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Cytokine Profiling in Iranian Patients with COVID-19; Association with Clinical Severity

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ABSTRACT

Background: SARS-CoV-2, the virus that causes coronavirus disease 2019 (COVID-19), is recognized for the first time in Wuhan, China. The cytokine storm is a known factor causing major clinical symptoms in COVID-19 patients leading to death.

Objective: To investigate and compare the serum levels of different cytokines in COVID-19 patients with different clinical severity.

Methods: Concentrations of serum cytokines, including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, and GM-CSF, were measured in 61 COVID-19 patients and 31 normal controls with ELISA. We investigated the correlation between the levels of these cytokines and clinical severity, CRP level, neutrophil and lymphocyte count in patients with COVID-19.

Results: Our data indicated that the levels of IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-γ, and GM-CSF, but not IL-10 were significantly increased in COVID-19 patients compared to normal controls. Statistical analysis showed that the level of IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-γ, and GM-CSF were higher in severe COVID-19 patients than those of mild cases. The concentrations of all mentioned cytokines were negatively associated with the absolute count of lymphocytes, and positively correlated with the CRP level and the absolute count of neutrophils.

Conclusion: The current study suggests that high levels of various cytokines correlate with the disease severity and immunopathogenesis of COVID-19.

Keywords: COVID-19, Cytokine storm, Disease severity, Iran

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INTRODUCTION

Over the last two decades, three novel β-coronaviruses were identified as the outbreak cause of viral pneumonia (1, 2). Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV-2), the virus that causes coronavirus infectious disease 2019 (COVID-19), was recognized for the first time in the city of Wuhan in China's Hubei province (3). The prevalence of COVID-19 started in December 2019 and then rapidly spread around the world, and now became an urgent public health concern (4). The prevalence of COVID-19 was announced as a pandemic on March 11, 2020 by World Health Organization (WHO), with a mortality rate of approximately 2.3% (5). Globally, SARS-CoV-2 has infected more than 16 million people at the time of submission of this article and claimed nearly 700,000 lives (6). It was on 19 February 2020 which the first Iranian COVID-19 case was confirmed and reported in Qom province. Until now, 286,523 confirmed cases were reported in Iran, and unfortunately over 15,000 patients died (6). The main clinical and laboratory characteristics of COVID-19 patients are fever, dry cough, fatigue, Chills, Muscle pain, Headache, Loss of taste or smell, Lymphopenia, elevated C-reactive protein (CRP), acute respiratory distress syndrome (ARDS), and pneumonia (1-3). Until now, there is no perfect therapy available for COVID-19, and therapeutic approaches for severe COVID-19 remain limited. However, several antiviral and immunosuppressive drugs are used in these patients, but no significant impacts have been reported on clinical outcomes (7, 8).

Innate arm of the host immune system, as the first line of antiviral immunity, is crucial for initial recognition of virus and triggering of the immune response (9). So far, our knowledge about the basic innate immune response to SARS-CoV-2 is limited. Generally, in the scenario of RNA viruses like SARS-CoV-2, these processes of immunity are triggered via the interaction of pattern recognition receptors (PRRs) of immune cells with viral pathogen-associated molecular patterns (PAMPs) like single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA) (10). Following activation of PRRs, downstream signaling pathways induce the production of various pro-inflammatory cytokines and chemokines (10, 11). Previous data about other coronaviruses have established the foundation for our knowledge regarding immune responses to these viruses (12, 13). Nevertheless, in many other ways, antiviral immunity against SARS-CoV-2 is different from those observed with other coronavirus infections (10, 14). So, it is necessary to explore the antiviral immunity against SARS-CoV-2 and hyper-inflammation mechanisms to better identify therapeutic approaches for COVID-19 patients. Primary evidences suggest that the critical outcome in SARS-CoV-2 infection often results from lung injury which are related with the excessive and prolonged cytokine and chemokine responses, also defined as the "cytokine storm" or "cytokine release syndrome" (15). Cytokine storm happens during the over-activation of the immune system by infection and other factors, resulting in release of various cytokines into the bloodstream, and finally extensive and harmful side effects on multiple organs (11, 16). It is widely assumed that the cytokine storm induced by SARS-CoV-2 infection is a key factor of lung damage, leading in ARDS observed in severe cases of COVID-19 patients (1, 15). In consequence, proper regulation of the cytokine storm in COVID-19 patients via immunomodulatory drugs and cytokine blockade by monoclonal antibodies, as well as reducing the infiltration of inflammatory cells in the lung, are crucial strategies to increase the success rate of the treatment and reduce the mortality rate of COVID-19 patients (10, 17).

