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Research Article

Levels of Cytokines in SARS-CoV-2 Infected Patients

Almina Shafiq[1,7], Muhammad Roman[2], Faheem Shahzad[1], Romeeza Tahir[1], Khursheed Javaid[1], Muhammad Bilal Sarwar[3], Muhammad Asif Naveed[4], Maria Zahid[5], Seema Aftab[6], Shah Jahan[1*]

[1]Department of Immunology University of Health Sciences Lahore, Pakistan.
[2]Department of Microbiology University of Health Sciences Lahore, Pakistan.
[3]Life Sciences, Pakistan Council of Scientific & Industrial Research, Pakistan.
[4]Department of Haematology, University of Health Sciences Lahore, Pakistan.
[5]Department of Molecular Pathology, Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan.
[6]Department of Paediatrics, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan.
[7]Department of Biomedical Laboratory Sciences, University of Management and Technology Lahore, Pakistan.
*Correspondence: shahjahan@uhs.edu.pk

Abstract

Cytokine dysregulation is the proposed mechanism for Coronavirus disease 2019 (COVID-19). This study aimed to evaluate the serum levels of interleukin (IL)-2, tumor necrosis factor (TNF)- α, IL-1β, interferons (IFN)-α, IFN-β, IFN-γ, and IL-10 in patients infected with SARS-CoV-2. The study was conducted in 120 adult patients with COVID-19 and compared with 40 age and gender-matched healthy subjects as controls. The age range in both groups was 18–70 years. The patients were classified into the mild/moderate group (60 patients) and the severe group (60 patients). Serum samples were collected from all participants and tested for cytokine levels by enzyme-linked immunosorbent assay (ELISA) method. Statistical analysis was performed using the one-way analysis of variance (ANOVA). Serum cytokines in SARS-CoV-2 patients, which indicate the host's immune responses against the coronavirus inflammation, seem different from what has been observed with other viral pathogens. The difference in the serum levels of IL-2, TNF-α, INF-α, INF-β, and IL-10 between the two groups was insignificant. However, higher levels of IL-2, TNF-α, and INF-β are reported in mild COVID-19 patients compared to healthy individuals and severe COVID-19 patients. Varying levels of cytokines were detected in the COVID-19 group than in the control group, suggesting distinct immunoregulatory mechanisms involved in COVID-19 pathogenesis.

Keywords: COVID-19, SARS-CoV-2, inflammation, cytokines, interleukins, coronaviruses

1. Introduction

Coronaviruses (CoVs) are a large family of respiratory viruses that can cause diseases ranging from the common cold to Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) (Chen et al. 2019). In December 2019, the emergence of novel coronavirus-induced pneumonia in Wuhan, China, posed a serious and urgent threat to public health worldwide. On 11 February 2020, the World Health Organization (WHO) officially renamed 2019-nCoV as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and designated the disease caused by it as coronavirus disease 2019 (COVID-19) (Song, Li, et al. 2020). The outbreak of COVID-19 has put health

authorities on high alert in China and across the globe. It has brought the world to a standstill.

Coronaviruses are a group of positive-sense, single-stranded RNA viruses with the largest genome among known human RNA viruses and a likely ancient origin, named as such because of the envelope spinous processes that resemble a corona. SARS-CoV-2 is a member of the β-CoV lineage(Song, Li, et al. 2020). It has been revealed that SARS-CoV-2 has a genome sequence 75% to 80% identical to the SARS-CoV and has more similarities to several bat CoVs. However, its direct origin is still a question(Zhou et al. 2020).

SARS-CoV-2 manifests a faster transmission rate than two previous pandemics SARS-CoV in 2003 and MERS-CoV in 2012. A high rate of person-to-person transmission of SARS-CoV-2 in family and hospital settings is observed(Li et al. 2020). Its clinical features included fever, fatigue, dry cough, shortness of breath, and acute respiratory distress syndrome (ARDS), which can lead to severe respiratory illness(Chen et al. 2019). Significant complications include respiratory failure, secondary infections, and systematic manifestations such as sepsis, septic shock, and multiple-organ dysfunction(Huang et al. 2020). According to WHO classification, COVID-19 infection can be categorized as mild, moderate, or severe. Mild COVID-19 shows respiratory symptoms without evidence of pneumonia or hypoxia, while the presence of clinical and radiological evidence of pneumonia defines moderate or severe infection (WHO, 2020).

