ISSN No:-2456-2165

Neonatal Lymphocyte, Neutrophil, Platelet, C-Reactive Protein, Procalciton in from Positive and Negative COVID-19 Mothers

Wiyani, Bugis Mardina Lubis, Nelly Rosdiana
Department of Pediatrics, Faculty of Medicine,Universitas Sumatera Utara
Jl. Dr. Mansyur No.5, Medan 20155,
Sumatera Utara, Indonesia

Abstract:- Babies under one year old are vulnerable to COVID-19. However, the vulnerabilities of neonates born to infected mothers are lack studied. Newborns from mothers with suspected or confirmed cases of COVID-19 must be clinically examined for SARS-CoV-2. Laboratory examination is recommended for disease diagnosis and progression. This research assessed the lymphocyte, neutrophil, thrombocyte, c-reactive protein (CRP), and procalcitonin (PCT) in neonates born from COVID-19 positive and negative mothers. A retrospective cross-sectional study involving 113 neonates and 110 neonates from COVID-19 positive and negative mothers was carried out in Haji Adam Malik hospital Medan, Indonesia. All data was collected from the medical record. Mann-Whitney statistical test was carried out to assess the difference in lymphocyte, neutrophil, thrombocyte, CRP, and PCT values between neonates born from COVID-19 positive and negative mothers. Lymphocyte values, platelet counts, CRP, and PCT were higher in negative group than positive group, but no difference between the two groups (p=0.322, p=0.364, p=0.925), except for PCT level with p<0.001. Neutrophil values were lower in the negative group than the positive group, but there was no significant difference between the two groups (p=0.551). There was a difference in the PCT values between newborns born from COVID-19 positive and negative mothers.

Keywords:- *COVID-19, diagnosis, neonates, blood work*

I. INTRODUCTION

Corona virus is a 30kb wide large enveloped virus with single stranded, non-segmented positive sense ribonucleic acid (RNA) genome. SARS-CoV-2 is the largest genomic RNA virus, which is enveloped in a single stranded RNA-virus of 60 – 140 nm diameter spherical/elliptical shape.[1] Corona virus possesses RNA that is capable of both homologous and non-homologous mutation and genetic recombination, allowing easier transmission between the hosts and species.[2]

SARS-CoV-2 is part of the corona virus, with other infection-causing pathogens including the Middle East Respiratory Syndrome (MERS) and SARS.[3] The main pathways for infection are through respiratory route and direct contact. The spectrum for disease severity ranges from mild to severe symptoms, while the majority of cases have been reported as mild symptoms.[4] Studies show that babies under 1 year old are vulnerable to COVID-19 when in contact with infected family members; there is however little understanding on the vulnerabilities of neonates born to infected mothers.[5] There is less than 4% cases of COVID-19 in neonates globally.[6]

According to the data from the World Health Organisation (WHO), SARS-CoV-2 have an incubation period averaging 5-6 days between exposure and symptom onset, lasting up to 14 days.[6] Most recent studies showed that vertically transmitted SARS-CoV-2 infection from mothers to newborns ranges from 3% to 8%.[7] A study by Wang et al. reported positive RT-PCR result from throat swab testing at 36 hours after birth. False negative results could be linked to the viral load.[8] Another study by Vivanti et al. about SARS-CoV-2 transmission through placentas from mothers to their babies reported a significantly higher viral load in the placenta tissues than in the amniotic fluids, mother's blood, or newborn's blood. When RT-PCR provided positive SARS-CoV-2 results, nasopharyngeal and rectal swabs need to be collected from the neonates in the 1st hour of life, and on 3rd day and 18th day after birth.[9] A study by Kulkarni et al. reported the detection of SARS-CoV-2 presence in nasopharyngeal swabs, umbilical cords, and placenta in the first 12 hours of life in newborns.[10] Patane et al. diagnosed COVID-19 in 2 newborns; the first newborn showed positive for SARS-CoV-2 24 hours after birth, while the second newborn showed positive results on the 7th day of birth (the newborns were isolated from the infected mothers in this period).[11] Penfield et al. reported the presence of SARS-CoV-2 gene in membrane and placenta samples of three pregnant mothers. However, the nasopharyngeal swab, done twice, in the newborns showed negative results for SARS-CoV-2 infection.[12] Dong et al. reported high immunoglobulin (IgM), interleukin 6 (IL-6) and interleukin 10 (IL-10) levels in newborns with SARS-CoV-2 in the first 2 hours after birth, but nasopharyngeal swab returning negative results.[13]

