

COMPLETE BLOOD COUNT FEATURES IN PREGNANT WOMEN WITH COVID-19 CONFIRMED AT ULIN REFERRAL HOSPITAL OF SOUTH KALIMANTAN

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Abstract

Coronavirus disease 2019 (COVID-19) outbreak has been a leading cause of mortality for nearly 3 million people worldwide since the first case detected in Wuhan, China, in December 2019. In Indonesia, the case fatality rate (CFR) for this disease has reached 2.7%. Meanwhile, South Kalimantan province recorded a high CFR of 2.9% until April 12th, 2021. One of the most vulnerable groups who are susceptible to COVID-19 infection in pregnant women due to their immunocompromised condition. Blood tests have an important role in the early diagnosis of the disease, considering the information regarding the inflammatory process. This study aimed to evaluate complete blood count features in the diagnosis of COVID-19 infection in pregnant women. This was a retrospective study conducted in Ulin Referral Hospital of South Kalimantan from April – October 2020. Total 66 pregnant women with positive rapid antibody tests were included. Hematological parameters were compared in patients with and without confirmed COVID-19 infection. Continuous variables were analyzed Independent T-test, and Mann Whitney U and categorical variables were analyzed by chi-square test. Then to determine the risk ratio, univariate analysis with Binary Logistics Regression. Comparisons made according to reverse transcriptase-polymerase chain reaction (RT PCR) results revealed that a statistically significant difference ($p < 0.05$) was found between the test groups regarding leukocyte (12.800/ul) [OR: 1.167, 95% CI: 1.036- 1.314, $P = 0.011$], lymphocyte (14.24±6.31%) [OR: 0.914, 95% CI: 0.848-0.986, $P = 0.019$], and neutrophil (78.64±7.71%) [OR: 1.069, 95% CI: 1.006-1.135, $P = 0.031$]. The study concluded that leukocytosis, lymphocytopenia, and neutrophilia are associated with confirmed COVID-19 infection in pregnant women. Further studies should investigate the prognostic role of hematological parameters, especially in pregnant women with COVID-19, considering the risk of fatal complications and outcomes.

Keywords: COVID-19, Leukocytosis, Lymphocytopenia, Neutrophilia, Pregnant women



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ABSTRACT

Coronavirus disease 2019 (COVID-19) outbreak has been a leading cause of mortality for nearly 3 million people worldwide since the first case detected in Wuhan, China, in December 2019. In Indonesia, the case fatality rate (CFR) for this disease has reached 2.7%. Meanwhile, South Kalimantan province recorded a high CFR of 2.9% until April 12, 2021. One of the most vulnerable groups susceptible to COVID-19 infection in pregnant women due to their immunocompromised condition. Blood tests have an important role in the early diagnosis of the disease, considering the information regarding the inflammatory process. This study aimed to evaluate complete blood count features in the diagnosis of COVID-19 infection in pregnant women. This was a retrospective study conducted in Ulin Referral Hospital of South Kalimantan from April – October 2020. Total 66 pregnant women with positive rapid antibody tests were included. Haematological parameters were compared in patients with and without confirmed COVID-19 infection. Variable analysis using independent T-test and Mann Whitney U, while categorical variables were analyzed by chi-square test. Then to determine the risk ratio, univariate analysis with Binary Logistics Regression. Comparisons made according to reverse transcriptase-polymerase chain reaction (RT-PCR) results revealed that a statistically significant difference ($p < 0.05$) was found between the test groups regarding leukocyte (12.800/ul) [OR: 1.167, 95% CI: 1.036-1.314, $P = 0.011$], lymphocyte (14.24±6.31%) [OR: 0.914, 95% CI: 0.848-0.986, $P = 0.019$], and neutrophil (78.64±7.71%) [OR: 1.069, 95% CI: 1.006-1.135, $P = 0.031$]. The study concluded that leukocytosis, lymphocytopenia, and neutrophilia are associated with COVID-19 infection in pregnant women; further studies should investigate the prognostic role of haematological parameters, especially in pregnant women with COVID-19 considering the risk of fatal complication and outcomes.

