

Associations between IL-17A G/A-rs2275913 and IL 23R A/G-rs11209026 gene polymorphisms and severe coronavirus disease 2019 (COVID-19)

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Abstract

Severe COVID-19 disease was linked to a severe proinflammatory response and cytokine storm interleukin 17 (IL-17) is one of these cytokines, was associated with severe acute lung injury and multiorgan dysfunction. Single nucleotide polymorphisms (SNPs) in genes coding IL-17 can affect level of IL-17 hence its role in diseases. Also, SNPs in IL-23 R which control IL-23 is the main activator of IL-17 production. This study aimed to determine whether the IL-17A (G/A-rs2275913), IL-23R (A/G-rs11209026) SNPs and serum levels of IL-17 were related to the risk of severe COVID-19. This case-control study included 120 confirmed COVID-19 patients, divided into two categories according to the severity of the disease and 74 normal subjects as controls. COVID-19 patients were SARS-CoV-2 positive by a reverse transcription-polymerase chain reaction and subjected to full clinical examinations, routine laboratory tests, and radiographic evaluations. The IL-17 levels were assessed using ELISA method, and genotyping of IL-17A (197 A/G; rs2275913) and IL-23R rs11209026 (A/G) was performed by the TaqMan Genotyping Assay. There were no differences in the distribution of IL-17A or IL-23R genotypes between COVID-19 groups and the control group ($p=0.93$ and $p=0.84$, respectively). Severe COVID-19 patients had significantly higher IL-17 serum levels than non-severe COVID-19 ($p=0.0001$). The GG genotypes of IL-17A were significantly higher in severe COVID-19 patients ($p=0.004$). Multivariate logistic regression analysis revealed that AG, GG genotypes of IL-17 and IL-17A were independent predictors of COVID-19 disease severity ($p<0.0001$, $p=0.06$ and $p=0.04$, respectively). ROC curve analysis for IL-17, as predictor of severe COVID-19 disease revealed a sensitivity of 87.9% and specificity of 66.1% at a cutoff point of 114 pg/ml with AUC = 0.799. In conclusion, these findings indicated that IL-17 may be considered a marker of severe COVID-19. IL-17A SNPs may have a role in COVID-19 severity. IL-23R SNPs had no role in COVID-19.

Keywords: COVID-19, SARS-CoV-2, single-nucleotide polymorphism, IL-17A G/A-rs 2275913, IL-23R.

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Introduction

The severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) that causes the corona virus disease-2019 (COVID-19) was originally identified in Wuhan, China.¹ The disease rapidly spread throughout the globe, with a total of 410,565,868 confirmed cases and 5,810,880 deaths in early 2022.²

The majority of COVID-19 infections have mild-to-moderate symptoms. Individuals who have the disease's severe variant had greater levels of pro inflammatory cytokines, such as interleukin (IL)-1B, IL-6, IL-8, IL-17, and granulate-macrophage colony-stimulating factor (GM-CSF) compared to those with mild infections. This hypercytokinemia is responsible for the development of the cytokines storm syndrome in patients with severe COVID-19.^{3,4}

CD4-T-helper 17 (Th17) cells help the host to defend against extracellular infections by producing IL-17A and IL-17F and directing the migration of neutrophils and macrophages to the affected tissues. Thus, there is evidence that Th17 cells contribute to the pathogenesis of a number of inflammatory and autoimmune illnesses.⁵⁻⁷ These cells are stimulated by IL-6, IL-21, and IL-23, which are secreted by antigen-presenting cells (APCs).⁸

In comparison to moderate cases, severe COVID-19 individuals had greater serum levels of IL-17, according to recent research studies.^{3,9} Additionally, IL-17 was found to be associated with severe pulmonary inflammatory changes in patients with acute respiratory distress syndrome (ARDS).⁵ Influenza A virus subtype, H1N1, was previously discovered to produce acute lung damage that was IL-17-dependent. Studies suggested that blocking the action of this cytokine might reduce the incidence of SARS-CoV-2-related organ damage.^{10,11} Several polymorphisms that could alter the expression of IL-17, such as rs2275913 (G>A) located on the 6p12.1 chromosome in the IL-17 gene, were identified.¹²

IL-23 have essential function in maintaining the Th17 cells. The IL-23 receptor (IL-23R) comprises two subunits: the IL-23R subunit, which is expressed by the IL-23R gene, and the IL-12Rb1 subunit, which is expressed by the IL-

