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Research Article

Correlation between Proinflammatory Cytokines and Severity of COVID-19 within Palestinian Population

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Abstract

COVID-19 was characterized by cytokine storm and endothelial dysfunction in severely ill patients. As the severity of the infection was correlated with ethnicity, this study aimed to assess the correlation between proinflammatory cytokine serum level and COVID-19 symptoms within the Palestinian population. In a cross-sectional study, serum samples of 27 non-hospitalized patients and 63 hospitalized patients SARS-CoV-2 infected patients were tested for total antibodies, IL-6, TNF-α, IFN-γ, and IL-1β using the ELISA test. Results showed that the most common symptoms within patients were joint pain, cough, and fever (73.3%, 69.7%, and 50%, respectively). Serum total antibodies (IGs) levels in non-hospitalized patients were higher than in hospitalized patients ((44.7 COI and 9.2 COI). TNF-α and IL-6 were lower in non-hospitalized patients than hospitalized patients (48 ± 17.9 pg/ml, 193.3 ± 350.5 pg/ml respectively). On the other hand, IFN-γ in non-hospitalized patients (1 ± 2 IU/ml) was significantly higher than hospitalized patients (0.4 ± 0.26 IU/ml). IL-1β was slightly lower in hospitalized patients (8.8 ± 13.6 pg/ml) compared to non-hospitalized patients (12.5 ±

24.5 pg/ml). Common mild symptoms of COVID-19 were negatively associated with proinflammatory cytokines serum level. In conclusion, as with other populations worldwide, IL-6 and TNF- α are playing a significant role in the complications of SARS-CoV-2 infection. Monitoring the two cytokines is crucial for the management and treatment of complicated consequences of COVID-19.

Keywords: COVID-19; Total Antibodies; Proinflammatory Cytokines; SARS-CoV-2.

1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a worldwide emerging situation, initially reported in December 2019 in Wuhan, China, then affected countries worldwide increased, and it was declared a pandemic by the WHO [1]. The SARS-CoV-2 infection has a heterogenous disease course. It may be asymptomatic in the majority of the cases and can be mild to severe respiratory illness. However, more severe cases can be observed, such as multi-organ dysfunction syndromes, sepsis, and septic shock [2]. Morbidity and mortality in COVID-19 patients are accompanied by the secretion of an excessive storm of proinflammatory cytokines caused by the virus. Excessive production of proinflammatory cytokines is responsible for exacerbating acute respiratory distress syndrome and the extensive tissue damage responsible for multiple organ failures and death. Therefore targeting proinflammatory cytokines in severe complications in COVID-19 patients could increase patient survival rates and reduce mortality [3].

Transfusion of plasma from patients recovered from SARS-CoV-2 infection becomes an immuno-classical treatment for virus neutralization in severe COVID-19 cases. But for the shortness in plasma, the researchers created specific antibodies to neutralize virus particles simultaneously with modulating proinflammatory cytokines such as type I interferon, IL-6, and TNF-α that can reduce the severity of infection [4]. It has been shown that there is a correlation between the level of proinflammatory cytokines and ethnic groups. COVID-19 illness and hospitalization were varied among races due to genetic variation and chronic disease association [5]. This study investigates the correlation between cytokines level and specific antibody level with the symptom severity within the Palestinian population.

2. Material and Methods

2.1 Study design and participants

An observational cross-sectional study was conducted on COVID-19 positive patients. The study was conducted at the Martyrs medical military complex-Corona Hospital- Nablus, which is a government-run care facility for managing COVID-19 patients in the North of the West Bank of Palestine. Patients are known to have SARS-CoV-2 infection according to the Palestinian Ministry of Health (MOH) recommendations on SARS-CoV-2 diagnosis (RT-PCR). Ninety COVID-19 patients were enrolled in the study. Sixty-three of them were hospitalized COVID-19 cases with moderate or severe symptoms (Oxygen saturation was less than 92 and needed for supplemental Oxygen or has other organ failures) and categorized as the hospitalized patients (HP). Twenty-seven were asymptomatic or with mild symptoms and classified as non-hospitalized patients (NHP).