In the current study, the serum levels of different immune cytokines were measured in COVID-19 patients and their correlation with their clinical symptoms and disease severity was evaluated. Furthermore, the

correlation between the concentration of different cytokines with the levels of CRP and absolute count of lymphocyte and neutrophil were also analyzed. We hope these findings will help to more identify the nature of host immune response against SARS-CoV-2 and immunopathogenesis of COVID-19 infection.

MATERIALS AND METHODS

Patients and Controls

In this study, 61 hospitalized COVID-19 patients (37 males and 24 females, mean age of 60.2 years (SEM: ±2.10)) were enrolled after admission to the Imam Khomeini Hospital affiliated to Mazandaran University of Medical Sciences. Thirty-one sex-and age-matched healthy volunteers (17 males and 14 females, mean age of 54.2 years (SEM: ±1.73)) were also enrolled in the study. The diagnosis of COVID-19 was performed based on the protocols introduced by the Iranian Ministry of Health and Medical Education applying a combination of laboratory findings, clinical symptoms, and CT-scan (14). All enrolled COVID-19 patients were positive for SARS-CoV-2 confirmed by Real-Time PCR (RT-PCR) assay. All hospitalized COVID-19 patients during a period of 1.5 months (April and May 2020) were enrolled in this study. The exclusion criteria for healthy control group was malignancy, active respiratory infection, severe systemic disease, infection to other microbial agents (tuberculosis, HIV, adenovirus infection, syphilis, flu infection, and other respiratory viral infections), and other chronic diseases including cardiovascular dysfunction and also lung, liver, renal and hematological disorders. We were not able to perform SARS-CoV-2 PCR test for our control group to exclude any possible asymptomatic patients. The patients were clinically classified into mild and severe groups. The following criteria was applied to define the severe patient: (1) pulse oximeter oxygen saturation (Spo2) ≤90% at rest; (2) breathing rate ≥24 times/min; (3) ratio of partial pressure of oxygen (PaO2) to fraction of inspired oxygen (FiO2) ≤300 mmHg; and (4) PaO2 <60 mmHg (14). The basic characteristics of mild (n=39), and severe (n=22) patients are shown in Table 1. Five milliliter of peripheral blood samples were collected centrifuged, and the resultant serum was frozen at -80 ° C until measurement of cytokines. Sampling from the patients group was performed at a maximum of 48 hours after hospitalization. Before sampling, written informed consent letters were obtained from all participants, and the Ethical Committee of Mazandaran University of Medical Sciences approved the study (IR.MAZUMS.REC. 1399.7302).

Laboratory Testing

Medical laboratory data were obtained by the clinical laboratory department of Imam Khomeini Hospital. The most updated results for laboratory findings were also collected during the hospital admission and disease progression. RT-PCR test was performed for all patients by the Health Center of Mazandaran University of Medical Sciences in Sari. Table 2 represents the laboratory findings in COVID-19 patients.

Cytokine Measurements

The level of various serum cytokines including Tumor Necrosis Factor-α (TNF-α), Interferon-γ (IFN-γ), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8 (chemokine (C-X-C motif) ligand 8 (CXCL8)), and IL-10 were measured by ELISA according to the manufacturer's instructions. IL-4 and IL-10 ELISA kits were from Invitrogen (Carlsbad, California, USA), IL-2 ELISA kit was from R&D Systems (Minneapolis, Minnesota, USA), and all others were from Sanquin (Amsterdam, The Netherlands). Finally, the absorbance of plate was recorded at 450 nm to 630 nm as reference wavelength with Multi-scan plate reader (Synergy H1 BioTek, Winooski, USA).

Table 1: Demographics and characteristics features of patients infected with COVID-19

	Number (%)			
	All patients (n=61)	**Mild (n=39)**	**Severe (n=22)**	**P value***
Characteristics				
Age, mean (SEM), Y	60.18 (2.10)	57.64 (2.53)	64.68 (3.60)	0.1
Sex				
Male	37 (60.6 %)	26 (66.6 %)	11 (50 %)	0.2
Female	24 (39.3 %)	13 (33.3%)	11 (50 %)	
Signs and symptoms				
Fever	54 (88.5 %)	32 (82 %)	22 (100 %)	0.04
Dry cough	44 (72.1 %)	28 (71.7 %)	16 (72.7 %)	0.99
Dyspnea	36 (59 %)	22 (56.4 %)	14 (63.6 %)	0.7
Fatigue	46 (75.4 %)	29 (74.3 %)	17 (77.2 %)	0.9
Myalgia	27 (44.2 %)	17 (43.5 %)	10 (45.4 %)	0.9
Sputum production	8 (13.1 %)	6 (15.3 %)	2 (9 %)	0.6
Haemoptysis	2 (3.2 %)	1 (2.5 %)	1 (4.5 %)	0.9
Anorexia	30 (49.1 %)	18 (46.1 %)	12 (54.5 %)	0.5
Pharyngalgia	13 (21.3 %)	8 (20.5 %)	5 (22.7 %)	0.9
Diarrhea	10 (16.3 %)	7 (17.9 %)	3 (13.6 %)	0.7
Nausea	9 (14.7 %)	6 (15.3 %)	3 (13.6 %)	0.9
Vomiting	5 (8.1 %)	3 (7.6 %)	2 (9 %)	0.9
Headache	14 (22.9 %)	10 (25.6 %)	4 (18.1 %)	0.7
Dizziness	14 (22.9 %)	8 (20.5 %)	6 (27.2 %)	0.5

