Original Article

Comparison Study of the Inflammatory Biomarkers and Cytokine Levels in COVID-19 Patients

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Abstract

Background: ACOVID-19, caused by the novel coronavirus SARS-CoV-2, presents with varying severity, from mild symptoms to severe disease, including ARDS and multi-organ failure. Identifying early biochemical markers to predict the severity of COVID-19 is crucial for improving patient outcomes. **Aims and Objectives:** The study aimed to identify distinct patient groups with COVID-19 and healthy controls using cluster analysis of multiple biomarkers. Specifically, it sought to determine if early detected levels of ferritin, D-dimer, and C-reactive protein (CRP) are associated with the severity of COVID-19. **Materials and Methods:** Biochemical profiles of sixty patients with SARS-CoV-2 RNA-positive testing and thirty healthy controls were collected and analyzed. Two-dimensional automated hierarchy clustering was performed on all biomarkers. The study focused on alterations in the biochemistry markers ferritin, D-dimer, and CRP. Additionally, levels of inflammatory cytokines, particularly interleukin-6 and tumor necrosis factor-alpha, were measured. **Results:** Ferritin, D-dimer, and CRP levels were significantly higher in COVID-19 patients compared to the control group. Early detected levels of these biomarkers were associated with a higher incidence of severe COVID-19. Elevated levels of inflammatory cytokines, especially interleukin-6 and tumor necrosis factor-alpha, were also observed in COVID-19 patients, indicating the presence of a cytokine storm, which is a major cause of organ dysfunction and mortality in severe cases. **Conclusion:** Our findings suggest that elevated levels of ferritin, D-dimer, and CRP are indicative of severe COVID-19. Early detection of these biomarkers can help predict the severity of the infection and guide clinical management. The study also underscores the significant role of inflammatory cytokines in the pathogenesis of COVID-19, contributing to the understanding of the cytokine storm that leads to severe disease outcomes.

Keywords: Coronavirus 2019, C-reactive protein, D. dimer, interleukin-6, serum ferritin, tumor necrosis factor-alpha

Introduction

The World Health Organization (WHO) proclaimed coronavirus 2019 (COVID-19) to be a pandemic in March 2020. COVID-19 is the most current pandemic and is brought on by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) coronavirus.[1] Since then, 216 countries and regions have reported cases of COVID-19, and as of February 2020, 502,906 deaths had been confirmed in those cases. Clinical traits of SARS-Covid-2, SARS-Covid-1, and MERS-CoV are similar. A small fraction of COVID-19 patients get severe pneumonia, multiorgan failure, acute respiratory distress syndrome, or even death, even though the majority of patients have a mild influenza-like illness or are asymptomatic.[2] The reason why some people develop serious illnesses and others do not is still a mystery. Comorbidities and laboratory markers have been considered for risk categorization.[3,4] There is growing evidence that, in critically sick patients, hyperinflammation is linked to hyperferritinemia, D-dimer, and C-reactive protein (CRP). These findings suggest that the pathophysiology of COVID-19 disease may be significantly influenced by a phenomena cytokine storm. In the event of a pandemic, laboratory biomarkers that forecast the severity of COVID-19 are essential because resource allocation must be carefully planned, particularly in the case of respiratory support

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preparation.[5] In this investigation, the association between ferritin, CRP, D-dimer, tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-6 with COVID-19 severity will be described. IL-6 is a glycoprotein that excreted by several cell types, including macrophages, monocytes, eosinophils, hepatocytes, and glial cells. The IL-6, a pro-inflammatory cytokine, encourages the development of neutrophils, cytotoxic T-lymphocytes, and natural killer cells.[6] When cells are stimulated by IL-1 or TNF-α, or when toll-like receptors-4 are activated, IL-6 production release is induced.[7]

TNF-α is a pro-inflammatory cytokine that regulates cell proliferation, differentiation, and apoptosis. It is located on chromosome 6p21.33 inside the human leukocyte antigen III region.[8] Macrophages are one of the main sources of TNF-α. It is mostly produced by active macrophages and monocytes, numerous cells, including T- and B-cells, osteoblasts, smooth muscle cells, endothelium, epithelial, and tumor cells, can also release it.[8] This study aimed to evaluate the IL-6 and TNF alpha levels in COVID-19 patients and to have an insight if there is a connection between the IL-6 and TNF-α with inflammatory biomarkers in COVID-19 patients and the correlation with the severity of the disease.

