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Comparison of TNF-α, IL-6 and IFN-γ Proinflammatory Cytokine Levels of COVID-19 Patients in Bengkulu City

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ABSTRACT
Coronavirus disease-19 or COVID-19 pandemic was happening all over the world and is the main concern of every country in the context of preventing, handling and controlling the disease, including in Indonesia. Severe Acute Respiratory Syndrome Virus 2, or SARS-Cov 2, is the official name of the virus that causes COVID-19. The virus has a fast and easy transmission rate and can cause death so serious control is needed in prevention and management of COVID-19 disease. Previous studies showed that the level of anti-SARS-CoV-2 IgG antibodies in the patient's body increased from the first week to the third week. Then COVID-19 patients who have recovered still show reactive levels of anti-SARS-CoV-2 IgG antibodies for up to three months after recovery, while antibody levels of anti-SARS-CoV-2 IgM levels are no longer reactive in COVID-19 patients who have been cured. healed. Apart from IgG and IgM, several other components of the immune system are also important to study. The increase in circulating cytokine levels in the patient's body is often referred to as a cytokine storm. According to the findings of several studies, the amount of cytokines, particularly the proinflammatory cytokines IL-6, TNF-α, and IFN-γ, is correlated with the severity of COVID-19 disease. Therefore, this study attempts to analyze the comparison of proinflammatory cytokine levels in COVID-19 patients who are being treated and those who have recovered.

KEYWORDS : COVID-19, Cytokine, IFN-γ, IL-6, SARS-Cov-2, TNF-α

INTRODUCTION

Global pandemics have been brought on by COVID-19, or coronavirus disease 2019. The angiotensin-converting enzyme (ACE) receptor, which also serves as the receptor for the SARS-Cov virus that caused earlier epidemics, is bound by the glycoprotein Spike component of this virus to infect humans (del Rio, Carlos; Malani, 2020; Hamming et al., 2004; Li et al., 2003). The clinical symptoms of COVID-19 infection are almost the same as the symptoms of other respiratory diseases such as fever, cough, myalgia, dyspnoea, and pneumonia (Huang et al., 2020). Blood samples showed T-cell and B-cell responses against SARS-Cov-2 approximately one week after COVID-19 symptoms appeared (Tay et al., 2020). IgG and IgM seroconversion can occur simultaneously or sequentially and both reach a plateau within 6 days after seroconversion (Long et al., 2020). IgM seroconversion generally increases from day 9 while IgG increases on day 11 after symptoms appear (Xiang et al., 2020).

Numerous studies indicate that SARS-CoV-2 infection triggers a hyperinflammatory response that exacerbates COVID-19 disease and results in mortality (Del Valle et al., 2020). Rising levels of several cytokines associated with infection and immune conditions are referred to as cytokine storms. Interleukin (IL-1, IL-2, IL-6), granulocite-macrophage colony-stimulating factor (GM-CSF), interferon (IFN-γ), and tumor necrosis factor (TNF-α) are among them. These interact with complement and the coagulation system to cause hemophagocytic

lymphohistiocytosis (HLH), respiratory failure (ARDS), disseminated intravascular coagulation (DIC), and multiorgan disease.

Previously, research was conducted on the level of antibodies to immunoglobulin M (IgM) and immunoglobulin G (IgG) in COVID-19 patients who were being treated and had recovered in Bengkulu City. In this study managed to collect serum from 48 research respondents. In this study, the researchers wanted to analyze the profile of IL-6, TNF-, and IFN-γ cytokines in the patient's serum, especially their correlation with the severity of COVID-19 in Bengkulu City. In contrast to previous studies, this study also compared the levels of proinflammatory cytokines in patients who were being treated and patients who had recovered.

MATERIALS AND METHODS

Research Design

The population of this study were patients with confirmed COVID-19 in Bengkulu City in October-November 2020. The inclusion criteria for research subjects were patients who were confirmed positive on the RT-PCR examination. The study's independent variables were the levels of the cytokines IFN-γ, TNF-α, and IL-6. While the dependent variable in this study is the severity of COVID-19 patients.

