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Interleukin-37 is down-regulated in serum of patients with severe coronavirus disease 2019 (COVID-19)

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ABSTRACT

Pro-inflammatory and anti-inflammatory cytokines are indicated to play a prominent role in mediating the immunopathogenesis of coronavirus disease 19 (COVID-19). Interleukin (IL-37) is one of the anti-inflammatory cytokines that has been proposed to be involved in disease progression but the data are not overwhelming. Therefore, a case-control study was performed to analyze IL-37 levels in serum of 100 patients with severe COVID-19 and 100 blood donors (control group). Median age was significantly higher in COVID-19 cases than in controls. Stratification by gender, body mass index and ABO and Rh blood group systems showed no significant differences between patients and controls. Chronic diseases (cardiovascular and diabetes) were observed in 57.0% of patients. Serum levels of IL-37 and vitamin D were significantly decreased in patients compared to controls. The low level of IL-37 was more pronounced in males, overweight/obese cases, blood group B or AB cases, Rh-positive cases, and cases with no chronic disease. Low producers of IL-37 were more likely to develop COVID-19 (odds ratio = 2.66; 95% confidence interval = 1.51–4.70; corrected probability = 0.015). Receiver operating characteristic curve analysis demonstrated that a low serum level of IL-37 was a good predictor of COVID-19. Spearman's rank correlation analysis showed that IL-37 and vitamin D were significantly correlated. In conclusion, IL-37 was down-regulated in serum of patients with severe COVID-19 compared to controls. This down-regulation may be associated with an increased risk of disease. Gender, body mass index, blood groups and chronic disease status may also affect IL-37 levels.

1. Introduction

Coronavirus disease 19 (COVID-19) is a recent respiratory infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Since the outbreak in Wuhan, China on 29 December 2019, the infection has become a pandemic, and so far (May 19, 2021), 222 countries have been affected by the disease with 165,346,216 confirmed cases, including 3,426,310 deaths (2.1%) [2]. There is no effective therapeutic protocol and vaccine strategies have not been well developed [3]. However, a number of risk factors have been linked to the development of COVID-19 and associated comorbidities, including age, gender, ABO blood group types, immune-related mediators and others [4]. Understanding these factors may reshape therapy and vaccine plans.

Cytokines are among the immune-related mediators that have been proposed to play a prominent role in mediating the pathogenesis of COVID-19 [5]. Cytokines are soluble low molecular weight factors produced by many types of cells with pleiotropic effects that influence and regulate various functional aspects of innate and adaptive immunity through pro-inflammatory and anti-inflammatory actions [6]. When SARS-CoV-2 infect the respiratory system, mild or severe acute respiratory syndrome can develop and be accompanied by dysregulated

Abbreviations: ALP, Alkaline phosphatase (ALP); ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; AUC, Area under the curve; BMI, Body mass index; BUN, Blood urea nitrogen; CI, Confidence interval; COVID-19, Coronavirus disease 19; ESR, Erythrocyte sedimentation rate; Hb, Hemoglobin; Hc, High producer control; Hp, High producer patient; IFN, Interferon; IL, Interleukin; IQR, Interquartile range; Lc, Low producer control; Lp, Low producer patient; OR, Odds ratio; *p*, Probability; *pc*, Corrected probability; RBG, Random blood glucose; ROC, Receiver operating characteristic; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.; SC, Serum cholesterol; SD, Standard deviation; SUA, Serum uric acid; TGF, Transforming growth factor; TNF, Tumor necrosis factor; WBC, White blood cell count.

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production of a number of cytokines, including interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IL-33, IL-25, IL-37 and IL-38 [7]. In this context, the cytokine storm phenomenon has been presented as a potential cause of acute respiratory distress syndrome and multiple organ failure in cases with severe COVID-19, although the evidence is not conclusive [8]. IL-37 is one such cytokine that has been proposed to have a role in immunopathogenesis of COVID-19 but the data in this regard are not overwhelming [9,10].

