverse	AAGGTGCTGGTGACTGTTGG

2.10. Primer sequence matching

Detecting Primer prepared gene (*IL-17*) was assessed for SNPs which included (rs 763780 and rs2275913) primers sequences were designed according to their reference sequence (rs) in the database of NCBI (National Center for Biotechnology Information).

The Primer 3plus, V4, and University Code of Student Conduct (UCSC) programs were used to design the primers and synthesized by Alpha DNA, SENC (Montreal) and stored lyophilized. The sequences of primers used in the experiments in this study are shown in tables (3-8). Primer sequences were matched by the bioinformatics programs NCBI, table Figure (3-2).

2.11. IL-17 assay

The level of (IL-17) was assessed in the sera of patients and controls using ELISA (enzyme-linked immunosorbent assay) technique. by using an ELISA kit produced by (Sunlong Biotech Company / China).

2.12. Statistical analysis

The Statistical Analysis System- SAS (2012) application was employed to determine how various factors affected the research parameters (17). The T-test and Least Significant Difference (LSD) test (Analysis of Variation, ANOVA) were used to compare between means significantly. In this study, a meaningful comparison between percentage (0.05 and 0.01 likelihood) was made using the Chi-square test (17).

Hardy-Weinberg equilibrium was calculated using a web tool (18).

3. Results and Discussion

In this study, COVID-19 infection in 100 Iraqi patients was classified depending on the severity of their infection (mild or moderate, severe and critical) involving males and females between the ages of 20 and 92, and taking place between November 2021 and March 2022 Based on a nasopharyngeal swab, SARS-CoV-2 was identified in all samples using real-time PCR and 70 individuals control group (healthy people) There was a statistical difference (high significant different) (p <0.01) of IL-17 levels between the patients and the control group. As seen in table 3, Control group (67.99 ±2.05 ng/L), Mild or Moderate (147.60 ±3.34 ng/L), Severe (218.15 ±6.27 ng/L), Critical (283.97 ±5.59 ng/L).

٢٠٦ Table 3. Comparison between different Types of severity in IL-17

Type of severity	Mean ± SE of IL-17 (ng/L)
Control	67.99 ±2.05 d
Mild or Moderate	147.60 ±3.34 c
Sever	218.15 ±6.27 b
Critical	283.97 ±5.59 a
LSD value	11.476 **
P-value	0.0001
This means that having the different letters in the same column differed significantly. ** (P≤0.01).	

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٢٠٨ Pulmonary inflammation due to COVID-19 is attributed to the release of specific pro-
٢٠٩ inflammatory cytokines, such as IL-17, leading to a cytokine storm (19, 20). Interestingly, Girija,
٢١٠ Shankar (21) described an increase in the numbers of Th17 cells in the peripheral blood of
٢١١ patients presented with SARS-CoV-2. This finding strongly suggests an amplifier role for IL-
٢١٢ 17A in the inflammatory response, since it triggers the production of other pro-inflammatory
٢١٣ cytokines, i.e., IL-1, IL-6, and TNF-α. Moreover, AL-Suhail and Ali (2021) observed a reduction
٢١٤ in the population of lymphocytic subsets, together with an elevation in Th17 cell number and
٢١٥ cytokines released by Th17 in these patients, consolidating the notion of a severe inflammation-
٢١٦ derived immune response. Raised responses of Th17 or improved pathways of IL-17 were
٢١٧ noticed in MERS-CoV and SARS-CoV patients as well (22). The death of lymphocytes might
٢١٨ happen if inflammatory cytokines are released out of control. Primary data approved that
٢١٩ lymphocyte deficiency could be induced by specific pro-inflammatory cytokines such as TNFα,
٢٢٠ IL-6, and others (23). Overall, the response of Th17 participates in the onset of the cytokine
٢٢١ storm in pulmonary viral infection, including SARS-CoV-2, leading to tissue damage and
٢٢٢ probable promoting pulmonary edema; consequently, targeting the Th17 pathway may be
٢٢٣ beneficial to patients presented with a Th17-dominant immune pattern (24).

A robust inflammatory response that results in a broad array of inflammatory mediators is a vital component of COVID-19 pathogenesis; one of them is interleukins. Excessive pro-inflammatory cytokine production during illness progression causes a cytokine storm, encouraging the severe development of acute organ damage (25). The fundamental mechanism is that SARS-CoV2 may quickly activate pathogenic T helper cell type 1 (Th1) cells to generate pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-10, and interleukin-17 (IL-17) (26). Interestingly, Ye, Wang (27) reported that the severity of COVID-19 infection is associated with increased concentrations of IL-10 and IL-6 alongside a decline in the cell number of $CD4^+$ T and $CD8^+$ T cells. Multiple studies show that those with severe COVID-19 infections had more significant quantities of IL2, IL6, IL 17, and IL 10 than those with mild and moderate COVID-19 infections.