Cytokine Detection

Serum was extracted from the patients in order to test the levels of the cytokines IL-6, TNF-α, and IFN-γ. Serum was inactivated at 56 for 30 min and stored at -20 before being examined. Cytokines IL-6, TNF-α, and IFN-γ were examined using the Beads Multiplex Assay methods.

Data analysis

Patient characteristics data were collected from medical record data. Some of the characteristics analyzed were age, gender, and symptoms. Statistical analysis was performed using SPSS software. The nonparametric Kruskal-Wallis differential test and Spearman correlation tests were used in statistical analysis to examine the impact of cytokine levels on patient characteristics.

RESULTS

Research Subject Characteristics

Recruitment of research subjects has been carried out based on data from COVID-19 patients who have been confirmed negative on PCR examination. Subject recruitment has been running for 1 month. The research sample used in this study amounted to 27 samples consisting of COVID-19 patients. The characteristics of this research subject explained in Table 1.

Table 1. General Characteristics of Research Subjects

Subject Characteristic		n	%
Gender	Male	13	48,15
	Female	14	51,85
Age	21-30	2	7,41
	31-40	3	11,11
	41-50	9	33,33
	51-60	8	29,63
	>60	5	18,52
Symptoms	Fever	22	81,48
	Cough	19	70,37
	Fatigue	17	62,96

Diarrhea	2	7,41
Nausea	11	40,74
Sore	3	11,11
Heartburn	6	22,22
Anosmia	2	7,41
Ageusia	5	18,52
Chest Congestion	19	70,37
Unconcious	3	11,11
Insomnia	2	7,41

Analysis of Differences in TNF-α Levels in Severity Degree of COVID-19

Statistical analysis of differences in TNF-α levels at several levels of severity of COVID-19 patients in Bengkulu City was carried out using the Kruskal-Wallis Test. The results of the test showed a p value = 0.032, which means there is a significant difference in TNF-α levels in COVID-19 patients with mild, moderate and severe severity (Figure 1). The mean TNF-α levels showed the highest TNF-α levels in severe COVID-19 patients (24.29 pg/ml), while the lowest TNF-α levels were in mild COVID-19 patients (15.54 pg/ml) (Table 2).

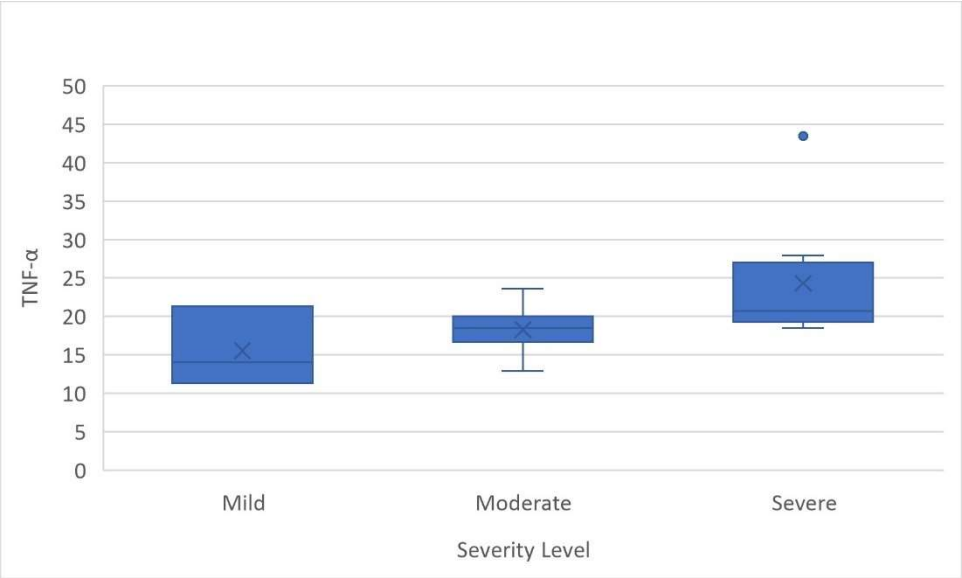

Figure 1. Comparison of the TNF-α levels in different severity degree of COVID-19 patients. p = 0.032.

