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Original Research

Interleukin-6 role in the severity of COVID-19 and intensive care unit stay length

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Abstract: Evaluation of cytokine production in COVIID-19 disease, in which the cytokine storm is one of the most important pathological features in complicated cases, especially interleukin 6 as a pre-inflammatory cytokine that exacerbates the immune response, could help determine the pathophysiology of the disease. Examining the level of this cytokine along with other related factors can help to better understand the pathogenesis of this disease. In this cross-sectional study, 48 patients with COVID-19 whose disease was confirmed by swap testing were evaluated. The demographic information of the individuals, the symptoms of the disease, and the ward in which they were admitted were recorded. Blood samples were taken from patients to test for interleukin-6 levels by electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics). Due to the lack of specific treatment protocols for patients and the use of supportive treatments based on meeting the nutritional needs for all patients, blood albumin levels and nutritional status of patients were also evaluated using Subjective Global Assessment (SGA) Form. Their calorie intake was assessed by calculating the number of calories received based on the type of nutrition and compared to the required amount calculated through the Harris-Benedict equation. 48 laboratory-confirmed 2019-nCoV infected patients were included in the study with the mean age of 46.4 ± 8.3 years. 21 patients were admitted to the intensive care unit (ICU). There was no significant difference between the ICU admitted and patients admitted inward in terms of demographic characteristics, and history of previous diseases ($p > 0.05$). The average interleukin 6 (IL-6) in patients was 72.3±34.4 pg/ml. ICU admitted patients had higher IL6 levels (p=0.001). The mean interleukin 6 level was 89.04±34.1 pg/ml in patients admitted for less than 7 days and it was significantly higher (119.2±28.3) in patients hospitalized for more than 7 days (p=0.001). there was no significant difference in terms of nutritional status and albumin level between ICU admitted and ward admitted patients (p >0.05). Our study shows that there may be possible associations of IL6 and disease severity and ICU stay length.

Key words: COVID-19; IL-6; 2019-nCoV; Intensive care unit.

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), formerly known as 2019 novel Coronavirus (2019 nCoV), is a single-stranded RNA virus that causes coronavirus disease in 2019 (COVID-19). A comparison of the genetic sequence of this virus and other examples of the virus has shown similarities with the SARS and bats coronaviruses and therefore may be the primary source of the virus in bats. This pandemic virus started in Wuhan, China, in December 2019. Early symptoms of Covid-19 include pneumonia, fever, muscle aches, and fatigue. To date, no successful vaccine or antiviral drug has been clinically validated and available for Coronavirus virus-2019 infections (1). Several studies have already shown that increased rates of plasma proinflammatory cytokines have been correlated with lung inflammation and extreme SARS lung damage. Cytokine release syndrome (CRS) is a generalized immune response that can be caused by injuries, characterized by a sudden rise in the amount of pro-inflammatory cytokines (2, 3). Interleukin 6 (IL-6) is a multifunctional cytokine that regulates immune responses and acute phase reactions and plays a very important role in the host's defense mechanism. IL-6 is not constantly produced by normal cells, but its expression is enhanced by a variety of cytokines, lipopolysaccharides, or viral infections (5). The product of the IL-6 gene expression is a single-chain protein, depending on its cellular origin, with a molecular weight of 21 to 28 kDa. This protein is produced by various cells such as macrophages, helper T cells, and B lymphocytes and works in a wide range of tissues and due to the nature of the target cell, processes functions as well as growth induction or growth inhibition and differentiation (6). Therefore, measuring IL-6 levels in serum and other body fluids provides

more details about pathogenic conditions. Body fluids of patients with local bacterial or viral infections have shown to contain very high amounts of activated IL-6 (7). Measurement of IL-6 levels seems to be a clinical and research necessity in the diagnosis and treatment of such diseases as well as COVID-19. Since there is not yet any specific treatment regimen for COVID-19 and most centers rely on supportive care, and so far no study has been done to investigate the nutritional status, calorie intake, and hypoalbuminemia in COVID-19 patients (8). This study was designed to evaluate IL-6 levels in these patients along with the nutritional status.

