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Original Research Article

High neutrophils to lymphocytes ratio in maternal blood serum as risk factor for preterm premature rupture of membrane

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ABSTRACT

Background: Neutrophil-to-lymphocyte ratio has been extensively studied as a prognostic factor for various diseases based on systemic inflammation. Premature rupture of membranes is an obstetric problem that does not only occur in term pregnancies but can also occur in preterm pregnancies. One of the main etiologies for premature rupture of membranes is inflammation. Knowing the difference in the NLR between preterm premature rupture of membranes and without PPROM is important to increase understanding of the crucial role of NLR in predicting the incidence of PPROM.
Methods: This analytic case-control study compared NLR values in maternal blood serum between PPROM and without PPROM. This research was conducted in the emergency delivery room and obstetrics and gynecology outpatient clinic at Prof. dr. I.G.N.G. Ngoerah Hospital Denpasar from February to June 2022.
Results: A high NLR in maternal blood serum may be a risk factor for PPROM. Patients with a high NLR had a 4.5 times greater likelihood of experiencing PPROM than those with a low NLR (OR=4.5; 95% CI=1.4-13.83; p=0.007).
Conclusions: A high NLR in maternal blood serum is a marker of inflammation with an increased risk of 4.5 times for the occurrence of PPROM.

Keywords: Inflammation, Neutrophil-to-lymphocyte ratio, Preterm premature rupture of membranes

INTRODUCTION

Premature rupture of membranes (PROM) is an obstetrical problem still frequently encountered in daily practice. PROM occurs not only in term pregnancies but also in preterm pregnancies. One of the main etiologies for preterm rupture of membranes is inflammation.[1] Under these conditions, an immune system-mediated response affects the number of leukocyte subtypes circulating in the circulation, in which the number of neutrophils increases while the number of lymphocytes decreases. The dynamics of the number of leukocyte subtypes is then calculated in the form of a ratio, a parameter known as the value of the neutrophil-to-lymphocyte ratio (NLR). Based on systemic inflammation, NLR has been extensively studied as a prognostic factor in various diseases.[2,3] The incidence of premature rupture of membranes ranges from 5% to 10% of all births, while preterm PROM occurs in around 2-5% of all pregnancies.[4] In developing countries, the incidence of preterm birth varies; for example, in India, around 30%; South Africa, around 15%; Sudan, 31%; and Malaysia, 10%. In Indonesia, unfortunately, there is no national

incidence of prematurity. However, the incidence of babies with Low Birth Weight (LBW) can roughly reflect the incidence of prematurity. The national incidence of LBW in almost all Indonesian hospitals is 27.9%. In Indonesia, the incidence of PROM ranges from 4.5-7.6% of all pregnancies.[5] In 2016, cases of premature rupture of membranes were reported in 212 out of 1450 deliveries (14.62%), with 179 cases of them (84.43%) occurring at term gestational age and the remaining 33 cases (15.57%) occurring at term preterm pregnancy.[6] Taking into account the high incidence and prevalence of preterm premature rupture of membranes and the accompanying complications, both maternal and neonatal complications, efforts need to be made to screen for the incidence of preterm premature rupture of membranes early so that appropriate intervention can be carried out and reduce the incidence of these complications. Because PROM is a disease with inflammation as one of its pathophysiological bases, determining the difference in NLR between preterm premature rupture of membranes (PPROM) and without PPROM is important to increase understanding of the crucial role of NLR in predicting the incidence of PPROM. So far, there have been no studies comparing the NLR between a group of preterm pregnancies with premature rupture of membranes and a group of preterm pregnancies without premature rupture of membranes. Therefore, the author is interested in researching the relationship between the ratio of neutrophils to lymphocytes in maternal blood serum as a risk factor for preterm premature rupture of membranes.