Methodology

This study, is a cross-section, was conducted from December 2020 to March 2021. Five milliliters of whole blood were taken from each of the sixty confirmed COVID-19 cases and the thirty healthy controls. According to the WHO criteria of severity which represented by adolescent or adult with one or more of the following symptoms in addition to clinically obvious pneumonia (fever, cough, and dyspnea), is regarded to have a severe disease. Breathing more than 30 times/min; acute respiratory discomfort; and 90% SpO_2 in room air. Even though the diagnosis is typically made based on clinical considerations., a moderately infected adult who exhibits the typical symptoms of pneumonia (fever, cough, dyspnea, and rapid breathing) but does not exhibit symptoms of a severe infection, such as SpO_2 levels that are lower than 90% on room air. SpO_2 in the air of the room is 90%. Illness without signs of pneumonia or hypoxia, as well as COVID-19 symptoms. Thirty patients had severe COVID-19, and thirty others had moderate. The study included all patients who were over the age of 18 years and had a positive nasopharyngeal swab-reverse transcription-polymerase chain reaction result who had been hospitalized in the COVID-19 ward in Salahuddin province. There were 60 patients in total, of which 54 were men and 6 were women. The sera of all samples (patients and control) were tested for CRP, D–Dimer, and serum ferritin. An antigen–antibody complex forms when the detector antibody in the buffer binds to the antigen in the sample and migrates to the nitrocellulose matrix, where they are recognized by the other immobilized antibody on the test strip. This test uses a sandwich immunodetection approach. As antigen concentrations in the sample increase, antigen–antibody complexes grow. The fluorescence signal from the detector antibody gets brighter as a result. The automated fluorescent immunoassay system-6 (AFIAS-6) apparatus next processes this signal to calculate the concentration of CRP, D- Dimer, or ferritin present in the sample, an enzyme-linked immunosorbent assay test (Mindray, China) to detect the concentration of cytokines (IL-6 and TNF-α).

Results

The current study includes sixty individuals with SARS-CoV-2 infection and thirty healthy controls. According to the descriptive statistics, the average disease duration for COVID-19 patients was 7–14 days, with a maximum disease duration of 20 days. After this period, the symptoms started to fade, and the patients were discharged from the hospital. The remaining parameter levels were taken at each one's peak; according to the control reference range, the results showed that D-Dimer had a mean value of 204 ng/ml, S. ferritin had a mean value of 76.37 ng/ml, and CRP 4.2. mg/dl. The analyzed parameters were divided into two groups by the two-step cluster analysis. Cluster 1 was categorized as a severe case with 50% of the patients, whereas cluster 2 was categorized as a nonsevere case with 50% of the patients. The import test for the measuring predictors of severe COVID-19 was as follows: D. Dimer was the most significant variable, followed by CRP and ferritin. Despite the grading of the importance of the variables, every single one of them still seemed to be linked to the disease. The levels of ferritin, CRP, and D-dimer are all related to how severe the COVID-19 infection is. In patients with severe coronavirus infection, the mean of these parameters increased significantly and reached 269.1 ng/dl, 86.2 mg/dl, and 705.7 ng/dl for ferritin, CRP, and D-Dimer, respectively, whereas in nonsevere patients, the mean ferritin, D-Dimer, and CRP were 244.4 ng/dl, 661.3 ng/dl, and 51.4 mg/dl, respectively.

As illustrated in Figures 1-3 and Table 1, the concentrations of biomarkers denote CRP, D-dimer, and S. Ferritin, respectively. The D. Dimer results demonstrated the high frequency of the data displayed in cluster 2. The frequency of CRP was higher in both clusters, and the frequency of ferritin was similarly higher in both clusters.

