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Original Article

Association of IL-17F Gene Polymorphism and Its Serum Level with SARS-CoV-2 Infection

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Abstract

OBJECTIVE: Although multiple studies have addressed the clinical outcomes of coronavirus disease, little data exist regarding the definition of immune and inflammatory profiles associated with this infection. Its clinical manifestations often worsen in association with hypercytokinemia (elevated interleukin 8 and interleukin 17). We conducted this research to elucidate the effect of interleukin 17 levels and interleukin 17F gene polymorphism on the severity and outcomes of coronavirus disease.

MATERIAL AND METHODS: Ninety patients with confirmed coronavirus disease and 30 healthy controls were enrolled. Coronavirus disease cases were classified into nonsevere, severe, and critical according to the World Health Organization definition. Approximately 10 mL peripheral blood sample was collected from all patients and controls by venipuncture in-plane and ethylenediaminetetraacetic acid tube. Enzyme-linked immunosorbent assay kits were used for calculating serum interleukin 17 levels, whereas real-time polymerase chain reaction was used for genotyping using the 5'-nuclease allelic discrimination assay for single nucleotide polymorphisms genotyping.

RESULTS: As regards interleukin 17 levels, there was a significant elevation of interleukin 17 in coronavirus disease cases compared to control healthy persons ($P < .001$). Moreover, serum interleukin 17 levels tended to be significantly higher with increased disease severity ($P = .004$). Patients with critical diseases expressed a significant rise of interleukin 17 compared to severe ($P = .03$) and nonsevere cases ($P = .02$). We noted no significant difference between the critical, severe, and nonsevere cases regarding different interleukin 17F genotypes.

CONCLUSION: Coronavirus disease is associated with elevated levels of interleukin 17, which tended to be considerably higher with disease severity. However, different interleukin 17F genotypes do not affect either the predisposition or the severity of coronavirus disease.

KEYWORDS: Interleukin 17, COVID-19, IL-17F, genotyping, gene polymorphism
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INTRODUCTION

In 2019, coronavirus disease (COVID-19) emerged as a worldwide pandemic that is caused by "severe acute respiratory syndrome coronavirus 2" or SARS-CoV-2. The mainstay management of this infection is based on symptomatic management (antipyretics for fever and oxygen therapy for desaturation), while mechanical ventilation is only indicated for patients with respiratory failure.[1]

The current literature is rich with studies, reports, and clinical trials describing the microbiological, epidemiological, clinical, and outcome characteristics of this infection. Nonetheless, this clinical entity is associated with immunological and inflammatory changes that significantly impact patient outcomes. These changes need to be well-defined for a better understanding of disease pathophysiology.[2]

The proinflammatory T-helper 17 (Th17) cells, which are a subtype of CD4+ T-cells), play a crucial role in the human immune system, especially against extracellular pathogens. In fact, these subtypes of immune cells secreted interleukin (IL) 17A and IL-17F. In addition, it organizes inflammatory cell recruitment in the infected area, including macrophages and neutrophils.[3] Therefore, abnormal Th17 regulation must have a role in the pathogenesis of numerous autoimmune and other inflammatory diseases.[4]

COVID-19 patients with worsened clinical presentation usually express a significant rise of proinflammatory cytokines in their serum (cytokine storm or cytokine release syndrome). These cytokines include IL6, 8, 17, and 1B. It is believed that the upregulation of Th17 cytokines (including IL-17 and IL-17F) is the main mediator of immunopathology and subsequent respiratory distress in such patients.[3]

Genetic polymorphism has been found to play an essential role in understanding the basis of many diseases, including pathophysiology, spread, and prevention. Single nucleotide polymorphism (SNP) is a common type of the previous

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phenomena, and it has a crucial impact on disease pathogenesis. It has a clear impact on organism attachment to the host cell, host susceptibility, resistance to diseases, and disease severity.[5]

The current investigation was done to evaluate the effect of IL-17 levels and IL-17F gene polymorphism on the severity and outcomes of COVID-19.

MATERIAL AND METHODS

A total of 90 patients with confirmed COVID-19 admitted at our quarantine hospital were enrolled in this case–control study, in addition to 30 healthy controls. Patients below 18 years old were excluded. This was done after gaining approval from the Mansoura University Scientific and Ethical Committee (code number: R.21.03.1275). Informed written consent was taken from all enrolled patients.

Nasopharyngeal swabs were used to confirm COVID-19 by identifying the specific RNA of the SARS-CoV-2 virus. First, the extraction kits (Qiagen, Foster City, USA) were used to isolate viral RNA. Then, it was identified via the real-time SARS-CoV-2 kit according to the manufacturer's instructions.

