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Original article

Cytokines profile and their related genotypes in COVID-19: Correlation with disease severity and outcome in Egyptian patients

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ABSTRACT

Background and Aim: COVID-19-related pulmonary inflammation is linked to elevated plasma levels of a group of proinflammatory cytokines. We aim to identify the association between IL-6 rs1800795, IL-17 rs2275913, and IL-37 rs3811046 gene polymorphisms and COVID-19 severity and prognosis. **Methods:** Two hundreds adult COVID-19-confirmed patients (100 patients with non-severe and 100 patients with severe or critical COVID-19) and 100 healthy individuals were enrolled in this cross-sectional study. Clinical and laboratory evaluations were performed, including liver and kidney functions, complete and differential blood counts, C-reactive protein, and D-dimer. Genotyping for IL-6 (rs1800795), IL-17 (rs2275913), and IL-37 (rs3811046) was conducted using allelic discrimination real-time PCR assay via TaqMan probes. The levels of IL-6, IL-17, and IL-37 were estimated by enzyme-linked immunosorbent assay (ELISA). **Results:** Serum levels of IL-6 and IL-17 were increased while IL-37 declined with ongoing COVID-19 severity. IL-6 rs1800795 genotypes and alleles did not differ significantly between the studied groups. Meanwhile, IL-17 rs2275913 GA (heterozygous) and AA (homozygous) genotypes and A allele showed significantly higher frequencies in the control group compared to those in the patients' groups and were proposed as protective factors against COVID-19 occurrence and increased severity. Notably, IL-37 rs3811046, GT and TT variants, and T allele were more prevalent in the patients' groups than in the control group and might be related to both disease occurrence and progression. **Conclusion:** Both GG genotype and G allele of IL-17 (rs2275913) and TT genotype and T allele of IL-37 (rs3811046) and their serum levels are potential risk factors for COVID-19 infection and severity, making them excellent disease management targets.

Introduction

Although the scientific community has made considerable efforts to uncover the molecular base of signs and symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the signs and symptoms of the world pandemic of

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coronavirus disease 2019 (COVID-19) and the underlying physiopathology remain incompletely understood [1,2]. The signs and symptoms of SARS-CoV-2 are incredibly diverse. These symptoms range from less serious (mild and moderate) non-specific ones, such as dry cough, fever, anosmia, and diarrhea, with mild pneumonia in certain cases, to more severe, potentially deadly ones, such as severe pneumonia that is associated with disrupted gas exchange, in addition to dyspnea and tachypnea. Surprisingly, multiple organ failure, shock, and lung dysfunction in patients with severe disease account for about 5% of cases. Patient reports of persistent symptoms occasionally occur [3].

However, it has been well known that COVID-19-related pulmonary inflammation is linked to elevated plasma levels of a group of proinflammatory cytokines, including interleukin-6 (IL-6), interleukin-17 (IL-17), interleukin-12 (IL-12), tumor necrosis factor (TNF), and interferon specifying a distinctive feature recognized as cytokine storm (CS) [4,5]. The coronavirus family has been associated with increased CS-induced acute lung damage resulting in death, as was previously observed for MERS-CoV (Middle East Respiratory Syndrome) and SARS-CoV infections. Defining the impact of cytokines throughout COVID-19 is the key to effective patient management [6, 7].

Indeed, some publications indicate higher levels of Th17 cells as well as circulating IL-17 in the peripheral blood of patients with severe SARS-CoV-2 infection [8, 9]. This clinical data is particularly significant, considering IL-17 stimulates the production of other proinflammatory mediators including IL-1, IL-6, and TNF-α, which may be crucial in tissue injury along with matrix metalloproteinases [10].

Numerous articles about the virus pathogenesis have been published since the COVID-19 pandemic began. However, much remains to be understood. For instance, it is currently unknown why some people remain asymptomatic while others develop serious illnesses [9]. The solutions to these issues and the identification of variables influencing SARS-CoV-2 pathogenicity and the severity of the disease will help in creating effective treatment plans and improved infection prevention. Additionally, effective resource allocation toward patients at high risk for deterioration is critical, considering the diversity of COVID-19 and the scarcity of medical resources in severely impacted areas. In the current work, we aimed to identify the association between IL-6 rs1800795, IL-17 rs2275913, and IL-37 rs3811046 gene polymorphisms and COVID-19 disease severity to help predict prognosis and clinical care as regards which variables allow the prediction of patients with a high risk of severe presentation and bad outcome.

Patients and methods

Study design and participants

This cross-sectional study was carried out as a collaboration between Tropical Medicine, Medical Microbiology and Immunology, Medical Biochemistry and Molecular Biology, Public Health and Community Medicine, Clinical Pathology, Chest, and ICU Departments and the Central Laboratory of Faculty of Medicine, Menoufia University. Two hundreds adult COVID-19-confirmed patients and 100 healthy volunteers were enrolled in the present study between March 2021 and January 2022. All patients in this study had a clinical suspicion of COVID-19, and the positive polymerase chain reaction (PCR) (nasopharyngeal and oropharyngeal samples) verified the diagnosis.