Regarding IL-6 and TNF-α, Table 2 and Figures 4 and 5 display the mean of each category. The mean levels of both

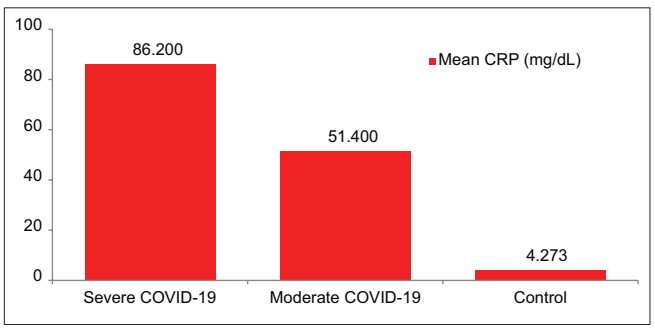

Figure 1: C-reactive protein titer in the three clusters

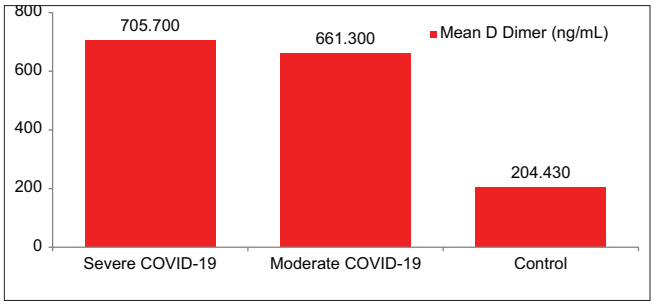

Figure 2: D. Dimer level in the three clusters

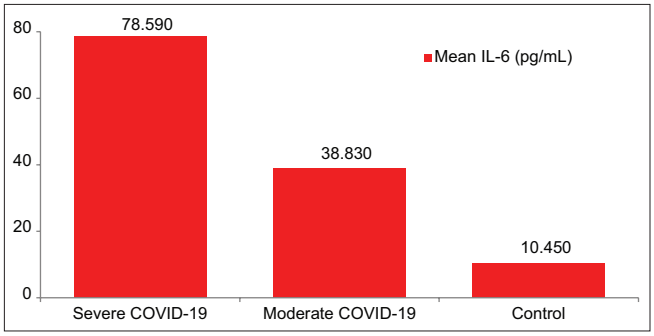

Figure 4: IL-6 frequency in the three clusters. IL-6: Interleukin-6

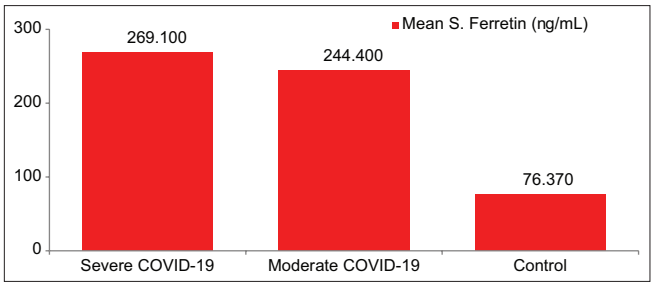

Figure 3: Serum ferritin level in the three clusters

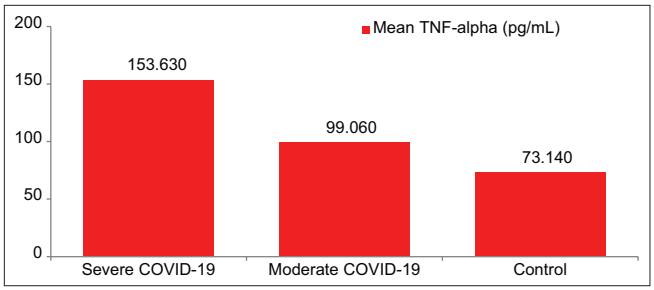

Figure 5: TNF-α frequency in the three clusters. TNF: Tumor necrosis factor-alpha

IL-6 and TNF-α in the three studied groups showed significant differences (p = 0.001; p = 0.001 respectively). patients with sever levels of IL-6 that were 78.59 ± 38.15 pg/ml and 38.83 ± 30.07 pg/ml for moderate group compared to 10.45 ± 10.95 pg/ml in healthy controls. Patients with severe COVID-19 had levels of 153.63 ± 87.30 pg/ml of TNF-α, whereas those moderate infections were 99.06 ± 31.75 pg/ml and 73.14 ± 13.476 pg/ml for the control group as shown in Tables 1 and 2.