12Rb1 gene. A single-nucleotide polymorphism (SNP) called rs11209026 causes the substitution of Arg to Gln at position 381 in the IL-23R gene (IL-23R381Gln).¹³ Data suggested that this IL-23R variation influenced the serum cytokine concentrations of IL-17 and its associated pathway. Additionally, this SNP was linked to several autoimmune disorders, such as Crohn's disease,¹ psoriasis,¹⁵ and rheumatoid arthritis.^{16,17} The current study intended to determine whether the IL-23R (A/G rs11209026) and IL-17A (G/A-rs2275913) SNPs and serum levels of IL-17 were related to the risk of severe COVID-19.

Subjects and Methods

Study design

The current case-control study was carried out at the COVID-19 isolation hospital and the Medical Microbiology and Immunology Department in the Sohag University from March 2021 to July 2021. The study included 120 patients with COVID-19 who were hospitalized to the isolation hospital and 74 normal subjects as controls. The COVID-19 groups were subjected to complete clinical examinations, routine laboratory tests, and radiographic evaluations and their infection with SARS-CoV-2 was confirmed by the reverse transcription-polymerase chain reaction (RT-PCR).

Ethical consideration

The study protocol was reviewed and approved by the Medical Research Ethics Committee of the Faculty of Medicine, Sohag University (No. Soh-Med-21-04-35, dated April 2021). An informed consent was taken from each participant before enrolled in the study.

Study participants were subjected to full history taking and routine laboratory testing at the hospital laboratory, according to their routine protocols. Complete blood count (CBC) by (Genius, KT 6200, China), Random blood sugar by (Fia Biomed, Germany), Liver function tests: ALT, AST, Serum C-reactive protein, and serum creatinine by COBAS C 311 (Roche Diagnostics International Limited, Switzerland), serum ferritin by mini-VIDAS system kit

(Biomerieux, USA) and D-dimer (Wondfo Finecare FIA Meter, India). Patients' data were obtained from hospital records. All such information for the study groups were collected and recorded in a data sheet.

Depending on the severity of the disease, the COVID-19 patients were divided into two categories, according to the Egyptian protocol of COVID-19, which was based on symptoms, clinical examination, and chest radiography. Severe COVID-19 patients were presented with a respiratory rate of more than 30 times per minute, a room oxygen saturation of <93 at rest, and a chest computed tomography scan showing at least 50% lung involvement or progressive lung lesions within 24 to 48h from the first computerized tomography (CT) on admission^[18,19].

IL-17 assay

A blood sample (5 ml) was collected from each study participant in a plain tube and centrifuged at 3070 xg for 20 min. The serum was collected from the upper part of the tube, transferred to a 1.5 ml Eppendorf tube, and stored at -20°C until used. Subsequently, serum IL-17 was assessed using a commercial enzyme-linked immunosorbent assay (ELISA) kits (Sinogeneclon Biotech, Co., Ltd, China), according to the manufacturer's instructions.

Genotyping

For extraction of DNA, a peripheral blood sample was collected in tubes containing an anticoagulant (ethylene-diamine-tetra-acetic acid). Genomic DNA was isolated from the mono nuclear cell layer's using a commercial kit (QIAamp DNA Blood Mini Kit, QIAGEN, Germany), according to the manufacturer's instructions. DNA was kept at 20°C until used for SNPs.

SNPs within IL-17A (197 A/G; rs2275913) and IL-23R rs11209026 (A/G) were detected by TaqMan Genotyping Assays (C_15879983_10 and C_1272298_10, supplied by Applied Biosystems, USA) according to the manufacturer's instructions. The reaction volume (25µL) contained 12.5 µL of the TaqMan Universal PCR Master Mix (2X) and 1.25 µL of the TaqMan Gene Expression Assay (20X);

DNase-free water (7.25 µLs) and 4 µLs of the extracted genomics DNA. The tests were performed using an RT-PCR machine (Step One Real-time PCR system, Applied Biosystems, USA). The following amplification protocol was used: 10 min at 95°C for AmpliTaq Gold enzyme activation followed by 45 cycles each of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and primer extension at 72°C for 5 min. After the PCR amplification step, the endpoint analysis for the allelic discrimination was performed using a RT-PCR system (Applied Biosystems 7500 Real-Time PCR System, ThermoFisher, USA). Plotting fluorescence (Rn) values, based on the signals from each well, was done by the Sequence Detection System (SDS) software using the fluorescence measurements received during the plate reading. The alleles present in each sample were identified by the displayed fluorescence signals.