2.2 Variables and data collection

Patient demographic and clinical information, including signs and symptoms, chronic illnesses, and

the date of potential exposure to the virus, were gathered from patient clinical records for hospitalized patients and direct interviews for asymptomatic non-hospitalized patients, after the patients or their relatives signed an agreement-consent form to use data in this study. Venous blood of about 5 ml blood sample was drawn in-plane tube from each study participant by a qualified lab technician and transported to the research laboratory at the faculty of Medicine and Health Sciences at An-Najah National University. Serum samples were kept at -80°C in sterile microtubes until the time of ELISA, following the manufacturer's instructions. Samples were taken from all patients before any anti-microbial or anti-inflammatory treatment administrations.

2.3 Total antibodies (IGs) and proinflammatory cytokines measurement

All serum samples were tested for IGs using the ELISA test (Elecsys® Anti-SARS-CoV-2 of the Roche Diagnostics Ltd). According to the manufacturer's recommendations. Results were considered positive above Cut of index 1 for total antibodies. In addition, proinflammatory cytokines serum concentration was tested by R&D system-Quantikine ELISA for IL-6 and TNF-α and DRG system ELISA for IFN-γ and IL-1β following the manufacture instructions.

2.4 Analysis and ethical consideration

All statistical analyses were done with IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY: IBM Corp). Continuous variables were expressed as mean ± standard deviation (SD). Counts and percentages described categorical variables. The Institutional Review Board (IRB) of An-Najah National University and the Ministry of Health Research Committee approved the study. Informed consent was obtained from each patient involved in this study or from a member of the patient family. No identifying data were collected during the study, and the data were to be only available to the research team.

3. Results

3.1 Background characteristics

The age of the study population ranged between 15 and 90 years old, with an average of 57.5 years old. Sample include 56 (62.2%) males and 31 (34.4%) females. The majority were residents in cities (62.2%) and non-smokers (84.4%). 73.3% suffered from chronic disease, 51% had diabetes, and 61% had hypertension. Previous yearly infection influenza comprised 32.2% of the sample, and only 7.8% received the annual influenza vaccine.

3.2 Symptoms associated with COVID-19

The symptoms of COVID-19 infection were varying in the incidence among patients. Joint pain was the most encountered symptom, constituting approximately 73.3%, followed by cough; 69.7%, and fever; 50 %. Other symptoms patients experience but at low incidence were weakness; 26.7%, headache; 25.6%, shortness of breath; 23.3%, chest pain; 22.2%, muscle pain; 20%, diarrhoea; 20%, nausea; 17.8%, losing sense of smell; 18.9%, losing sense of taste; 16.7% and sore throat; 14.6% (Figure 1).

3.3 Correlation between severity of COVID-19 and total IGs and cytokines serum level

Serum IGs levels in NHP (44.7 ± 56.6 COI) were significantly higher than HP (9.2 ± 14.7 COI), ($p=0.012$). TNF-α and IL-6 were significantly ($p<0.001$) lower in NHP compared to HP (48 ± 17.9 pg/ml, 193.3 ± 350.5 pg/ml respectively). In the contrary, IFN-γ, in NHP (1 ± 2 IU/ml) was significantly higher than HP (0.4 ± 0.26 IU/ml)

(P=0.001). With no significant difference (p=0.827), IL-1β was slightly lower in HP (8.8 ± 13.6 pg/ml) compared to NHP (12.5 ± 24.5 pg/ml). (Table-1)

3.4 Correlation between Cytokine serum levels and common symptoms

Cytokine's level did not show any difference in the absence or presence of nausea, vomiting, diarrhea, shortness of breath, weakness, chest pain, and joint pain. Meanwhile, the TNF-α concentration was significantly higher in the absence rather than the presence of sore throat, headache, muscle pain, loss of taste (Figure 2 A). It was similar with and IL-6 except for a significant elevation in the presence of cough (Figure 2 B). On the other hand, IFN-γ was higher in the present than in the absence of the symptoms, with significant elevation in the presence of headache, muscle pain, and loss of taste (Figure 2 C). Finally, the IL-1β level was significantly higher in the presence of headache and slightly with muscle pain and the absence of other symptoms (Figure 2 D).