Inflammatory, immune cells cytokines and adhesion molecules are crucial factors involved in damaging lungs. Studies have shown that in addition to direct viral damage, uncontrolled inflammation contributes to disease severity in COVID-19(Del Valle et al. 2020). A high serum level of chemokines and other cytokines is referred to as the term cytokine storm. To date, most studies have focused on the direct measurement of those cytokines and chemokines in the peripheral blood, but in the context of the rapidly changing environment after virus infection, we do not have a well-rounded understanding of the cause of the vigorous inflammatory response.

However, some researchers found cytokine storm of interleukin (IL)-2, IL-4, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and C-reactive protein (CRP) is absent in 25 patients who were admitted to the ICU with confirmed infection of SARS-CoV-2. All of these indicate the severity of COVID-19 symptoms is not directly associated with circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and CRP (Luo et al. 2021). In addition, the body's immune damage is closely related to the viral load, and the degree of the cytokine storm is an essential connecting point. Therefore, we designed this study to evaluate the cytokines levels in COVID-19-infected patients and compared them with healthy volunteers.

2. Materials & Methods

This comparative analytical study was performed in the University of Health Sciences (UHS) resource laboratory in Lahore, Pakistan. Samples were taken from Mayo Hospital, University of Health Sciences COVID laboratories, University of Veterinary & Animal Sciences, Chughtai Lab, and Mughal Eye Hospital Lahore. The written consent forms for patients were taken before inducting them into the study. The sampling technique was non-probability purposive sampling. A total of one hundred and twenty COVID-19 patients as well as age and gender-matched healthy individuals from the same

Table 1. Concentration of IL-2 (pg/ml)

	Median	Interquartile range (Q1 – Q3)	Independent-samples Kruskal-Wallis test
Healthy Individuals	155.39400	71.25000-218.97925	0.55
Mild/Moderate COVID-19 Symptoms	186.35850	95.03550-221.19300	
Severe COVID-19 Symptoms	175.41350	105.60800-235.62200	

geographical and ethnic origin participated in this study. COVID-19 patients in this study were confirmed as SARS-CoV-2 positive by a reverse transcription polymerase chain reaction. All the COVID-19 patients who have acute respiratory symptoms with any of the symptoms like cough, sore throat, shortness of breath, coryza (head cold), anosmia (loss of sense of smell), with or without fever were placed in mild COVID-19 patients' group. While active COVID-19 patients with clinical signs of pneumonia, dyspnoea, respiratory frequency ≥ 30/min, blood oxygen saturation (SpO2) ≤ 90%, PaO2/FiO2 ratio or P/F [the ratio between the blood pressure of the oxygen (partial pressure of oxygen, PaO2) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO2)] < 300, and/or lung infiltrates > 50% within 24 to 48 hours were placed in severe COVID-19 patients' group. All individuals below 18 years, immunocompromised, on immune-modifying therapy, active respiratory infections, malignancies, and other chronic diseases were excluded. Five ml of venous blood from each subject was drawn in EDTA and gel tubes. Samples were transported to the Department of Immunology, UHS, in an ice box. The samples were centrifuged at 800g at 4^0 C for 10 min, and the supernatant was stored at -80^0 C.

2.1. Quantification of Cytokines

Cytokine levels were evaluated by enzyme-linked immunosorbent assay (ELISA). Thermofisher, BioRad, and Fine kits were used for the determination of cytokines levels. Analyses were performed according to the manufacturer's instructions for each ELISA kit. Absorbance was read by an ELISA microplate reader at wavelength 450 nm.

2.2. Statistical Analysis

Data were analyzed in SPSS software (Version.25). The data were compared using one-way analysis of variance (ANOVA) and Tukey's posthoc analysis. For non-parametric data, median and interquartile ranges were applied to determine the differences in cytokines levels. The p-value was determined by applying the independent-samples Kruskal- Wallis test. A p-value of < 0.05 was considered statistically significant. Graphs were produced using GraphPad Prism 8 software.