Statistical analysis

The STATA statistical software (Stata Statistical Software: Release 14.2 College Station, TX: StataCorp LP.) was used for data analysis. The mean, standard deviation, median, and interquartile range were used to illustrate quantitative data. ANOVA was used to compare the means of the three groups and was followed by the Bonferroni post hoc test. When the data was not normally distributed, the Mann-Whitney test and Kruskal Wallis test were applied to compare two groups. The Chi square test was used to evaluate qualitative data that was given as numbers and percentages. The Hardy-Weinberg equilibrium (HWE) proportion tests were performed to analyze the genotype distributions. The receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic ability (the optimal cutoff, sensitivity, specificity, positive and negative predictive values) of studied parameters for severe COVID-19. Spearman's correlation tests were used for the correlation between IL-17 serum level and other laboratory findings in COVID-19 patients. From the analysis of logistic regression, odds ratios were determined. If the *p* value was less than 0.05, it was deemed significant.

Results

Patient characteristics

A total of 120 patients with COVID-19 and 74 age and sex matched normal control subjects were included in the study. Of the study patients, 62 patients (51.7%) were diagnosed with non-severe COVID-19, and 58 (48.3%) with severe COVID-19. Patients with severe COVID-19 had significantly cardiac disorders and chronic liver diseases compared to those in the

non-severe COVID-19 category ($p=0.02$ and $p=0.003$, respectively) (Table 1).

Comparisons of the laboratory findings between groups revealed that the ALT and AST, and D-dimer levels, IL-17 serum level in patients with severe COVID-19 infection were significantly higher than those in the non-severe group (Table 1). Also, significant correlations were observed between the elevated serum levels of IL-17 and levels of Hb, serum ferritin, and random blood glucose (Table 2).

Table 1. Clinical and laboratory characteristics of the study group.

Variable	Controls N=74	Non-severe COVID-19 N=62	Severe COVID-19 N=58	<i>p</i> value	<i>p</i> ₁ value	<i>p</i> ₂ value	<i>p</i> ₃ value
Age/year							
Mean ± SD	56.23±10.32	56.63 ± 15.28	57.97±13.56	NS	NS	NS	NS
Median	59 (46:59)	58 (45:58)	62 (48:62)				
(IOR)							
Gender							
Female	38 (51.35%)	28 (45.16%)	20 (34.48%)	NS	NS	NS	NS
Male	36 (48.65%)	34 (54.84%)	38 (65.52%)				
Comorbid conditions							
DM		24 (38.71%)	30 (51.72%)				
Hypertension		28 (45.16%)	34 (58.62%)				NS
Chest disorders		12 (19.35%)	12 (20.69%)				NS
Cardiac disorders		10 (16.13%)	20 (34.48%)				0.02
Chronic liver disease		2 (3.23%)	12 (20.69%)				0.003
Lab finding							
WBCS (10 ⁹ /l)							
Mean ± SD	7.77±2.22	12.47±5.68	13.82±5.37	0.0001	0.0001	0.0001	NS
Median	7.5 (6:7.5)	11.9	15.3 (8.1:15.3)				
(IOR)		(6.6:11.9)					
Lymphocyte (%)							
Mean ± SD	24.53±7.74	39.76±23.37	35.11±27.23	0.01	0.001	NS	NS
Median	22 (19:22)	39.65	33.9 (7:33.9)				
(IOR)		(15:39.6)					
Hemoglobin (g/dl)							
Mean ± SD	11.86±1.13	12.07±1.63	12.74±2.19	0.01	NS	0.01	NS
Median	11.8	12 (11:12)	12.6 (11.2:12.6)				
(IOR)	(11:11.8)						
Platelets(10 ³ /μl)							
Mean ± SD	263.46±69.35	265.76±114.17	242.13±80.69	NS	NS	NS	NS
Median	250	212 (188:212)	247 (197:247)				
(IOR)	(210:250)						
ALT (U/L)							
Mean ± SD	8.37±1.16	31.63±17.73	83.10±175.65	0.0001	0.0001	0.0001	0.0001
Median	8 (7:8)	25 (21:25)	42.5 (30:)				
(IOR)							

Table 1. Continued.