According to the WHO's most recent update, "COVID-19 Clinical Management: Living Guidance, 25 January 2021," patients have been categorized into two groups. Group I (non-severe COVID-19 cases) guaranty 100 patients, not fulfilling either the critical or severe COVID-19 parameters (criteria). Group II included both patients who met the severe or critical criteria and comprised 100 patients. The presence of any of the following criteria defines severe COVID-19: oxygen saturation < 90% on room air; signs of severe respiratory distress in adults (accessory muscle use, inability to complete whole sentences, and respiratory rate > 30 breaths per minute); in addition to signs of pneumonia. However, critical COVID-19 patients included those with the criteria for acute respiratory distress syndrome (ARDS), sepsis, septic shock, or other conditions that would generally require the provision of life-sustaining therapies such as mechanical ventilation (invasive or non-invasive) or vasopressor therapy.

Upon attending the COVID-19 isolation room at the Faculty of Medicine, Menoufia University Hospital during the study period, patients with clinical suspicion of COVID-19 were evaluated. After clinical, laboratory, and

radiological evaluation, patients were categorized into either non-severe, severe, or critical. For non-severe cases, a prescription of outpatient treatment was provided and follow-up was done by telephone and/or at the outpatient clinic for COVID-19. However, those with severe or critical presentations were admitted to COVID-19 quarantine ward or COVID-19 ICU. Upon admission, baseline clinical, laboratory, and radiological data were recorded. During hospitalization, daily assessment of disease progression, treatment response, and outcomes were also verified. Peripheral blood samples were collected by sterile venipuncture. EDTA-blood samples were preserved for genomic DNA extraction. Moreover, serum aliquot was preserved frozen at −20 °C for estimating IL-6, IL-17, and IL-37 levels. All required data for this research were obtained from the patients' medical records.

One hundred patients were identified as non-severe, and 100 patients were severe or critical COVID-19 cases. One hundred additional healthy individuals, healthy controls (HC), who matched the patients' age and gender were enrolled. Patients with a known history of hepatic, renal, or cardiac diseases and patients with coagulation disorders were excluded from the study.

Sample size calculation:

El-Shabrawy et al. [11] documented that IL-6 was 14.5 ± 11.52 in moderate COVID-19 patients and 55.9 ± 25.32 in severe COVID-19 patients, at 95% confidence interval and marginal error of 0.05, study power of 95%, and a ratio between the studied groups of 1:1. The estimated minimum sample size was 91 cases which was rounded to 100 subjects per each group and equal number of healthy controls.

Ethical consideration:

After outlining the study's purpose, each participant was notified about the research and to provide their informed written consent before taking part in the study. The work obtained approval from the Menoufia University Ethical Committee and was conducted in line with the Helsinki Declaration.

Methods

Sampling and immunochemical tests:

Peripheral blood samples were collected by sterile venipuncture. EDTA-blood samples were preserved for genomic DNA extraction and subsequent single nucleotide polymorphisms (SNPs) analysis for IL-6 rs1800795, IL-17 rs2275913, IL-37 rs3811046, and complete blood count (CBC) (Sysmex XN1000, Japan).

Samples in plain tubes were allowed to clot, and the serum was separated. An aliquot was preserved frozen at −20 °C until estimating IL-6, IL-17, and IL-37 levels using enzyme-linked immunosorbent assay (ELISA) kits (SunRed, China) as per the manufacturer's directions. Serum ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine were measured using Beckman Coulter (Au 680) chemistry autoanalyzer (Beckman, USA). The coagulation profile and D-dimer were conducted by BFT II Analyzer (Dade Behring Marburg GmbH, Germany). The CRP was estimated using a Nephstar protein analysis system (Goldsite, China).

DNA extraction and genotyping:

DNA extraction was performed from peripheral blood utilizing a GeneJET Whole Blood Genomic DNA Purification Mini-Extraction Kit (Thermo Fisher Scientific, USA). Genotyping for IL-6 (rs1800795), IL-17 (rs2275913), and IL-37 (rs3811046) was performed using real-time PCR and allelic discrimination assays utilizing a TaqMan probe (Applied Biosystems, USA). The primers, probes, and master mix (40×) were provided by Thermo Scientific. The probe sequences [VIC/FAM] are illustrated in the following table1.