What's more, intensive care unit hospitalized COVID-19 patients developed increased levels of IL-17 in comparison to the control patients (28). Therefore, it is necessary to grasp these essential inflammatory cytokines to comprehend the increased mortality in extreme instances caused by a cytokine storm (29).

There is no-significant Relationship between SNP80 and IL-17 level (ng/L) with different types of severity and there is no-significant Relationship between SNP13 and IL-17 level (ng/L) with different types of severity ($p \geq 0.05$) both of them. The prevalence of the AA genotype at rs2275913 and the severity of COVID-19 were significantly correlated (P value = 0.021), while there was no significant correlation between the frequency of GA genotype and severity of COVID-19 ($p > 0.05$). The frequency of the TC, CC genotype at rs 763780 and the severity of COVID-19 was significantly correlated (P value = 0.0001, $p< 0.05$) both of them, while no significant correlation between the frequency of TT genotype and severity of COVID-19 ($p> 0.05$) As seen in the tables (4) and (5).

Table 4. Relationship between SNP80 and IL-17 level (ng/L) with different types of severity

	Type of severity

Genotype / SNP80	Control	Mild or Moderate	Sever	Critical
TT	63.51 ±2.84	132.54 ±9.86	237.02 ±18.35	303.82 ±20.41
TC	79.38 ±0.98	151.89 ±6.29	213.04 ±7.25	283.93 ±5.08
CC	68.16 ±0.00	146.40 ±3.32	237.24 ±5.80	274.13 ±12.41
LSD value	17.03 NS	39.74 NS	42.91 NS	48.031 NS
P-value	0.079	0.337	0.511	0.381
NS: Non-Significantly.				

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٢٥٧ Table 5. Relationship between SNP13 and IL-17 level (ng/L) with different types of severity

Genotype / SNP13	Type of severity			
	Control	Mild or Moderate	Sever	Critical
GG	62.09 ±3.08	148.93 ±6.19	217.51 ±13.64	284.56 ±9.24
GA	77.46 ±1.78	145.08	222.73	293.64

		±2.98	±8.85	±5.57
AA	61.56 ±0.00	155.16 ±0.00	211.01 ±10.48	266.21 ±15.48
LSD value	18.57 NS	42.196 NS	61.02 NS	49.82 NS
P-value	0.081	0.659	0.703	0.423
NS: Non-Significantly.				

The prevalence of the AA genotype at rs2275913 and the severity of COVID-19 were significantly correlated (P value = 0.021, $p < 0.05$) and Odd ratio= 3.9 more risk factor from other, while no significant correlation between the frequency of GA genotype and severity of COVID-19 ($p > 0.05 = 0.3$), risk was found among A-allele carriers of rs2275913 at IL-17, (P value = 0.01,$p < 0.05$) and Odd ratio= 1.8, control group and Patients Group, as seen in table (6).

Table 6. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs13 between Patient group and Healthy group

------ polymorphism rs13	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=70)	Patients Group (n=100)		
Codominant				
GG	**55.7 % (n=39)**	**٤٢,٠ % (n=٤٢)**	**---**	**1.00 (Reference)**
GA	**38.5 % (n=27)**	**41.0 % (n=٤١)**	**0.3**	**1.4 (0.7-2.7)**
AA	**5.8 % (n=4)**	**١٧,٠ % (n=17)**	**0.021**	**3.9 (1.2-12.7)**
Dominant				
GG	**55.7 % (n=39)**	**٤٢,٠ % (n=٤٢)**	**---**	**1.00 (Reference)**
GA+AA	**٤4.3 % (n=31)**	**58.0 % (n=58)**	**0.079**	**1.7 (0.9-3.2)**
Recessive				
GG+GA	**94.2 % (n=66)**	**83.0 % (n=83)**	**---**	**1.00 (Reference)**
AA	**5.8 % (n=4)**	**١٧,٠ % (n=17)**	**0.03**	**3.3 (1.0-10.5)**
Allele				
G	**75.0 % (n=105)**	**62.5 % (n=125)**	**---**	**1.00 (Reference)**
A	**25.0 % (n=35)**	**37.5 % (n=75)**	**0.01**	**1.8 (1.1-2.9)**

There was no significant correlation with the mild group for both genotypes GA, AA p-value 0.5,0.8 respectively (p-value > 0.05) as seen in the table 7.