Keywords: COVID-19, Leukocytosis, Lymphopenia, Neutrophilia, Pregnant women

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by a new type of coronavirus. Since its first report in Wuhan, China, in December 2019, WHO has recorded 137,866,311 confirmed cases of COVID-19, with the death toll reaching 2,965,707 worldwide. As of April 12, 2021, Indonesia reported 1,571,824 confirmed cases, and 43,073 of them died with a case fatality rate (CFR) of 2.7%,^{1,2} while the province of South Kalimantan had a positive number of 30,479 cases, making South Kalimantan one of the 12 provinces with the most confirmed cases in Indonesia. Death cases in South Kalimantan reached 1,029 or 2.9% of the number of confirmed cases.³



COVID-19 has passed its first year since it was declared a global pandemic and entered a new phase, namely vaccination. As of April 14, 2021, a total of 734,121,870 doses of vaccine have been administered worldwide.⁴ Nevertheless, the presence of COVID-19 remains a concern for the wider community, especially for at-risk groups such as pregnant women.⁵ Wendy N et al. mentioned that pregnant women were more susceptible to infection with COVID-19 because this population is immunocompromised. Decreased lymphocytes, natural killer cell (NKG2A) inhibitory receptors and increased angiotensin-converting enzyme-2 (ACE2), interleukin (IL)-8, IL-10, interferon-induced protein (IP)-10 characterize both normal pregnancies and COVID-19, so it can be concluded that pregnancy is a risk factor for COVID-19 infection. However, there is not enough data to show that pregnant women are more susceptible to being infected with COVID-19.^{6,7}

Data and research on COVID-19 in pregnant women are still limited. There is not much literature and research that discusses COVID-19 infection in pregnant women. COVID-19 causes various clinical symptoms, ranging from asymptomatic, mild flu symptoms to severe symptoms that cause respiratory failure. Early detection and diagnosis of COVID-19 are essential to avoid delays in treatment. Complete blood count can describe the inflammatory status of COVID-19 patients. Besides that, these tests are widely available, inexpensive, and easy to perform. Several research results show that complete blood count has a role in the early detection of COVID-19 infection.^{8,9} Lymphopenia accompanied by mild thrombocytopenia and an increase in the neutrophil-lymphocyte ratio (NLR) is a feature of the results of routine blood tests that are often found in COVID-19 patients. Neutrophilia, increased prothrombin time (PT), and prolonged activated partial thromboplastin time (aPTT) are other parameters that can also be seen in COVID-19 patients. The number of leukocytes has been reported to vary in several studies.^{9,10}

Routine blood tests can also predict the course and outcome of a disease. Elevated proinflammatory chemokines and cytokines correlated significantly with the clinical degree and mortality caused by COVID-19 infection.¹¹⁻¹⁴ Elevated simple biomarkers, such as C-reactive protein (CRP) and ferritin, were observed frequently in the acute phase. In addition, a consistent decrease in lymphocyte levels and a significant increase in neutrophils in COVID-19 cases have also been widely reported. Thus, the neutrophil-lymphocyte ratio (NLR) is considered significant as an indicator of the diagnosis and progression of the disease.^{11,15-17} Systemic immune inflammation index (SII, the platelet count multiplied by the neutrophil count divided by the count of lymphocytes) is a predictive parameter that is also considered significant for predicting clinical outcomes and the need for ventilators in COVID-19 patients.¹⁸ In pregnant women, the immune system is physiologically decreased. It is assumed that the immune response to infection is also different.^{7,19} Some of these clinical phenomena describe the very complex pathogenesis of COVID-19 disease. In contrast, research on the parameters of laboratory results of COVID-19 patients in the group of pregnant women is still limited.⁷ In this study, we analyzed the feature of complete blood count in pregnant women with rapid reactive antibody confirmed COVID-19.

METHODS

This study is a retrospective study using secondary data from the medical records of 66 pregnant patients with the results of rapid-test reactive COVID-19 antibody screening who were treated at the Ulin Regional General Hospital (RSUD) Ulin Banjarmasin as one of the COVID-19 referral hospitals in South Kalimantan. The period from April to October 2020 was followed by an oro-nasopharyngeal reverse-transcriptase polymerase chain reaction (RT-PCR) swab examination. Rapid reactive antibody was defined as the result of a patient's rapid antibody to



one or more of the immunoglobulin (Ig) M reactive, reactive IgG, and total reactive Ig. A confirmed diagnosis of COVID-19 is defined as tested positive for the COVID-19 virus as evidenced by RT-PCR examination. This research was approved by the Ethical Research Committee of Ulin General Hospital, Banjarmasin.