3. Results

In the current study, we evaluated the serum cytokines levels in COVID-19 patients (mild/moderate and severe patients) with healthy individuals. The Shapiro-Wilk test was applied to check the distribution of data and was found to be non-parametric. By

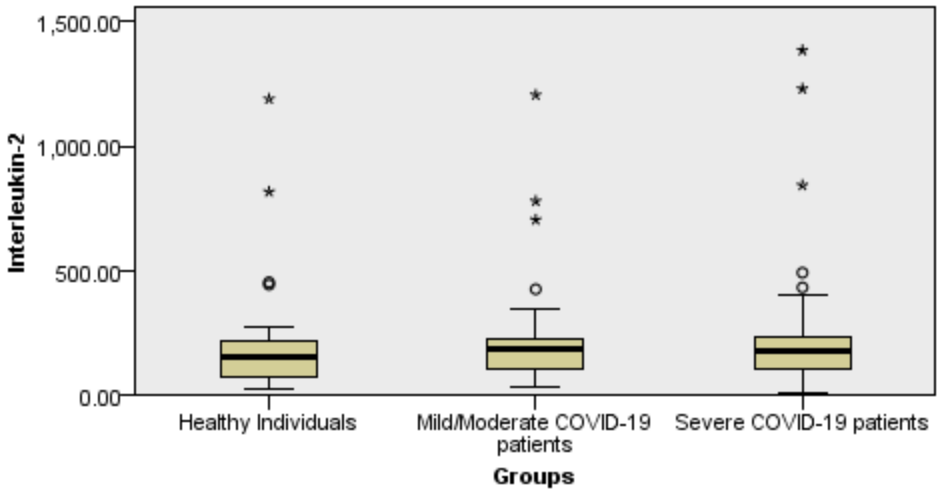

Figure 1. Levels of interleukin-2 in the three groups.

applying the independent Kruskal-Wallis test, the p-value of IL-2 is found to be not significant (p=0.55). There were no significant differences in the mean and interquartile ranges of the three groups. However, the median level of IL-2 was slightly increased in mild patients (186.36) as compared to healthy individuals and severe COVID-19 patients (155.39, 175.41) (Table: 1 and Figure 1)

There are no significant differences in the mean and Interquartile ranges of 03 groups concerning TNF-α with the p-value of 0.615 though the median level of TNF-α is slightly increased in mild COVID-19 patients as compared to healthy individuals and severe COVID-19 diseased patients (Table: 2 and Figure 2)

We have found no significant differences for INF-α and INF-β in the three groups with (p=0.223 and p= 0.533, respectively). The median level of INF-α is slightly higher in healthy individuals (20.81) as compared to mild and severe COVID-19 patients (11.66 and 13.65, respectively) (Table: 3 and Figure 3). The level of INF-β was higher in mild COVID-19 patients compared to healthy individuals and severe COVID-19 patients (Table 4 and Figure 4). IL-10 results are also not significantly different (p=0.269). There are no significant differences in the median levels of IL-10 in the 03 groups, with (6.45, 6.78, and 7.44) (Table: 5 and Figure 5). These results are summarized in Table 6.

4. Discussion

The secretion of multiple cytokines, also termed cytokine release syndrome (CRS), is closely related to the development of clinical symptoms. For example, IFN-γ can cause fever, chills, headaches, dizziness, and fatigue; TNF-α can cause flu-like symptoms similar to IFN-γ, with fever, general malaise, and fatigue, but can also cause vascular leakage, cardiomyopathy, lung injury, and acute-phase protein synthesis (Chen et al. 2020). This cytokine release may lead to the characteristic symptoms of severe CRS, such as diffuse intravascular coagulation (DIC) (Anka et al. 2021). It is likely to cause cardiomyopathy by promoting myocardial dysfunction, often observed in patients with CRS (Han et al. 2020). In addition, endothelial cell activation may also be a hallmark of severe CRS. Endothelial dysfunction can lead to capillary leakage, hypotension, and coagulopathy (Sherwani and Khan 2020). These studies

Table 2. Concentration of TNF-α (pg/ml).

	Median	Interquartile range	p-value
Healthy Individuals	275.04050	204.25000-349.53725	0.615 (Retain null hypothesis)
Mild/Moderate COVID-19 Symptoms	301.49200	179.91300-380.24625	
Severe COVID-19 Symptoms	275.09350	191.01550-358.96250	

argue that virus-induced immunopathological events play a crucial role in fatal pneumonia observed after CoV infections.