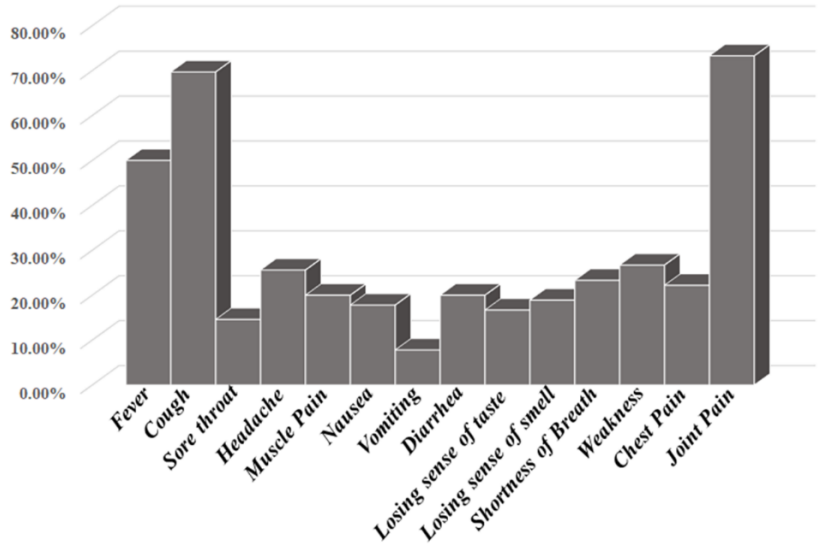

Figure 1: Common symptoms distribution within study population. Columns represent the percentage of patients experience symptoms during COID-19.

	Hospitalized (n=63)	**Non-Hospitalized (n=27)**	**P-Value***
Total Igs (COI)	9.2 (±14.7)	44.7 (±56.6)	0.012
TNF-α (pg/ml)	193.3 (±350.5)	48.0 (±17.9)	<0.001
IL-6 (pg/ml)	18.2 (±34.6)	4.2 (±0.88)	<0.001
IFN-ϒ (IU/ml)	0.4 (±0.26)	1.0 (±2.0)	0.001
IL-1β (pg/ml)	8.8 (±13.6)	12.5 (±24.5)	0.827

Table 1: Total IGs, TNF-α, IL6, IFN-γ, IL-1β levels between hospitalized and non-hospitalized COVID-19 patients. *Mann-Whitney U test.

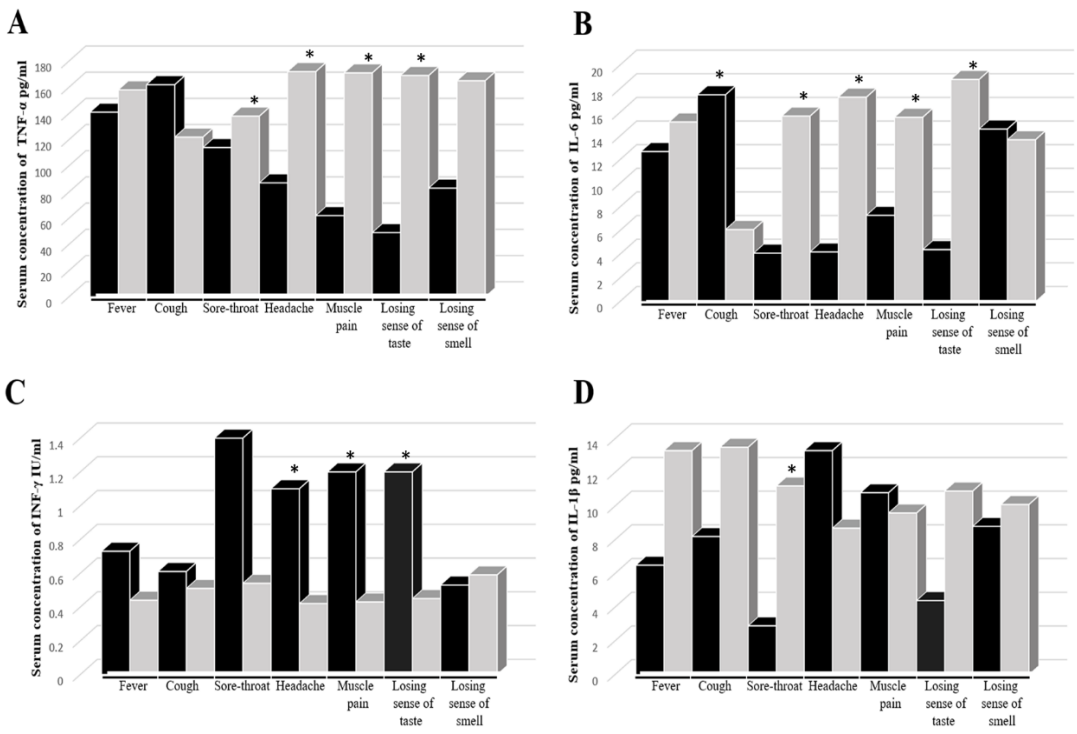

Figure 2: Ccorrelation between the common symptoms and cytokine serum concentrations. Columns represent the serum concentration of TNF-α (A), IL-6 (B), IFN-γ (C) and IL-1β (D) in the presence (Black) and absence (Grey) of common symptoms. *P. Value <0.05.