Discussion

In order to explain the high levels of cytokine production that are responsible for inducing immunopathological reactions during infectious processes, the term "cytokine storm" was developed.[9] Among the inflammatory mediators released by immune cells, the cytokines interferon, IL-1, IL-6, TNF, and transforming growth factor are highlighted, and altered levels are associated with different clinical features either metabolically triggered or a result of an infection.[10,11] What is the relationship between COVID-19, cytokines, inflammation, and spices? Accordingly, this investigation assessed the levels of TNF-α and IL-6, together with their relationships to CRP, D-dimer, and S. Ferritin, in individuals with clinical COVID-19 that was classified as moderate or severe. Patients in the current study had significantly higher CRP levels than those control group (P = 0.001). CRP was noticeably higher in the severe group compared to controls and moderate patients (P = 0.001). There is a link between the mild group and the controls (P = 0.001). Similar findings were made by Ahnach *et al*., who found that individuals with severe COVID-19 had significantly higher CRP levels (P = 0.000). It was also a separate discriminator of serious illness at admission in comparison to other biological indicators.[12] Accordingly, research from 2021 found that the severe group's median values did not fall inside the usual range. These patients had significantly higher CRP values (P = 0.001).

Liu *et al*.'s studyin 2020 found that more severe COVID-19 cases had significantly higher CRP levels.[13] This is consistent with a study by Qin *et al*., 2020, which discovered elevated CRP titer in patients with acute COVID-19, suggesting that this biomarker can be utilized to monitor disease development.[14] Sahu *et al*. (2020) published a study concluding that CRP levels remained elevated in individuals with poor prognoses, suggesting that CRP could serve as a valuable biomarker for predicting mortality (P=0.05), and found that the C-reactive protein (CRP) level at admission is a straightforward indicator that can independently predict the early detection of COVID-19 severity.[12] This particularly sensitive acute inflammatory protein serves as a biomarker for infection, inflammation, and tissue damage. Studies have shown a correlation between CRP levels and inflammation levels. It can encourage phagocytosis and activate the complement system. In addition, CRP binds to germs and quickens the phagocytosis clearance process.[15] High levels may be associated with to production of inflammatory cytokines in acute COVID-19 individuals. Although cytokines fight microbes, an immune system overreaction can harm lung tissue. Inflammatory cytokines and tissue damage lead to its production in COVID-19 patients.[16] As our understanding of COVID-19, the authors found the frequency of thrombotic episodes and the use of decreasing mortality with heparin. The scientists reported in autopsy reports, there are several thromboembolic occurrences, both clinical cases and

Table 1: Descriptive statistics of C reactive protein, D Dimer, and serum ferritin in patients with COVID-19

	Mean±SD (range)			*P*
	Severe COVID-19	Moderate COVID-19	Control	
CRP (mg/dL)	86.2±69.4 (12.0-298)	51.4±44.7 (9.0-189)	4.27±3.15 (0.5-11)	0.001*
D Dimer (ng/mL)	705.7±328.8 (240-1654)	661.3±286.0 (195-1398)	204.4±46.49 (111-259)	0.001*
Serum ferretin (ng/mL)	269.1±161.2 (18-783)	244.4±134.6 (19-635)	76.37±60.11 (19-232)	0.001*

*ANOVA-test significant difference between more than two independent means at the 0.05 level. SD: Standard deviation, CRP: C-reactive protein

Table 2: Descriptive statistics of interleukin-6 and tumor necrosis factor-alpha, in patients with COVID-19

	Mean±SD (range)			*P*
	Severe COVID-19	Moderate COVID-19	Control	
IL-6 (pg/mL)	78.59±38.15 (28.8-183.4)	38.83±30.07 (10.8-151.4)	10.45±10.95 (0.797-53.6)	0.001*
TNF-alpha (pg/mL)	153.63±87.30 (10.62-477.6)	99.06±31.75 (10.47-188.3)	73.14±13.476 (38.48-110.4)	0.001*