According to the World Health Organization definition, COVID-19 cases were classified into nonsevere, severe, and critical.[6] Critical infection was established if the patient developed acute respiratory distress syndrome, sepsis, septic shock, or the need for vasopressors. Severe infection was defined when the patient had less than 90% oxygen saturation on room air, respiratory rate >30 breaths per minute, or signs of severe respiratory distress. In addition, outcome assessment was recorded, including improved clinical data and mortality.

The control group included health service personnel and blood donors who had no systemic comorbidities and were free from any respiratory illness for the past year. Laboratory investigations were ordered to ensure infection clearance, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The subject was enrolled as a control when CRP was negative, and ESR was lower than 20 mm/h.

Measurement of IL-17 Levels and Genotyping

Approximately 10 mL peripheral blood sample was collected from every patient (before the beginning of any therapy) and from the healthy controls, by venipuncture, in both plane and ethylenediaminetetraacetic acid (EDTA) tubes.

Enzyme-linked immunosorbent assay kits (Bioassay Technology Laboratory) were used to quantify serum IL-17 levels (ng/L).

A crucial quality control step in genetic experiments is to undertake Hardy–Weinberg equilibrium (HWE) estimates. Variations from HWE in the group may reflect significant challenges involving selection bias, population stratification, and genotyping errors.[7]

Real-time polymerase chain reaction (PCR) was used for genotyping using the 5'-nuclease allelic discrimination (TaqMan) SNP Genotyping Assays (Applied Biosystems; Thermo Fisher Scientific, Foster City, USA). To identify IL-17F polymorphism, we utilized TaqMan Universal PCR Master Mix at rs763780 loci using StepOne Real-Time (Applied Biosystems) Figure 1.

The SNP ID is C__2234166_10 for IL-17F rs763780, and the chromosomal location is Chr.6:52101739. The context sequence [VIC/FAM] was GTGGATATGCACCTCTTACT GCACA[C/T] GGTGGATGACAGGGGTGACGCAGGT.

Two TaqMan® probes: One probe labeled with VIC® dye detects the Allele 1 (C) sequence AND the other probe labeled with FAM™ dye detects the Allele 2 (T) sequence.

The protocol used was managed blindly with a final volume of 20 µL containing 5 µL of DNA template (200 ng/µL), 1 µL of TaqMan SNP Genotyping Assay Mix, and 10 µL of TaqMan Universal PCR Master Mix. Thermal cycling conditions were performed as follows: initially, denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and at 60°C for 30 seconds, and final extension was set at 60°C for 2 minutes. In each run, the proper negative controls were used.[8]

Allelic discrimination was carried out by measuring fluorescence intensity at the endpoint. The results of the measurement were analyzed using SDS software version 1.7 (Applied Biosystems, Foster, Calif, USA), and the genotype was determined.

Each sample is interpreted according to the 2 alleles and genotypes (homozygous or heterogygous), an increase in either FAM or VIC dye fluorescence indicates homozygosity for FAM- or VIC-specific alleles (C:C or T:T), and an increase in the fluorescence of both dyes indicates heterozygosity (C:T).

Statistical Analysis

All data were tabulated and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp, Armonk, NY, USA). As the alleles and genotype frequencies are considered categorical data, they were presented as numbers and percentages. Comparing 2 or more groups with categorical data was performed by the chi-square test (Fisher's exact test/Monte-Carlo test as a correction). Heredity equilibrium was assessed by Hardy–Weinberg test. Pearson's chi-square goodness-of-fit test was used to determine if SNP genotype frequencies in controls were in Hardy–Weinberg equilibrium (HWE). The SHEsis software was used to calculate

MAIN POINTS

- The mainstay management of COVID-19 is based on symptomatic control.
- The proinflammatory T-helper 17 (Th17) cells, a subtype of CD4+ (T-cells), play a crucial role in the human immune system.
- COVID-19 patients with worsened clinical presentation usually express a significant rise of proinflammatory cytokines including IL-17.
- The current investigation was done to evaluate the effect of IL-17 levels and IL-17F gene polymorphism on the severity and outcomes of COVID-19 infection.

