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Cytokine profile and disease severity in patients with COVID-19

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ABSTRACT

Cytokine dysregulation is the proposed mechanism for Coronavirus disease 2019 (COVID-19). The aim of this study was to evaluate the serum levels of interferon (IFN)-γ, interleukin (IL)-5, IL-8, Il-9, IL-17, TGF-β and IFN-γ in patients infected with SARS-CoV-2. The study was conducted between 63 adult patients with COVID-19 and compared with 33 age and gender-matched healthy subjects as controls. The age range in both groups was 50–70 years. The patients were classified into mild group (33 patients) and severe group (30 patients). Serum samples were collected from all participants and tested for the cytokine levels by ELISA (enzyme-linked immunosorbent assay) method. Statistical analysis was performed using the one-way ANOVA. The mean serum levels of IFN-γ, TGF-β, IL-17 and IL-8 in the COVID-19 patients were significantly higher than those observed in the control group. A comparison of between the mild and severe groups showed significant differences in TGF-β levels. The mean concentration of serum IL-5 and IL-9 in patients with COVID-19 did not differ from those in the control group. Systemic IL-17 levels correlated positively and significantly with TGF-β in patients with COVID-19. Th1 (IFN-γ), Treg (TGF-β), and Th17 (IL-17) cytokines concentration were increased in COVID-19 patients. Interferon-γ and IL-17 are involved in inducing and mediating proinflammatory responses. Our data suggest that TGF-β can be used as a predictive factor of disease severity in patients with COVID-19.

1. Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1]. It was first identified in the Wuhan Province of China, in December 2019, and provoked a pandemic which is considered a life-threatening disease [2].

Acute respiratory distress syndrome (ARDS) can develop in patients with COVID-19. Pro-inflammatory cytokines play a central role in many respiratory viral infections by coordinating and activating the adaptive immune response and therefore are of great importance in the pathology of the disease [3]. When there is an uncontrolled anti-inflammatory response, it may lead to involvement of lung tissue in the course of the disease and ARDS and / or a systemic response to multiple organs.[4]. The balance between the different arms of the immune system can lead to disease clearance with minimal side effects, while imbalance can lead to tissue damage [5,6].

Higher serum levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-2 and IL-6) and chemokines (IL-8) have been observed in many patients with severe COVID-19 compared with individuals with mild disease [7,8]. Therefore, imbalance in the T-helper-cell (TH) subsets (TH1/TH2/TH17) and regulatory T-cells (Tregs) is suggested to contribute in the pathogenesis of COVID-19. CD4+ T cells are divided into different subtypes based on their cytokine production, including Th1 cells (producing IFN-γ, IL-2, and TNF-α), Th2 cells (IL-4, IL-5, IL-9, IL-13), Th17 cells (IL-17, IL-22), and Treg (TGF-β, IL-10) among others [9]. Because the respiratory system is integrated with circulatory system [10,11], the study measured the serum levels of TH1 (IFN-γ), TH2 (IL-5, IL-9), TH17 (IL-17) and Treg (TGF-β) cytokines in patients infected with SARS-CoV-2. The correlation of these cytokines with the disease severity was also shown.

2. Material and methods

2.1. Study design

Sixty-three adult patients with COVID-19 and thirty-three age and gender-matched healthy subjects as controls from the same

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geographical area (Arak city in Markazi province located in the central western part of Iran) and same geographical (west of Iran) and ethnic origin (Fars) participated in the study. The age range in both groups was 50–70 years.