A total mixture of 20 µl composed of 1.5 µl of the primer/probe, 10 µl of the master mix, 3.5 µl of nuclease-free water and 5 µl of purified DNA. The cycling was as follows: 10 min of initial denaturation step at 95 °C, then 40 cycles of 15 s for denaturation at 94 °C, 60 s for primer annealing at 50 °C and 2 min at 72 ◦C for primer extension. The ABI 7500 real-time PCR software, version 2.0.1, was utilized for analyzing the results. Data were analyzed by the software accompanying the ABI7500 real-time PCR **(Figure 1).**

Statistical analysis of the data

The data was gathered and tabulated before being analyzed using SPSS (Statistical Package for Social Science) version 20.0. (SPSS Inc., Chicago, IL, USA). Categorical data were described as frequency and percentage and compared between the studied groups using Chi-square test ($\chi 2$) and Fisher's exact test accordingly. Student's *t*-test was utilized for testing the difference between two groups with normally distributed quantitative data.

Meanwhile, in the not normally distributed one, Mann Whitney U test was used between two groups, and Kruskal Wallis test was used between multiple groups. Odds ratio was used to evaluate the risk of gene mutation on the outcome of the disease, and p value less than 0.05 was considered significant. Hardy-Weinberg equilibrium [HWE] was evaluated in patients and controls.

Results

Table 1 demonstrated a significantly lower age in the non-severe COVID-19 group (group 1) compared to both the severe COVID-19 group (group 2) and the control group. Regarding gender distribution, the females predominated in group 2 compared to group 1 and controls. Furthermore, diabetes mellitus and hypertension were also more common in group 2 than in group 1. As for laboratory investigations of the involved cases, the severe group showed lower hemoglobin levels, lymphocyte percentage and INR. However, the same group showed a higher segmental percentage, urea, creatinine, CRP, ALT, AST, and PT. In addition, serum ferritin, LDH and D-Dimer were significantly higher in the severe group compared to the non-severe group.

The presenting clinical data were more evident in the severe than the non-severe groups, with fever, cough, and dyspnea as the most prominent symptoms in approximately 100% of severe patients. Meanwhile, a runny nose was observed in 25% of the non-severe cases with no affection observed in the severe group **(Table 2)**. Serum levels of IL-6 and IL-17 were significantly raised with increasing disease severity. Both cytokines recorded the lowest levels in the control group and increased successively in group 1 and group 2, while IL-37 was downgraded with increasing disease severity **(Table 3).**

The genotype distribution of IL-6 rs1800795, IL-17 rs2275913, and IL-37 rs3811046 were consistent with Hardy-Weinberg equilibrium in controls and severe cases, while IL-17 rs2275913 varied significantly in the patients' group and IL-6 rs1800795 in mild and moderate cases. When comparing IL-6 rs1800795 genotypes and alleles, there was no significant difference between the three studied groups. Meanwhile, IL-17 rs2275913 GA and AA genotypes and A allele were of a significantly higher frequency in controls compared to patients group 1 and group 2. Regarding IL-37 rs3811046, GT and TT variants and T allele were more prevalent in the patients' groups than in the control group **(Table 4)**.

The IL-17 GA genotype was a protective factor for the acquisition of SARS-CoV-2 infection (between control and group 1) with an odds ratio of 0.49 (CI: 0.24–0.89) and a prognostic factor determining COVID-19 severity (between group 1 and group 2) with an odds ratio of 0.18 (CI: 0.08–0.41) **(Table 5)**. Similarly, AA genotype had the same effect with odds ratios of 0.46 (CI: 0.16–1.35) and 0.13 (CI: 0.03–0.62), respectively. The same effect was also observed with its allele (A). Regarding IL-37, both GT and TT genotypes increased the risk of disease occurrence (between control and group 1) with odds ratios of 3.88 (CI: 2.04–7.36) and 7.84 (CI: 2.11–29.08), respectively. Importantly, both genotypes related directly to ongoing disease severity (between group 1 and group 2) with odds ratios of 6.07 (CI: 3.1–11.9) and 22.62 (CI: 6.33–80.83), respectively. Serum IL-6 did not change significantly regarding different IL-6 rs1800795 genotypes in any of the studied groups. Serum IL-17 significantly decreased in GA and AA variants, and serum IL-37 decreased in GT and TT variants in all studied groups as illustrated in **(Figure 2).**

Table I: Probes sequences for the target SNPs.

Target SNPs	Probes sequences
IL-6 rs1800795	ACTTTTCCCCCTAGTTGTGTCTTGC[C/G]ATGCTAAAGGACGTCACATTGCACA
IL-17 rs2275913	TGCCCTTCCCATTTTCCTTCAGAAG[A/G]AGAGATTCTTCTATGACCTCATTGG
IL-37 rs3811046	CTGCGTCTGACTGCAGACCCGGCTG[G/T]AAGCCCCCTGGAACCAGGCCCAAGC

Table 1. Sociodemographic criteria and laboratory investigations among the studied groups.