Variable	Controls N=74	Non-severe COVID-19 N=62	Severe COVID-19 N=58	<i>p</i> value	<i>p</i> ₁ value	<i>p</i> ₂ value	<i>p</i> ₃ value
AST (U/L)							
Mean ± SD	8.03±5.50	36.94±26.78	93.97±122.50	0.0001	0.0001	0.0001	0.0001
Median (IOR)	6 (5:6)	26 (19:26)	60 (29:60)				
CRP (mg/dl)							
Mean ± SD	2.19±1.36	25.91±22.01	34.18±30.37	0.0001	0.0001	0.0001	NS
Median (IOR)	2 (1:2)	19.4 (12:19.4)	24 (12.6:24)				
Ferritin (ng/ml)							
Mean ± SD	75.19±26.14	180.43±183.12	626.06±1596.20	0.0001	0.0001	0.0001	0.01
Median (IOR)	78 (54:78)	116 (74:116)	202.4 (86:202.4)				
D-dimer (ng/ml)							
Mean ± SD	29.52±58.49	617.40±326.25	581.53±820.40	0.0001	0.0001	0.0001	NS
Median (IOR)	0.59 (0.4:0.59)	611 (480:611)	526.5 (1.8:526.5)				
Random blood sugar(mg/dl)							
Mean ± SD	93.54±18.84	159.20±90.48	184.97±102.02	0.0001	0.0001	0.0001	NS
Median (IOR)	90 (80:90)	115 (100:115)	183 (120:183)				
Creatinine (mg/dl)							
Mean ± SD	0.59±0.13	2.27±6.23	1.24±0.45	0.0001	0.0001	0.0001	NS
Median (IOR)	0.6 (0.5:0.6)	1 (0.86:1)	1.1 (0.96:1.1)				
Serum level of IL-17(pg/ml)							
Mean ± SD	10.15±1.24	194.68±264.07	484.16±340.73	0.0001	0.0001	0.0001	0.0001
Median (IOR)	9.6 (9.6:9.6)	85.95 (56:85.95)	439.96 (160:439.96)				

p compared the three group, *p*₁ compared controls and non-severe cases, *p*₂ compared controls and severe case, *p*₃ compared non-severe and severe cases. *P* > 0.05 is not significant (NS).

Table 2. Correlation between IL-17 serum level and other laboratory findings in COVID-19 patients.

Variable	Spearman Correlation coefficient (<i>rho</i>)	<i>p</i> -value
WBCS	-0.01	NS
Hemoglobin	0.23	0.01
PLTs	-0.01	NS
ALT	0.14	NS
AST	0.16	NS
Random blood sugar	0.23	0.01
D-dimer	-0.08	NS
Ferritin	0.31	0.001
CRP	0.15	NS
Creatinine	0.16	NS

P > 0.05 is not significant (NS).

There was no significant difference in the distribution of IL-17 and IL-23R genotypes between severe and non-severe COVID-19 and control groups. Also, application of HWE did not

result in deviation of genotype distribution in severe and non-severe COVID-19 patients and controls (Table 3).

Table 3. Genotypic distribution and allele frequency of IL-17A polymorphisms and IL-23R polymorphism in the COVID-19 patients and control groups.

Genotype	COVID-19 patients		Controls		<i>p</i> *
	No. (%)	<i>P</i> _{HWE}	No. (%)	<i>P</i> _{HWE}	
IL-17A					
AA	33 (27.50%)	NS	22 (29.73%)	NS	NS
AG	58 (48.33%)		34 (45.95%)		
GG	29 (24.17%)		18 (24.32%)		
G allele vs. A allele	116 (48.33%) vs. 124 (51.67%)		70 (47.30%) vs. 78 (47.30%)		NS
IL-23R					
AA	27 (22.50%)	0.0002	10 (13.51%)	NS	NS
AG	37 (30.83%)		30 (40.54%)		
GG	56 (46.67%)		34 (45.95%)		
G allele vs. A allele	149 (62.08%) vs. 91 (0.38)		98 (0.66%) vs. 50 (33.78%)		NS

*p*_{HWE}: *p* value based on Hardy-Weinberg equilibrium test, *p** based on χ^2 , OR odds ratio, CI confidence interval, *P* > 0.05 is not significant (NS).