In viral infections, the aberrant release of proinflammatory factors leads to lung epithelial and endothelial cell apoptosis, which damages the lung microvascular and alveolar epithelial cell barrier, leading to vascular leakage, alveolar edema, and hypoxia. The uncontrolled production of proinflammatory factors, including IL-6, IL-1β, and granulocyte-macrophage-colony stimulating factors and chemokines such as CCL2, CCL-5, IP-10, and CCL3, together with reactive oxygen species, cause ARDS leading to pulmonary fibrosis and death (Pum et al. 2021). Based on our current study, we propose the possibility that blockade may constitute a novel therapeutic strategy for severe and critical COVID-19 patients (Han et al. 2020).

Various studies of COVID-19 patients have detected elevated levels of cytokines associated with severe respiratory symptoms. However, the severity of COVID-19 symptoms is not directly associated with circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and CRP. Although anti-inflammatory agents are believed to combat severe COVID-19 patients, we suggest that anti-inflammatory drugs should be used very carefully based on our observation. This indicates that COVID-19 patients have severe clinical characteristics independent of circulating levels of inflammatory cytokines in peripheral blood, including IL-2, IL-4, TNF-α, IFN-γ, and CRP. (Luo et al. 2021). These results are in line with our study.

IL-10 is a type 2 cytokine that inhibits the production of proinflammatory cytokines (e.g., IFNγ, TNFα, IL-1β, and) in various cell types and prevents dendritic cell maturation by blocking IL-12. However, IL-10 can have immunostimulatory effects, including stimulating IFNγ production by CD8+ T cells. It is also a powerful factor for the growth and differentiation of B cells, mast cells, and thymocytes. Various authors have detected this interleukin in patients with COVID-19 and related its levels to disease severity and progression, as in the case of other cytokines, and it has been reported to have possible prognostic value. Some authors indicate that IL-10 may be hyper-expressed in anti-SARS-CoV-2 immunity, being higher in patients of advanced age with respect to a "hyperinflammatory response," possibly related to the reduction of T-cell receptors in the elderly. As with other cytokines, IL-10 levels were higher in patients with COVID-19

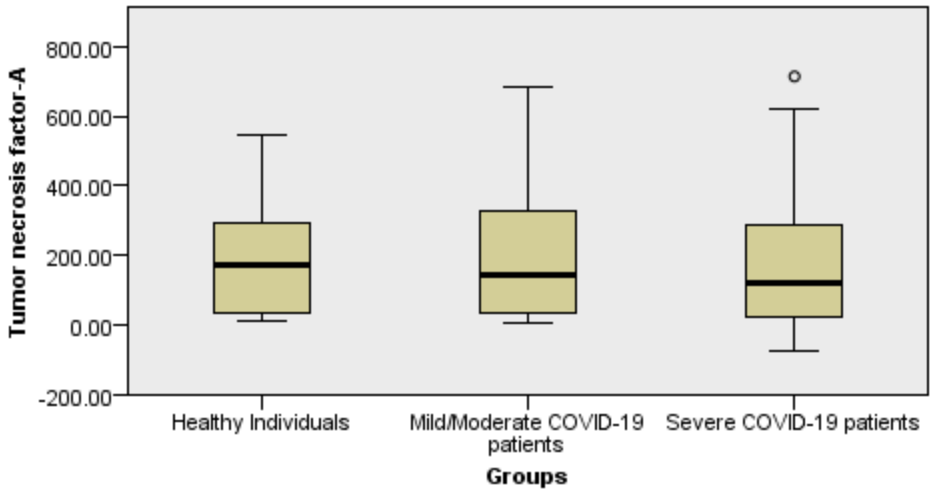

Figure 2. Levels of TNF-α in the three groups.

than in those with SARS-CoV or MERS. (Merza et al. 2021).