All COVID-19 patients in this study were confirmed as SARS-CoV-2 positive by RT-PCR. Patients were grouped based on the severity of the disease. There were 21 (33.3%) patients with comorbidities, including diabetes, cardiovascular disease, malignancy and chronic lung disease. Compared to mild group, the proportion of comorbidities, were higher in severe group. COVID-19 mild group includes patients who showed oxygen saturations higher than 95% along the disease evolution and did not required ICU admission. A total of 33 patients were included in this group (17 females, 16 males). COVID-19 severe group included those patients with oxygen saturations lower than 93%, and arterial blood oxygen partial pressure (PaO2)/oxygen concentration (FiO2) ≤ 300 mm Hg and required intubation and admission to the ICU [12]. Number of patients in this group was 30 (14 females, 16 males). In severe group, treatment mainly includes Lopinavir/ritonavir (Kaletra), hydroxychloroquine and azithromycin. Thirty healthy individuals (15 female, 15 male), without a history of serious illness or infection during the past month, were used as control group. Five ml of blood was taken from each participant between 8 a.m. and 10 a.m. and serum separated by centrifuge and immediately stored at –80 °C until analysis. Blood samples were taken within 48 h after admission to the ICU for severe group and on the day of admission for mild group. The research was approved by the Research Ethics Committee of Arak University of Medical Sciences (AUMS).

2.2. Cytokines assay

ELISA kits for human IL-17 (sensitivity: < 1 pg/ml), IL-5 (sensitivity: < 1 pg/ml), IL-9 (sensitivity: 0.5 pg/mL) and TGF-β (sensitivity: < 1 pg/ml) were purchased from Peprotech (Rocky Hill, NJ, USA) and ELISA kits for human IFN-γ (sensitivity: ≤ 2 pg/mL) and IL-8 (sensitivity. 2.0 pg/mL) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Analyses were performed according to the manufacturers' instruction for each ELISA kit. Absorbance was read by StatFax-2100 microplate reader (Awareness Technology Inc., USA).

2.3. Statistical analysis

Data were analyzed in SPSS software (SPSS Inc, Chicago, Illinois, USA). The data were compared using one-way ANOVA and Tukey's post-hoc analysis. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of cytokines levels to identify with maximum sensitivity and specificity for detection of COVID-19 severity. Correlations were evaluated by calculating Pearson's correlation coefficients. All data is presented as the mean ± SEM. A p value of < 0.05 was considered statistically significant. Graphs were produced using GraphPad Prism 8 software.

3. Results

There was no significant difference between the patients and control groups concerning age and sex distribution. Demographic and clinical characteristics of the patients are presented in Table 1.

The mean level of IL-17 was significantly increased in mild cases compared to the severe group and control group (Fig. 1a). Also, there was no difference in IL-8, IL-17 and IFN-γ levels between severe and mild groups (Figs. 1a, 1b and 1e). IL-8, IL-17 and IFN-γ levels were significantly higher in mild group than in control group (Figs. 1a, 1b and 1e). The mean IL-5 and IL-9 levels were not significantly different between these three groups (Figs. 1c and 1d). IFN-γ was increased significantly in severe and mild groups compared to healthy control group (Fig. 1e). Severe group had significantly higher serum TGF-β concentrations than mild group (Fig. 1f).

Table 1
Demographic and clinical characteristics of COVID-19 patients.

	Total(n = 63) Number (%)	**Severe**(n = 30) Number (%)	**Mild**(n = 33) Number (%)
Age, median, years	62 (50 to 70)	66 (59 to 72)	60 (48 to 67)
Sex			
Male	32 (50.8)	16 (53)	16 (48)
Female	31 (49.2)	14 (47)	17 (52)
Comorbidity			
Hypertension	20 (31.7)	14 (46.7)	6 (18.2)
Diabetes mellitus	14 (22.2)	11 (36.7)	3 (9.1)
Cardiovascular disease	7 (11.1)	5 (16.7)	2 (6.1)
Chronic pulmonary disease	4 (6.3)	3 (10)	1 (3)
Malignancy	1 (1.6)	1 (3.3)	0 (0)
Other	8 (12.7)	5 (16.7)	3 (9.1)
Symptoms at admission			
Fever	40 (63.5)	24 (80)	16 (48.5)
Cough	33 (52.4)	18 (60)	15 (45.5)
Dyspnea	27 (42.8)	21 (70)	6 (18.2)
Fatigue	10 (15.9)	7 (23.3)	3 (9.1)
Smoker	14 (22.2)	8 (26.7)	6 (18.2)
Previous corticosteroid use	1 (1.6)	1 (3.3)	0 (0)
Need to mechanical ventilation	9 (14.3)	9 (30)	0 (0)