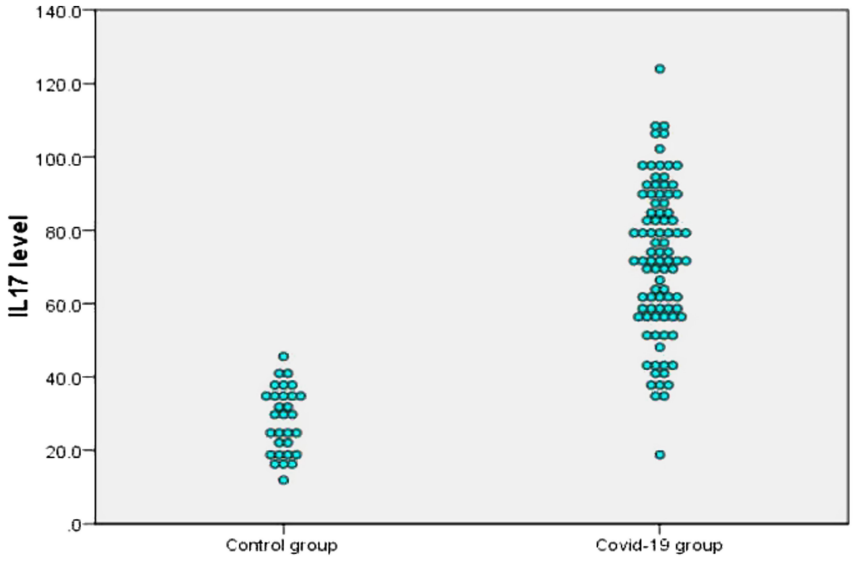

Figure 1. Real-time fluorescence curves of real-time PCR assay targeting analysis for IL-17F rs6505162.

haplotype frequencies and measure linkage disequilibrium (LD) between IL37 gene SNPs. This is the LD coefficient (D′). D′ ranges from 0 (no LD) to 1.0 (complete LD). The odds ratio (with the 95% CI) was performed by logistic regression. In the case of multiple comparisons, the *P*-value was corrected using the Bonferroni test. To specify optimal cutoff values of IL-17 level, receiver-operating characteristic (ROC) curve analysis was applied. All tests were performed using two-tailed probabilities with considering the $P \leq .05$ as the cutoff point for significance.

RESULTS

Ninety patients with confirmed COVID-19 (median age 61 (19-79) years) were enrolled. 57.7% of them were males. In addition, 4.5% were classified as nonsevere COVID-19, 53.3% had severe disease, and 42.2% had critical COVID-19. In-hospital mortality was reported in 56.7% of the studied patients (Table 1).

As regards IL-17 level, there was a significant elevation of IL-17 in COVID-19 cases compared to control healthy persons (mean values 71.8 ± 20.5 vs. 28.1 ± 8.9, respectively, $P < .001$) (Figure 2). Moreover, a positive correlation was noted between IL-17 levels and disease severity ($P = .004$). Cases with the critical disease significantly elevated IL-17 compared to cases with severe and nonsevere disease ($P = .03$ and .02, respectively). Patients with severe disease had comparable IL-17 values to nonsevere cases ($P = .337$) (Table 2).

Table 1. Clinical Severity of COVID-19 and Its Outcome among Studied Patients

Parameter		n, (%)
COVID-19 clinical manifestation	Nonsevere	5 (5.6%)
	Severe	48 (53.3%)
	Critical	37 (42.1%)
Outcome	Improved	39 (43.3%)
	Dead	51 (56.7%)

ROC analysis was conducted to identify the optimal IL-17 level for the prediction of COVID-19. IL-17 best cutoff value for the prediction of COVID-19 was 37.2 ng/L. Therefore, the area under the curve (AUC) was 0.979 ($P \leq .001$) (Table 3 and Figure 3).

The genetic characteristics of the studied SNPs followed the data of the National Center for Biotechnology Information

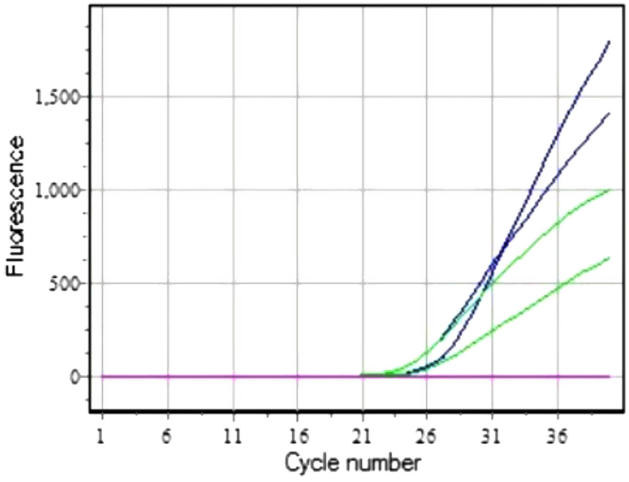

Figure 2. IL-17 levels (ng/L) in COVID-19 patients and control.