On average IgM antibodies are reported to appear 3-7 days after initial, where higher levels are found in blood samples taken 2 hours after birth. IgM antibodies can be transmitted to the fetus through the placenta. Higher levels of the antibodies showing possibility for newborns to have been infected during pregnancy.[13] Newborns from mothers with suspected or confirmed cases of COVID-19 must be clinically examined and tested for SARS-CoV-2. Babies with COVID-19 may be asymptomatic or show respiratory and gastrointestinal symptoms. SARS-CoV-2-infected

ISSN No:-2456-2165

newborns can cause respiratory disorders, thrombocytopenia, abnormal heart functions, and/or death according to the severity of the disease.[14]

The Indonesian Paediatric Society (IDAI) recommends laboratory examinations to allow COVID-19 diagnosis and its progression, such as complete blood work, C-reactive protein (CRP), procalcitonin (PCT), blood cultures, coagulation markers, heart function, kidney function, creatine kinase, troponin I, serum ferritin, IL-6, as well as lymphocyte analysis, including additional supporting tests such as lactate, blood gas, and electrolyte.[15]

Wang et al. examined two newborns with SARS-CoV-2 infected mothers. The first baby was born full-term through vaginal delivery, showing positive RT-PCR test results with lowered lymphocyte levels (lymphopenia) and slightly increased cardiac enzyme levels. The second baby had negative RT-PCR test results and did not show signs of poor conditions.[8] Ferrazzi et al. conducted laboratory tests on 19 neonates whose mothers were SARS-CoV-2 positive, where four neonates were reported to have increasing white blood cell counts and CRP levels.[16]

At this moment there is still little data and studies that compared biomarkers such as lymphocyte, neutrophil, thrombocyte, c-reactive protein (CRP), and procalcitonin in neonates born from mothers confirmed to be COVID-19 positive. This study was conducted to examine the aforementioned biomarkers in neonates from COVID-19 positive mothers and neonates from COVID-19 negative mothers in Haji Adam Malik hospital Medan, Indonesia.

II. MATERIAL AND METHODS

This research was a retrospective cross-sectional study to compare lymphocyte, neutrophil, and platelet counts, as well as C-reactive protein (CRP) and procalcitonin (PCT) values in neonates from COVID-19 positive mothers vs. COVID-19 negative mothers. The research was conducted in the isolated neonatology treatment ward at Haji Adam Malik hospital Medan, between August 2021 to December 2021. The research subjects were newly born babies admitted to the isolated neonatology treatment ward during the research period. Babies with incomplete laboratory data and babies who were referred to Haji Adam Malik hospital Medan were not included in the research. The sample size for this research was calculated with the following formula:

$$n1 = n2 = 2\left[\frac{SD(Z\alpha + Z\beta)}{x1 - x2}\right]^2$$

$$= 2\left[\frac{33(1.96 + 1.28)}{43 - 26}\right]^2$$

$$= 79.11 \approx 80$$

Where, n1 was the number of neonate samples from COVID-19 negative mother, n2 was the number of neonate samples from COVID-19 positive mother, Alpha (α) was one-type error, set at 5% (one-sided hypothesis), $Z\alpha$ was alpha standard value, set at 1.96, beta (β) was two-type error, set at 10%, Zβ was beta standard value, set at 1.28, SD was standard deviation of 33, as the combination of newborns from COVID-19 positive and COVID-19 negative mothers, X1 was average lymphocyte values in neonate from COVID-19 negative mothers (X1 = 43), X2 was average lymphocyte values in neonate from COVID-19 positive mothers (X2 = 26).

Based on the above equation, the minimum sample size for each group in this study was 80; i.e. 80 babies born from COVID-19 negative mothers, and 80 babies born from COVID-19 positive mothers.

