

RATIO OF PLATELET-LIMFOSITES AND LEUKOSIT LEVEL AS INDICATORS OF BABY FEEDBACK (APGAR SCORE) ON PREMATURE RUPTURE OF MEMBRANE CASES IN ULIN GENERAL HOSPITAL

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Abstract: Premature rupture of membranes (PROM) has the potential effect to cause complications such as premature infants, neonatal sepsis, and intrauterine infection (chorioamnionitis). Some inflammatory markers have been investigated to detect PROM at their early stage and prevent the complications. Platelet lymphocyte ratio (PLR) and leukocytes are a simple, affordable inflammatory marker, and have been used to predict a variety of inflammatory conditions. This study aims to see the relationship between PLR and leukocytes with APGAR Score in the case of PROM. This study was an analytic observational study with a retrospective cross sectional design. Data from 80 samples showed PLR and leukocytes had no significant effect on APGAR value ($p > 0.05$).

Keywords: Platelet Lymphocyte Ratio, Premature rupture of membranes, APGAR score

INTRODUCTION

Premature Rupture of Membrane's (PROM) incidence ranges from 10% to 30% of all births. PROMs are grouped into two based on the timing of occurrence, PROM or Premature Rupture of Membrane (PROM) and preterm PROM or Preterm Premature Rupture of Membrane (PPROM). PROM has the potential to cause complications of premature infants, neonatal sepsis and the risk of intrauterine infection (chorioamnionitis).^{1,2}

The incidence of PROM in Ulin General Hospital occurred as 126 cases in 2016. The incidence of chorioamnionitis infection increases from 6-10% to 40% if PROM occurs > 24 hours. While the risk of neonatal infection increased to 2x fold.²³ Therefore, the need for diagnosis as early as possible in order to avoid serious complications to both fetal and maternal. Various factors play a role in the pathogenesis of PROM / PPRM occurrence; especially the inflammatory factor plays an important role in the occurrence of membrane rupture.³

Several studies for inflammatory markers in PROM use maternal blood leukocyte levels, CRP, leukocyte levels in urine, differential count, and platelet lymphocyte ratio. Platelet lymphocyte ratio (PLR) is a simple inflammatory marker, affordable and already used to predict a variety of conditions. PLR is thought to be a marker that is more sensitive to inflammation and is a prognostic factor in breast, ovarian, and colorectal cancer.⁴

Another PLR study as an inflammatory marker was proposed by Farah in 2017 who reported a correlation between Platelet lymphocyte ratio infection and the incidence of *Helicobacter pylori* infection in 200 patients performed by biopsy in the gastrointestinal tract.⁵ The study was randomized controlled trial and the correlation was found to be directly proportional and significant. In the study, PLR was called a simple and widely available marker but found to be in line with infection.

Turgut et al⁵ reported platelet participation in endothelial destruction, angiogenesis, and hypoxia in various conditions. While research conducted by Toprak et al 2017 shows a close relationship between PLR exceeding 117,14 found many in the incidence of preterm PROM.³ The study of PLR in Indonesia is still not done much. Therefore, this is a preliminary study to be one of the references to health services in Indonesia that facilities are not yet equally distributed. Routine blood examination with type count in Indonesia is quite easy and still affordable by financing.

As stated earlier that the risk of neonatal infection increases with the course of PROM disease. Furthermore, the inflammatory process of PROM which has the potential to cause neonatal sepsis has several risk factors. Significant risk factors for neonatal sepsis by Patil, 2014, evidence of inflammation in chorion placenta, gestational age less than 34 weeks, APGAR Score less than 6 in the first 5 minutes, and clinical signs of amnionitis.^{1,6} APGAR score use and age separation a pregnancy under 34 weeks is a simple factor for early assessment of the risk of neonatal sepsis.

RESEARCH METHODS

This was an analytic observational study with retrospective cross sectional design in patients of premature rupture of membranes treated in the Maternity Hospital of Ulin Hospital from 1 January 2016 to 1 January 2017 with purposive sampling from medical records.