Table 2. IL-17 Levels and Clinical Severity of COVID-19 among Studied Patients

	Nonsevere Group (n = 5)	Severe Group (n = 48)	Critical Group (n = 37)	*P*
IL-17 (ng/L), mean ± SD	53.3 ± 17.04	68.04 ± 19.5	79.2 ± 19.7	$P^1 = .337$
				$P^2 = .020$
				$P^3 = .031$
				$P = .004$

P^1, comparison between nonsevere and severe; P^2, comparison between nonsevere and critical; P^3, comparison between severe and critical; *P*, comparison between 3 groups; SD, standard deviation.

Table 3. IL-17 Level for Prediction of COVID-19

	AUC	SE	*P*	95% CI	Cutoff (ng/L)	Sensitivity (%)	Specificity (%)
IL-17	0.979	0.011	<.001	0.958-1.00	37.2	96.7	83.3

AUC, the area under the curve.

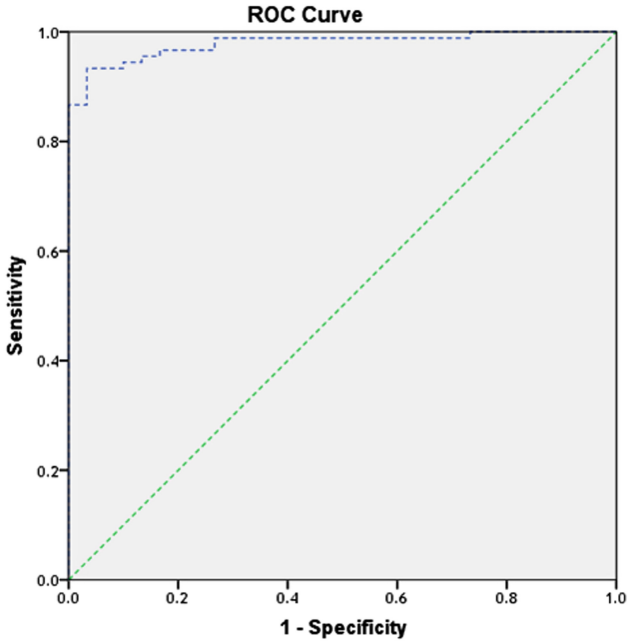

Figure 3. ROC analysis to identify the optimal IL-17 level for the prediction of COVID-19.

Table 4. Genetic Features of Studied SNPs According to the National Center for Biotechnology Information (NCBI)

SNP ID	rs763780
Alleles	T/C
Ancestral Allele	T
Cytogenetic location	6p12.2
Gene	Interleukin-17F
Nucleotide change	T to C substitution at the third exon of the IL-17 F gene in the coding region (+7488)
Amino acid change	His-to-Arg (H161R) substitution in position 161

(NCBI), as shown in Table 4. When the Hardy–Weinberg equation was applied at random from the population, it revealed that rs763780 genotypes in COVID-19 patients and control healthy persons were in Hardy–Weinberg equilibrium (HWE) (*P* = .9 and .6) (Table 5).

Table 6 shows the distribution of rs763780 genotype variants alleles in cases compared to controls. We did not detect any significant differences between them regarding genotypes and alleles.

Regarding different genotypes in the cases group, no significant differences were seen between either nonsevere, severe, or critical COVID-19 patients (*P* = .7). Also, no significant differences were noted between improved and dead patients (*P* = .4) (Table 7).

DISCUSSION

In this study, we assessed the effect of IL-17 levels and IL-17F gene polymorphism on the prediction, severity, and outcomes of COVID-19. We noticed a significant elevation of IL-17 in coronavirus disease cases compared to control healthy persons. Moreover, serum IL 17 levels tended to be significantly higher with increased disease severity—also, no significant difference between the critical, severe, and nonsevere cases regarding different IL-17F genotypes.

In the same context, Huang et al[9] noted a significant increase in IL-17 levels in COVID-19 patients who needed intensive care unit (ICU) admission, compared to others who did not, as well as healthy controls. Additionally, Liu et al[10] detected a positive correlation between serum IL-17 levels and disease severity, including COVID-19, middle east respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In this study, IL-17's best cutoff value for the prediction of COVID-19 was 37.2 ng/L. However, a cutoff value of >25 ng/mL was determined by Fathy et al[11] to differentiate between the patients' groups and the control group with 93.94%

Table 5. Assessment of Hardy–Weinberg Equilibrium for Studied IL-17F Genes

		Control (n = 30)		COVID-19 Patients (n = 90)	
Frequency		**Observed**	**Expected**	**Observed**	**Expected**
rs763780	TT	29	29.0	82	82.2
	CT	1	1.0	8	7.6
	CC	0	0.0	0	0.2
	P (HW)	0.962		0.659	

HW, Hardy–Weinberg.