Research data collection and laboratory results were in the form of lymphocyte, neutrophil, thrombocyte, C-reactive protein (CRP), and procalcitonin (PCT) examinations, taken from the medical records of the research subjects.

A. *Research ethics*

Ethical clearance had been conducted through the Medical Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara/Haji Adam Malik hospital prior to the start of the research (No. 929/KEP/USU/2021).

B. *Data Analysis*

Univariate analysis was carried out to describe the characteristics of each research variable in a frequency distribution table. Bivariate analysis was carried out to test the hypothesis and obtain the relative risk. The relative risk was calculated to identify the risk factors studied in this research. The analysis was also carried out to assess the relationship between independent and dependent variables, where p-values were obtained by using Mann-Whitney statistical test. p value ≤ 0.05 was significant, where relative hypothesis could be accepted. Whereas p value > 0.05 was not significant, and absolute hypothesis could be accepted.

III. RESULTS AND DISCUSSION

A. *Research subject data characteristic*

This research involved all newborns treated in the isolated neonatology treatment ward at Haji Adam Malik hospital Medan, Indonesia. All research subjects were divided into 2 groups, such as 113 newborns born from COVID-19 positive mothers and 110 newborns born from COVID-19 negative mothers. All the research subjects had met the inclusion criteria.

There were more male neonates than female in both groups, where over half of the babies born were male. All of the research subjects were ≤ 24 hours old. There was no significant difference in gender between the positive and negative group (p=0.970). All newborns from COVID-19 positive mothers reached ≥ 36 weeks' gestation. Meanwhile, in the newborns from COVID-19 negative mothers group, there were 1 (0.9%) newborn below 28 weeks' gestation, 2 (1.8%) newborns between 28-32 weeks' gestation, and 1 (0.9%) newborn between 32-36 weeks' gestation. Kruskal-Wallis test showed no significant differences between the two groups on gestational age (p = 0.244).

ISSN No:-2456-2165

Demographic characteristic	Mother status for COVID-19		p
	Positive (n=113)	Negative (n=110)	
Gender, n (%)			
Male	65 (57.5)	63 (57.3)	0.970[a]
Female	48 (42.5)	47 (42.7)	
Age, n (%)			
0 – 24 hour(s)	113 (100)	110 (100)	
Gestational age			
< 28 weeks	0	1 (0.9)	0.244[b]
28 - 32 weeks	0	2 (1.8)	
32 - 36 weeks	0	1 (0.9)	
≥ 36 weeks	113 (100)	106 (96.4)	

Table 1: Subject characteristics data distribution

[a]Mann-Whitney, [b]Kruskal-Wallis

B. Difference in Lymphocyte, Neutrophil, Thrombocyte, CRP, and PCT values

Table 2 shows the examination results for lymphocyte, neutrophil, thrombocyte, CRP, and PCT values for newborns born from COVID-19 positive and COVID-19 negative mothers.

Laboratory tests	Mother status for COVID-19		p*
	Positive (n=113)	Negative (n=110)	
Lymphocyte, %			
Average (SD)	20.47 (7.81)	22.48 (10.96)	0.322
Median (Min – Max)	19.8 (2.2 – 57.1)	20.5 (5.6 – 76.6)	
Neutrophil, %			
Average (SD)	66.8 (11.04)	65.27 (13.88)	0.551
Median (Min – Max)	68.3 (16.06 – 85.1)	67.65 (0.8 – 85.9)	
Thrombocyte, thousand/µL			
Average (SD)	255.72 (99)	267.25 (112.48)	0.364
Median (Min – Max)	270 (8-499)	274.5 (7-721)	
CRP, mg/dL			
Average (SD)	0.83 (0.37)	0.84 (0,39)	0.925
Median (Min – Max)	0.7 (0.2 – 2.8)	0.7 (0.7 – 2.8)	
PCT, ng/mL			
Average (SD)	1.28 (4.01)	1.57 (1.86)	<0.001
Median (Min – Max)	0.52 (0.07 – 41.45)	0.95 (0.02 – 9.07)	

Table 2. Lymphocyte, neutrophil, thrombocyte, CRP and PCT values

*Mann-Whitney

The average lymphocyte value for newborns from COVID-19 positive mothers was 20.47%, with lowest value of 2.2% and highest value of 57.1%. In newborns from COVID-19 negative mothers, lymphocyte values appeared to be higher with an average of 22.48%, lowest value of 5.6% and highest value of 76.6%. There were however, no significant differences in lymphocyte values in newborns born from COVID-19 positive mothers and COVID-19 negative mothers (p = 0.322).