Population was obtained from all patients treated in the maternity room of Ulin Hospital Banjarmasin Periode January 1, 2016 - January 1, 2017. Inclusion criteria include: 1). Patients receiving diagnosis of PROM enforced by Doctors in charge. 2).

Medical records include data required in this study: platelet content, lymphocyte, APGAR score, baseline maternal profile (gestational age, parity, maternal age). Exclusion criteria include: 1) Medical record data is not listed completely. 2). Patients have congenital

hematologic abnormalities listed in the medical record.

Research procedure is levels of leukocytes, platelets and lymphocytes are determined by taking medical record data. PLR is calculated from the ratio between platelets with lymphocytes. The hematology processing method used is by

using Mindray BC 1800 hematology machine for 10 seconds. Meanwhile, *APGAR score* is determined from *APGAR scores*. The first minute scores are listed according to the assessment of Doctor / medical assistant of childbirth. *APGAR score* assessment is outlined in Figure 1.

APGAR SCORING SYSTEM				
	0 Points	1 Point	2 Points	Points totaled
Activity (muscle tone)	Absent	Arms and legs flexed	Active movement	
Pulse	Absent	Below 100 bpm	Over 100 bpm	
Grimace (reflex irritability)	Flaccid	Some flexion of Extremities	Active motion (sneeze, cough, pull away)	
Appearance (skin color)	Blue, pale	Body pink, Extremities blue	Completely pink	
Respiration	Absent	Slow, irregular	Vigorous cry	
				↓
				Severely depressed 0-3
				Moderately depressed 4-6
				Excellent condition 7-10

Figure 1. APGAR SCORE

After data collection is completed, data is entered into SPSS 18 for multiple linear regression test. If the test is classical assumption test value (multicollinearity, autocorrelation, heteroscedasticity, and normality) and feasibility test (F test and t test) is fulfilled then data interpretation is done. This research is done with value $\alpha = 0,05$.

RESULTS AND DISCUSSION

A total of 80 patients with PROM diagnosis met the inclusion criteria in this study. There are 92.5% of patients aged less than 35 years, 7.5% others aged 35 years or older. According to parity, 51.2% of patients were primipara, 48.8% of patients were multiparous, and by mode of delivery 74.8% of patients gave birth normal, and another 26.2% gave birth surgically. The distribution of patients by age, parity, and mode of delivery can be seen in Table 1.

Table 1. Distribution of age, parity, and mode of delivery

Characteristics	Total (percentage)
Age : < 35 years old	74 (92,5 %)
Age : 35 years old or more	6 (7,5 %)
Parity : Primipara	41 (51,2 %)
Parity : Multipara	39 (48,8 %)
Mode of delivery : normal	59 (74,8 %)
Mode of delivery : Operative	21 (26,2 %)
Total	80

Diagnosis of PROM <12 hours was found in as many as 50 patients, and 30 patients had PROM > 12 hours (Table 2). The mean comparison of platelet lymphocyte ratio (PLR), leukocytes, and

APGAR Score group of patients with PROM <12 hours and PROM > 12 hours is shown in diagrams 1, diagrams 2, and diagrams 3.

Table 2. Distribution of patients, average PLR, average leucocyte, and average APGAR (total 80 samples).

	PROM < 12 hours	PROM > 12 hours
Number of patients	50	30
Average PLR	135,8	128,4
Average Leucocyte	11216	12940
APGAR	7,2	6,5

The unpaired T test was conducted to determine whether the ratio of PLR, leukocyte and APGAR scores between the two groups was significant. Previously, the

normality test of each data group, if the data is not normally distributed, non-parametric test of Mann Whitney was tested (Table 3).

Table 3. Result of normality test and differential test of PLR, Leucocyte, and APGAR in group of patients with PROM < 12hours and PROM >12 hours.