*Significant difference among more than two independent means using ANOVA-test at 0.05 level. TNF: Tumor necrosis factor, IL: Interleukin, SD: Standard deviation

histopathological series. Based on we might state that these and coagulation indicators, COVID-19 is prothrombotic disease. As stated by scientists discovered platelet abnormalities, altered blood flow, and vascular endothelium abnormalities. Malfunction that resulted in numerous different thrombosis within COVID-19.[16]

The current study is comparable to the study of Berger *et al*., which involved of 2377 patients; adults were hospitalized with COVID-19 who had D-dimers more than 2000 ng/mL, which regarded a risk of critical illness of 66%, hematological thrombotic event of 37.8%, acute renal injury for about 58.3%, and death for about 47% (the critical disease being regarded as death, mechanical ventilation, or stay in intensive care).[17] The study of 29 randomized studies, which represented 4328 patients with COVID-19, found that severe patients had higher average D-dimer levels at admission, also patients who needed critical care and those who died.[17] Well-known study discovered that patients with SARS-CoV-2 infection who had elevated D-dimers at the beginning of their hospitalization were at an increased risk of developing severe disease and dying.[17] During their initial hospitalization, founded that at a number of SARS-CoV-2 infected individuals and discovered a substantial correlation between serum ferritin and overall mortality, regardless of age. A worse prognosis is typically associated with an earlier transition to intensive, respiratory, or even dialysis care. This brief laboratory report intended to determine whether we could pinpoint any pertinent function for risk prognosis utilizing additional tests that are frequently requested in positive SARS-CoV-2 hospitalized patients. There is significant correlation between S. ferritin and COVID-19 disease. Both the severe and mild COVID-19 in this study had observably higher levels of IL-6 ($P = 0.001$). The conclusions of Liu's inquiry were approved by his coworkers in 2020.[13]

Other researchers found that 67.9% of patients had higher IL-6 levels on admission. The percentage of patients in the severe category who had elevated IL-6 levels was significantly higher ($P = 0.001$)[13] and that is similar to our study. In addition, the Coomes e*t* a*l*.'s study from 2020 discovered that individuals with COVID-19 had noticeably higher IL-6 levels, which were connected to worse clinical outcomes, based on the comparability of data between investigations. The severe group had considerably higher blood levels of IL-6 than the nonsevere group. In studies describing the immunological features of severe cases of COVID-19, significant levels of circulating cytokines, especially IL-6, as well as high concentrations of various inflammatory indicators as complete blood count values, have been documented.[18]

TNF was strongly correlated with the presence of coronavirus. This finding might point to a particular cytokine profile that was present at this location during the symptomatic stage of SARS-CoV-2 infection. According to Costela-Ruiz *et al*. 2020, patients with high systemic levels of IL-6 and TNF were associated with an excessive and harmful immune response that contributed to COVID-19-related morbidity and mortality. In addition, even in severely ill COVID-19 patients who were hospitalized, IL-6 and TNF-α normal range was found. Similarly, data on the use of anti-TNF-α are indicative of a therapeutic benefit in patients with COVID-19. ACompared to other immune-suppressing medications, collected data from patients already receiving anti-TNF medications suggest a lower rate of COVID-19 bad health or even and death.[19,20] Clinical severity, lung fibrosis, and mortality in COVID-19 patients are predicted by pro-inflammatory cytokines. The cytokine storm, or the unchecked production of cytokines, associated with morbidity and mortality in COVID-19 patients. Other cytokines, however, have higher levels and protective effects of these cytokines enhance the chance of survival.

Conclusion

The spread of the COVID-19 disease is driving up the cost of healthcare globally. Due to the mutable nature of diseases and complications, solid data must be acquired to diagnose the patient's condition in the right way and predict repercussions.

Our findings imply that early assessed levels of (S. ferritin, D-dimer, and CRP) may be linked with a lower risk of developing COVID-19 severity. IL-6 and TNF-α are a reliable indicator of severe illness in COVID-19 patients. The results of the current investigation could help clinicians and healthcare professionals in identifying COVID-19 patients who may be seriously ill or in need of immediate attention.

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Conflicts of interest

There are no conflicts of interest.

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