Table 7. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs13 between Patient Mild group and Healthy group

------ polymorphism rs13	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=70)	Patients (Mild) Group (n=34)		
Codominant				
GG	55.7 % (n=39)	٥٠,٠ % (n=١٧)	---	1.00 (Reference)
GA	38.5 % (n=27)	4٤.١ % (n=١٥)	٠,٥	1.2 (0.5-2.9)
AA	5.8 % (n=4)	٥,٩ % (n=2)	0.8	1.1 (0.1-6.8)
Dominant				
GG	55.7 % (n=39)	٥٠,٠ % (n=١٧)	---	1.00 (Reference)
GA+AA	٤4.3 % (n=31)	50.0 % (n=17)	0.5	1.2 (0.5-2.8)
Recessive				
GG+GA	94.2 % (n=66)	94.1 % (n=32)	---	1.00 (Reference)
AA	5.8 % (n=4)	٥,٩ % (n=2)	0.9	1.0 (0.1-5.9)
Allele				
G	75.0 % (n=105)	69.1 % (n=47)	---	1.00 (Reference)
A	25.0 % (n=35)	30.9 % (n=19)	0.5	1.2 (0.6-2.3)

The frequency of the AA genotype and the severity of COVID-19 showed a significant correlation, showing an increased risk in the severe group (p-value= 0.005, p<0.05) and Odd ratio= 7.0 more risk factor from other while no significant correlation between the frequency of GA genotype and severity of COVID-19 (p value= 0.19, p >0.05) and Control group, risk was found among A-allele carriers of rs2275913 at IL-17, (P value = 0.003,p< 0.05) and Odd ratio= 2.5 as seen in table 8.

Table 8. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs13 between Patient Sever group and Healthy group

------ polymorphism rs13	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=70)	Patients (Sever) Group (n=33)		
Codominant				
GG	55.7 % (n=39)	٣٣,٣ % (n=١١)	---	1.00 (Reference)
GA	38.5 % (n=27)	4٢.٤ % (n=١٤)	0.19	1.8 (0.7-4.6)
AA	5.8 % (n=4)	٢٤,٣ % (n=8)	0.005	7.0 (0.1-2.8)
Dominant				
GG	55.7 % (n=39)	٣٣,٣ % (n=١١)	---	1.00 (Reference)
GA+AA	٤4.3 % (n=31)	67.7 % (n=22)	0.03	2.5 (1.0-5.9)
Recessive				
GG+GA	94.2 % (n=66)	76.7 % (n=25)	---	1.00 (Reference)
AA	5.8 % (n=4)	٢٤,٣ % (n=8)	0.011	5.2 (1.4-19.0)
Allele				
G	75.0 % (n=105)	54.5 % (n=36)	---	1.00 (Reference)
A	25.0 % (n=35)	45.5 % (n=30)	0.003	2.5 (1.3-4.6)

The frequency of the AA genotype and the severity of the COVID-19 critical group were significantly correlated, showing increased risk in the critical group. (p-value= 0.023 p<0.05) and Odd ratio= 4.8 more risk factor from other, risk was found among A-allele carriers of rs2275913 at IL-17, (P value = 0.003,p< 0.05) and Odd ratio= 2.5 as seen in table 9.

Table 9. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs13 between Critical Patient group and Healthy group

------ polymorphism rs13	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=70)	Patients (Crit.) Group (n=33)		
Codominant				
GG	55.7 % (n=39)	٤٢,٤ % (n=١٤)	---	1.00 (Reference)
GA	38.5 % (n=27)	36.3 % (n=١٢)	0.2	1.2 (0.4-3.0)
AA	5.8 % (n=4)	٢١,٣ % (n=7)	0.023	4.8 (0.1-1.9)
Dominant				
GG	55.7 % (n=39)	٤٢,٤ % (n=١٤)	---	1.00 (Reference)
GA+AA	٤4.3 % (n=31)	57.6 % (n=19)	0.2	1.7 (0.7-3.9)
Recessive				
GG+GA	94.2 % (n=66)	78.7 % (n=26)	---	1.00 (Reference)
AA	5.8 % (n=4)	٢١,٣ % (n=7)	0.02	4.4 (1.1-16.4)
Allele				
G	75.0 % (n=105)	54.5 % (n=36)	---	1.00 (Reference)
A	25.0 % (n=35)	45.5 % (n=30)	0.003	2.5 (1.3-4.6)

The frequency of the TC and CC genotype at rs 763780 and the severity of COVID-19 had a significant correlation (P value = 0.0001,p< 0.05) both of them and Odd ratio= 11.4, 17.1 respectively more risk factor from other, risk was found among C-allele carriers of rs763780 at IL-17, (P value = 0.0001,p< 0.05) and Odd ratio= 5.0 as seen in table 10.