	Group I (non-severe) N = 100	Group II (Severe & critical cases) N = 100	Controls N = 100	Test	P value
Age Mean ±SD Median (Range)	 51.61±16.80 47 (25 – 80	 64.60±13.23 62.0 (42 – 88)	 61.4±16.01 69 (36 – 80	4.22^{t} 1.54^{t} 19.25^{t}	$<0.001^{1}$ 0.120^{2} $<0.001^{3}$
Sex Male Female	 62 (62.0) 38 (38.0)	 48 (48.0) 52 (52.0)	 70 (70.0) 30 (30.0)	1.43^{c} 10.0^{c} 10.33^{c}	0.230^{1} 0.002^{2} 0.006^{3}
History of DM	26 (26.0)	76 (76.0)	……	50.02^{c}	$<0.001^{1}$
History of HTN	23 (23.0)	72 (72.0)	……	48.14^{c}	$<0.001^{1}$
Hb (gm/dl) Mean ±SD Median (Range)	11.25±1.46 11 (8.5 – 14)	10.77±1.69 11 (7.5 – 14)	12.5 ± 0.7 12.5 (11.5 – 14)	7.7^{t} 9.46^{t} 2.14^{t}	$<0.001^{1}$ $<0.001^{2}$ 0.03^{3}
WBCs (×1000/ul) Mean ±SD Median (Range)	 8.81±4.51 8.75 (3.8 – 25)	 9.91±7.03 9 (3.8 – 32)	 4.6 ± 0.6 4.3 (4 – 6)	4.55^{U} 5.02^{U} 0.16^{U}	$<0.001^{1}$ $<0.001^{2}$ 0.87^{3}
Lymphocytes (%) Mean ±SD Median (Range)	 14.87±4.0 15 (7 – 23)	 13.12±4.14 13 (5 – 22)	 32.4 ± 4.8 33 (20 – 40)	36.4^{t} 30.4^{t} 3.04^{t}	$<0.001^{1}$ $<0.001^{2}$ 0.003^{3}
Neutrophils (%) Mean ±SD Median (Range)	 77.37±9.35 78 (38 – 90)	 81.28±5.73 80 (70 – 90)	 51.5 ± 5.0 52 (42 – 60)	24.4^{t} 40.59^{t} 3.56^{t}	$<0.001^{1}$ $<0.001^{2}$ $<0.001^{3}$
Platelet count (×1000/ul) Mean ±SD Median (Range)	 251.24±76.01 231.5 (129 – 430)	 264.24±95.16 230 (140 – 450)	 364.8 ± 51.7 378 (233 – 432)	8.54^{t} 7.89^{U} 0.59^{U}	$<0.001^{1}$ $<0.001^{2}$ 0.56^{3}
CRP (mg/l) Mean ±SD Median (Range)	 48.76±38.92 45.5 (0 – 169	 102.16±54.55 96 (24 – 196)	 3.7 ± 1.4 4 (1 – 6)	18.21^{U} 33.64^{U} 8.02^{U}	$<0.001^{1}$ $<0.001^{2}$ $<0.001^{3}$
Serum creatinine (mg/dl) Mean ±SD Median (Range)	 1.01±0.31 1 (0 – 2.1)	 1.14±0.25 1.1 (0.8 – 1.9)	 0.8 ± 0.15 0.8 (0.3 – 0.9)	6.10^{t} 11.66^{t} 3.21^{t}	$<0.001^{1}$ $<0.001^{2}$ 0.002^{3}
Blood urea (mg/dl) Mean ±SD Median (Range)	 40.51±13.99 40 (25 – 100)	 48.32±20.07 45 (25 – 102	 21.6 ± 4.6 20 (12 – 31)	10.45^{U} 11.22^{U} 3.57^{U}	$<0.001^{1}$ $<0.001^{2}$ $<0.001^{3}$
ALT (IU/L) Mean ±SD Median (Range)	 36.43±22.49 28 (18 – 120)	 58.56±60.65 40 (18 – 250	 25.3 ± 7.1 25 (17 – 36)	2.01^{U} 5.23^{U} 3.47^{U}	0.03^{1} $<0.001^{2}$ 0.001^{3}

AST (IU/L) Mean ±SD Median (Range)	 37.26±25.05 30 (20 – 140)	 54.84±48.28 38 (18 – 200	 19.1±5.5 18(9-25)	2.89 U 3.62 U 4.78 U	0.003[1] <0.001[2] <0.001[3]
PT (seconds) Mean ±SD Median (Range)	 12.89±0.94 13 (12 – 15)	 13.56±1.10 14 (11 – 15)	 12.6 ± 0.69 13 (11 – 13.5)	2.49 t 7.39 t 4.62 t	0.01[1] <0.001[2] <0.001[3]
INR Mean ±SD Median (Range)	 1.02±0.08 1 (0.88 – 1.30	 0.99±0.06 1.0 (0.80 – 1.1)	 0.997 ± 0.005 1 (0.99 – 1)	2.87 t 1.16 t 2.72 t	0.005[1] 0.25[2] 0.007[3]
Serum ferritin (ng/ml) Mean ±SD Median (Range)	 473.7±266.71 352.5 (300 – 1500)	 894.2±414.37 800 (200 – 2000)	 277 ± 39.9 295.5 (190 – 335)	8.95 U 8.63 U 18.57 U	<0.001[1] <0.001[2] <0.001[3]
LDH (IU/L) Mean ±SD Median (Range)	 438.0±206.51 340 (280 – 1000)	 866.0±384.74 750 (350 – 1800)	 207.8 ± 45.8 199 (105 – 276)	1 0.12 U 9.10 U 21.33 U	<0.001[1] <0.001[2] <0.001[3]
D- dimer (mg/L) Mean ±SD Median (Range)	 0.63±0.62 0.40 (0.1 – 3.0)	 1.41±0.79 1 (0.5 – 3.5)	 0.23 ± 0.08 0.20 (0.10 – 0.40)	12.63 U 8.44 U 39.57 U	<0.001[1] <0.001[2] <0.001[3]