METHODS

This study was an analytic case-control study that compared the value of the NLR in maternal blood serum between PPROM (case group) and without PROM (control group). This research was conducted in the emergency department and obstetrics and gynecology outpatient clinic at Prof. Dr. IGNG Ngoerah Hospital Denpasar. The research was done from February 2022 to June 2022. The sample was 56 pregnant women with a gestational age of 20 weeks to less than 37 weeks who came to the emergency delivery room and obstetrics and gynecology outpatient clinic at Prof. Dr. IGNG Ngoerah Hospital Denpasar, divided into 28 samples for case group and 28 sample for control group. The inclusion criteria for the case group were pregnant women with a diagnosis of preterm premature rupture of membranes (PROM) with a gestational age between 20 weeks to 36 weeks 6 days, singleton and live pregnancy. Inclusion criteria for the control group were pregnant women with a diagnosis of normal preterm pregnancy without premature rupture of membranes, gestational age between 20 weeks to 36 weeks 6 days, singleton and live pregnancy, no uterine contractions, and intact membranes. The exclusion criteria for this study were pregnant women with multiple pregnancies, a history of previous preterm labor, pregnant women with polyhydramnios, active smokers, acute intrauterine infections, and patients who had undergone invasive procedures such as amniocentesis. Signed informed consent was taken from patients who were willing to take part in the research.

Data analysis

The research data were processed using the software IBM SPSS version 26.0. All data obtained in this study were analyzed descriptively based on age, parity, and gestational age, and the results were described in the table. The normality test was carried out with the Kolmogorov-Smirnov test. A homogeneity test was performed using Levene's test. The comparative test was performed using the independent t test and the Mann-Whitney test. Risk factor analysis was carried out by correlating the incidence of PPROM with an increase in NLR.

RESULTS

This study involved 56 pregnant women using a case-control study design that compared the value of the NLR in maternal blood serum between PPROM and without PPROM. The (Table 1) compares the distribution based on age, parity, and gestational age.

Table 1: Mean distribution by age, parity, and gestational age in both groups.

Variable	Preterm PROM (N=28)		Preterm without PROM (N=28)		P value
	Mean	SD	Mean	SD	
Age (years)	27.32	5.5	29.75	6.7	0.117
Parity	0.96	0.962	1.54	1.37	0.149
Gestational age	30.96	3.57	31.25	4.6	0.520

Table 2: Maternal serum neutrophil-to-lymphocyte ratio as a risk factor for preterm premature rupture of the membrane.

NLR	With PPROM	Without PPROM	OR	CI 95%	P value
High	18	8	4.5	1.4-13.83	0.007
Low	10	20			

There was no significant difference in age between the two groups (p=0.117). In the PPROM, the mean age of the maternal was 27.32±5.5 years, while in the maternal without PPROM, the average age was 29.75±6.7 years, with the lowest age being 17 years old and the oldest being 43 years old. There was no significant difference in parity status between the two groups (p=0.149), where the mean parity in PPROM was 0.96±0.962, while in the maternal without PPROM was 1.54±1.37. There was no significant difference in gestational age between the two groups (p=0.520). Determination of the cut-off value of the NLR in maternal blood serum, which was used as the limit value for the risk factor for PPROM, was obtained from a study conducted by Zhan *et al* with a cut-off value of 4.59. The sensitivity and specificity of the cut-off were 43% and 87%, respectively.[7] The (Table 2) shows that a high NLR in maternal blood serum may be a risk factor for PPROM. The analysis found that high NLR in maternal blood serum had a 4.5 times greater likelihood of experiencing PPROM (OR=4.5, CI 95%=1.4-13.83, p=0.007).