4. Discussion

Cytokine profiles and immune response in COVID-19 patients were deeply investigated in many countries worldwide due to the correlation between the severity of the disease and cytokine storm [6]. As in any other viral infection, proinflammatory cytokines and chemokines play an essential role in immune-pathology during viral infections, leading to hyperinflammatory responses in the pathogenesis of COVID-19 disease [7, 8]. However, the incident, severity, and complications of COVID-19 were varied between races and ethnic backgrounds [5, 9]. This variation refers to the differences in the immune repose and cytokine production between races [10, 11] which could be due to allelic distribution and genotype frequency, such as IL-2 and IL-6 [12].

Reports indicated the elevation of serum level of several proinflammatory cytokines, including IL-1β, TNF-α, IL-6, and T-cell cytokine IFN-γ in COVID-19 infection in general despite the severity of the disease [8, 13, 14]. The correlation with the severity of COVID-19 illness was confirmed with IL-6 [15, 16] but with variable results for other cytokines. This variation in results leads us to study the relationship of clinical symptom severity with the level of four cytokines within Palestinian COVID-19 patients. Our data showed a significant correlation between the severity of COVID-19 and the elevation level of IL-6 and TNF-α, with a nonsignificant elevation of IFN-γ, with no deference in IL-1β level between HP and NHP. Our study is in close corroboration with De

Valle et al who suggested that IL-6 and TNF-α can be independent predictors of the disease severity [17]. This is, however, in contrast with Chen et al who demonstrated an increase in the IL-6 levels, but the concentration of TNF-α, IL-1, remained unaltered in severely affected patients [18]. The difference in TNF-α concentrations could be due to the race/ethnic background and the pathological condition, and the difference in sampling times. However, further research is needed for this topic.

The role of antibodies in the severity of COVID-19 is less clear. Normally, viral elimination requires cell-mediated immunity, but humoral immunity plays an essential role in eliminating viruses and infected cells such as antibody-dependent cell cytotoxicity (ADCC), opsonization and phagocytosis via innate immune cells. However, two COVID-19 cases of patients with X-linked gamma globulinemia were challenged with antibodies acquired and survived SARS-CoV-2 infection without severe complications [19]. Some studies have demonstrated and suggested a pathogenic role for antibodies in primary infection through enhancement and increased inflammation [20], although this is thought to be not enough to explain the prevalence of severe cases of infection [21]. As such, the beneficial, neutral, or harmful role of antibodies in active coronavirus infection remains controversial. Our results didn't show any correlation with the severity of the infection.

On the contrary with the severity of COVID-19, our results showed that IL-6 and TNF-α were neutral or negatively associated with the common symptoms except for cough, and IL-6, which was associated with losing sense of smell. Previous results suggested a significant role of IL-6 and TNF-α smell and taste dysfunction [22, 23]. This deference may be due to the difference in the population where those studies compared serum level of cytokines and chemokines in correlation with SARC-CoV-2 infection with healthy subjects, no correlation with the severity of the disease. This study has some limitations, including a limited sample size and a lack of time to follow up on patient changes. These factors may have played a role in the lack of a significant association of some of our findings, however they do not change the absence of any correlation between specific clinical severe symptoms and cytokine levels.

5. Conclusion

IL-6 and TNF-α have been playing a major role in the complications and severity of SARS-CoV-2 infection within Palestinians like other populations worldwide. But the difference was regarding the relationship of cytokines and common symptoms of infection. Therefore, monitoring IL-6 and TNF-α serum levels during disease stages is critical to managing and treating complicated consequences of COVID-19.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, [W. Basha], upon reasonable request. A preprint of this article has previously been published [24].

Conflict of Interest

Walid Basha, Zaher Nazzal, Yousef El-Hamshary, Anwar Odeh, Lama Hijjawi, Mahmoud Doden, Ahmad Musa, and Saad Ruzzeh declare that they have no conflict of interest. Walid Basha as team leader has received research grants from the An-Najah National University for research development grant.

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