*P values indicate differences between mild and severe patients. P<0.05 was considered significant. IQR: interquartile range; Y: year..

Table 2: Laboratory findings of patients infected with COVID-19

	Median (IQR)			
	All patients (n=61)	**Mild (n=39)**	**Severe (n=22)**	**P value***
WBC (10^3/µl)	6.3 (4.5-10.1)	6 (4.5-8.5)	7.1 (4.4-10.7)	0.3
Hb (g/dL)	11.4 (9.9-12.7)	11.7 (10-12.7)	11.1 (9.8-12.8)	0.2
PLT (10^3/µl)	199 (154-147)	214.5 (170-249.5)	178 (117.3-233.8)	0.04
Lymph (%)	10.4 (6.5-18.7)	16.5 (9.5-20.9)	6.3 (4.1-9.1)	<0.0001
Neut (%)	81.2 (70.9-88.8)	75.3 (67.4-82.5)	88.7 (84.1-92.1)	<0.0001
PT (Sec)	12 (12-13.9)	12 (12-13)	13.4 (12-15)	0.0004
PTT (Sec)	32 (27-38)	30 (26-35)	34.5 (28.7-40)	0.07
Albumin (g/dL)	3.4 (3.1-3.8)	3.6 (3.2-3.9)	3.1 (2.7-3.4)	0.0008
Total bilirubin (mg/dL)	1 (0.7-1.2)	0.8 (0.6-1)	1.2 (0.9-1.6)	<0.0001
LDH (IU/L)	535 (420-694)	489 (400-609)	631 (533-729)	0.006
BUN (mg/dL)	35 (26.5-60)	31 (25.5-51)	49.5 (29.2-79.2)	0.03
Cr (mg/dL)	1.2 (1-1.5)	1.1 (1-1.3)	1.4 (1.1-2.1)	0.07
ALT (U/L)	37 (28-57.5)	33 (26.2-42)	54 (28.7-67.7)	0.01
AST (U/L)	43.5 (33-65.2)	41.5 (29.7-50)	59.5 (35.7-75.2)	0.05
CRP (mg/dL)	65 (30.5-95.5)	41.3 (24-65)	95.5 (84.7-129.1)	< 0.0001
ESR (mm/h)	54 (35-64)	50 (29-62)	58 (48.2-76.5)	0.02
Serum ferritin (ng/mL)	745 (376.5-1142)	431 (332.3-761.5)	928 (806-1201)	0.08

*P values indicate differences between mild and severe patients. P<0.05 was considered significant. IQR: interquartile range; WBC: white blood cell; Hb: hemoglobin; PLT: platelet count; Lymph: lymphocyte; Neut: Neutrophil; PT: prothrombin time; PTT: partial thromboplastin time; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; Cr: creatinine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Statistical Analysis

GraphPad Prism version 8.0 software was used to statistical analyses and preparation of graphs (GraphPad Software, Inc., San Diego, CA, USA). After determining the data normality with Kolmogorov–Smirnov test, the parametric two-tailed Student's t-test was used to calculate the mean difference between the patients and control groups. Pearson correlation analysis was used to calculate the correlation coefficients. P-values less than 0.05 were considered significant.

RESULTS

Profiling of Serum Cytokines in COVID-19 Patients

Serum concentration of a variety of immune system cytokines including IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α, and GM-CSF was measured in all COVID-19 patients and normal controls. As expected and shown in Figure 1, our data indicated that COVID-19 patients produced higher levels of pro-inflammatory cytokines including IL-1β (P<0.0001), TNF-α (P<0.0001), IL-6 (P<0.0001), IL-2 (P=0.0001), IFN-γ (P=0.003), and GM-CSF (P=0.02) in comparison to normal controls. IL-4 as a Th2 cytokine was also found higher in COVID-19 patients than that of controls (P=0.004). Nevertheless, no significant difference was observed between the production level of inhibitory cytokine IL-10 by COVID-19 patients and normal controls (P=0.09). Additionally, the level of IL-8 (CXCL8) as neutrophil chemotactic factor was significantly increased in COVID-19 patients when compared with normal group (P=0.003).