DISCUSSION

In this study, the mean age of the maternal without PPROM was 27.32 years, and the mean age of the maternal with PPROM was 29.75 years (p value = 0.117). There was no significant difference in age between mothers with PPROM and those without PPROM. Mostly, the age of mothers with PPROM was 20-35, followed by >35 years and <20 years of age. The age of 20-35 years is the childbearing age for women. In a study by Torika et al., similar results were reported. In their report, the pregnant women with PROM in term and preterm pregnancies were mostly at the age of 20-35 years.[8] The mean parity of women with PPROM was 0.96, and that of women without PPROM was 1.54 (p value = 0.149). It indicated that there was no significant difference in parity between the two groups. Hackenhaar et al. stated that mothers with PROM were mostly found in primigravid.[9] Another study by Movahedi et al. also showed that the highest incidence of PPROM occurred in primigravid (69.7%).[10] Previous research by Budijaya and Negara also showed that the incidence of PROM in the primigravid was 41.05%.[6] On the other hand, Manuaba and Varney stated that women who have given birth several times had a higher risk. Women who had experienced PROM in previous pregnancies and were too close in the birth period had more risk of PROM in subsequent pregnancies. Multiparities had a higher risk of occurrence of PPROM because of the faster cervix opening than Nulliparities. Therefore, PROM can occur earlier. The infection can cause biomechanical disturbance in the amniotic membranes by proteolytic formation. It makes the membranes rupture easier. In multiparities, due to a history of previous labor, the connective tissue is looser than in nulliparities due to the increased cervical damage. Therefore, there is no resistance to the amniotic membrane.[11,12] The average gestational age in the PPROM and without PPROM was 30.96 and 31.25, respectively (p value = 0.520). It showed no significant difference in gestational age between the two groups. Mostly, the gestational age was in the range of 30-36 weeks, followed by 24-29 weeks. This is by research by Locatelli et al which stated that PPROM occurred in less than 1% in 24-27 weeks of gestational age, 2-5% in 28-33 weeks of gestational age, and 3-8% in 34-36 weeks of gestational age.[13] However, other studies by Ozel et al and Toprak et al found no significant difference between gestational age and the occurrence of PPROM.[14,15] PPROM has a significant association with preterm delivery. Despite various etiologies, PROM is closely related to infection or inflammation. In cases of PPROM, there are increased levels of IL-6, IL-1β, and TNF-α. However, only a few cases are preceded by clinical signs and symptoms of acute infection. In contrast to clinical infection, subclinical infection is characterized by tissue infiltration by neutrophils, macrophages, and lymphocytes without clinically significant findings of infection. Such subacute infection can be proven by histological evidence of chorioamnionitis and positive amnion culture results.[15,16] It is known that cytokines and chemokines produced by the early inflamed choriodecidua circulate into the maternal bloodstream, leading to changes in circulating leukocyte subtypes. Strong host immune response resulting from increased local production of proinflammatory cytokines and chemokines (such as IL-1β, IL-6, IL-10, TNF-α, G-CSF, prostaglandins, and leukotrienes) causes neutrophilia. On the other hand, lymphocytopenia is caused by inflammation-induced mechanisms such as impaired antigen presentation, activation of negative costimulatory signals, and production of immunosuppressive factors. They all lead to a significant decrease in T-helper lymphocytes in the early phase of the inflammation response.[17]

In the presence of systemic disturbance such as trauma, infection, stress, or ischemic injury, the body response is regulated by the neuroendocrine and the innate immune system and mediated by the adaptive immune system (cellular and humoral). On the injury site, inflammation cells will recognize the site and recruit specific leukocyte subpopulations to the tissue to initiate the destructive process. It will lead to systemic inflammation characterized by fever, leukocytosis, increased acute phase proteins, and inflammation mediators (cytokines, chemokines). In this systemic inflammation response, the leukocyte subtypes that play an important role are monocytes, lymphocytes, and neutrophils. This response is characterized by an increase in circulating neutrophils and a decrease in lymphocytes.[18] Neutrophilia and lymphocytopenia are physiological responses of the innate immune system to various disorders and stressors, including systemic inflammation, malignancy, major trauma, and malnutrition. Several factors, including hormones, chemokines, and cytokines, induce the mechanisms that cause lymphocytopenia. They regulate the quantity and activity of lymphocytes and indicate the intensity of inflammation and the resistance and adaptability of the immune system. Furthermore, neutrophilia is caused by delaying neutrophil apoptosis

and stimulation of stem cells by growth factors (G-CSF).[19] At the onset of inflammation, until it reaches its peak in the first 6 hours, there is an increase in the number of neutrophils. In acute inflammation, circulating neutrophils can rapidly increase 10-fold from 5000/µl to 30,000/µl. This increase is due to the migration of neutrophils from the spinal cord to the peripheral blood circulation and delays in the process of apoptosis.[18] In PPROM and imminent preterm delivery, cytokines released from the inflamed choriodecidual area can cause changes in the leukocyte subtypes. Lymphocytopenia is common in chronic inflammation due to increased lymphocyte stress and apoptosis.[20]