Table 6. Distribution of rs763780 Genotype Variants and Alleles in all COVID-19 Patients Versus Control

				Relative Risk of COVID-19			
		Control (n = 30)	COVID-19 Patients (n = 90)	OR	95% CI		*P*
TT	Count (%)	29 (96.7%)	82 (91.1%)	1	–	–	*R*
CT	Count (%)	1 (3.3%)	8 (8.9%)	2.82	0.339	23.608	.336
CC	Count (%)	0 (0.0%)	0 (0.0%)	0.357	0.006	18.43	.609
CT+CC	Count (%)	1 (3.3%)	8 (8.9%)	2.82	0.339	23.608	.336
T	Count (%)	59 (98.3%)	`172 (95.5%)	2.74	0.336	22.405	.346
C	Count (%)	1 (1.6%)	8 (4.5%)				

OR, odds ratio.

Table 7. Comparison of Clinical Severity of COVID-19 and Outcome Regarding Different Genotypes in COVID-19 Patients

Parameter		TT Group (n = 82)	CT Group (n = 8)	*P*
COVID-19 clinical severity	Nonsevere	5 (6.1%)	0 (0.0%)	.714
	Severe	44 (53.7%)	4 (50.0%)	
	Critical	33 (40.2%)	4 (50.0%)	
Outcome	Improved	37 (45.1%)	2 (25.0%)	.458
	Dead	45 (54.9%)	6 (75.0%)	

sensitivity and 65% specificity. Fatemeh et al[12] determine cutoff value of 169.2 pg/mL for o differentiating mild from severe COVID-19.

COVID-19 has different inflammatory responses between individuals. A possible genetic background could explain this phenomenon. Multiple polymorphisms have been reported in the rs2275913 locus IL-17A gene based on the tested population. At the same previous locus, the AG genotype is present in the populations of Norway, Poland, Finland, Czechia, Japan, India, Iran, and China, whereas the GG genotype is present in the population of Egypt, Tunisia, Germany, Turkey, Netherlands, Spain, Brazil, and Mexico. On the other hand, other studies handling the rs763780 locus noted that all populations had the TT genotype.[13] Therefore, we proposed to investigate rs763780 loci polymorphism and its association with the COVID-19 severity and outcome.

Though, there is uncertainty regarding the fact that IL-17 upregulation is a direct consequence of COVID-19 infection. Recent studies discovered that COVID-19 open reading frame 8 (ORF8) causes activation of the IL-17 signaling pathway via binding to the IL-17 receptor. In previous experimental research, mice infected with SARS-CoV2 ORF8 pseudo-virus decreased pulmonary and hepatic inflammation when treated with IL-17RA blocker (antibody).[14]

In this study, the tested genotypes and alleles showed no significant difference between cases and controls. Also, in comparison between gene polymorphism and COVID-19 severity and outcome among studied groups, no statistically significant difference could be detected.

However, in the study conducted by Karcioglu Batur and Hekim,[13] rs763780 locus TT and TC genotypes showed a significant correlation with COVID-19 prevalence, whereas rs2275913 locus AG genotype showed a significant correlation with COVID-19-related mortality. This was noticed in the tested population from many countries, including Egypt.

Xie et al[15] noted that patients with gene polymorphisms causing decreased IL-17 production had better 30-day survival rates. Contrarily, patients with polymorphisms causing increased IL-17 production were associated with increased disease severity and related mortality.[15]

The main limiting element of this study is the number of patients involved. Further investigations must be done to identify the relationship between IL gene polymorphisms and COVID-19 outcomes. These future investigations must be analyzed to predict potential drugs blocking IL-17RA and reducing IL-17-mediated systemic inflammatory response associated with COVID-19 infection. This would help us to improve disease outcomes.

Ethics Committee Approval: This study was approved by the Ethics Committee of Mansoura University (code number: R.21.03.1275.).

Informed Consent: Written and verbal informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – H.S., S.Y.; Design – A.H., S.S.; Supervision – E.A., H.M.; Resources – G.S., E.A.; Materials – H.S, S.Y.; Data Collection and/or Processing – A.H., S.S.; Analysis and/or Interpretation – E.A. Literature Search – A.H., S.S.; Writing – A.H., H.S.; Critical Review – A.H.

Declaration of Interests: The authors have no conflict of interest to declare.

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