The GG genotype of IL-17 and the G allele were significantly higher in patients with severe COVID-19 infection compared to those with non-severe infections (*p*=0.004 and *p*=0.001,

respectively). However, no difference was observed in IL-23R genotype and alleles between the three groups (Table 4).

Table 4. Genotype distribution and allele frequency of IL-17A (rs2275913) and IL-23R (rs11209026) in the study groups.

Variable	Controls N=74	Non-severe COVID-19 N=62	Severe COVID-19 N=58	<i>p</i>	<i>p</i> 1	<i>p</i> 2	<i>p</i> 3
IL-17 A genotypes							
AA	22 (29.73%)	24 (38.7%)	9 (15.5%)	0.03	NS	NS	0.004
AG	34 (45.95%)	29 (46.8%)	29 (50%)				
GG	18 (24.32%)	9 (14.5%)	20 (34.5%)				
Allele							
A	78 (47.30%)	77 (62.10%)	47 (40.52%)	0.004	NS	0.049	0.001
G	70 (47.30%)	47 (37.90%)	69 (59.48%)				
IL-23R genotypes							
AA	10 (13.51%)	17 (27.4%)	10 (17.2%)	NS	NS	NS	NS
AG	30 (40.54%)	20 (32.3%)	17 (29.3%)				
GG	34 (45.95%)	25 (40.3%)	31(53.4%)				
Allele							
A	50 (33.78%)	54 (43.55%)	37 (31.90%)	NS	NS	NS	NS
G	98 (0.66%)	70 (56.45%)	79 (68.10%)				

p compared the three group, *P*₁ compared controls and non-severe cases, *P*₂ compared controls and severe case, *P*₃ compared non-severe and severe cases. *P* > 0.05 is not significant (NS).

Also, there was no difference in IL-17 serum levels among the COVID-19 patients with IL-17A (197 A/G; rs2275913) genotypes. However, there was a significant difference in the mean

IL-17 serum levels among the control group with different IL-17A (197 A/G; rs2275913) genotypes (Table 5).

Table 5. Serum level of IL-17 among the different genotypes of IL-17 and IL-23R.

Genotypes of IL-17	Serum level of IL-17 Mean ± SD, median (IOR)	p value
In controls		
Genotypes of IL-17		
AA	10.58±1.09, 10.8 (9.6:10.8)	0.04
AG	10.16±1.26, 9.6 (9.6:9.6)	
GG	9.6±1.23, 9.6 (8.4:9.6)	
In non-severe COVID-19		
Genotypes of IL-17		
AA	201.88±274, 103 (69.63:103.29)	NS
AG	186.17±283, 70 (52:70)	
GG	202.94±183.52, 67 (61.7:67)	
In severe COVID-19		
Genotypes of IL-17		
AA	415.16±392, 254 (200:254)	NS
AG	453.90±300, 439.96 (160:439.96)	
GG	559.07±375.31, 477.3 (240.88:477.3)	
Genotypes of IL-23	Serum level of IL-17 Mean ± SD, median (IOR)	p value
In controls		
Genotypes of IL-23		
AA	9.96±1.27, 9.6 (9.6:9.6)	NS
AG	10.36±1.20, 10.8 (9.6:10.8)	
GG	10.02±1.28, 9.6 (9.6:9.6)	
In non-severe COVID-19		
Genotypes of IL-23		
AA	197.47±293.14, 102 (70:102)	NS
AG	155.20±186.98, 70.5 (47.05:70.5)	
GG	224.37±300.10, 83 (61.7:83)	
In severe COVID-19		
Genotypes of IL-23		
AA	699.35±358.82, 673.7 (431.1:673.65)	NS
AG	379.52±245.41, 254 (200:254)	
GG	472.12±357.84, 379 (140.88:379)	

P > 0.05 is not significant (NS).

Uni-variate regression analysis indicated that 5 factors including: presence of hepatic problems, presence of cardiac problems, elevated serum ferritin level, elevated serum level of AST, high serum level of IL-17, IL-17A AG, GG genotypes were correlated with severe COVID-19. Multivariate logistic regression analysis revealed that elevated serum levels of AST, IL-17 and IL-17A (AG, GG) genotypes were independent

predictors of the severity of COVID-19 infections (Table 6). The ROC curve analysis was performed to determine the IL-17 serum level which best differentiate severe COVID-19 patients from non-severe COVID-19 patients, revealed a cutoff point more than 114 pg/ml with a sensitivity of 87.9% and specificity of 66.1%, Figure 1.

