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# TNF-α serum Level between SARS-CoV-2 Infected Pregnant women with normal pregnant women in RSUD Dr. Soetomo Surabaya

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## ABSTRACT

**Introduction:** Pregnant women infected with SARS-CoV-2 experienced increased pro-inflammatory cytokines, including TNF-α, resulting in a cytokine storm condition. This study compares TNF-α levels between pregnant women infected and not infected with Covid-19 in the 3rd trimester.
**Methods:** This observational research was conducted with cross sectional design. Our study included pregnant women who came to delivery room/inpatient at RSUD Dr. Soetomo Surabaya before or during the labor period between May and June in 2021. The inclusion criteria were 3rd trimester gestational age or > 28 weeks with SARS-RT-PCR nasopharyngeal swab result. The exclusion criteria were all significant pregnancy complications. The pregnant woman coming to the delivery room will be screened for COVID-19, and blood samples will be examined using the flow cytometry method for the cytokine Th1: TNF-α.
**Results:** There were no significant differences in TNF-α levels in pregnant women who were infected and not infected with COVID-19 (median 3.42 (7.24) pg/ml vs. 2.70 (3.06) pg/ml. p=0.138). There were also no significant differences in TNF-α levels in pregnant women with symptomatic vs. asymptomatic COVID infection (3.21(3.97) vs. 2.41(2.71) pg/ml; p=0.314).
**Conclusion:** This study revealed no significant difference in TNF-α serum level between SARS-CoV-2 infected pregnant women with normal pregnant women.

**Keywords:** Covid-19, Cytokines, TNF-, Pregnant women
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## INTRODUCTION

Pregnant women are a vulnerable population to be infected with COVID-19 with more severe clinical manifestations and a high risk of abnormal fetal growth and development.[1] COVID-19 infection in pregnancy carries the risk of excess pro-inflammatory conditions in the first and third trimesters. The immune response is back to the pro-inflammatory state in the third trimester to support the labor process.[2] Infiltration of neutrophils and macrophages in the myometrium increases pro-inflammatory cytokines (IL-1β and IL-8) and changes in the decidua in the form of an increase in TNF.[3] Increased levels of TNF- can cause cell apoptosis, organ failure, to septic shock, which will cause pregnancy complications. Some literature states that TNF- is a key pro-inflammatory cytokine in COVID-19 mortality.[4] TNF- is one of the cytokines whose overproduction is associated with a worse prognosis in SARS-CoV and MERS patients.[5] Elevated TNF is said to cause septic shock and multiple organ failure. Myocardial damage and circulatory failure were found in some COVID-19 patients.[6]

Research on anti-cytokine therapy (especially TNF-inhibitors) in COVID-19 patients has been widely carried out and has reduced the aggravation process.[4] In addition, several observational studies state that anti-TNF- therapy can reduce adverse outcomes and reduce mortality in COVID-19 compared to other anti-immune therapies. Anti-TNF-α has long been used as a safe therapy, including in high-risk populations and various preparations.[4,5] There are not many observational studies of TNF- in pregnant women with COVID-19 infection. Based on the above background, this study was conducted to know the difference in TNF-α levels in pregnant women who are infected and not infected with COVID-19

## METHODS

This study was conducted using observational analysis and a *cross-sectional design* with ethics obtained from the Health Research Ethics Committee of RSUD Dr. Soetomo Surabaya with registration number 0099/KEPK/XI/2020. We collected data between May and June in 2021 on Pregnant women who came to the delivery room/inpatient at RSUD Dr. Soetomo Surabaya before or during In labor period. We divide subjects into two groups, the sample and the control group. The inclusion criteria are 3rd trimester gestational age or > 28 weeks, SARS-CoV-2 PCR swab examination is available. The exclusion criteria are pregnancy with complications, including hypertension in pregnancy, chronic hypertension, diabetes mellitus, obesity, autoimmune disease,

kidney disease, intrauterine fetal death (IUFD), childbirth, and diagnosed with other infectious diseases. The patient will have 20cc of blood taken at arrival for blood sampling and 6cc into the yellow tube. Blood samples will be sent and centrifuged and then examined using the Facs Calibur in the Clinical Pathology laboratory of RSUD Dr. Soetomo.

The sample size is calculated based on unpaired numerical, analytical research formula. From the calculation, the result is 21.06, so the required sample size for each group is rounded up to a minimum of 21 samples. The research sample was by *consecutive sampling, a sampling technique in which every subject meeting the inclusion criteria* is selected until the required sample size is achieved. Sample possibility 10% is issued so that the sample is taken as many as 25 samples for each group. The instrument used is the *specimen kit* BD CBA (*Cytometric Bead Array*)Human Th1/Th2 *Cytokine kit* II CAT No. 551809 with Facs *Calibur*,2 PCR machine *Roche Cobas Z480* and 1engine *Lepgen 96*, and brand reagents *Maccura* and *SD Biosensor*. The research took place from May to July 2021. The data analysis technique was a non-parametric study using the Mann-Whitney Test.