IL-37, formerly named IL-1F7, is a new member of the IL-1 family of cytokines. This family plays a critical role in mediating the activation of innate and adaptive immune responses [11]. Eleven members of the IL-1 family have been described (IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37 and IL-38), and their role in controlling inflammatory responses has been identified [12]. Various normal immune and non-immune cells and tissues express IL-37 including, stimulated B cells, natural killer cells, monocytes, epithelial cells, skin keratinocytes, thymus, lymph nodes, lung and bone marrow [13]. IL-37 is widely recognized as an anti-inflammatory cytokine that has inhibitory effects on inflammatory responses by affecting the release of pro-inflammatory cytokines [14]. Regarding this point, IL-37 has been considered a key player in a variety of inflammatory and autoimmune diseases (for instance, inflammatory respiratory diseases, inflammatory bowel diseases, rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, psoriasis and multiple sclerosis), and studies have shown dysregulated expression of IL-37 under these conditions [15,16]. Besides, IL-37 has been reported to have important role in immunity against various infectious agents, including viruses, bacteria and fungi [17]. In COVID-19 patients, a significant association between IL-37 plasma levels and a clinical prognosis has been recently been identified. Moreover, it has been indicated that a severe clinical prognosis can be predicted due to the absence of an IL-37-mediated response [10]. Additionally, Conte and colleagues recently proposed the therapeutic potential of IL-37 in COVID-19 [9]. In line with these proposals, this study sought to investigate serum level of IL-37 in severe cases of COVID-19 in order to determine the risk and predictive significance of this cytokine in disease progression. The correlation between IL-37 serum level and some demographic and laboratory characteristics of patients was also analyzed.

2. Subjects and methods

2.1. Populations studied

A case-control study was performed on 100 cases with severe COVID-19 and 100 healthy controls according to the guidelines of the Ethics Committee of the Iraqi Ministry of Health and Environment. Patients were admitted to Al-Karkh District hospitals in Baghdad during the period from October 1 to November 15, 2020, and were enrolled in the study 4–5 days after their admission to hospital. COVID-19 was molecularly diagnosed by detecting the virus RNA in nasal-pharyngeal swabs. The ExtractNow Virus RNA Swab Kit (Minerva Biolabs GmbH) was used to isolate the viral RNA, which was subjected to reverse real-time polymerase chain reaction (rRT-PCR) to detect the RNA of SARS-CoV-2 using the RealLine SARS-CoV-2 kit (Bioron Diagnostics GmbH), and instructions of manufacturers were followed. Chest computed tomography (CT) scan was also applied to confirm diagnosis. Only severe cases were included. The world health organization Interim Guidance was followed to define the case as a severe COVID-19 [18]. According to this guidance, if the patient suffered from one of the following conditions: severe respiratory distress, respiratory rate ≥30 breaths/minute and pulse oxygen saturation (SpO2) ≤ 93% on resting state, the case was considered to be in severe illness. Data regarding age, gender, body mass index (BMI), ABO and Rh blood group systems, chronic diseases (cardiovascular and diabetes), erythrocyte sedimentation rate (ESR), white blood cell count (WBC), hemoglobin (Hb), platelets, random blood glucose (RBG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum creatinine, blood urea nitrogen (BUN), serum uric acid (SUA), serum cholesterol (SC), triglycerides and vitamin D (25OHD3) were recorded for each patient. Data on age, gender, BMI, ABO and Rh blood group systems, ESR, RBG and vitamin D were also recorded in the controls (Table 1). The control group included blood donors, and their serum was negative for the panel of anti-infectious pathogen antibodies (including anti-COVID-19 IgG and IgM antibodies) at the Central Blood Bank (Baghdad).

2.2. Immunoassay of IL-37

An enzyme-linked immunosorbent assay (ELISA) kit was used to determine serum level of IL-37, and instructions of manufacturers were followed (Cat. No E1947Hu, Bioassay Technology Laboratory, China). The standard curve range of the kit was 7–400 ng/L, and the sensitivity was 4.56 ng/L.

2.3. Statistical analysis

Normality testing was applied to continuous variables (Kolmogorov-Smirnov and Shapiro-Wilk tests). Normally-distributed variables were given as mean ± standard deviation (SD). Skewed variables were given as median and interquartile range (IQR: 25–75%). Significant differences between medians were assessed using Mann–Whitney *U* test (to

Table 1
Baseline characteristics of COVID-19 cases and controls.