Table 6. Logistic regression analysis of factors that predict severity of COVID-19 infection.

Variable	Uni variate		Multivariate	
	OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Age/year	1.01 (0.98:1.03)	NS	0.995 (0.956:1.04)	NS
Males vs. females	1.56 (0.74:3.27)	NS	2.26 (0.71:7.23)	NS
Presence of cardiac problems	2.73 (1.15:6.51)	0.02	1.34 (0.35:5.08)	NS
Presence of hepatic problems	7.82 (1.67:36.70)	0.009	8.64 (1.19:62.89)	0.03
Serum ferritin	1.002 (1.00:1.003)	0.007	1.002 (0.999:1.005)	NS
ALT	1.03 (1.01:1.05)	0.001	0.998 (0.969:1.03)	NS
AST	1.03 (1.01:1.04)	<0.0001	1.02 (1.00:1.04)	0.03
D-dimer	0.999 (0.999:1.001)	NS	0.999 (0.998:1.001)	NS
RBS	1.00 (0.998:1.001)	NS	0.999 (0.993:1.006)	NS
IL-17 level	1.003 (1.001:1.005)	<0.0001	1.00 (1.001:1.005)	<0.0001
IL-17A				
AA	1		1	
AG	2.67 (1.06:6.71)	0.037	3.83 (0.93:15.84)	NS
GG	5.93 (1.98:17.77)	0.001	5.33 (1.09:26.02)	0.04
IL-23				
AA	1		1	
AG	1.45 (0.52:3.98)	NS	3.61 (0.74:17.63)	NS
GG	2.11 (0.82:5.41)		2.06 (0.46:9.20)	NS

P > 0.05 is not significant (NS).

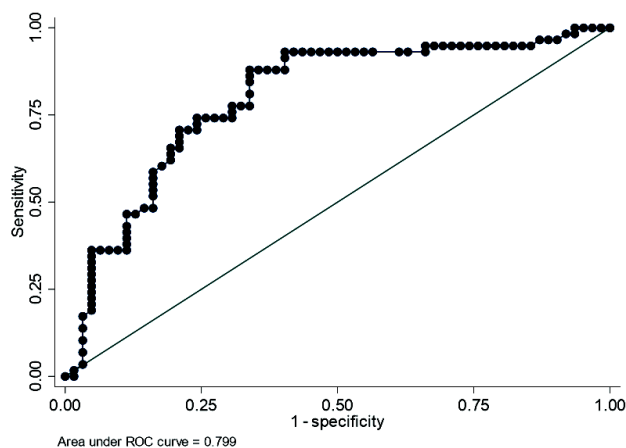


Figure 1. ROC curve analysis to evaluate the performance of Serum IL-17A in detecting severe COVID-19. The area under curve (AUC) is 0.799 (95% CI 0.716:0.866), *p*<0.0001, cutoff point>114 (sensitivity=87.9%, specificity=66.1%, PPV=70.8% NPP=85.4).

Discussion

In the present study, a group of 120 COVID-19 patients and 74 normal controls were recruited to investigate the role of the IL-17 serum level, IL-17A (G/A-rs2275913) and IL-23R (A/G rs11209026) SNPs in the severity of COVID-19 disease. The IL-17 was shown as an important proinflammatory cytokine that involved in several chronic inflammatory and autoimmune diseases.^{7,20,21}

In this study, severe COVID-19 patients had significantly higher serum levels of IL-17 than the non-severe COVID-19 patients. IL-17 is one of the several cytokines generated during corona virus infection. It is important for neutrophil activation and recruitment especially in the lungs, which lead to the development of severe COVID-19. Several previous studies reported elevated levels of IL-17 along with other cytokines in seriously ill COVID-19 patients.^{3,22,23} Additionally, IL-17 has been linked to ARDS, caused by other viruses such as influenza, the Middle East respiratory syndrome-coronavirus (MERS-coV)²⁴ and, Dengue virus.²⁵ Some studies suggested that blocking the IL-17 pathway could prevent the occurrence of fatal SARS in patients with corona virus infection.^{26,27} However, a recent study on COVID-19 patients reported no relation between IL-17 and disease severity.²⁸

In the present study, significantly high levels of AST, D-dimer, and serum ferritin were detected in the severe COVID-19 group compared to the non-severe group. They were reported as biomarkers of a severe inflammatory stage and are frequently associated with the establishment of a cytokine storm in severe COVID-19 infection.^{22,29,30} Furthermore, in the current study, serum levels of IL-17 were significantly correlated with low levels of Hb and high levels of AST, CRP, serum creatinine, serum ferritin, and random blood glucose.