The average neutrophil value for newborns from COVID-19 positive mothers was 66.8%, with lowest value of 16.06% and highest value of 85.1%. In newborns from COVID-19 negative mothers, neutrophil values appeared to be lower with an average of 65.27%, lowest value of 0.8% and highest value of 85.9%. There were however, no significant differences in neutrophil values in newborns born from COVID-19 positive mothers and COVID-19 negative mothers (p = 0.551). In line with several studies, leukocyte and lymphocyte values were observed to be either normal or lower than normal. Lymphopenia is very commonly observed and considered as a primary indicator of COVID-19.[15]

The average thrombocyte value or platelet counts for newborns from COVID-19 positive mothers was 255.72 thousand/µL, with lowest value of 8 thousand/µL and highest value of 499 thousand/µL. In newborns from COVID-19 negative mothers, thrombocyte values appeared to be higher with an average of 267.25 thousand/µL, lowest value of 7 thousand/µL and highest value of 221 thousand/µL. There were however, no significant differences in thrombocyte values in newborns born from COVID-19 positive mothers and COVID-19 negative mothers (p = 0.364).

The systemic study by Hoang et al involving 7780 pediatric patients with COVID-19 reported the comprehensive blood tests that showed 69.6% of all the research subjects had normal leukocyte values, where 15.2% of the population in each group experienced leukocytosis, leukopenia and thrombocytopenia. Moreover, the majority of patients had normal neutrophil values.[17]

The average c-reactive protein (CRP) value for newborns from COVID-19 positive mothers was 0.83 mg/dL, with lowest value of <0.7 mg/dL and highest value of 2.8 mg/dL. In newborns from COVID-19 negative mothers, CRP values appeared to be almost similar with an average of 0.84 mg/dL, lowest value of <0.7 mg/dL and highest value of 2.8 mg/dL. There were however, no significant differences in CRP values in newborns born from COVID-19 positive mothers and COVID-19 negative mothers (p = 0.925).

The average procalcitonin (PCT) value for newborns from COVID-19 positive mothers was 1.28 ng/mL, with lowest value of 0.07 ng/mL and highest value of 41.45 ng/mL. In newborns from COVID-19 negative mothers, PCT values appeared to be higher with an average of 1.57 ng/mL, lowest value of 0.02 ng/mL and highest value of 9.07 ng/mL. The analysis carried out via the Mann-

Whitney test showed significant differences in PCT values in newborns born from COVID-19 positive mothers and COVID-19 negative mothers ($p < 0.001$). The study by Kulkarni et al reported that the increase in procalcitonin (PCT) values is a marker for inflammatory. The average PCT values in newborns from COVID-19 positive mothers was 1.28 ng/mL, with lowest value of 0.07 ng/ml and highest value of 41.45 ng/mL. In newborns from COVID-19 negative mothers, the average value was lower at 1.57 ng/mL, with lowest value of 0.02 ng/mL and highest value of 9.07 ng/mL.[10] Inflammation markers in the form of increased procalcitonin (PCT) > 0.5 ng/ml were found in patients infected by either virus or bacteria,[17] and normal procalcitonin (PCT) values can be found in the majority of infection cases.[18] Neonates with symptoms need to be evaluated for the basis of symptoms and findings during examination. Comprehensive tests on blood count, heart function, kidney function, coagulation parameter (if needed), and other relevant tests need to be considered and performed according to the clinical condition of the neonates.[19] Normal procalcitonin (PCT) values were found in a majority of infection cases, where PCT values > 0.5 ng/mL indicating bacterial coinfection. The increase in liver enzymes, muscle enzymes, and myoglobin in conjunction with d-dimer levels were reported in severe cases, alongside normal or increasing CRP values.[18]

IV. CONCLUSION

There was a significant difference in procalcitonin values (PCT) between newborns from COVID-19 positive and COVID-19 negative mothers ($p < 0.001$).