Several studies have examined the relationship between NLR and the occurrence of PPROM. In 2019, Ozel et al. found that the NLR in the PROM was higher compared to the imminent premature delivery and control groups. The increase in NLR is also proportional to the increased risk of neonatal sepsis and C-reactive protein (CRP) levels, with a sensitivity and specificity of 69.7% and 72%, respectively.[14]

Determination of the cut-off value of the NLR in maternal blood serum as a risk factor for the occurrence of PPROM was obtained from a study conducted by Zhan et al. The cut-off value was 4.59, with a sensitivity and specificity of 43% and 87%, respectively. The results of this study indicated that a high NLR in maternal blood serum may be a risk factor for PPROM. It was found that 18 samples had a high NLR, while 10 samples had a low NLR in the PPROM. On the other hand, eight samples had a high NLR, while the other 20 samples had a low NLR in maternal without PPROM (p value=0.007). It indicated a significant association between a high NLR in maternal blood serum and the occurrence of PPROM. The odds ratio was 4.5 (95% CI: 1.4-13.83), which means that materials with high NLR in the blood serum have a 4.5 times greater chance of experiencing PPROM (p=0.007).[21] This is done by Toprak et al which showed a significant increase in NLR in the PPROM compared to spontaneous preterm delivery.[15] A year earlier, Akkar et al reported that NLR was significantly increased in preterm delivery compared to the term delivery.[22] Furthermore, Daglar et al showed that NLR was significantly higher in women with preterm delivery with PROM.[23] The analysis found that high NLR in maternal blood serum had a 4.5 times greater likelihood of experiencing PPROM (OR=4.5; CI 95%=1.4-13.83; p=0.007). As a comparison, the researchers also analyzed the levels of white blood cells (WBC) in maternal blood serum, which were examined using previous research data that had also been conducted with a cut-off value of 9.63 with a sensitivity and specificity of 58% and 83%, respectively. It showed that the WBC count, % neutrophils, absolute count of neutrophils, and NLR in PROM were higher than the normal group.[21] In this study, the outcome of preterm pregnancy with or without PROM was not affected by high or low levels of WBC (OR 1.00; 95% CI 0.255-3.926; p<0.05). Balciuniene et al presented 137 adults with PROM before 34 weeks of gestational age, showing much greater WBC count and neutrophils in the PROM group with histological findings of chorioamnionitis. WBC, CRP, and NLR levels were higher in PROM with histological chorioamnionitis (p value=0.001). WBC, CRP, and NLR levels predicted HCA in the area under the curve (AUC) of 0.81, 0.81, and 0.89, respectively. Even though the AUC of the NLR was statistically larger than that of the WBC, there was no marked difference between the AUC of the NLR and the CRP.[24] Kim et al stated that the ability of NLR to predict preterm labor is second to cervical length.[17] Gezer et al reported that high NLR at the time of hospitalization was an independent risk factor for preterm delivery with previous PROM in women between 34 and 37 weeks of gestational age.[25] In a similar study, Ozel et al found high NLR in PPROM.[14] Lakshmi et al concluded that NLR monitoring can be carried out during the second and early third trimesters as a routine practice among high-risk mothers because it can significantly assist in the early prediction of PPROM and help minimize the bad outcomes of maternal and neonate.[26] In the presence of inflammation in the tissue, inflammatory cells recruit leukocytes to the site of infection. Depending on the strength and intensity of the inflammation and the resistance and adaptability of the immune system, neutrophilia and lymphocytopenia develop and are maintained.[27] Increasing NLR stimulates neutrophil progenitor cells and lymphocyte apoptosis by various hormones and cytokines.[28,29] In the study of Gezer et al NLR >6.2 on the admission proved useful as a cut-off point for predicting preterm birth with a sensitivity and specificity of 65.1% and 62.5%, respectively.[25]

CONCLUSION

It can be concluded that a high NLR in maternal blood serum is a marker of inflammation with a higher risk of 4.5 times for the occurrence of PPROM. NLR, a marker of the body's response related to innate immune response, is thought to occur also in various stressful events in pregnancy. In this case, it is related to the inflammation response that occurs in the PPROM. NLR is a relatively cheap, easy, and simple examination. It is quite reliable in measuring the index of the systemic inflammation response. Therefore, it has been widely studied as a predictor and prognostic factor for the severity of several diseases, including the occurrence of PROM.

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