DM: diabetes mellitus, HTN: hypertension, Hb: hemoglobin concentration, WBCs: white blood cells, CRP: C reactive protein, ALT: alanine transaminase, AST: aspartate aminotransferase, PT: prothrombin time, INR: international normalized ratio, and LDH: lactate dehydrogenase.
t=student t test, c= chi squared test, U= Mann Whitney U test
1= comparing control group with Group I
2= comparing control group with group II
3 = comparing group I and group II

Table 2. Presenting symptoms among COVID-19 patients groups.

	Group I (non-severe) N = 100	Group II (Severe & critical cases) N = 100	X^2 Test	P value
Fever	72 (72.0)	100 (100%)	33.91	<0.001
Cough	86 (86.0)	100 (100%)	15.05	<0.001
Sputum	27 (27.0)	76 (76.0)	48.06	<0.001
Dyspnea	48 (48.0)	100 (100)	70.27	<0.001
Hemoptysis	0 (0.0)	4 (4.0)	4.08	0.12
Cyanosis	0 (0.0)	24 (24.0)	27.27	<0.001
Myalgia	30 (30.0)	24 (24.0)	0.91	0.34
Bone ache	46 (46.0)	36 (36.0)	2.07	0.15
Anosmia	66 (66.0)	32 (32.0)	23.13	<0.001
Loss of taste	42 (42.0)	12 (12.0)	22.83	<0.001
Vomiting	27 (72.0)	8 (8.0)	12.5	<0.001
Diarrhea	61 (61.0)	24 (24.0)	28.01	<0.001
Conjunctivitis	20 (20.0)	56 (56.0)	27.5	<0.001
Chills	8 (8.0)	20 (20.0)	5.98	0.01
Runny nose	25 (25.0)	0 (0.0)	28.57	<0.001

Table 3. Serum levels of interleukin-6, interleukin-17, and interleukin-37 among the studied groups.

	Group I (non-severe) N = 100	Group II (Severe & critical cases) N = 100	Control group (HC) N = 100	t-Test	P value
IL-6 (ng/L)					
Mean ±SD	169.25±53.89	210.03±62.77	15.0±3.27	28.57	<0.001[1]
Median	185	220	14.5	31.03	<0.001[2]
Range	30 – 260	60 – 350	10 – 21	4.93	<0.001[3]
IL-17 (pg/ml)					
Mean ±SD	456.15±131.61	591.75±96.93	210.47±47.09	17.44	<0.001[1]
Median	435	600	216	35.32	<0.001[2]
Range	200 – 700	400 – 725	130 – 266	8.21	<0.001[3]
IL-37 (pg/ml)					
Mean ±SD	113.47±16.47	101.73±22.32	136.74±10.97	11.79	<0.001[1]
Median	112.5	99	134	14.09	<0.001[2]
Range	67 – 141	63 – 163	120 – 156	4.22	<0.001[3]

1 = comparing control group with group I.
2 = comparing control group with group II.
3 = comparing group I and group II.

Table 4. Distribution of IL-6 (rs1800795), IL-17 (rs2275913), and IL-37 (rs3811046) genotypes and alleles among the studied groups.