Receiver operator characteristic (ROC) curve analysis of cytokines to differentiate mild from severe COVID-19 shown on Table 2. The AUC of TGF-β and IL-8 which was used to predict the severity of COVID-19 were 0.653 and 0.652, respectively ($p < 0.05$). The optimum cut point of TGF-β and IL-8 were 134.4 pg/mL and 17.22 pg/mL, respectively.

ROC curve analysis for TGF-β and IL-8 was also shown on Fig. 2.

Our study revealed a positive correlation between TGF-β levels and IL-17 values in mild COVID-19 patients (Table 3).

4. Discussion

Cytokines and their receptors play a pivotal role in the pathogenesis of viral infections [13]. Serum concentrations of the proinflammatory cytokines are elevated in patients with sepsis. Some researchers have also suggested the role of cytokine storm in the severity of COVID-19 disease [14]. Higher serum levels of proinflammatory cytokines (TNF-α, IL-1, and IL-6) were observed in patients with severe COVID compared to those with mild disease, similar to the results in SARS and MERS [15,16]. This study was undertaken to evaluate serum levels of proinflammatory cytokines in patients with COVID-19.

Thl (T helper I) cells, NK (Natural killer cells) and CD8+ T cells are the main sources of IFN- γ [17]. The increase in IFN-γ production indicates a Th1 cells response in COVID-19 patients. Developing the Th1 response to eradicate viral infection is one of the immune system's strategies [18]. A strong IFN-γ response can lead to a better outcome in patients with COVID-19. By negatively regulating the production of Th2 cytokines, IFN-γ shifts the Thl/Th2 balance away from a Th2 response. An increase in Th2 cytokine level (IL-5 and IL-9) were observed in COVID-19 patient groups compared to controls, although the increase was not determined to be statistically significant [19,20].

The mean level of IL-17 was significantly increased in mild cases compared to the severe group and control group. Th17 cells are the major source of Interleukin 17 (IL-17). IL-17 stimulates the secretion of IL-8, which is a potent neutrophil chemoattractant. Therefore, IL-17 plays an important role in regulating neutrophil responses. Neutrophils are important in host defense against a number of pathogens. Most importantly, neutrophils have been shown to play a role in several immune system conditions, including acute lung damage [21].

Transforming growth factor β (TGF-β), a pleiotropic cytokine with potent regulatory and inflammatory activity, which is important for balancing the immune response [22], was increased significantly in patients with severe COVID-19. SARS-CoV-2 virus infection increases