Association of Cytokines Concentration with Clinical Severity of COVID-19 Patients

To further examine whether the cytokines profile is contributed to disease severity, we next analyzed and compared our results between mild and severe forms of SARS-CoV-2-infected patients (Figure 2). Our

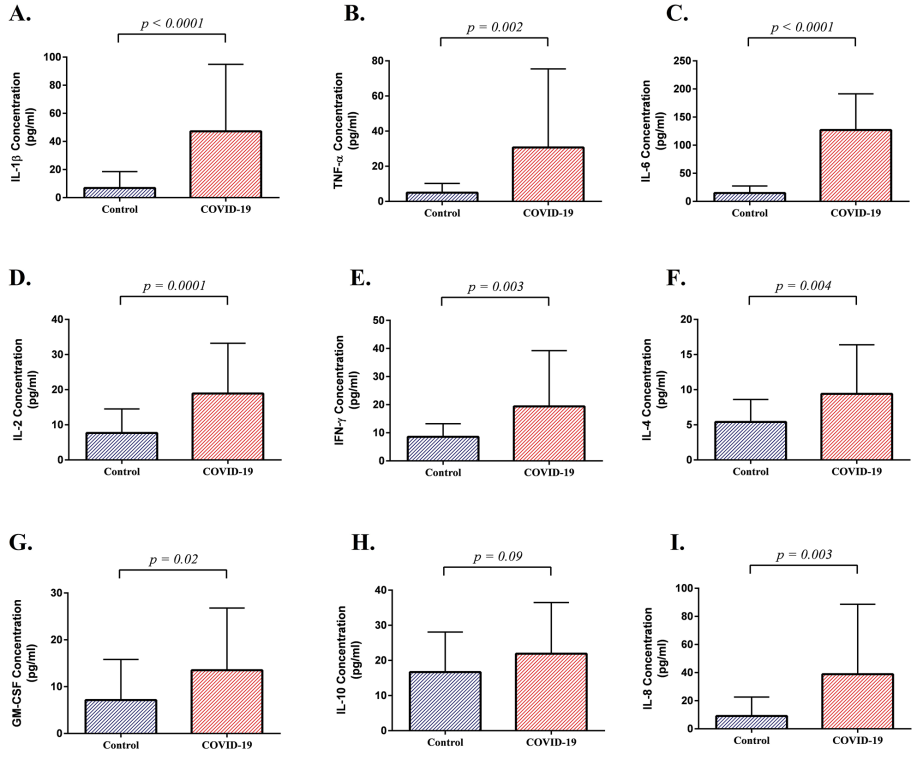

Figure 1: The serum concentration of various cytokines in COVID-19 patients and normal controls. The serum concentrations of IL-1β (A), TNF-α (B), IL-6 (C), IL-2 (D), IFN-γ (E), IL-4 (F), GM-CSF (G), IL-10 (H), and IL-8 (I) from patients with COVID-19 and controls were measured by ELISA. The results are expressed as the Mean±SD. P values < 0.05 were considered significant.