	Group I (non-severe) N = 100	Group II (Severe & critical cases) N = 100	Control group (HC) N = 100	Test	P value
IL-6 rs1800795 genotypes				X^2	
CC	3 (3.0)	4 (4.0)	2 (2.0)	0.56	0.76[1]
CG	12 (12.0)	22 (28.0)	15 (15.0)	2.51	0.28[2]
GG	85 (85.0)	74 (68.0)	83 (83.0)	3.85	0.15[3]
IL-6 rs1800795 alleles				X^2	
C	18 (9.0)	30 (15.0)	19 (9.5)	0.03	0.86[1]
G	182 (91.0)	170 (85.0)	181(90.5)	2.81	0.09[2]
				3.41	0.06[3]
IL-17 rs2275913 genotypes				X^2	
GG*	75 (75.0)	89 (79.0)	58 (58.0)	6.49	0.040[1]
GA	19 (26.0)	9 (17.0)	32 (32.0)	24.77	<0.001[2]
AA	6 (6.0)	2 (4.0)	10 (10.0)	6.77	0.030[3]
IL-17 rs2275913 alleles				X^2	
G*	169 (84.5)	187 (93.5)	148 (74.0)	6.7	0.010[1]
A	31 (15.5)	13 (6.5)	52 (26.0)	27.9	<0.001[2]
				8.27	0.004[3]
IL-37 rs3811046 genotypes				X^2	
GG*	42 (42.0)	28 (28.0)	76 (76.0)	24.77	<0.001[1]
GT	45 (45.0)	47 (47.0)	21 (21.0)	49.4	<0.001[2]
TT	13 (13.0)	25 (25.0)	3 (3.0)	6.63	0.036[3]
IL-37rs3811046 alleles				X^2	
G*	129 (64.5)	103 (51.5)	173 (86.5)	26.17	<0.001[1]
T	71 (35.5)	97 (48.5)	27 (13.5)	57.27	<0.001[2]
				6.94	0.008[3]

1 = comparing control group with group I.
2 = comparing control group with group II.
3 = comparing group I and group II.

Table 5. Risk evaluation of IL-6 (rs1800795), IL-17 (rs2275913), and IL-37 (rs3811046) genotypes and alleles among the studied groups.

	Control (HC) N = 100	Group I (non-severe) N = 100	Test (p value)	Odds ratio (95%CI)	Group II (Severe & critical cases) N = 100	Test (p value)	Odds ratio (95%CI)
IL-17 genotypes							
GG*	58 (58.0)	75 (75.0)			89 (79.0)		
GA	32 (32.0)	19 (26.0)	5.40 (0.02)	0.49 (0.24 – 0.89) [1]	9 (17.0)	19.1 (<0.001)	0.18 (0.08 – 0.41)
AA	10 (10.0)	6 (6.0)	2.05 (0.15)	0.46 (0.16 - 1.35) [2]	2 (4.0)	8.73 (0.005)	0.13 (0.03 – 0.62)
IL-17 alleles							
G*	148 (74.0)	169 (84.5)	6.7(0.01)	0.52 (0.32 – 0.86)	187 (93.5)	27.90 (<.001)	0.20 (0.1 – 0.38)
A	52 (26.0)	31 (15.5)			13 (6.5)		
IL-37 genotypes							
GG*	76 (76.0)	42 (42.0)			28 (28.0)		
GT	21 (21.0)	45 (45.0)	18.0(<0.001)	3.88(2.04 – 7.36)[1]	47 (47.0)	29.77(<0.001	6.07(3.1 – 11.9)
TT	3 (3.0)	13 (13.0)	12.14(<0.001)	7.84 (2.11 – 29.08) [2]	25 (25.0)	35.7(<0.001)	22.62(6.33- 80.83)
IL-37 alleles							
G*	173 (86.5)	129 (64.5)			103 (51.5)		
T	27 (13.5)	71 (35.5)	26.17(<0.001)	3.53(2.14 – 5.8)	97 (48.5)	57.27((<0.001)	6.03(3.69 – 9.86)

* = reference genotype.
1 = comparing heterozygous genotype with reference genotype.
2 = comparing homozygous variant genotype with reference genotype.

Figure 1. Allelic discrimination Plot for IL-17 gene polymorphism (rs2275913).