Table 10. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs80 between Patient group and Healthy group

polymorphism rs80	Frequencies (%)		P value	Odd ratio (95% CI)
	Patients Group (n=)	Control group (n=)		
Codominant				
TT	13.0 % (n=13)	65.7 % (n=46)	---	1.00 (Reference)
TC	٥٨,٠ % (n=58)	25.7 % (n=18)	0.0001	11.4 (0.5-25.6)
CC	٢٩.0 % (n=29)	8.6 % (n=6)	0.0001	17.1 (0.5-5.0)
Dominant				
TT	13.0 % (n=13)	65.7 % (n=46)	---	1.00 (Reference)
TC+CC	٨٧,٠ % (n=87)	34.3 % (n=24)	0.0001	12.8 (0.5-2.7)
Recessive				
TT+TC	71.0 % (n=71)	٩١,٤ % (n=٦٤)	---	1.00 (Reference)
CC	٢٩.0 % (n=29)	8.6 % (n=6)	0.0001	4.3 (1.6-11.1)
Allele				
T	42.0 % (n=84)	91.7 % (n=110)	---	1.00 (Reference)
C	58.0 % (n=116)	8.3 % (n=30)	0.0001	5.0 (3.0-8.2)

The frequency of the TC and CC genotype at rs763780 was significantly correlated with the COVID-19 severity (P value = 0.0001,p< 0.05) and Odd ratio= 17.7, 56.8 respectively more risk factor from other both of them, risk was found among C-allele carriers of rs763780 at IL-17 in mild group, (P value = 0.0001,p< 0.05) and Odd ratio = 8.2 as seen in table 11.

Table 11. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg equilibrium law of ----- gene polymorphism rs٨٠ between Patient Mild group and Healthy group

polymorphism rs80	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=)	Patients Mild Group (n=)		
Codominant				
TT	65.7 % (n=46)	٨,٨ % (n=٣)	---	1.00 (Reference)
TC	25.7 % (n=18)	٤٤.١ % (n=١٥)	0.0001	17.7 (0.4-6.8)
CC	8.6 % (n=6)	٤٧,١ % (n=16)	0.0001	56.8 (0.1-25.2)
Dominant				
TT	65.7 % (n=46)	٨,٨ % (n=٣)	---	1.00 (Reference)
TC+CC	34.3 % (n=24)	91.2 % (n=31)	0.0001	19.8 (0.5-7.1)
Recessive				
TT+TC	٩١,٤ % (n=٦٤)	62.9 % (n=18)	---	1.00 (Reference)
CC	8.6 % (n=6)	٤٧,١ % (n=16)	0.0001	9.4 (0.3-2.7)
Allele				
T	42.0 % (n=84)	30.0 % (n=21)	---	1.00 (Reference)
C	58.0 % (n=116)	70.0 % (n=47)	0.0001	8.2 (0.4-1.5)

٣١٩ TC and CC genotypes were shown to be significantly correlated among C-allele carriers of the rs
٣٢٠ 763780 at IL-17, show increase risk in sever group (P value = 0.0001, p< 0.05) TC genotype and
٣٢١ (P value = 0.04, p< 0.05) CC genotype, (p> 0.05) Odd ratio= 16.6, 5.7 respectively more risk
٣٢٢ factor from other both of them, risk was found among C-allele in mild group, (P value =
٣٢٣ 0.0001,p< 0.05) and Odd ratio= 3.4 as seen in table 12.

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٣٢٦ Table 12. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg
٣٢٧ equilibrium law of gene polymorphism rs٨٠ between Patient Sever group and Healthy group

٣٢٨

polymorphism rs80	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=)	Patients Sever Group (n=33)		
Codominant				
TT	65.7 % (n=46)	١٢,١ % (n=٤)	---	1.00 (Reference)
TC	25.7 % (n=18)	٧٨,٨ % (n=٢٦)	0.0001	16.6 (0.5-5.4)
CC	8.6 % (n=6)	٩,١ % (n=3)	0.04	5.7 (0.1-3.2)
Dominant				
TT	65.7 % (n=46)	١٢,١ % (n=٤)	---	1.00 (Reference)
TC+CC	34.3 % (n=24)	87.9 % (n=29)	0.0001	13.8 (0.4-4.4)
Recessive				
TT+TC	٩١,٤ % (n=٦٤)	90.9 % (n=30)	---	1.00 (Reference)
CC	8.6 % (n=6)	٩,١ % (n=3)	0.9	1.0 (0.2-4.5)
Allele				
T	42.0 % (n=110)	51.5 % (n=34)	---	1.00 (Reference)
C	58.0 % (n=30)	48.5 % (n=32)	0.0001	3.4 (1.8-6.4)