The sampling technique in this study used a purposive sampling method with inclusion criteria of pregnant women with rapid-test reactive antibody screening results who were treated at Ulin Hospital, had diagnostic swab results with RT-PCR, and had complete blood count data. The data observed included initial patient data on admission to the hospital, which consisted of demographic data, namely age, obstetric status, the severity of disease at the beginning of hospital admission (asymptomatic / asymptomatic, mild, moderate [pneumonia (+), SaO₂ 93 % room air], severe [SaO₂ < 93% room air], critically ill [ARDS, PaO₂/FiO₂ < 300 mmHg]), obstetric status, initial clinical symptoms, and laboratory data, namely haemoglobin (Hb), hematocrit (HCT), platelets, leukocytes, lymphocytes, neutrophils, Neutrophil Lymphocyte Ratio (NLR; the ratio between absolute neutrophil count and absolute lymphocyte count), Absolute Lymphocyte Count (ALC) and systemic immune-inflammation index (SII; thrombocyte count × neutrophil count/lymphocyte count).

The data were tabulated using Microsoft Excel and analyzed using SPSS 21. The Shapiro-Wilk test determined the normality of the data distribution. The Chi-square test was used to analyze patient categorical variables, which were expressed in categorical form. Analysis of parametric variables used an independent sample T-test and was presented as mean and standard deviation. Nonparametric variables were analyzed using the Mann-Whitney U test, then to determine the risk ratio, univariate analysis was performed using the Binary Logistics Regression test. The variable is said to have a significant difference if it is obtained p-value < 0.05.

RESULT

This study had a total sample of 66 pregnant women with rapid-test reactive antibodies, 27 (41%) samples confirmed negative for COVID-19, and 39 (59%) samples for pregnant women confirmed positive for COVID-19. The details of the sample characteristics obtained can be seen in table 1.

Table 1. Characteristics of Research Sample

Variable	Negative (n=27)	Positive (n=39)	p-value
Age (year)	30 ± 1.23	30 ± 1.06	0,6 ^l
Parity			
0-1	16 (59,2)	17(43,5)	
2-3	10 (37,03)	17(43,5)	0,22 [□]
4-5	1(3,7)	4(10,2)	
>5	0 (0)	1(2,5)	
Disease degree			
Asymtomatic	21 (77,8)	25 (64,1)	
Mild	2 (9,4)	4(10,2)	
Moderate	1 (3,7)	3(7,6)	0,82 [□]
Severe	1(13,7)	2 (5,1)	
Critical	2 (9,4)	5 (12,8)	
Early Symptom			
Dyspneu	3 (11,1)	7 (17,9)	0,44
Cough	4 (14,8)	9 (23,07)	0,40
Sore Throat	1 (3,7)	2(5,12)	0,76



Cold	1 (3,7)	0 (0)	0,23
Nausea	1 (3,7)	2(5,12)	0,75
Fatigue	0 (0)	1 (2,56)	0,42
Headache	0 (0)	1 (2,56)	0,42
Subfebrile	4(14,8)	6(15,3)	0,94

Mean \pm SD, n (%); ¹Unpaired T-test; [□]Chi-square test

In table 1, it can be seen in both groups that the average age of the sample is 30 years, indicating that the sample of this study is productive age with a dominant parity status of 0-3 (90.1%). The majority of disease degrees were asymptomatic (69.7%), critical (10.6%), and mild (9.1%). The most common complaints were cough (19.7%), shortness of breath (15.1%), and subfebrile (15.1%). Comparisons made according to the patient's RT-PCR results showed that there was no statistically significant difference in age, parity status, disease degree, or initial symptoms with positive or negative COVID-19 test results with p-value > 0.05.

Table 2. Comparison of complete blood count results for COVID-19 negative and positive patients based on RT-PCR examination

Variable	Negative ‡	Positive ‡	p-value
Haemoglobin (g/dl)	12,08 \pm 1,14	11,54 \pm 1,85	0,186 ¹
Leukocytes ($\times 10^9/L$)	11,2 (6,5-11,2)	12,8 (10,4-16,9)	0,001 [□]
Haematocrite (%)	36,8(35-38,7)	34,6 (32-38,1)	0,094 [□]
Platelet ($\times 10^9/L$)	282 (212-322)	290 (257-366)	0,37 [□]
Neutrophil (%)	73,74 \pm 9,74	78,64 \pm 7,71	0,035 ¹
Lymphocyte (%)	18,7 \pm 7,98	14,24 \pm 6,31	0,02 ¹
NLR	8,1(5,8-9,8)	8,19 (7,11-10,2)	0,024 [□]
ALC	1800 (1196-2132)	1786 (1393-2115,6)	0,634 [□]
SII	8100 (5900-9800)	16400 (14200-20400)	0,067 [□]

‡Mean \pm Standard Deviation/Median (interquartile range: Q1 – Q3)

¹Unpaired T-test; [□]Mann-Whitney Test

Haemoglobin (Hb), neutrophils, and lymphocytes have normal data distribution, so the unpaired T-test analysis model is used. Leukocytes, hematocrit, platelets, NLR, ALC, and SII, had an abnormal distribution of data, so the Mann-Whitney Test was used.