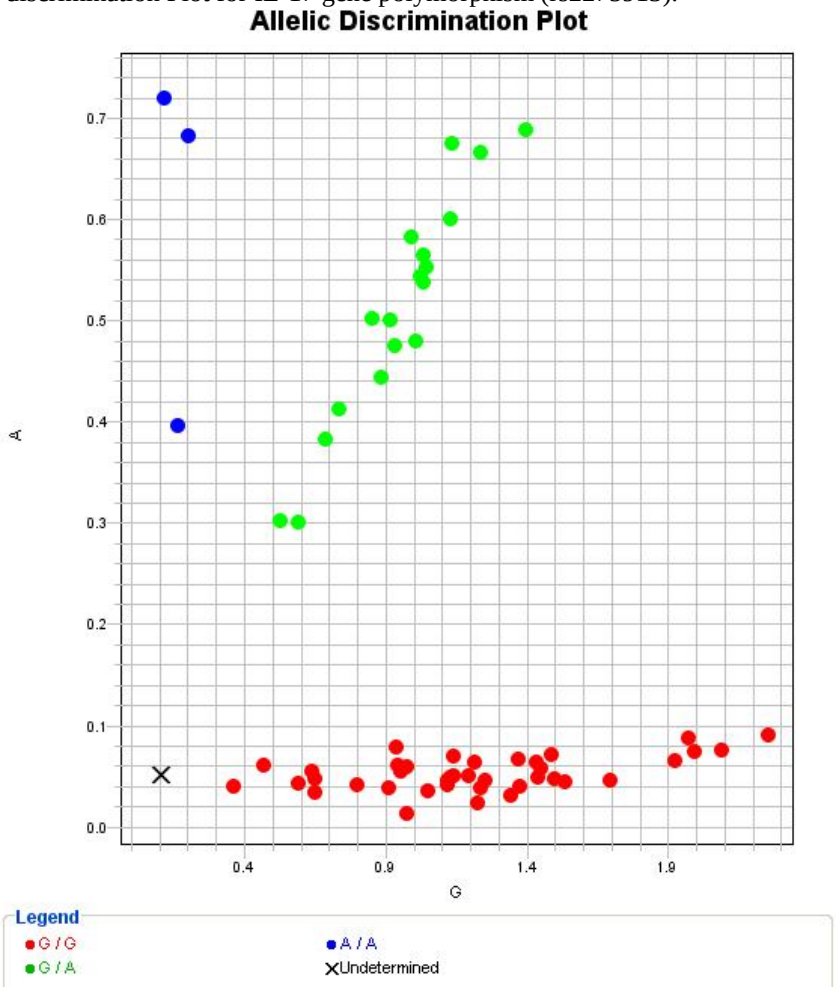

Figure 2. Relation between IL-6 (rs1800795), IL-17 (rs2275913), and IL-37 (rs3811046) genotypes and serum levels of related cytokines.

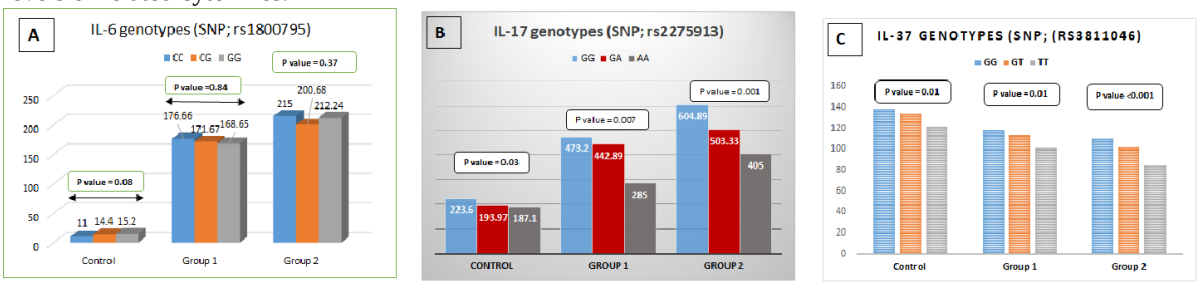

Figure 2A: Relation between IL-6 (rs1800795) genotypes and IL-6 serum levels
Figure 2B: Relation between IL-17 (rs2275913) genotypes and IL-17 serum levels.
Figure 2C: Relation between IL-37 (rs3811046) genotypes and IL-37 serum markers.

Discussion

The COVID-19 infection is a significant healthcare emergency. The manifestation of COVID-19 varies from asymptomatic infection to severe pneumonia and acute respiratory distress syndrome (ARDS) with elevated concentrations of cytokines [10]. In COVID-19, the cytokine storm is a prime feature and results in apoptosis in various organs with repressed virus recognition by receptors [12]. Moreover, interleukins, the driving force in the acute phase response, are considered prognostic markers in sepsis and organ damage in COVID-19 infections [13].

In that regard, we intended to evaluate the association of IL-6 rs1800795, IL-17 rs2275913, and IL-37 rs3811046 SNPs and their serum levels with COVID-19 severity as predictors of disease prognosis. Our results indicated a significantly increasing trend in serum levels of IL-6 and IL-17, with decreasing levels of IL-37 across our groups. IL-6 rs1800795 variants were non-significant between patients and controls. However, IL-17 rs2275913 GG genotype (wild type) and G allele had a higher frequency in patients compared to controls, with higher levels of serum IL-17 than those reported with AA genotypes and alleles in all groups. Regarding IL-37 rs3811046 SNP, GT and TT variants (heterozygous and mutant) and T allele were more prevalent in patients than in controls and correlated with lower levels of serum IL-37 in the studied cases.

Although IL-6 rs1800795 variants did not significantly vary between patients and controls, serum IL-6 levels were significantly elevated in patients compared to healthy individuals, with increasing levels in severe cases. Similarly, increased IL-6 blood levels were reported in severe cases compared to non-severe ones, and increased IL-6 has been associated with bad consequences in cases with severe COVID-19 infection [14,15]. Elevated IL-6 levels have also been associated with increased mortality rates in COVID-19 infection [16]. IL-6 controls cathepsin L synthesis, which is needed by COVID-19 infection to affect epithelial cells and increase the expression of the angiotensin II receptors in cell entry [17,18]. An instant primary assessment of IL-6 was recommended on hospital admission of COVID-19 as it could predict disease progression [16]. Meanwhile, IL-6 rs1800795 polymorphism was analyzed in other studies of COVID-19 pneumonia and found to be correlated with the disease progression, with the CC variant giving significantly increased IL-6 levels among the studied patients [19]. In addition, the G allele of rs1800795 was described as a protective marker in pneumonia-induced sepsis. Moreover, this variant has been correlated with the clinical stage of the disease and has a critical impact on IL-6 levels [20].