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٣٣٠ Among C-allele carriers of rs 763780 at IL-17, a strong link between the frequency of the TC
٣٣١ and CC genotypes and the severity of COVID-19 risk was discovered. This increased risk was
٣٣٢ seen in both the TC and CC critical groups (P value = 0.0001, p< 0.05), Odd ratio = 9.2, and 15.3
٣٣٣ more risk factors than others, as seen in the table 13.

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٣٣٥ Table 13. Comparison of the genotype and Allele frequencies detected by Hardy-Weinberg
٣٣٦ equilibrium law of ----- gene polymorphism rs٨٠ between Patient Crit. group and Healthy group

٣٣٧

polymorphism rs80	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=)	Patients Crit. Group (n=33)		
Codominant				
TT	65.7 % (n=46)	١٥,٢ % (n=٥)	---	1.00 (Reference)
TC	25.7 % (n=18)	٥٤,٥ % (n=١٨)	0.0001	9.2 (0.2-2.8)
CC	8.6 % (n=6)	٣٠,٣ % (n=10)	0.0001	15.3 (0.3-6.0)
Dominant				
TT	65.7 % (n=46)	١٥,٢ % (n=٥)	---	1.00 (Reference)
TC+CC	34.3 % (n=24)	84.8 % (n=28)	٠,٠٠٠١	10.7 (0.3-3.1)
Recessive				
TT+TC	٩١,٤ % (n=٦٤)	69.7 % (n=23)	---	1.00 (Reference)
CC	8.6 % (n=6)	٣٠,٣ % (n=10)	٠,٠٠٧	4.6 (1.5-14.1)
Allele				
T	42.0 % (n=110)	42.5 % (n=28)	---	1.00 (Reference)
C	58.0 % (n=30)	57.5 % (n=38)	0.0001	4.9 (2.6-9.3)

This study is the first to document a link between IL-17 (rs763780, rs2275913) polymorphisms and ARDS susceptibility and COVID-19 prognosis. The current research found a link between two functional polymorphisms of IL-17, rs2275913 and rs763780, and the risk of ARDS and COVID-19 prognosis. These findings suggest that these two genetic variations can serve as promising indicators for COVID-19 risk and prognosis prediction.

The clinical syndrome known as ARDS has several etiological agents and complex pathogenesis (30). Although the exact pathologic pathways are still unclear, it is well known that ARDS is an inflammatory disease and that immune dysregulation disorder may play a substantial role in the development of inflammation (31). The pro-inflammatory cytokine IL-17 has received much attention. The IL-17 family has several expression forms, among them IL-17A-F, among

which IL-17A can broadly trigger the inflammatory response and is important in a number of inflammatory disorders—indicating that IL-17 disruption may contribute to the early stages of ARDS development and the increase in COVID-19 severity (32). Additionally, increased levels of IL-17A in the blood and lungs have been linked to ARDS-related organ failure, an increased alveolar neutrophil proportion, and alveolar permeability (33). IL-17A polymorphisms, such as rs2275913 and rs763780, have influenced how many inflammatory disorders begin. The *IL-17A* gene, linked to ARDS risk and outcome, has the variant rs2275913. People with the wild G-allele of rs2275913 had ARDS susceptibility that was at least as low as those with the mutant A-allele, suggesting that the former mutant A-allele is the pathogenic one (AA genotype) and the later wild G-allele is the protective one (GG, GA genotype). The IL-17F gene contains the variant rs763780. The risk and prognosis of ARDS were affected by the SNP of rs763780 ($P>0.05$) (34). Individuals with the wild T-allele, on the other hand, had decreased ARDS susceptibility, demonstrating that the former mutant C-allele functioned as the pathogenic one while the latter wild T-allele played the protective role. The mutant C-allele carriers are at increased risk of having the ARDS (TC, CC) genotype (TT genotype). Additionally, the research presented here showed that IL-17 SNPs can modify IL-17 serum levels. Results showed no correlation between SNPs and blood levels of IL-17, indicating that rs2275913 and rs763780 were active polymorphisms with no ability to affect IL-17 production. Studies were also needed to explain the molecular processes that led to the connection between SNPs and IL-17 expression and the development of ARDS susceptibility and prognosis.

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