ACKNOWLEDGMENT

The author would like to thank all the staff in the Faculty of Medicine, Universitas Sumatera Utara for the extensive support during the study.

REFERENCES

[1.] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China 2019. N Engl J Med.2020;382(8):727-33.
[2.] Fehr AR, Perlman S. Coronavirus: an overview of their replication and pathogenesis. Methods Mol Biol.2015; 1282:1-23.
[3.] Fan C, Lei D, Fang C, Li C, Wang M, Liu Y, et al. Perinatal Transmission of COVID-19 Associated SARS-CoV-2: Should We Worry?. Clin Infect Dis. 2021;72(5):862-4
[4.] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet.2020;395(10229):1054-62
[5.] Wei M, Yuan J, Liu Y, Fu T, Yu X, Zhang ZJ. Novel Coronavirus Infection in Hospitalized Infants Under 1 Year of Age in China. *JAMA*.2020;323(13):1313-14.
[6.] Qiao J. What are the risks of COVID-19 infection in pregnant women?. *Lancet*.2020;395(10226):760-2.
[7.] Khan S, Liu J, Xue M. Transmission of SARS-CoV-2, Required Developments in Research and Associated Public Health Concerns. Front Med.2020;7(310):1-8.
[8.] Wang S, Guo L, Chen L, Liu W, Cao Y, Zhang J, Feng, L. A case report of neonatal COVID-19 infection in China. Clin Infect Dis. 2020;71(15):853-7.
[9.] Vivanti AJ, Vauloup-Fellous C, Prevot S, et al. Transplacental transmission of SARS-CoV-2 infection. *Nat Commun*. 2020;11(3572):1-7.
[10.] Kulkarni R, Rajput U, Dawre R, et al. Early-onset symptomatic neonatal COVID-19 infection with high probability of vertical transmission. *Infection*. 2021;49(2):339-43.
[11.] Patanè L, Morotti D, Giunta MR, et al. Vertical transmission of coronavirus disease 2019: severe acute respiratory syndrome coronavirus 2 RNA on the fetal side of the placenta in pregnancies with coronavirus disease 2019-positive mothers and neonates at birth. *Am J Obstet Gynecol MFM*. 2020;1-5
[12.] Penfield CA, Brubaker SG, Limaye MA, J Ratner, dkk. Detection of SARS-COV-2 in Placental and Fetal Membrane Samples. Am J Obstet Gynecol. MFM.2020;1-3
[13.] Dong L, Tian J, He S, Zhu C, dkk. Possible Vertical Transmission of SARS-CoV-2 From an Infected Mother to Her Newborn. JAMA.2020;323:1846-8.
[14.] Li N, Han L, Peng M, Lv Y, Ouyang Y, Liu K, Yue L, Li Q, Sun G, Chen L, Yang L. Maternal and Neonatal Outcomes of Pregnant Women With Coronavirus Disease 2019 (COVID-19) Pneumonia: A Case-Control Study. Clin Infect Dis. 2020;71(16):2035-41.
[15.] Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis T, Politou M, et al. Hematological findings and complications of COVID-19. Am J Hematol.2020;95(7):834-47.
[16.] Ferrazzi E, Frigerio L, Savasi V, et al. Vaginal delivery in SARS-CoV-2-infected pregnant women in Northern Italy: a retrospective analysis. *BJOG*. 2020;127(9):1116-21.
[17.] Hoang A, Chorath K, Moreira A, Evans M, Burmeister-Morton F, Burmeiter F, dkk. COVID-19 in7780 pediatric patients: a systematic review. Eclinical Medicine. 2020. 24(2020):1-19.
[18.] Rekomendasi Nasional untuk Diagnosis dan Pengobatan pneumonia yang disebabkan oleh SARS-CoV-2 (edisi ke-4). Komisi Kesehatan Nasional dan Kantor Administrasi Nasional Pengobatan Tradisional Cina. 2020.
[19.] Queensland Government. Queensland Clinical Guidelines: Maternity care for mothers and babies during the COVID-19 pandemic, COVID-19 and breastfeeding Guideline. Queensland Health. 2020;1-33.