Characteristic		Cases; n = 100	Controls; n = 100	*p*-Value
Age; years		57.0 (49.0–67.0)	34.0 (27.0–39.5)	**<0.001**
Gender	Male	78 (78.0)	76 (76.0)	0.737
	Female	22 (22.0)	24 (24.0)	
Body mass index; kg/m^2	Normal weight	20 (20.0)	30 (30.0)	0.102
	Overweight/ obese	80 (80.0)	70 (70.0)	
ABO system	A	30 (30.0)	34 (34.0)	0.469
	B	25 (25.0)	26 (26.0)	
	AB	11 (11.0)	5 (5.0)	
	O	34 (34.0)	35 (35.0)	
Rh system	Positive	88 (88.0)	89 (89.0)	0.825
	Negative	12 (12.0)	11 (11.0)	
Chronic diseases	Cardiovascular	21 (21.0)	0 (0.0)	
	Diabetes	13 (13.0)	0 (0.0)	
	Cardiovascular/ diabetes	23 (23.0)	0 (0.0)	
	No disease	43 (43.0)	100 (100.0)	
Erythrocyte sedimentation rate; mm/hour		60.0 (40.0–116.0)	8.0 (5.0–21.0)	**<0.001**
White blood cell count × 10^9/L		12.6 ± 8.1	Not assessed	
Hemoglobin, g/dL		13.4 ± 2.5	Not assessed	
Platelets × 10^9/L		287.0 ± 125.0	Not assessed	
Random blood glucose; mg/dL		237.0 (149.5–462.6)	95.5 (87.0–108.5)	**<0.001**
Alanine aminotransferase; U/L		43.6 ± 30.8	Not assessed	
Aspartate aminotransferase; U/L		34.9 ± 17.3	Not assessed	
Alkaline phosphatase; IU/L		100.9 ± 72.7	Not assessed	
Serum creatinine; mg/dL		1.0 ± 0.5	Not assessed	
Blood urea nitrogen; mg/dL		57.8 ± 34.1	Not assessed	
Serum uric acid; mg/dL		5.2 ± 2.3	Not assessed	
Serum cholesterol; mg/dL		187.8 ± 107.3	Not assessed	
Triglycerides; mg/dL		178.6 ± 104.5	Not assessed	
Vitamin D (25OHD3); ng/mL		45.5 (33.7–63.7)	50.9 (46.9–56.6)	**<0.001**

Values were given as median followed by interquartile range (25–75%) in parentheses (non-parametric variable), mean ± standard deviation (parametric variable) or number followed by percentage in parentheses (categorical variable). *p*: Probability of Mann-Whitney *U* test (to compare non-parametric variables) or Pearson Chi-square test (to compare categorical variables). Significant *p*-value is indicated in bold.

compare two groups) or Kruskal-Wallis test (to compare more than two groups). Categorical variables were expressed as number and percentage frequency, and significant differences were assessed using Pearson Chi-square test. Patients and controls were classified into low (L) and high (H) producers according to the median of IL-37 (≤median and >median). Accordingly, a coding system was applied; Lp and Lc for low producer patients and controls, and Hp and Hc for high producer patients and controls, respectively. The four codes were tabulated to calculate odds ratio (OR) and 95% confidence interval (CI). The predictive significance of IL-37 in severity of COVID-19 was estimated by analyzing the receiver operating characteristic (ROC) curve, and results were expressed as area under the curve (AUC), cut-off value, sensitivity and specificity. Bivariate Spearman rank correlation test was used to analyze the correlation between variables. A probability (p) ≤ 0.05 was considered statistically significant. Bonferroni principle was applied to correct the p-value (pc). The statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was used to perform these analyses.