Furthermore, IL-17 was reported to be elevated in intensive-care COVID-19 patients versus controls [21]. IL-17 was assessed in ARDS patients and associated with expanded alveolar neutrophils ratio and organ damage [22]. Earlier investigations have defined increased levels of IL-17 in severe COVID-19 patients compared to mild and moderate cases [23], which agrees with our finding of increased serum IL-17 in COVID-19 patients compared to controls and in severe cases versus mild and moderate cases. The T cells of patients with COVID-19 pneumonia showed an enhanced ability to generate IL-17, and increased IL-17A is allied with elevated proinflammatory biomarkers and apoptosis [24,25]. Regarding IL-17 rs2275913 polymorphism, the current analysis detailed the prevalence of the GG genotype in COVID-19 patients with higher levels of serum IL-

17. Meanwhile, the controls had a higher frequency of GA and AA genotypes with reduced IL-17 concentrations. An earlier study confirmed these findings and described an inverse relationship between mortality rates and prevalence of the AG genotype [22]. Moreover, IL-17 gene polymorphisms in ARDS showed elevated 30-day survival with genetic variants that had declined IL-17 levels, while genetic variants with increased IL-17 levels reported lower survival [26]. The increased IL-17 level aggravates LPS-induced generation of TNF, IL-1β, and IL-6, confirming the impact of IL-17 as a regulator of the inflammatory process [27].

The polymorphism of IL-37 rs3811046 on exon 2 is a missense mutation with amino acid substitution (Gly/Val) [28]. Our results revealed that GT and TT variants and T allele of IL-37 rs3811046 SNP were more prevalent in the patients' groups compared to the controls and reported lower levels of serum IL-37. The rs3811046 has not been studied much with disease association. Recently and in agreement with these findings, the TG genotype was reported to increase the risk of COVID-19 infection [29]. A previous study by Ahmed and Ad'hiah demonstrated reduced IL-37 concentrations in severe COVID-19 cases compared to controls, and reduced IL-37 levels were associated with increased disease risk [30].

IL-37 was identified as an anti-inflammatory cytokine with the ability to repress the generation of various proinflammatory cytokines, such as IL-1, IL-6, IL-17, and TNF-α [31]. Furthermore, Conti and colleagues in 2020 detailed the ability of IL-37 to suppress IL-1 members [32]. Moreover, IL-37 could increase the generation of transforming growth factor-β (TGF-β), which has immunosuppressive properties [33]. All might explain the elevated concentrations of the proinflammatory cytokines in severe COVID-19 cases with widespread lung injury [34]. The reduced IL-37 might result in augmented production of proinflammatory cytokines, which is a primary pathophysiological process of COVID-19 progression [30]. Circulatory IL-37 levels remarkably declined in patients with community-acquired pneumonia on admission, and these levels were steadily reduced analogous to the severity score [35]. The rs3811046 was investigated in other diseases; the rs3811046 G allele was significantly correlated with a declined risk of Graves' disease in a female, with T allele prevalence in patients compared to controls [27]. Additionally, rs3811064 showed a significant association with periodontitis [36].

We acknowledge that our study has some limitations. The cross-section nature of this study does not allow following up the same patients as they pass through different disease stages. The second limitation of our study is its small sample size and single center this needs larger studies to be done to validate our results.

Conclusion: Both GG genotype and G allele of IL-17 (rs2275913) and TT genotype and T allele of IL-37 (rs3811046) and their serum levels are potential risk factors for COVID-19 infection and severity, making them excellent disease management targets.

Abbreviations

ALT: Alanine transaminase.

AST: Aspartate aminotransferase.

CRP: C reactive protein.

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

COVID-19: coronavirus disease 2019.

CS: cytokine storm.

DM: diabetes mellitus.

HTN: hypertension.

HB: hemoglobin concentration.

INR: international normalized ratio.

LDH: lactate dehydrogenase.

IL: interleukin.

MERS-CoV: Middle East Respiratory Syndrome.

PT: prothrombin time.

PCR: polymerase chain reaction.

WBCs: white blood cells.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

This analysis was reported to the Local Research Ethical Committee of the Faculty of Medicine, Menoufia University (IRB TROP19-3).

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed significantly to the work reported, whether it be in the ideation, study design, implementation, data collection, analysis,

and interpretation, or all of these areas. They also participated in writing, revising, or critically evaluating the article, gave their final approval for the version to be published, decided on the journal to which the article has been submitted, and agreed to be responsible for all aspects of the work.

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