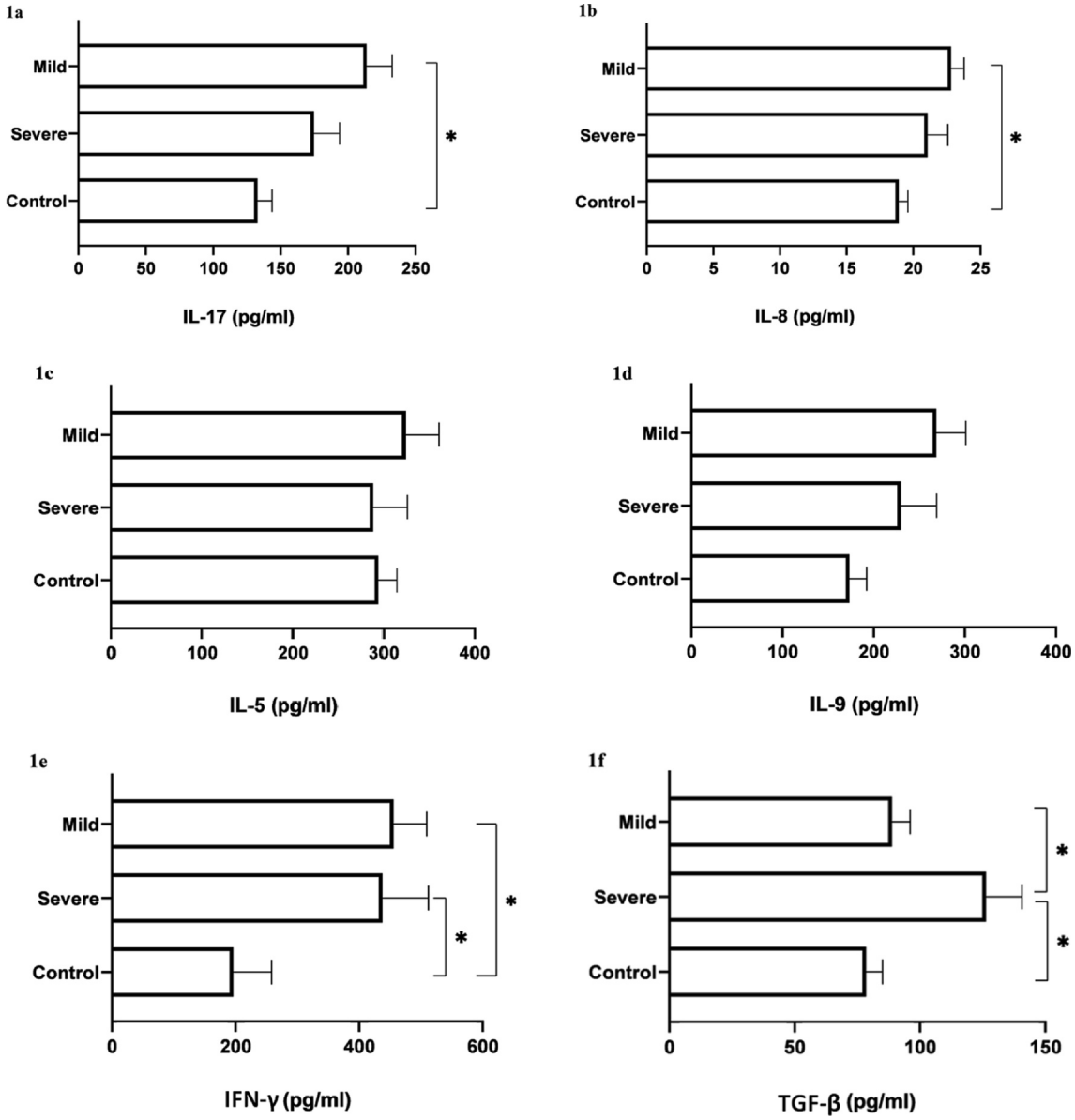

Fig. 1. Mean cytokine level changes in serum of different groups. Comparison of cytokines serum concentration of severe and mild groups with healthy controls. The data are given as mean (standard error of the mean). * $p < 0.05$.

Table 2
ROC curve analysis of cytokines to differentiate mild from severe COVID-19.

Variables	AUC	Sensitivity (%)	Specificity (%)	95% CI	p value
TGF-β (Cut off: 134.4 pg/mL)	0.653	35.48	88.24	0.512–0.786	0.034
IL-8 (Cut off:17.22 pg/mL)	0.652	43.75	88.24	0.516–0.787	0.034
IL-17 (Cut off: 224.1 pg/mL)	0.642	93.55	38.24	0.507–0.777	0.049
IL-5 (Cut off:246.3 pg/mL)	0.626	67.74	60.61	0.485–0.768	0.083
IL-9 (Cut off: 196 pg/mL)	0.610	60	67.86	0.453–0.767	0.170
IFN-γ (Cut off:357.9 pg/mL)	0.561	60	57.58	0.416–0.706	0.405

the infiltration of immune cells into the lungs, which can release TGF-β into patients' blood [23].