Comparisons made according to the RT-PCR test results revealed that while no statistically significant difference was observed between test result groups (negative or positive) regarding haemoglobin, haematocrit, platelet, ALC, and SII (p > 0.05), a statistically significant difference (p < 0.05) was found between the test result groups regarding leukocyte, neutrophil, lymphocytes, and NLR. Leukocytosis ($12.8 \times 10^3/L$), lymphopenia (14.24 \pm 6.31%), neutrophilia (78.64 \pm 7.71%), and NLR value (8.19) was found in pregnant women patients with positive RT-PCR for COVID-19 (Table 2).

Table 3. Multivariate Logistics Regression Analysis

Variables	Odds Ratio	95% CI	p-Value
Leukocyte ($\times 10^3/L$)	1.167	1.036 - 1.314	0.011
Limfocyte (%)	0.914	0.848 - 0.986	0.019
Neutrophils (%)	1.069	1.006 - 1.135	0.031
NLR	0.778	0.974 - 1.220	0.134



In univariate logistic regression analysis (Table 3), leukocytes, lymphocytes, and neutrophils were significant. Leukocytes were found to be higher in the confirmed COVID-19 group. An increase in leukocytes significantly increased the odds of 1,167 times (95% CI: 1,036 - 1.314, $p = 0.011$) for a confirmed COVID-19 event. While the decrease in lymphocytes increased the chance of 0.914 times (95% CI: 0.848-0.986, $p = 0.019$), as well as high neutrophils also 1,069 times (95% CI: 1.006-1.135, $p = 0.031$) the chance of positive cases of COVID-19 in pregnant women.

DISCUSSION

Theoretically, physiological changes during pregnancy, such as reduced pulmonary residuals, diaphragmatic elevation, and airway mucosal oedema, as well as changes in the immune system, may lead to increased susceptibility to COVID-19 infection. However, it has not been proven that pregnant women are more susceptible to COVID-19 disease and have a worse outcome.⁷ In Indonesia, pregnant women who wish to give birth must undergo a COVID-19 screening, including a rapid antibody test, if suspected positive, they will be referred to complete service facilities.²⁰

Complete blood counts are carried out on every pregnant woman who comes to the hospital. In addition to being accessible, inexpensive, and widely used, this examination has an essential role in screening and early diagnosis of COVID-19 infection because it can indirectly describe the inflammatory condition of the body.⁸ Neutrophils are part of the innate immune cells that first respond to infection and indicate inflammation conditions. The decreased number of lymphocytes in COVID-19 disease has been associated with viral cytopathic effects, induction of pyroptosis by interleukin (IL)-1, and bone marrow suppression by proinflammatory cytokines. An increase in NLR is reported as an indicator of the COVID-19 infection process too.²¹

Based on data from 66 pregnant women with positive rapid reactive antibodies, only 39 patients (59%) were confirmed positive for COVID-19, with the majority asymptomatic as many as 25 patients (64.1%), four patients with mild degree (10.2%), two patients with severe degree (5.1%), and five patients with critical degree (12.8%). These results are consistent with a meta-analysis of 637 pregnant women with COVID-19, which found that 76.5% had mild disease, 15% had severe symptoms, and 7.7% were critically ill at the time of hospital admission.⁸ A case-control study involving 60 pregnant women in Wuhan also stated that most pregnant COVID-19 patients did not show fever or respiratory symptoms, and only 15% reported respiratory symptoms during hospitalization.⁹