## RESULT

This study recruited 25 pregnant women with COVID-19 and 25 without COVID-19. The characteristics of the maternal age in the infected group were not different than the uninfected (p=0.47).

The characteristics of gestational age and reference origin did not show significant differences (p=0,175) and (p=1,000). A total of 12 (48%) pregnant women from the COVID-19 positive group had clinical symptoms compared to 0 in the non-COVID group (p<0.0001). And 13 (52%) pregnant women in COVID-19 group had more pneumonia in chest x-ray compared to 2 (8%) in non-COVID-19 group (p=0.001). This result shows significant differences in the presence or absence of clinical symptoms and the appearance of pneumonia on chest radiographs for pregnant women who were infected and not infected with COVID-19 (Table 1).

There is no significant difference in TNF- levels in pregnant women who are infected and not infected with COVID-19 (mean (IQR): 3.42 (7.24) vs. 2.70 (3.06) pg/ml; p=0.138) (Table 2).

TNF- levels in women with COVID symptoms (3.21(3.97) pg/ml) were not different than those without symptoms (2.41(2.71) pg/ml) (Table 3).

**Table 1. Characteristics between groups of pregnant women infected and not infected with COVID-19**

| Variable | Pregnant Women Infected with COVID-19 (n=25) | Pregnant Women Are Not Infected With COVID-19 (n=25) | p-Value |
|---|---|---|---|
| **Maternal age** (mean ±SD) | 30.52 ± 5.18 | 31.56 ± 5.48 | 0.475[a] |
| < 35 years old (%) | 18 (72%) | 18 (72%) | |
| ≥ 35 years old (%) | 7 (28%) | 7(28%) | |
| **Gestational age** | | | |
| (median, IQR) | 37 (4) | 37 (3) | |
| 28 – 36 weeks(%) | 10 (40%) | 12 (48%) | 0.175[b] |
| 37 – 40 weeks (%) | 15 (60%) | 13 (52%) | 0.569[c] |
| **Referred from** | | | |
| Inside Surabaya (%) | 22 (88%) | 22 (88%) | |
| Outside Surabaya (%) | 3 (12%) | 3 (12%) | 1.000[c] |
| **Symptom** | | | |
| Yes (%) | 12 (48%) | 0 | |
| No (%) | 13 (52%) | 25 (100%) | 0.000[c] |
| **Chest Xray** | | | |
| Pneumonia (%) | 13 (52%) | 2 (8%) | 0.001[c] |
| Normal (%) | 12 (48%) | 23 (92%) | |

**Table 2. Analysis of TNF- levels in pregnant women infected and not infected with COVID-19**

| | Pregnant Women Infected with COVID-19 (n=25) | Pregnant Women Are Not Infected With COVID-19 (n=25) | P-value |
|---|---|---|---|
| TNF α (pg/ml) (Median,IQR) | 3.42 (7.24) | 2.70 (3.06) | 0.138[b] |

**Table 3. Analysis of TNF- levels based on the presence or absence of clinical symptoms of COVID-19 in pregnant women infected with COVID-19**

| | With Symptoms (n = 12) | Without symptoms (n = 13) | Nilai p |
|---|---|---|---|
| TNF α (pg/ml) (Median, IQR) | 3.21(3.97) | 2.41 (2.71) | 0.314[b] |

## DISCUSSION

Pregnant women are a vulnerable population to be infected with COVID-19 with more severe clinical manifestations, so there is a high risk of disturbing fetal growth and development.[1] According to research conducted by Wardhana et al. in 2021, more attention should be paid to the symptoms of Covid-19 because they are associated with lower gestational age and birth weight, poor clinical parameters, and the need for intensive care maternal mortality.[7] From April 2020 – April 2021 data at RSUD Dr. Soetomo Surabaya, from 175 pregnant women infected with COVID-19, 134 (76.5%) people aged 25-34 years, while the research of Wardhana et al. showed that from 23 patients, 18 (78.3%) were aged 20-35 years.[7] The main finding of this study was that there was no difference in inflammatory markers in third-trimester pregnant women who were infected compared to those who were not infected with COVID-19.