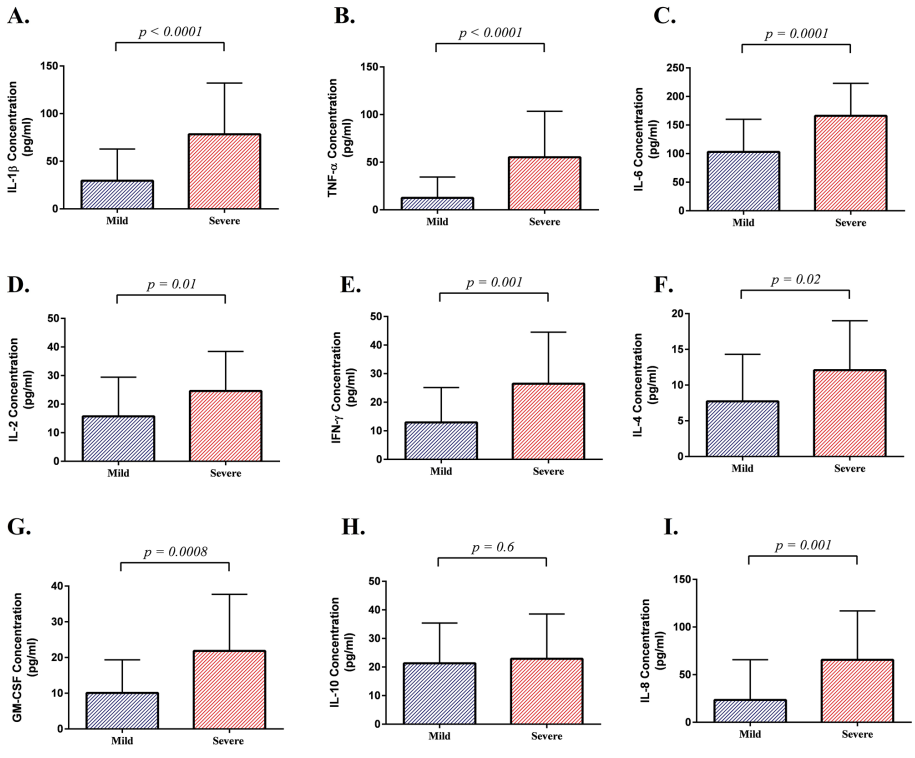

Figure 2: Cytokines concentration in mild and severe forms of COVID-19 patients. The serum concentrations of IL-1β (A), TNF-α (B), IL-6 (C), IL-2 (D), IFN-γ (E), IL-4 (F), GM-CSF (G), IL-10 (H), and IL-8 (I) were measured by ELISA. COVID-19 patients were clinically classified into mild and severe forms. The results are expressed as the Mean±SD. P values<0.05 were considered significant.

analysis showed that the concentration of pro-inflammatory cytokines including IL-1β (P<0.0001), TNF-α (P<0.0001), IL-6 (P=0.0001), IL-2 (P=0.01), IFN-γ (P=0.001), and GM-CSF (P=0.0008) was statistically higher in severe cases than that of mild group. The concentration of IL-4 was also found higher in severe group than that of mild group (P=0.02). IL-10 amount was similar in both mild and severe groups (P=0.6). In addition, compared to the mild cases, severe COVID-19 patients demonstrated significant elevated levels of IL-8 (P=0.001).

Correlation Analysis of Cytokines Profile with CRP Concentration in COVID-19 Patients

Since CRP concentration was introduced as a diagnostic biomarker for SARS-CoV-2 infection, we further analyzed the correlations between cytokines concentration and CRP amount. As shown in Figure 3, CRP concentration was positively correlated with the level of different cytokines including IL-1β (r=0.394, P=0.002), TNF-α (r=0.470, P=0.0004), IL-6 (r=0.469, P=0.0002), IL-2 (r=0.289, P=0.02), IFN-γ (r=0.403, P=0.002), and GM-CSF (r=0.269, P=0.04). Moreover, CRP concentration was also associated with the levels of IL-4 (r=0.249, P=0.07) and IL-8 (r=0.244, P=0.06), but the analysis was not statistically significant. No correlation was observed between the level of CRP and IL-10 concentration (r=0.032, P=0.8).

Correlation Analysis of Cytokines Profile with the Absolute Counts of Lymphocyte and Neutrophil in COVID-19 Patients

Correlation analysis was then applied to clarify the relationship between serum cytokine levels and the absolute counts of lymphocyte and neutrophil. Our results showed that the lymphocyte count was inversely correlated with the concentration of IL-1β (r=-0.374, P=0.005), TNF-α (r=-0.298, P=0.03), IL-6 (r=-0.294, P=0.03), IL-2 (r=-0.269, P=0.04), and IFN-γ (r=-0.269, P=0.04) (Figure 4 A-E). Negative associations were also seen between the lymphocyte absolute