Table 2. Statistical Comparative Analysis of TNF-α Levels in Different Severity of COVID-19.

Severity	n	Mean (pg/ml)	SE	SD	Median (pg/ml)	Minimum (pg/ml)	Maximum (pg/ml)	p-Value
Mild	3	15.54	3.00	5.19	14.05	11.26	21.32	0,032
Moderate	16	18.29	0.72	2.89	18.50	12.91	23.57	
Severe	8	24.29	2.96	8.36	20.69	18.50	43.51	

*Note : Normal score TNF-α: <8,8.

Analysis of Differences in IL-6 Levels in Severity Degree of COVID-19

The Kruskal-Wallis Test was also utilized in the statistical study of variations in IL-6 levels among COVID-19 patients in Bengkulu City at various severity levels. The test's findings revealed a p value of 0.001, indicating a substantial variation in IL-6 levels across patients with mild, moderate, and severe COVID-19 (Figure 2). The average difference in patients with mild, moderate and severe severity does look very significantly different (Table 3). The average IL-6 level in severe COVID-19 patients reached 106.06 pg/ml), in moderate patients it reached 26.63 pg/ml, while the IL-6 level in mild COVID-19 patients was only 1.61 pg/ml.

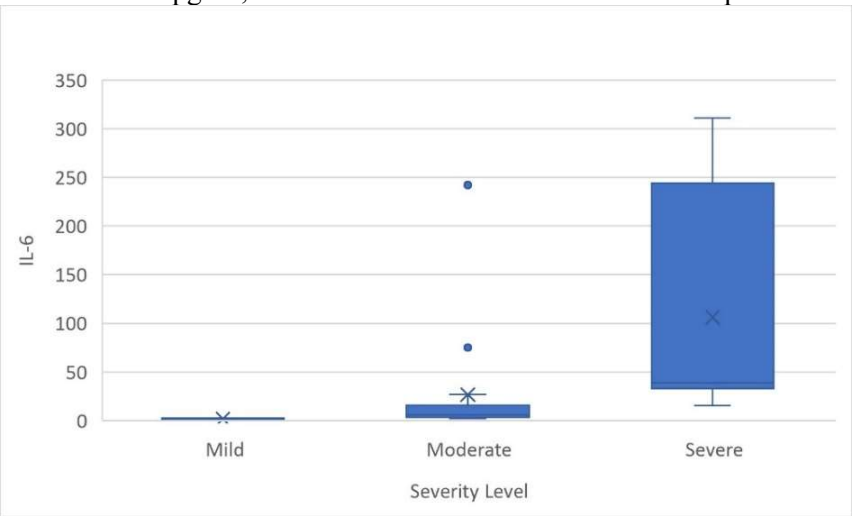

Figure 2. Comparison of the IL-6 levels in different severity degree of COVID-19 patients. p = 0.01.

Table 3. Statistical Comparative Analysis of IL-6 Levels in Different Severity of COVID-19.

Severity	n	Mean (pg/ml)	SE	SD	Median (pg/ml)	Minimum (pg/ml)	Maximum (pg/ml)	p-Value
Mild	3	1.61	0.39	0.67	1.26	1.19	2.39	
Moderate	16	26.63	15.06	60.24	5.62	1.89	242.16	0,01
Severe	8	106.06	44.04	124.57	38.62	15.27	311.51	

*Note : Normal score IL-6 : 0,7-12,5

Analysis of Differences in IFN-γ Levels in Severity Degree of COVID-19

Analysis of differences in IFN-γ levels in mild, moderate and severe patients also showed significantly different values (p=0.017) based on the Kruskal-Wallis test (Figure 3). The average IFN-γ levels in mild, moderate and severe COVID-19 patients were 1.19 pg/ml, 2.28 pg/ml and 3.16 pg/ml, respectively (Table 4). There was an increase in IFN-γ levels in cases of COVID-19 with increasing severity.