3. Results

3.1. Baseline characteristics

Median age was significantly higher in COVID-19 cases than in healthy controls (57.0 [IQR: 49.0–67.0] *vs.* 34.0 [IQR: 27.0–39.5] years; p-value <0.001). Stratification by gender (males and females), BMI (normal weight and overweight/obese), ABO blood group system (A, B, AB and O) and Rh blood group system (positive and negative) revealed no significant differences between patients and controls. Chronic diseases (cardiovascular, diabetes and cardiovascular/diabetes) were observed in 57.0% of COVID-19 cases (21.0, 13.0 and 23.0%, respectively). ESR and RBG were significantly elevated in cases compared to controls (60.0 [IQR: 40.0–116.0] *vs.* 8.0 [IQR: 5.0–21.0] mm/h; p-value <0.001 and 237.0 [IQR: 149.5–462.6] *vs.* 95.5 [IQR: 87.0–108.5] mg/dL; p-value <0.001, respectively), while serum level of vitamin D was significantly decreased (45.5 [IQR: 33.7–63.7] *vs.* 50.9 [IQR: 46.9–56.6] ng/mL; p-value <0.001). Other laboratory parameters (WBC, Hb, platelets, ALT, AST, ALP, serum creatinine, SUA and SC) were within the reference ranges. BUN (57.8 ± 34.1 mg/dL) and triglycerides (178.6 ± 104.5 mg/dL) were exceptions and their values were above the reference ranges (7–20 and <150 mg/dL, respectively) (Table 1).

3.2. IL-37 serum level

Median levels of IL-37 were significantly decreased in COVID-19 cases compared to controls (109.2 [IQR: 80.7–134.9] *vs.* 125.4 [IQR: 114.6–141.7] ng/L; p-value <0.001). This decrease was obvious in male cases (104.4 [IQR: 77.3–129.1] *vs.* 125.1 [IQR: 115.5–141.2] ng/L; pc-value < 0.001), overweight/obese cases (107.0 [IQR: 82.0–130.7] *vs.* 127.9 [IQR: 119.0–146.0] ng/L; pc-value < 0.001), cases with blood group B (104.1 [IQR: 72.2–120.1] *vs.* 132.6 [IQR: 121.4–151.7] ng/L; pc-value < 0.001) or AB (95.6 [IQR: 55.5–137.5] *vs.* 163.7 [IQR: 133.5–204.2] ng/L; pc-value = 0.018) and cases with Rh-positive blood group (107.0 [IQR: 78.7–130.7] *vs.* 125.4 [IQR: 113.7–142.0] ng/L; pc-value < 0.001) compared to the corresponding groups in controls. In the case of chronic diseases, the lowest levels of IL-37 were found in cases with diabetes and cases who had no chronic diseases, but the difference was no significant compared to other groups of chronic diseases (cardiovascular and cardiovascular/diabetes). However, the level of IL-37 was significantly decreased in cases with no chronic disease compared to controls (99.4 [IQR: 66.9–125.8] *vs.* 125.4 [IQR: 114.6–141.7] ng/L; p-value <0.001) (Table 2).

3.3. Estimation of OR and 95% CI

COVID-19 cases and controls were coded as low (≤median) and high (>Median) producers of IL-37 (Lp, Lc, Hp and Hc, respectively), and then the OR and its 95% CI were estimated. An increased risk of developing COVID-19 was recorded in low producers (OR = 2.66; 95% CI = 1.51–4.70; pc-value = 0.015). This increased risk was apparent in male cases (OR = 3.44; 95% CI = 1.78–6.64; pc-value = 0.005), overweight/obese cases (OR = 3.69; 95% CI = 1.88–7.25; pc-value = 0.002), cases with blood group B (OR = 8.60; 95% CI = 2.49–29.62; pc-value = 0.015), cases with Rh-positive blood group (OR = 2.70; 95% CI = 1.47–4.93; pc-value = 0.03) and cases with no chronic disease (OR = 4.21; 95% CI = 1.95–9.12; pc-value = 0.003) (Table 3).

Table 2
Median levels of IL-37 in serum of COVID-19 cases and controls.