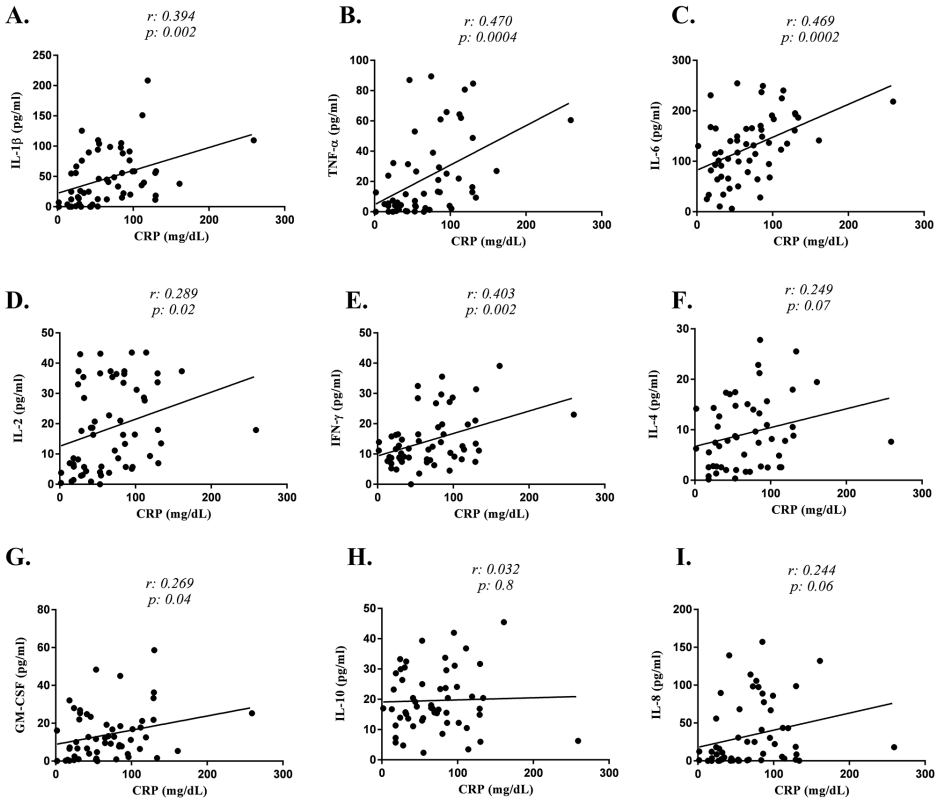

Figure 3: Correlation analysis of cytokine levels with CRP amount in COVID-19 patients. Correlations between the levels of IL-1β (A), TNF-α (B), IL-6 (C), IL-2 (D), IFN-γ (E), IL-4 (F), GM-CSF (G), IL-10 (H), and IL-8 (I) with CRP concentration are represented in COVID-19 patients.

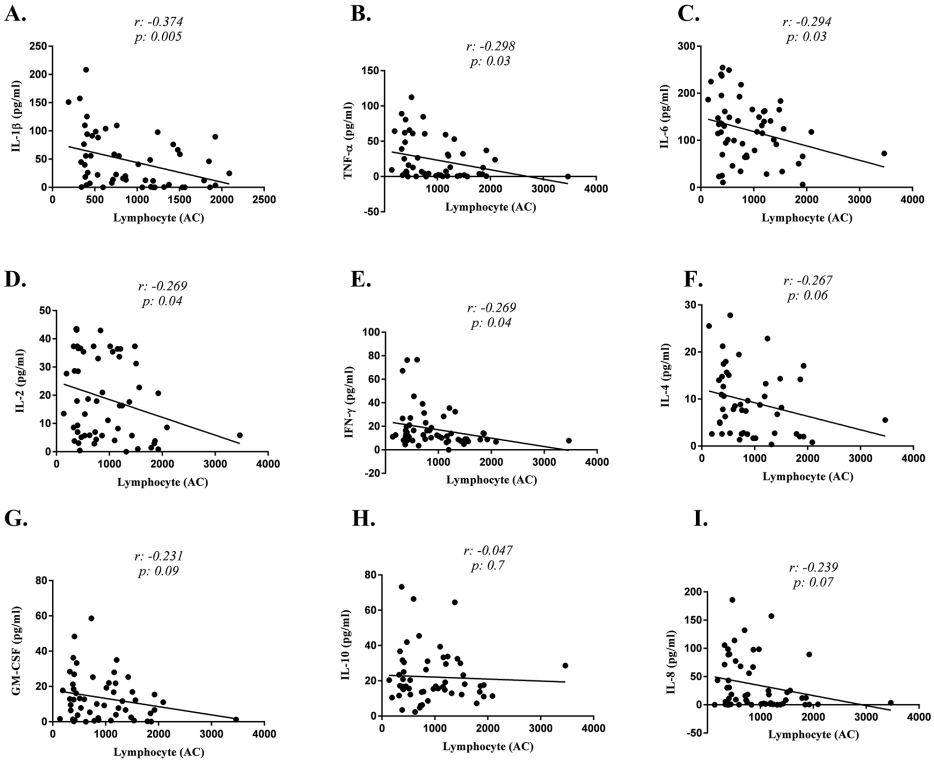

Figure 4: Correlation analysis of cytokine levels with the absolute count of lymphocytes in COVID-19 patients. Correlations between the levels of IL-1β (A), TNF-α (B), IL-6 (C), IL-2 (D), IFN-γ (E), IL-4 (F), GM-CSF (G), IL-10 (H), and IL-8 (I) with the absolute count of lymphocytes in COVID-19 patients are shown.

count and the amounts of IL-4 (r=-0.267, P=0.06), IL-8 (r=-0.239, P=0.07), and GM-CSF (r=-0.231, P=0.09), but the result was not significant. No correlation was observed between IL-10 concentration and lymphocyte absolute count (r=-0.047, P=0.7) (Figure 4 F-I). In addition, we next investigated the relationships between neutrophil absolute count and the levels of cytokine within COVID-19 patients. Interestingly, the neutrophil count was positively correlated with the concentration of IL-1β (r=0.267, P=0.04), TNF-α (r=0.432, P=0.001), and GM-CSF (r=0.275, P=0.03) (Figure 5 A-C), but was not associated with IFN-γ (r=0.022, P=0.8), IL-2 (r=0.115, P=0.3), IL-4 (r=0.111, P=0.4), IL-6 (r=0.040, P=0.7), IL-8 (r=0.049, P=0.7), and IL-10 (r=0.024, P=0.8) (Figure 5D-I).