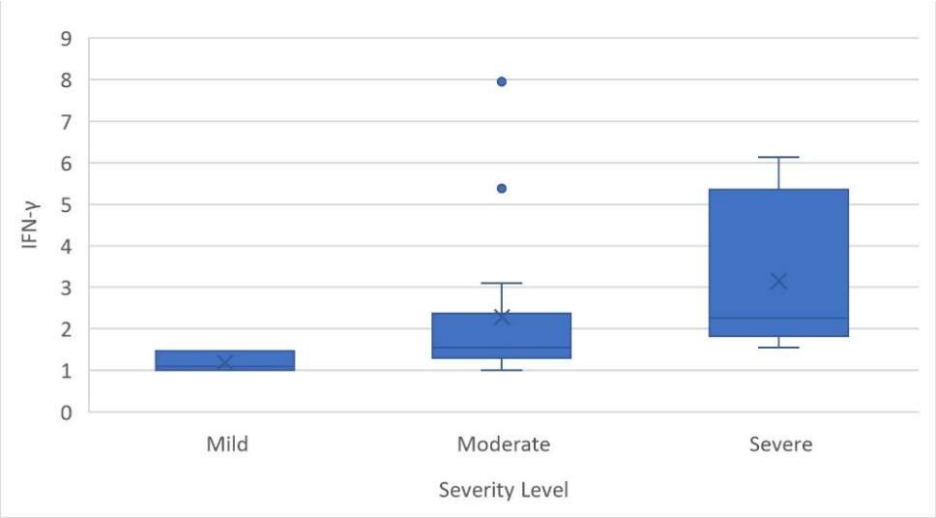

Figure 3. Comparison of the IFN-γ levels in different severity degree of COVID-19 patients. p = 0.017.

Table 4. Statistical Comparative Analysis of IFN-γ Levels in Different Severity of COVID-19.

Severity	n	Mean (pg/ml)	SE	SD	Median (pg/ml)	Minimum (pg/ml)	Maximum (pg/ml)	p-Value
Mild	3	1.19	0.14	0.25	1.1	1.00	1.47	
Moderate	16	2.28	0.46	1.85	1.56	1.00	7.95	0,017
Severe	8	3.16	0.67	1.88	2.27	1.56	6.13	

*Note : Normal score IFN-γ : <5,0.

Analysis of Correlation in TNF-α, IL-6, and IFN-γ Levels in Severity Degree of COVID-19

Spearman correlation test was conducted on the three variables of inflammatory cytokines TNF-α, IL-6, and IFN-γ against the severity of COVID-19. The correlation test results showed a link between the levels of the inflammatory cytokines TNF-α, IL-6, and IFN-γ with the severity of COVID-19 patients, with p values of 0.008, <0.001, and 0.003 correspondingly (Table 5). As a result, TNF-α, IL-6, and IFN-γ levels increase with more severe patients. The correlation coefficient of IL-6 cytokines showed a very strong positive correlation. The correlation coefficient of TNF-α and IFN-γ showed a strong positive correlation.

Table 5. Statistical Correlation Analysis of TNF-α, IL-6, and IFN-γ Levels in Different Severity of COVID-19.