In this study, the regression analysis results showed leukocytosis, lymphopenia, and neutrophilia in pregnant women with confirmed COVID-19. This finding is in line with Sun et al.¹⁹ Similar results were also found in the study of Liu et al., a retrospective study at Xinhua Hospital involving 59 pregnant women, which found significant laboratory results were leukocytosis and an increase in the NLR ratio.¹⁶ A retrospective study in Wuhan with 188 pregnant patients who were confirmed positive for COVID-19 compared to controls also had similar laboratory characteristics, namely, leukocytosis, increased NLR, and lymphopenia.²² In a meta-analysis by Jafari et al., patients who were not pregnant had lower leukocytes than the COVID-19 patients with pregnancy. The increase in leukocytes also occurred in COVID-19 cases with 27% of pregnancies, while in patients without pregnancy, only 14%.²³ Leukocytosis in COVID-19 cases was common in the patients in this study, which could be attributed to the relative immunosuppressed status of the patients with pregnancy and immunocompromised conditions during SARS-CoV-2 infection. A meta-analysis showed that bacterial or viral



coinfection was higher in COVID-19 with pregnancy. Therefore, laboratory differences such as leukocytosis in pregnant women suffering from COVID-19 are thought to occur due to coinfection. It is also related to pregnancy outcomes such as miscarriage, premature rupture of membranes, premature birth, intrauterine growth restriction (IUGR), and respiratory distress in infants.²³

The morbidity and mortality of COVID-19 cannot be separated from the presence of a cytokine storm that causes hyperinflammatory conditions that have the potential to cause damage to many organs.¹¹ In addition to excessive cytokine release, an increase in simple chemical markers such as C-reactive protein (CRP), ferritin, lymphopenia, and increased neutrophil-lymphocyte ratio (NLR) were also mostly found, so it is often used as a predictor of COVID-19 infection or a predictor of COVID-19 outcome.¹³ The mechanism of progressive lymphopenia in severe COVID-19 is still unclear. Redistribution of T cells through pulmonary recruitment and dysregulation of tumor-mediated apoptosis of necrotic factors (TNF) and direct cytopathic injury are possible mechanisms associated with lymphopenia.^{12,24}

The predictive value of leukocytosis and lymphopenia on the clinical degree of COVID-19 was investigated by Huang et al. in a meta-analysis involving 1289 patients. There was a significant association of leukocytosis and lymphopenia with severe COVID-19.²⁵ Orantes et al. reported that leukocytes and lymphocytes of patients who survived and died from COVID-19 showed a substantial difference in lymphocytes, but not crucial for leukocytes. This may be related to the Orantes et al. study sample, which tends to be homogeneous, focus on COVID-19 infection associated with pneumonia only.¹⁷ A retrospective study in Wuhan, China involving 115 confirmed COVID-19 patients showed a strong association of lymphopenia with the severity of the patient's clinical symptoms and a more extended hospital stay. It is recommended to routinely check lymphocyte levels in patients with COVID-19 as a predictor of severity of illness and prognosis.¹⁶ Apart from being a predictor of severity and prognosis, lymphopenia can also be used as a confirmed predictor of COVID-19 in pregnant women, as the results of this study.

Neutrophils are innate immune cells with a short circulating life. This leukocyte component plays an essential role in the initial process of the body's defense against infection. An increase in neutrophils has been reported in COVID-19 disease and is a significant laboratory marker for assessing the course of illness and the outcome of COVID-19, especially in the acute phase. In line with the results of this study, Wang et al. also reported a significant difference in the increase in neutrophils in the group of pregnant and non-pregnant women infected with COVID-19.^{22,26} Usul et al., in their research on simple laboratory results as confirmed predictors of COVID-19 involving 282 samples, showed contradictory results, namely leukopenia and neutropenia were potential predictors of confirmed COVID-19, while lymphocytes in their research results tended to increase, but not significantly. The difference in the results of this study is very likely related to the type of sample population used. In their study, the sample population was not pregnant women, the average sample age was 47 years, and other morbidity conditions were not adjusted for the sample in the study.⁸

This study is a retrospective study with a minimal number of samples. More extensive studies are needed to describe the COVID-19 population with pregnancy better. Further research is required to determine the association and cut-off parameters of laboratory variables in determining confirmed cases of COVID-19, which tend to be different in patients with pregnancy conditions. Comorbidities and other confounding factors are the limitations of this study.



CONCLUSION

Pregnant women are considered a group at risk of being infected with COVID-19 because of their immunocompromised condition. Screening and early diagnosis are essential to prevent delays in the treatment of COVID-19 in pregnant women. Complete blood count was not only easy, inexpensive, and widely used, but this examination has an essential role because it can indirectly describe the inflammatory condition of the body due to COVID-19 infection. In this study, several results were obtained from the complete blood count of pregnant women who played a role in positive confirmed cases of COVID-19, namely leukocytosis, lymphopenia, and neutrophilia.

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CERTIFICATE OF APPRECIATION

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