Characteristic		IL-37 median (IQR: 25% − 75%); ng/L		p-Value (pc)
		Cases; n = 100	Controls; n = 100	
All cases *versus* controls		109.2 (80.7–134.9)	125.4 (114.6–141.7)	**<0.001**
Gender	Male	104.4 (77.3–129.1)	125.1 (115.5–141.2)	**<0.001** **(<0.001)**
	Female	126.5 (103.0–144.0)	130.0 (108.4–143.4)	0.416 (1.0)
	p-value (pc)	**0.044** (0.176)	0.943 (1.0)	
Body mass index; kg/m^2	Normal weight	112.6 (64.4–144.6)	119.9 (101.2–137.5)	0.338 (1.0)
	Overweight/ obese	107.0 (82.0–130.7)	127.9 (119.0–146.0)	**<0.001** **(<0.001)**
	p-value (pc)	0.771 (1.0)	0.063 (0.252)	
ABO system	A	112.3 (66.9–158.9)	124.7 (112.5–139.0)	0.118 (0.708)
	B	104.1 (72.2–120.1)	132.6 (121.4–151.7)	**<0.001** **(<0.001)**
	AB	95.6 (55.5–137.5)	163.7 (133.5–204.2)	**0.003** **(0.018)**
	O	114.7 (92.7–133.2)	120.6 (109.8–140.9)	0.338 (1.0)
	p-value (pc)	0.469 (1.0)	**0.016** (0.096)	
Rh system	Positive	107.0 (78.7–130.7)	125.4 (113.7–142.0)	**<0.001** **(<0.001)**
	Negative	114.7 (93.0–164.2)	130.2 (119.2–137.6)	0.353 (1.0)
	p-value (pc)	0.287 (1.0)	0.809 (1.0)	
Chronic diseases	Cardiovascular	121.5 (92.7–129.1)	125.4 (114.6–141.7)	0.094 (0.47)
	Diabetes	99.0 (77.3–137.5)		**0.012** (0.06)
	Cardiovascular/ diabetes	120.1 (93.2–172.2)		0.316 (1.0)
	No disease	99.4 (66.9–125.8)		**<0.001** **(<0.001)**
	p-value	0.085 (0.425)		

IQR: Interquartile range; p: Probability of Mann-Whitney U test (to compare two groups) or Kruskal-Wallis test (to compare more than two groups); pc: The probability value is adjusted by the Bonferroni correction for multiple tests. Significant p-value is indicated in bold.

3.4. ROC curve analysis

ROC curve analysis demonstrated that the decreased serum level of IL-37 was a predictor COVID-19 with an AUC of 0.678 (95% CI = 0.600–0.757; p-value <0.001). At a cut-off value = 121.5 ng/L, the sensitivity and specificity of IL-37 were 62.0 and 62.5%, respectively. To exclude effects of chronic diseases, the ROC curve was reanalyzed to include only patients without chronic diseases. This time, the AUC was increased to 0.753 (95% CI = 0.657–0.848; p-value <0.001), and at a cut-off value of 118.4 ng/L, the sensitivity and specificity of IL-37 were 69.0% (Fig. 1).

Table 3
Analysis of IL-37 levels (≤Median and >Median) in serum of COVID-19 cases and controls stratified according to some characteristics of subjects.

Characteristic	IL-37 level: Number (%)				OR (95% CI)	*p*-Value (*pc*)
	Cases; n = 100		Controls; n = 100			
	≤Median	>Median	≤Median	>Median		
All cases *versus* controls	62 (62.0)	38 (38.0)	38 (38.0)	62 (62.0)	2.66 (1.51–4.70)	**0.001 (0.015)**
Gender						
Male	53 (67.9)	25 (32.1)	29 (38.2)	47 (61.8)	3.44 (1.78–6.64)	**<0.001 (0.005)**
Female	9 (40.9)	13 (59.1)	9 (37.5)	15 (62.5)	1.15 (0.36–3.72)	1.0 (1.0)
Body mass index						
Normal weight	13 (65.0)	7 (35.0)	17 (56.7)	13 (43.3)	1.42 (0.45–4.45)	0.769 (1.0)
Overweight/obese	49 (61.2)	31 (38.8)	21 (30.0)	49 (70.0)	3.69 (1.88–7.25)	**<0.001 (0.002)**
ABO system						
A	16 (53.3)	14 (46.7)	13 (38.2)	21 61.8)	1.85 (0.69–4.92)	0.315 (1.0)
B	19 (76.0)	6 (12.0)	7 (26.9)	19 (73.1)	8.60 (2.49–29.62)	**0.001 (0.015)**
AB	8 (72.7)	3 (27.3)	0 (0.0)	5 (100.0)	26.71 (1.47 to 485.08)	**0.026** (0.39)
O	19 (55.9)	15 (44.1)	18 (51.4)	17 (48.6)	4.25 (1.23–14.74)	0.811 (1.0)
Rh system						
Positive	55 (62.5)	33 (37.5)	34 (38.2)	55 (61.8)	2.70 (1.47–4.93)	**0.002** (**0.03**)
Negative	7 (58.3)	5 (41.7)	4 (36.4)	7 (63.6)	2.45 (0.49–12.21)	0.414 (1.0)
Chronic diseases						
Cardiovascular	10 (47.6)	11 (52.4)	38 (38.0)	62 (62.0)	1.84 (0.59–3.75)	0.466 (1.0)
Diabetes	9 (69.2)	4 (30.8)			3.67 (1.10–12.20)	**0.039** (0.585)
Cardiovascular/diabetes	12 (52.2)	11 (47.8)			1.78 (0.73–4.36)	0.244 (1.0)
No disease	31 (72.1)	12 (27.9)			4.21 (1.95–9.12)	**<0.001** (**0.003**)