Materials and Methods

The present study was a cross-sectional descriptive study. In this study, all patients diagnosed with swap tests and real-time polymerase chain reaction (RT-PCR) samples for COVID-19 were identified and examined. Inclusion Criteria were as follows: 1- laboratory-confirmed 2019-nCoV infection [based on standard WHO method], 2- The amount of serum albumin was examined during the week before the study, and the amount and rate of prescribing tube nutrition and parenteral nutrition were determined. Exclusion criteria included: Patients whose serum albumin levels were not measured during the week before the study and patient death. For each patient, information was collected using the history of the case, the latest tests and measurement of the middle arm circumference and the length of the ulna bone to determine the ideal height and weight. Information such as a history of hypertension, diabetes, heart disease and stroke, type of nutrition, type of formula, and dietary supplementation for each patient were recorded using the General Individual Assessment Form (SGA). The SGA form includes two sections: medical history and physical examination. The first part of this form, which is about the patient's medical history, has 5 components, including weight changes, dietary intake, gastrointestinal symptoms, the functional capacity of the patient, the presence of underlying hypercatabolic diseases (such as physical injuries, burns, inflammatory diseases, infections, and malignant tumors). The second part of the SGA form, which is about the physical examination, has three components, which include subcutaneous fat analysis, muscle analysis, the presence of edema or ascites. If any of the above components were normal, the A score was awarded. If it was mild to moderately affected, the B score was awarded, and the C score was awarded if it was severely affected. In the final scoring of the SGA form, if most of the components have scored A, the person has a normal nutritional status and if most of the components have received a B score, the person has a mild to moderate malnutrition and if most of the components have received a C score, the person has severe malnutrition (9). Calories received by each patient using the amount Calories received from antral and parenteral nutrition and its prescribed rate were calculated, and the calories required by each patient were determined using the Harris-Benedict formula, and then the percentage of calories received from the number of calories required for each patient was calculated, all by a nutrition specialist. After obtaining consent inform of patients who were admitted at least for last 5 days in the hospital, blood samples were taken and after centrifugation of 2-3 cc of the blood sample, its plasma was immediately frozen at a temperature of 20 C - and quickly sent to the laboratory for Evaluation of plasma levels of interleukin 6 with Roche-Hitachi (Elecsys 2010) device with electrochemiluminescence (ELC) method. The results of the last albumin test were recorded for each patient. All data were entered into SPSS after collection. For data comparison, we used the independent t-test for parametric data to compare two independent groups and the Mann-Whitney test for non-parametric data. a Chi-square test was used to evaluate the relationship between qualitative variables.

Results

48 admitted patients were identified as having laboratory-confirmed 2019-nCoV infection with the mean age of 46.4 ± 8.3 years. Among them, 21 patients were admitted to the ICU. 64.6% of the patients were male. 31.2% had a history of high blood pressure, 22.9% had a history of diabetes, and 14.5% had a history of cardiovascular disease. 72.9% of patients had Fever, 47.9% had Cough, 43.8% had Myalgia, 33.3% had Headache, 45.8% of Diarrhea, and 50.0% had Dyspnea at the referral to the emergency department. All patients had a fever. The average IL-6 in patients was 7.3 ± 3.4 (Table 1). There was no significant difference between the two groups in terms of demographic characteristics, and history of chronic diseases ($p > 0.05$). ICU admitted patients had higher IL6 levels (p=0.001).

Of the patients admitted to the ICU, 12 patients were hospitalized for less than 7 days and 9 patients were hospitalized for more than 7 days. Comparison of demographic characteristics, history of chronic diseases, and clinical symptoms are given in Table 2. The mean interleukin 6 was 89.04±34.1 in patients admitted for less than 7 days and it was significantly higher (119.2±28.3) in patients hospitalized for more than 7 days (p=0.001). There were no differences in terms of Albumin and Estimated received or required calories between the ICU and ward admitted patients (p >0.05).

There was no statistically significant difference between the two groups of critically ill patients in ICU in terms of demographic characteristics, history of chronic diseases and clinical symptoms ($\boldsymbol{p} > 0.05$), but there was a statistically significant difference between the two groups in terms of mean interleukin 6 and hospital stay length ($\boldsymbol{p} <0.05$). None of the correlation tests performed between variables was significant ($\boldsymbol{p} >0.05$).

Discussion

According to a decision by the World Health Organization (WHO), the new coronavirus, the seventh known virus in the family, has been called "SARS-Co-2" since Tuesday, February 11, 2020. It was also called "CoViD-19". COVID is a compound name for the three words corona, virus, and disease, and the number 19 refers to the year the disease was diagnosed (2019) (1-2). Interleukins are cytokines made by a variety of white blood cells that often affect other lymphocytes. These compounds play an important role

Table 1. Demographics and baseline characteristics of patients infected with 2019-nCoV.

	All patients (n=48)	ICU care (n=21)	Inward care (n=27)	*p*-value
Age, years	46.4±8.3	45.8±8.9	46.8±7.9	0.313
Sex, n (%)				
Men	31 (64.6%)	13 (61.9%)	18 (66.7%)	0.518
Women	17 (35.4%)	8 (38.1%)	9 (33.3%)	
Hypertension, n(%)	15 (31.2%)	7 (33.3%)	8 (29.2%)	0.844
Diabetes, n (%)	11 (22.9%)	5 (23.8%)	6 (22.2%)	0.801
Cardiovascular disease, n (%)	7 (14.5%)	4 (19.04%)	3 (11.1%)	0.946
Fever, n (%)	35 (72.9%)	16 (76.2%)	19 (70.4%)	0.374
Cough, n (%)	23 (47.9%)	9 (42.9%)	14 (51.9%)	0.515
Myalgia or fatigue, n (%)	21(43.8%)	11 (52.4%)	10 (37.0%)	0.257
Headache, n (%)	16 (33.3%)	6 (28.6%)	10 (37.0%)	0.224
Diarrhea, n (%)	7 (14.5%)	4 (19.04%)	3 (11.1%)	0.946
Dyspnea, n (%)	24 (50.0%)	12 (57.1%)	12 (44.4%)	0.829
Parenteral Nutrition, n (%)	19(39.5%)	19(90.5%)	0(0%)	0.065
Oral Nutrition, n (%)	29(60.5%)	2(9.5%)	27(100%)	
Estimated received calorie	1208.7±118.3	1235.2±119.5	1188.1±112.9	0.172
Estimated essential calorie	1746.34±218.4	1592.75±185.5	1865.88±334.5	0.0008
Calories received from calories required (%)	72.5±0.1	81.9±0.01	61.14±0.01	0.981
Albumin, g/dL	3.43±0.8	3.5±0.8	3.37±0.7	0.971
Il6, pg/ml	72.3±34.4	101.9±62.5	46.5±34.80	0.0001