In this study, there was no significant difference in TNF- levels in pregnant women who were infected and not infected with COVID-19. Although TNF- levels tended to be higher in pregnant women infected with COVID-19 than pregnant women not infected with COVID-19. A study conducted by Ying et al. compared inflammatory cytokines, including TNF- in COVID patients and healthy controls. Compared with healthy control samples, serum TNF levels were higher in COVID-19 patients.[8]

The increase in TNF- levels was not significant because it might be related to the day of blood sampling to measure TNF-α. The sampling process cannot be taken on an ideal cytokine storm period. Continuous monitoring of recovered COVID-19 patients showed that TNF- levels at 15-42 days and IL-4 at 29-42 days were significantly higher than those at 0-14 days[9]. In this study, the average sample was taken on days 1-5 of symptoms of COVID so that it had not entered the cytokine storm phase so that TNF- levels had not increased significantly compared to when sampling was carried out on days 15-42 of symptoms.

Research conducted by Merza MY et al. did not find a significant increase in TNF levels in positive covid patients compared to controls. TNF- levels that do not increase in positive covid patients can be caused by differences in sampling time, specific stages of the coronavirus life cycle in the host and differences in disease severity. TNF- production is downregulated or inhibited by T-helper production. TNFR2 expression is upregulated in activated Tregs and can be detected in activated conventional T cells, although to a lesser extent than inactivated Tregs.[10]

In this study, there was no significant difference in TNF-α levels from the symptomatic vs. asymptomatic COVID-19 pregnant women (p = 0.314). TNF- levels in women with symptomatic COVID tend to be higher than those without symptoms. These findings are in line with Ying et al., which showed that serum TNF- levels in asymptomatic COVID cases were significantly lower than in symptomatic COVID cases.[8] In the study conducted by Chan YH et al., pro-inflammatory cytokines and chemokines were expressed in IL-18, TNFSF8, TNFSF13B, TNFSF4, IL27, IL1RN TNFSF10, IFNG, CXCL8, CXCL10, CCL8, and CCL2 were increased in symptomatic COVID patients when compared with asymptomatic COVID.[11] However, Han H et al., no differences in inflammatory cytokine levels were observed between asymptomatic and symptomatic COVID-19 patients.[12] However, in Han et al.'s study, all symptomatic COVID-19 patients had only mild or moderate symptoms, none of which had severe symptoms. In addition, in the study of Karki R et al, serum levels of TNF- and IL-1α in moderate and severe cases of COVID-19 patients showed a non-significant increase because the host immune system response may be different from SARS-CoV-2 and may be due to infection from the other pathogen.[9] From some of the previously mentioned studies, it can be concluded that the relationship between TNF- and clinical symptoms in third-trimester pregnant women infected with COVID-19 is still unclear.

There were no significant differences in serum TNF-@ level in this study between pneumonia vs. non-pneumonia COVID-19 pregnant women. TNF- levels in women with pneumonia tend to be higher than in non-pneumonia pregnant women. Clinical examinations and radiological investigations were reviewed to determine the severity of the patient's COVID-19 illness. In a study conducted by Chen L et al. in 2020, 106 patients with 12 COVID-19 patients without pneumonia and 94 COVID-19 patients with pneumonia.[13] Compared with COVID-19 without pneumonia, COVID-19 with pneumonia had significantly higher serum interleukin (IL)-2R, IL-6, and tumor necrosis factor (TNF)-α. These results suggest that an increase in the tumor necrosis factor (TNF)-α cytokine is significantly associated with the presence of COVID-19 pneumonia. Cytokine storm is characterized by an exaggerated inflammatory reaction in which pro-inflammatory cytokines are increasingly released in response to infection. This process can result in tissue injury and an unfavorable prognosis. The lungs are the main target organ during SARS-CoV-2 infection. ARDS is the most important cause of death in COVID-19, so it is important to evaluate the role of cytokines in lung injury in COVID-19 patients and seek to find potential therapeutic targets for lung injury management in COVID-19 pneumonia.

## CONCLUSION

This study revealed no significant difference in TNF-α serum level between SARS-CoV-2 infected pregnant women with normal pregnant women. There was no significant difference in TNF- levels between pregnant women infected with COVID-19 with symptoms and without clinical symptoms. The normal pregnant adaptation may affect inflammatory response in Covid-19 infection.

## AUTHOR CONTRIBUTION

Margaretha Claudhya Febryanna: First author and researcher
Manggala Pasca Wardhana: Researcher
Muhammad Ilham Aldika Akbar: Researcher
Arif Rahman Nurdianto: Journal preparation and journal editor

## CONFLICT OF INTEREST

There is no conflict of interest in this journal publication

## FUNDING

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## ETHICS APPROVAL

This study had been approved by Health Research Ethics Committee of RSUD Dr. Soetomo Surabaya with registration number 0099/KEPK/XI/2020

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