DISCUSSION

Coronavirus disease 2019 caused by the SARS-CoV-2 induce rapid activation of the innate immune cells, particularly in patients with severe clinical presentation (18). It has been generally argued that cytokines and chemokines have been considered to play crucial roles in immunopathology during viral infections like SARS-CoV-2 (19, 20). The first line of defense against viral infection is a fast and well-coordinated innate immune response (21). Nevertheless, over-activation and dysregulation of the immune responses may cause immune malfunctions and multiple damages to the host (21, 22). It is now believed that cytokine release syndrome is the key mechanism of the lung injury and adverse clinical outcomes (15). In this study, most patients presented with fever, fatigue, cough, lymphopenia, neutrophilia, and elevated infection-related biomarkers like CRP which share similar clinical and laboratory characteristics with previous β-coronavirus infections (14, 23, 24). We report here a dysregulation of the immune system in COVID-19 patients. Our data indicated higher levels of IL-1β, TNF-α, IL-6, IL-8, IL-2, IFN-γ, IL-4, and GM-CSF

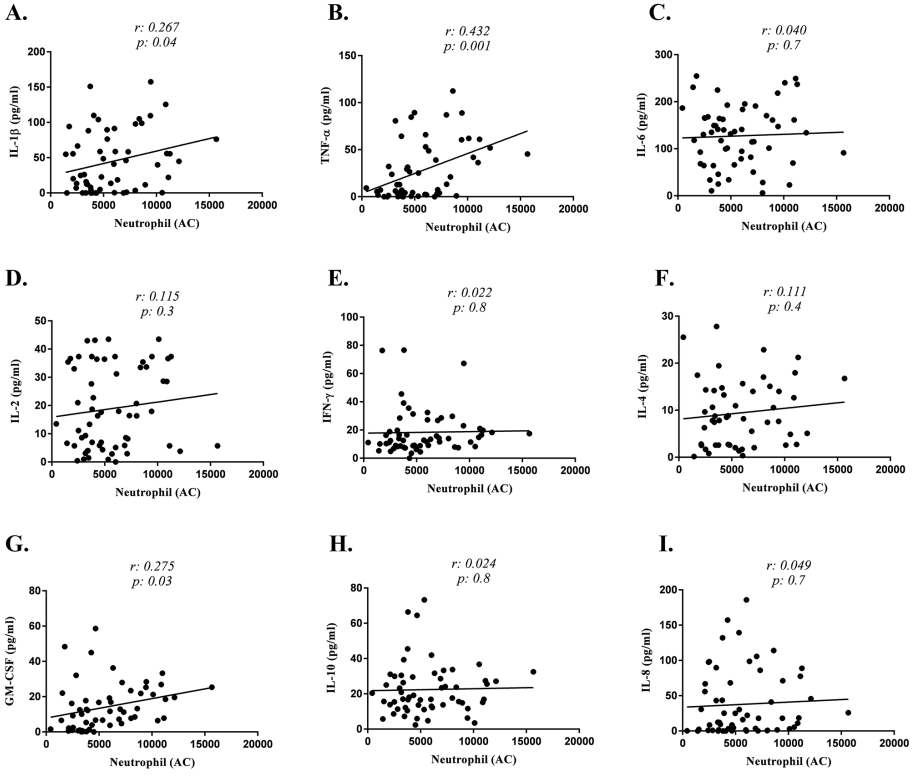

Figure 5: Correlation analysis of cytokine levels with the absolute count of neutrophils in COVID-19 patients. Correlations between the levels of IL-1β (A), TNF-α (B), IL-6 (C), IL-2 (D), IFN-γ (E), IL-4 (F), GM-CSF (G), IL-10 (H), and IL-8 (I) with the absolute count of neutrophils in COVID-19 patients are indicated.

in patients than those of normal group. Regarding clinical severity, the amount of all mentioned cytokines were higher in the severe cases compared to the mild group. These data and relevant evidences from COVID-19 patients showed that the immune system is impaired during the disease course and pro-inflammatory responses play vital roles in the pathogenesis of SARS-CoV-2.