OR: Odds ratio; CI: Confidence interval; *p*: Two-tailed Fisher exact probability; *pc*: The probability value is adjusted by the Bonferroni correction for multiple tests. Significant *p*-value is indicated in bold.

3.5. Spearman rank correlation analysis

Correlation analysis (Spearman's rank correlation) was performed between IL-37 serum level and the continuous variables presented in Table 1. Only the level of vitamin D was significantly correlated with the level of IL-37 (correlation coefficient = 0.611; *p*-value <0.001) (Fig. 2).

4. Discussion

The current sample of COVID-19 patients was initially analyzed for the characteristics presented in Table 1 to determine whether or not they are related to the risk of developing the disease. It was found that most patients were 50 years of age or older. In keeping with this observation, it was evident that the elderly were more likely to contract COVID-19, compared to younger adults, and to progress to more severe disease with an unfavorable prognosis [19]. Animal model studies also demonstrated that viral replication of SARS coronavirus was enhanced in experimentally infected aged mice, compared to younger mice, and associated with alveolar damage, pneumonia, and clinical disease [20]. This may indicate that age is a critical risk factor for the development of COVID 19 and progression to severe disease. Two explanations could be taken to explain the age-related consequences of COVID-19. First, aging is associated with quantitative and qualitative changes in the functions of the immune system; for instance, production of B and T cells in primary lymphoid organs is reduced and mature lymphocytes in secondary lymphoid tissues show declined function [21]. These changes not only influence susceptibility to COVID-19 but also disease progression and clinical outcome thereafter [22]. Second, the elderly tend to have an increased prevalence of chronic diseases (for example, diabetes and cardiovascular disease) worldwide [23]. Regarding this point, it was observed that more than 50% of the current patients had diabetes, cardiovascular disease, or both. Besides, RBG level was significantly increased in patients compared to controls, and serum levels of BUN and triglycerides exceeded the reference ranges. Evidence has been clearly demonstrated that people with comorbidities such as diabetes, cardiovascular disease, and nephropathy are susceptible to SARS-CoV-2 and are more likely to develop an acute course of COVID-19 [24]. It was also observed that 80% of COVID-19 patients were overweight/obese. Overweight and obesity are major known risk factors for the development of diabetes and cardiovascular disease [25]. It has become evident that these conditions (diabetes, cardiovascular disease and obesity) make individuals more susceptible to develop severe COVID-19 with increased morbidity and mortality [26]. This is due to the fact that these conditions are accompanied by several biological features (changes in immunological functions, athero-thrombotic state, chronic inflammatory state and accumulation of advanced glycation end products) that may complicate the clinical profile of SARS-CoV-2 infection [27]. This study also revealed that COVID-19 patients showed significantly lower levels of vitamin D compared to controls. In line with this finding, a meta-analysis of ten studies indicated that low levels of vitamin D may increase the risk of developing COVID-19 [28]. It is important to note that low levels of vitamin D are associated with diabetes, cardiovascular disease and obesity [29]. Therefore, these factors may act synergistically in determining the risk of developing COVID-19 infection, severity of COVID-19, and mortality from COVID-19.