Researchers have shown that coronavirus infection leads to an increase in regulatory T cells. Treg cells affect the magnitude of immunity and outcome of viral infections. The ability of viruses to induce proliferation and activation of Treg cells contributes to delayed clearance and persistence in the host [24,25]. The SARS-CoV-2 is likely to use TGF-β as a means to dampen the immune response allowing for viral persistence. Some reports also suggest that TGF-β may suppress the generation of Th17 cells [26].

The AUC of TGF-β, IL-8, IL-17, IL-5 and IFN-γ were below 0.750, thus leading to poor predictive value. The positive correlation between TGF-β and IL-17 levels in mild COVID-19 patients suggests that the TGF-β-IL-17 axis is very important in these patients. TGF-β plays an important role in differentiation of CD4+ T cells toward Tregs or Th17 cells. Due to the largely anti-inflammatory and cell-cycle inhibitory

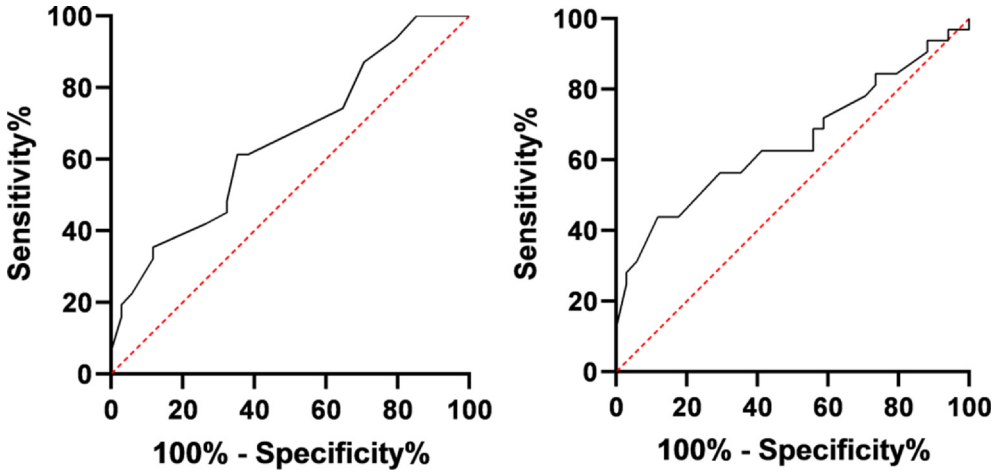

Fig. 2. ROC curves of TGF-β and IL-8 to predict the severe COVID-19.

Table 3
Correlation between TGF-β and IL-17.

Group	Severe	Mild	Control
Parameter	Interleukin-17, r		
TGF-β	0.320	0.494*	0.029

Correlation was performed by the Pearson analysis.
* $p < 0.05$.

nature of TGF-β, its role in Th17 cell differentiation remained controversial [27]. Because small sample sizes may affect statistical results, more research is necessary to explore these interactions.

5. Conclusions

Cytokines are thought to play an important role in immunity and immunopathology during SARS-CoV-2 infection, so that an increase in serum TGF-β levels in infected individuals predicts poor outcomes in patients with COVID-19. This study suggests that TGF-β can be used as a predictive factor of disease severity in patients with COVID-19. Therefore, measurement of cytokines may be an indicator of disease progression and provide more definitive approaches for treatment.

6. Authors' contributions

Ali Ghazavi designed the study, analyzed the data and revised the manuscript. Ali Ganji analyzed the results and revised the manuscript. Nafiseh Keshavarzian performed the ELISA. Somayeh Rabiemajd collected the samples based on the inclusion and exclusion criteria. Ghasem Mosayebi designed the study, wrote the manuscript, and revised it.

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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