IFN-γ is a type-II IFN produced by various lymphocyte cells, including CD4+ and CD8+ T cells, Treg cells, FoxP3+ CD8− T cells, B cells, and NK cells. Monocytes, macrophages, dendritic cells, and neutrophil granulocytes can also produce this cytokine. Although numerous cells can be the source of IFN-γ, it is mainly produced by T and NK cells. Mesenchymal stem cells can also secrete low IFN-γ levels to regulate hematopoiesis. IFN-γ participates in numerous immune and adaptive immunological functions and in inflammatory processes. It promotes macrophage activation and antigen presentation and is involved in anti-bacterial and anti-viral immunity and in signal transduction. Given its complex and varied roles, it is difficult to classify IFN-γ as a pro- or anti-inflammatory cytokine. Some found that serum IFN-γ levels were higher in patients with COVID-19 than in healthy individuals and proposed that the elevation of this and other cytokines might result from the activation of Th1 and Th2 cells. Elevated IFN-γ levels were associated with greater viral load and lung damage. Some studies found IFN-γ and IL-10 levels were higher in patients with infection by SARS-CoV-2 but did not differ between patients who required ICU admission and those who did not. These authors found that levels of this cytokine were lower in CD4+ T-cells from patients with severe versus mild symptoms and suggested that the infection may initially affect CD4+ and CD8+ T-cells, reducing the production of IFN-γ. (Ghazavi et al. 2021)

TNF-α is produced by various cell types, such as monocytes, macrophages, and T cells, among others. Alongside other cytokines, TNF-α regulates inflammatory processes, infectious diseases, and malignant tumors. It has been observed that serum TNF-α levels are elevated in patients with COVID-19 and are higher with more severe disease. Similar results were reported in a sample of 522 patients with COVID-19 and found an inverse relationship between TNF-α levels and T-cell counts. In contrast, some described normal TNF-α levels in patients with COVID-19. Others proposed that the administration of certolizumab, an anti-TNF-α antibody, might have beneficial effects on patients with COVID-19.

IL-2 plays a key role in the proliferation of T cells and the generation of effector and memory T cells. It is involved in adaptive immunity and increases glucose metabolism to promote T, B, and NK cell proliferation and activation.(Costela-Ruiz et al. 2020) detected elevated levels of IL-2 or its receptor IL-2R in patients with COVID-19, and it has been reported that these increases are directly proportional to the severity of the disease. To date, most studies have focused on the direct measurement of those cytokines and chemokines in the peripheral blood, but in the context of the rapidly changing environment after virus infection, we do not have a well-rounded understanding of the cause of the vigorous inflammatory response (Song, Zhang, et al. 2020).

However, some researchers found cytokine storm of IL-2, IL-4TNF-α), interferon-gamma (IFN-γ), and CRP is absent in 25 patients who were admitted to the ICU with confirmed infection of SARS-CoV-2. All of these indicate that the severity of COVID-19 symptoms is not directly associated with circulating levels of IL-2, IL-4, TNF-α, IFN-γ, and CRP (Luo et al. 2021). In addition, the body's immune pathological damage is closely related to the viral load, and the degree of the cytokine storm is an essential connection point. If plasma cytokines are monitored dynamically to assess the degree of cytokine storm in a timely and effective way, this may greatly benefit the care of critically ill patients. (Merza et al. 2021).

5. Conclusions

We report higher levels of IL-2, TNF-α, and INF-β in mild COVID-19 patients compared to healthy individuals and severe COVID-19 patients. Regarding INF-α, the median level is higher in healthy individuals compared to mild and severe COVID-19 patients. IL-10 levels were higher in severe COVID-19 patients. Varying levels of cytokines were found in the COVID-19 group than in the control group, suggesting distinct immunoregulatory mechanisms involved in COVID-19 pathogenesis. However, additional investigations needed to be performed `to understand the exact cellular mechanism of this disease.

Conflict of Interest

The authors declare no conflicts of interest.

Funding

The study did not receive any external funding.

Ethics Approval

This study was approved by the University of Health Sciences, Lahore, Pakistan.

Data Availability

All the data related to this study is available with the authors**.**

Consent Forms

The written consent forms for patients were taken before inducting them into the study. These consent forms are available with the authors.

Authors Contribution

AS, MR, SJ conceptualized the study and wrote the final manuscript, FS, RT, KJ, AS helped in the analysis and writing the first draft, did the experimental analysis, MB, MA, MZ, SA, contributed to manuscript writing and SJ supervised the whole project and wrote the final manuscript.

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