Cytokine	Correlation coefficient	p-value
IL-6	0.741	<0.001
TNF-α	0.502	0.008
IFN-γ	0.553	0.003

DISCUSSION

Based on the results of the provisional data that has been obtained, most of the symptoms and clinical manifestations of COVID-19 patients hospitalized generally include mild, moderate and severe manifestations. Then from the results of the cytokine examination, it was seen that there was generally an increase in IL-6, especially in COVID-19 patients who experienced mild, moderate and severe manifestations. For those with

mild manifestations and those who have recovered, they are generally normal. The increase in TNF- also occurred in all COVID-19 patients. Meanwhile, the concentration of IFN-γ in general in COVID-19 patients is still normal, except for some patients who have moderate and severe manifestations.

The levels of proinflammatory cytokines TNF-α, IL-6, and IFN-γ in mild, moderate, and severe COVID-19 individuals generally differ significantly. The results of the correlation test also showed that the higher the severity of COVID-19 patients, the higher the levels of proinflammatory cytokines TNF-α, IL-6, and IFN-γ.

Weakened immunity and the unchecked and increased release of pro-inflammatory cytokines, which causes the cytokine storm, are hallmarks of advanced COVID-19 syndrome (Rabaan et al., 2021). Pro-inflammatory cytokines and chemokines, which are linked to pulmonary inflammation and significant lung damage, are abundant in COVID-19 patients (Darif et al., 2021). When SARS-CoV-2 triggers an innate immune response, neutrophils and other immune cells immediately increase whereas T cells (CD4+ and CD8+) significantly decrease. However, one notable feature of SARS-CoV-2 infection has been identified as the decrease of T cells combined with an increase in IL-6 and IL-8 production (Zhang et al., 2020). Coagulation pathways are triggered during the immunological response to infection, resulting in an overabundance of proinflammatory cytokines and damage to several organs (Jose & Manuel, 2020).

The etiology of COVID-19 includes the cytokine release syndrome and its clinical implications. TNF-α, IL-17, IL-6, IL-8, and IL-1β are among the pro-inflammatory cytokines that aid in the onset of cytokine release syndrome (CRS). Among the severe consequences of excessive cytokine release are acute lung injury, cellular damage, altered microbiota, severe lymphopenia, and a decrease in regulatory T cells. (Darif et al., 2021).

T and B lymphocytes, monocytes/macrophages, dendritic cells, fibroblasts, and endothelial cells are among the cell types that express the glycoprotein interleukin 6 (IL-6). Hematopoiesis, inflammation, immune system modulation, and the CRS are all influenced by IL-6. Previous investigations found a favorable correlation between the severity of COVID-19 and a greater level of IL-6 in serum (X. Chen et al., 2020).

The most researched cytokine in the TNF superfamily, tumor necrosis factor (TNF-α), is also a crucial component in the pathogenesis of COVID-19 CRS (Darif et al., 2021). TNF-α causes bronchial hyperresponsiveness and airway inflammation in healthy individuals via increasing neutrophil recruitment. Moreover, this cytokine can result in T cells Apoptosis (Makwana et al., 2012). TNF-α plasma levels are elevated in severe COVID-19 cases, according to numerous investigations (G. Chen et al., 2020; Huang et al., 2020). Additionally, a rise in TNF was linked to the severity of the illness and inversely connected with a decrease in T cells (Diao et al., 2020).

IFN (type I, II, III) are involved in natural immunity to bacteria and viruses. Natural killer (NK) cells and macrophages both produce type II interferon (IFN-γ) in response to intracellular bacterial and/or viral infections (Fara et al., 2020). IFN-γ levels rose in COVID-19 in tandem with the viral load (Huang et al., 2020). The patient's condition worsened, the delayed peak coincided with a decrease in lymphocyte counts, and there was an increase in neutrophil infiltration of the lungs' alveoli (Zheng et al., 2020).

CONCLUSION

The concentration of IL-6 and TNF-α cytokines in COVID-19 patients increased significantly, especially in conditions of moderate and severe clinical manifestations. Meanwhile, the concentration of IFN-γ cytokines was generally still normal. There are significantly differential level of IL-6, TNF-α and IFN-γ in severity degree of COVID-19 patient. The high level of these cytokines caused more severity of COVID-19 disease.

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