In addition to the aforementioned risk factors, the study analyzed serum level of IL-37 in severe cases of COVID-19 to predict the significance of this cytokine in disease. IL-37 was significantly down-regulated in serum of patients compared to controls. A 2.66-fold increased risk of developing COVID-19 was associated with this down-regulation. IL-37 acts as anti-inflammatory cytokine, and through this mode of action, it inhibits the production of several constitutive or induced pro-inflammatory cytokines, such as IL-1α, IL-1β, IL-6, IL-17, IL-23, tumor necrosis factor (TNF)-α and interferon (IFN)-γ [14]. IL-37 can also increase the production of transforming growth factor (TGF)-β, which is a cytokine with immunosuppressive effects [30]. There is accumulating evidence indicating that COVID-19 is associated with cytokine release syndrome in cases with severe disease, and high levels of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6 and TNF-α) were reported in patients with pulmonary inflammation and extensive lung damage [5,31]. Therefore, low levels of IL-37 may consequence in up-regulation of pro-inflammatory cytokines, which in turn can contribute to immunopathogenesis of COVID-19. Two recent investigations proposed for the first time that pro-inflammatory cytokines associated with inflammation in COVID-19, particularly of IL-1 family, maybe inhibited by the anti-inflammatory cytokine IL-37 [9,32]. Later, Li and colleagues analyzed plasma levels of IL-37 in patients with COVID-19 prior to any treatment intervention, and found significantly elevated levels. But when IL-37 was analyzed in severe cases of COVID-19, a very low initial level was recorded. ROC curve analysis revealed 100% sensitivity for IL-

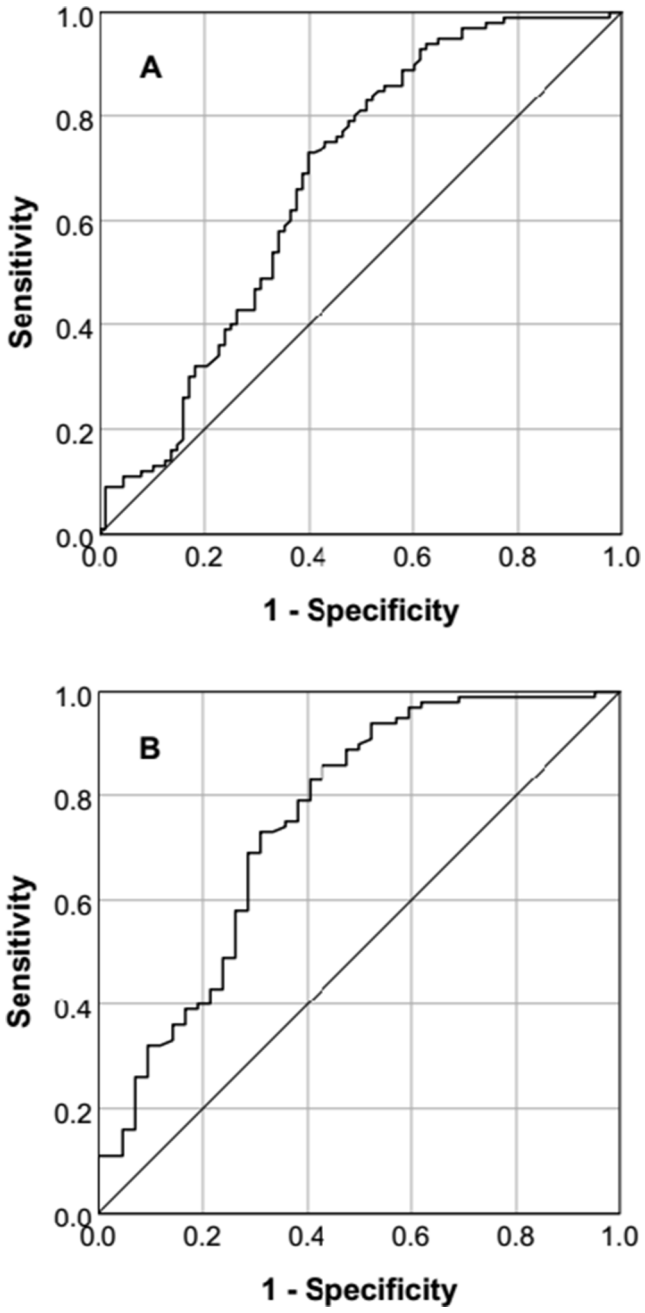

Fig. 1. Receiver operating characteristic (ROC) curve plots of IL-37 for predicting COVID-19 showing area under the curve (AUC). (A) All patients (AUC = 0.678; 95% confidence interval = 0.600–0.757; *p*-value <0.001; Cut-off value = 121.5 ng/L; Sensitivity = 62.0%; Specificity = 62.5%); (B) Patients without chronic diseases (AUC = 0.753; 95% confidence interval = 0.657–0.848; *p*-value <0.001; Cut-off value = 118.4 ng/L; Sensitivity = 69.0%; Specificity = 69.0%).