In the present study, there was no difference in genotypes and allele frequencies of IL-17A (G/A-rs2275913) and IL-23R (A/G rs11209026) between COVID-19 and the controls. Such observation indicated that occurrence of COVID-19 infection was not affected by these SNPs. However, there was a link between the IL-

17A (G/A-rs2275913) genes and the SNPs. The homozygous AA variant was significantly related to non-severe COVID-19, whereas the GG variant was significantly associated with the severe form of the disease ($p = 0.004$). Similar outcomes were reported in a study which detected association between ARDS and IL-17 genotypes in Chinese population.²⁴ The individuals with the wild-type GG genotype of rs2275913 at IL-17A were presented with severe illness, but those with the AA-homozygous and GA-heterozygous genotypes were protected from developing ARDS. The authors suggested that the A allele decreased the biological activity of the genes, resulting in protection from the ARDS.²⁴ However, Rushdy et al., 2022 reported no relation between the IL-17A rs2275913 gene polymorphism and the severity of COVID-19 disease in a sample of Egyptian population.²⁸

IL-23 was reported to be essential for the maintenance of Th17 cells and promotion of IL-17-based immune responses, as a positive link between the serum levels of IL-17A and IL-23R was demonstrated.¹⁷ The IL-23R SNP rs11209026 (A/G) include the wild allele GG and the minor allele AA which results in a missense mutation. The minor allele of the IL-23R SNP rs11209026 (AA genotype) provides significant protection against Crohn's disease and inflammatory bowel disease.^{7,14}

In the current study, no relationships were detected between the IL-23R: A/G rs11209026 SNPs and the IL-17 serum level. This outcome does not match that of the study by Hazlett et al., 2012, wherein a decrease in the serum level of IL-17 was reported among A allele carriers of IL-23 (rs11209026).¹⁷

Also, in the present study, the IL-17 serum level was not different among the different genotypes of IL-17A G/A-rs2275913 in all the study groups. Similar results were reported by some previous studies,^{28,31,32} and it was concluded that the SNPs may not affect the gene expression but could make a conformational change that affect its function. However, ElBassuoni et al., 2015, reported elevated serum level of IL-17 in genotypes GG and GA among diseased group.³³ On limitation of the present study, is the small sample size.

Therefore, to corroborate the results of the current investigation, future studies including more patients are necessary.

In conclusion, findings of the current study suggested that the SNPs at the IL-17 A: G/A- rs 2275913 gene was associated with the severity of COVID-19 infection. The homozygous AA variant was significantly associated with the non-severe disease, whereas the GG variant was associated with severe COVID-19. Thus, the different genotypes of IL-17A G/A-rs 2275913 can be proposed as markers of the severity of COVID-19 infections. Patients with severe COVID-19 had significantly higher serum levels of IL-17 than those with non-severe COVID-19. Thus, serum levels of this cytokine can be used as a biomarker along with D-dimer, serum AST, and serum ferritin, to assess the severity of COVID-19 infection.

Author Contributions

AMG, reviewed the protocol, laboratory work, shared in writing and reviewing the manuscript. NSS, reviewed the protocol, laboratory work, shared in writing and reviewing the manuscript, submitted the manuscript as the corresponding author. MMA, reviewed the protocol, collected data, participated in clinical assessment of the patients, statistical analysis, shared in writing and reviewing the manuscript. AAA, EKA, MGM; approved the protocol, participated in clinical assessment of the patients, and reviewed the manuscript. SBH and MAY reviewed laboratory work, shared in writing, and reviewing the manuscript. MFS, supervised laboratory work, shared in writing and reviewing the manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Medical Research Ethics Committee of the

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Informed consent

An informed consent was taken from each participant before enrolled in the study.

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