Table 2. Demographics and initial characteristics of patients infected with 2019-nCoV in the ICU.

	Days ICU care <7(n=12)	Days ICU care >7(n=9)	*p*. value
Age (years)	44.4±9.5	47.7±8.3	0.001
Men	7 (58.3%)	6 (66.7%)	0.464
Women	5 (41.7%)	3 (33.3%)	
Hypertension	4 (33.3%)	3 (33.3%)	0.946
Diabetes	3 (25.0%)	2 (22.2%)	0.780
Cardiovascular disease	3 (25.0%)	1 (11.1%)	0.780
Fever	9 (75.0%)	7 (77.8%)	0.780
Cough	5 (41.7%)	4 (44.4%)	0.819
Myalgia	7 (58.3%)	4 (44.4%)	0.819
Headache	4 (33.3%)	2 (22.2%)	0.282
Diarrhea	3 (25.0%)	1 (11.1%)	0.780
Dyspnea	6 (50.0%)	6 (66.7%)	0.258
Il6, pg/ml	89.04±34.1	119.2±28.3	0.0001
Albumin	3.5±0.8	3.4±0.7	0.764
Estimated received calorie	1228.1±117.1	1244.7±113.9	0.748
Estimated essential calorie	1493.6±179.5	1538.2±198.7	0.603
calories received from calories required (%)	82.2±0.01	81.5±0.01	0.918

in the immune system. Their number is very large and they are marked with a number. Interloquin-6 (IL6 / IL-6) is a multifunctional cytokine that regulates immune responses and acute phase reactions and plays a very important role in the host's defense mechanism. The human IL-6 gene is located in region 21 on the short arm of chromosome 7, and its genomic sequence is determined. IL-6 is not constantly produced by normal cells, but its expression is enhanced by a variety of cytokines, lipopolysaccharides, or viral infections. The product of the expression of the IL-6 gene is a single-chain protein, depending on the cellular origin; with a molecular weight of 21 to 28 kDa (2-5).

Our study shows that there may be possible associations of IL6 and disease severity and ICU stay length. Rostamian et al. (10) research found that IL-6 rates were substantially higher in hospitalized COVID-19 patients relative to healthy people (P <0.001). IL-6 was nearly 10-fold of the normal range in COVID-19 patients. They concluded that a dangerous amount of IL-6 is likely to endanger essential health factors and plays a key role in immune-mediated acute lung damage in CIOVID-19 patients. As well as their study, our patients had a high level of IL6 which was extremely higher in critically ill patients. Also in Coomes's study (11) in COVID-19 cases, IL-6 rates

were substantially elevated and correlated with negative clinical consequences.

Cytokine storms that occur after a COVID-19 infection are important causes of lethal immunopathology. A wave of cytokine and pre-inflammatory responses shows the host's extensive efforts to control infection. Recent laboratory studies have highlighted the importance of proper regulation of cytokines so that both insufficient and high levels of certain cytokines can be associated with adverse effects (2). COVID-19 infection is characterized by high levels of cytokines and pre-inflammatory, including interferon-gamma and IL-6. Severe cytokine response results in acute pneumonia, severe pulmonary leakage, and destruction of lung tissue structure (2).

Our study didn't reveal any association between the disease severity (being admitted in ICU) and albumin levels or received calories which may happen due to the optimal nutritional supportive care provided for all patients. Albumin appears to be associated with the level of inflammation and severity of some diseases more than a marker of nutritional status and calories received (12). While some studies suggest that serum albumin is a poor indicator of nutritional status because it is affected by many factors, including insulin, thyroid hormones, inflammatory cytokines, hypermetabolism, malabsorption, and increased intravascular fluid volume (13).

There may be a significant inverse relationship between serum albumin levels and inflammatory markers (14), but there wasn't such an association in our study. New and advanced genetic engineering technologies, such as genome editing, may be used to combat respiratory pandemic viruses (15).

As our study showed the relationship between disease severity and interleukin 6 levels, pulmonary immunopathology seen during COVID-19 infection appears to be one of the leading causes of COVID-19-related complications. Instead of eliminating viral agents, the host's antiviral defense mechanisms seem to be among the main causes of pulmonary immunopathology caused by COVID-19 infection. While there is a need for many other studies to evaluate the precise relationship of this cytokine with the COVID-19.

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