37 in differentiating severe from moderate cases. The authors expanded their study and included administration of recombinant human IL-37 to transgenic mice experimentally infected with SARS-CoV-2. It was found that lung inflammation was attenuated and respiratory tissue damage was alleviated in these mice [10]. Together, these data indicate the therapeutic potential of IL-37 in COVID-19. In other respiratory diseases, it has been shown that IL-37 alleviated airway inflammation and remodeling (asthma) [33] and ameliorated pneumonia by attenuating the secretion of pro-inflammatory cytokines (H1N1infection) [34]. Further, IL-37 has been described as having down-regulating effects on

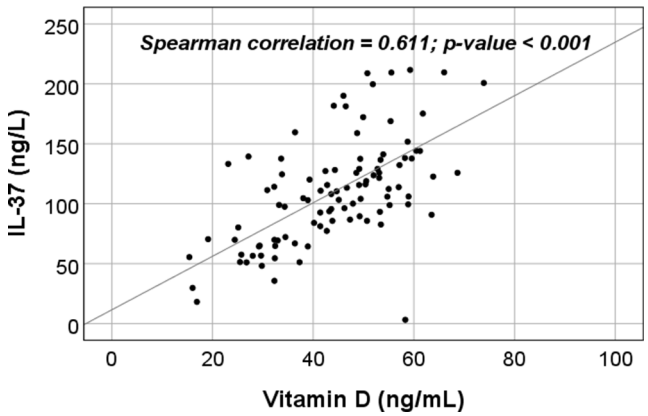

Fig. 2. Spearman rank correlation analysis between IL-37 and vitamin D in serum of COVID-19 patients.

the expression of pro-inflammatory cytokines to reduce inflammatory responses in several inflammatory diseases (ankylosing spondylitis, rheumatoid arthritis, systemic lupus erythematosus, and adult-onset Still's disease) [16,35].

The down-regulation of IL-37 was more pronounced in serum of male patients, overweight/obese patients, blood group B or AB patients and Rh-positive patients. There is no evidence to support or refute these results, but it appears that these groups of patients are more likely to have low levels of IL-37 in their serum, and thus may be more susceptible to severe COVID-19. It was also interesting to note that cases who had no chronic diseases showed low levels of IL-37. When the ROC curve was re-analyzed in these patients, a more predictive significance was obtained for IL-37 in COVID-19 compared to the analysis performed in all patients. The AUC was increased from 0.678 to 0.753, and better sensitivity and specificity were recorded (69.0%). Another point of interest was revealed by Spearman's rank correlation analysis that showed a strong positive correlation between IL-37 and vitamin D. In addition to their significantly low levels in serum of COVID-19 patients, both markers share the function of down-regulating pro-inflammatory cytokines, particularly IL-1, IL-6 and TNF-α, which are essential contributors to the uncontrolled systemic inflammatory response in COVID-19 patients [5,31,36]. Therefore, IL-37 and vitamin D are suggested to play key immunopathological roles in COVID-19 through synergistic effects. Besides, a simultaneous evaluation of the two markers may have predictive value for determining the clinical outcome of disease, as well as response to treatment. Accordingly, future analysis of pro-inflammatory and anti-inflammatory cytokines in cases with severe COVID-19 should take into account the presented factors (age, gender, BMI, blood groups, chronic disease status and vitamin D status) in order to gain a greater understanding of the role of these mediators in immunopathogenesis of COVID-19 and disease severity. It is important to note that only severe cases were included in this study, whereas moderate or critical patients were not included. This represented an important limitation of the study along with the small sample size of patients and controls examined.

5. Conclusions

IL-37 was down-regulated in serum of patients with severe COVID-19 compared to controls. This down-regulation may be associated with an increased risk of developing the disease. Gender, BMI, blood groups and chronic disease status may also influence the levels of IL-37.

CRediT authorship contribution statement

Aeshah A. Ahmed: Conceptualization, Visualization, Methodology, Investigation, Validation, Writing – review & editing. **Ali H. Ad'hiah:** Conceptualization, Visualization, Methodology, Investigation,

Supervision, Software, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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