Adaptive immune responses not only have important roles in virus clearance during viral infections, but also modulate and diminish the innate immune response and prevent additional damages to the host (17, 25). However, hyper-inflammatory responses induce T cell apoptosis and reduction during some viral infections leading to dysregulated of the inflammatory responses (24). While there is no strong data confirming the contribution of the pro-inflammatory cytokines and chemokines in lung pathology during COVID-19 infection, several studies have shown that changing in the levels of serum cytokines and chemokines are associated with the disease severity and adverse outcome, indicating a potential role for hyper-inflammatory responses in COVID-19 pathogenesis (19, 26). In this regard, other reports indicated in COVID-19 infection that the concentration of many pro-inflammatory effector cytokines, including IL-1β, TNF-α, IL-6, IL-8, GM-CSF, and Granulocyte colony-stimulating factor (G-CSF) as well as chemokines, such as Monocyte Chemoattractant Protein-1 (MCP-1), IFN-induced protein 10 (IP10), and Macrophage Inflammatory Proteins 1α (MIP1α, CCL3), are increased in patients and correlated with disease severity (1, 27). In addition, Th1, Th2, and Th17 cell responses are induced in the context of SARS-CoV-2 infection and the amounts of related cytokines like IFN-γ, IL-2, IL-4, and IL-17 are elevated (1, 3, 19). Consistent with these reports, here we found that the production of cytokines including IL-1β, TNF-α, IL-6, IL-8, IL-2, IFN-γ, IL-4, and GM-CSF are significantly elevated in patients and statistical analysis explained that their levels in severe patients are remarkably higher than those of mild patients. Although the concentration of IL-10 was higher in patients group than that of controls, the statistical analysis was not significant (P=0.09). However, most studies showed the elevated levels of IL10 in COVID-19 patients, particularly in severe cases (3, 26, 28). Due to the extent of the cytokine storm phenomenon or cytokine release syndrome (CRS) in COVID-19 patients which contributes to the disease immunopathology and elevated morbidity and mortality, increasing in IL-10 serum concentration is predictable and makes more sense. Contradictory results were also reported by Sadeghi et al. who showed decreasing of IL-10 mRNA in COVID-19 patients using RT-PCR (29). Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 causes abnormal and excessive immune responses like massive immune cell infiltration and cytokine storms that are related to severe lung pathology and death (12, 13, 30). For these reason, some patients with COVID-19 develop some complications such as ARDS, pneumonitis, shock, respiratory failure, and multiple organ damages (1, 3). Previous studies have found in MERS patients that increasing in concentration of serum cytokines and chemokines is associated with the higher frequency of monocytes and neutrophils in the peripheral blood and lung tissue, indicating that monocytes and neutrophils may play important roles in lung pathology of these patients (31, 32). Similar phenomena have been reported in SARS-CoV patients (12, 33). In line with these findings, it has been appeared from previous reports that dysregulated and exaggerated cytokine and chemokine responses by SARS-CoV-2 infection could play a crucial role in the pathogenesis of COVID-19. There are multiple checkpoint mechanisms which coordinate and regulate the magnitude and duration of an immune response. Any delay or malfunction of these regulatory responses may lead to the sustained cytokine release and corresponding pathological manifestations. Since all cytokines storm are not the same in the nature and affected tissues, understanding

the involved immune regulatory mechanisms could be helpful to manage the patients and find new therapeutic strategies (11). In this regard, early control of the cytokine storm by immunomodulators or cytokine antagonists together with the reduction in cellular infiltration of the lung are crucial strategies to improve the clinical success rate and diminish the related morbidity (34). Defining the various standard ranges for different cytokines and their relations to clinical findings can introduce these cytokines as invaluable clinical progression biomarkers (15).

It has been reported in several studies that COVID-19 patients display elevated levels of CRP as well as increasing in neutrophils and decreasing in lymphocytes compared to normal levels (1, 17). Later on, it was documented from some reports that inflammation represented by cytokine storms and CRP in COVID-19 patients could attribute to disease severity and death (1, 27). Accordingly, we next investigated the relationships between the production of cytokines with CRP levels and neutrophil and lymphocyte count within COVID-19 patients. Interestingly, the concentration of IL-1β, IL-2, IL-4, IL-6, IL-8, IFN-γ, and TNF-α was negatively associated with the absolute count of lymphocytes, and positively correlated with the CRP levels and the absolute count of neutrophils. We also noted that serum concentration of IL-10 had no correlations with the concentration of above cytokines. These data suggest that decreasing in lymphocytes and increasing in neutrophils observed in COVID-19 patients may be the result of the high serum levels of different immune cytokines.

In conclusion, the current study suggests that high levels of various cytokines are correlated with the disease severity and immunopathogenesis of COVID-19. These findings may contribute in a better understanding of the immune dysfunction in this infection. In addition, serum levels of cytokines and chemokines have been identified as prominent biomarkers for early dentification of severe patients and predict the clinical progression of COVID-19 patients.

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ETHICAL APPROVAL

This study was ethically approved by the Ethical Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1399.188).

Conflict of interest: None declared.

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