Variable	Significancy		
	<i>Shapiro-Wilk</i>	<i>T Independent test</i>	<i>Mann Whitney test</i>
Platelet lymphocyte ratio (PLR)	0.053	0.487	-
Leucocyte	0.30	0.036	-
APGAR	0.00	-	0.02

Platelet lymphocyte ratio (PLR) data has a Sig value. normality> 0.05, which means normal distributed data, PLR group difference of PROM group <12 hours and PROM > 12 hours tested with Independent T test (Unpaired) has significance value> 0.05, indicating no significant difference of PLR value between the two groups .eukocyte data was normal distribution and had significance value of independent T test <0.05, indicating there was significant

difference of leukocyte value between group of patients with PROM <12 hours and PROM > 12 hours. APGAR Score data is not normally distributed, so tested using non parametric test, in the form of Mann-Whitney test, got significance value <0.05, which means there is also significant difference value of APGAR Score between groups (Table 3).

Table 4. Saphiro Wilk Normality Test.

	PROM <12 hours	PROM >12 hours
Statistic	,991	,993
Df	50	30
sig	,974	,999

Table 5. Results of double linear regression test in PROM <12 hours group.

Independent Variable	VIF	Durbin Watson	Anova (sig)	Coefficients		R Square
				T	Sig	
PLR	1.030	2.089	0.426	0.817	0.418	0.036
Leucocyte	1.030			0.879	0.384	

Tabel 6. Results of double linear regression test in PROM >12 hours group.

Independent Variable	VIF	Durbin Watson	Anova (sig)	Coefficients		R Square
				T	Sig	
PLR	1.001	2.052	0.432	-1.289	0.208	0.060
Leucocyte	1.001			-0.316	0.754	

Premature rupture of amniotic membranes is one of pregnancy complication that often happens, and considered related to the increasing incidence of perinatal death, fetal distress during labor, and infection (maternal and fetal). Positive correlation between PROM and outcome of the baby mostly caused by PROM complication, such as oligohydramnion and chorioamnionitis, which its incidence strongly affected by onset of premature rupture of amniotic membrane.^{6,7}

PLR and leucocyte as inflammation marker in premature rupture of amniotic membranes had been known before. Studies reported by Toprak 2017 showed significant positive correlation between PLR in incidence of PROM with $p < 0.05$. Later, Toprak 2017 reported the specificity and sensitivity of PLR to diagnose premature rupture of membranes as much as 73.7% and 57.8%.³

The process of the relationship between KPD and inflammation has not been clear. This process begins with a localized and systemic system that causes tearing of the membranes. Inflammation can occur due to

microbial infection of the vagina. The microbial invasion can detect in 30% of cases of KPD by amniocentesis. This process will lead to the recruitment of inflammatory cells including white blood cells (leukocytes).² Leukocytes as a marker of inflammation have been widely investigated because of inflammatory markers in KPD. A study by Nili, 2015 that found a 20% increase in leukocyte levels associated with KPD incidence.¹⁶

In line with leukocytes, PLR has also long been recognized as one of the markers of systemic inflammation, particularly in chronic inflammatory diseases such as malignancy, rheumatoid arthritis, and arterial thrombosis.⁸⁻¹⁰ Studies show platelets due to reactive chronic inflammatory processes, resulting from inflammatory cytokines such as IL-1, IL-11, IL-6 which activates the thrombopoiesis process through megakaryocyte activation.¹¹⁻¹³ However, in acute inflammatory processes, infections caused by infection (viral or bacterial), platelet production due to the substance produced by the host interaction and agents, such as IFN- α , IFN- β , TNF- α , and

Lipopolysaccharide (LPS) undermine the process of megakariopoiesis and increase platelet replacement. Substance injection to humans has been shown to decrease platelet count. By complement, PLR can be used as one of the predictors of KPD related to APGAR Score infant.^{8, 14-15}

Furthermore, multiple linear regression tests were performed to find the correlation of two independent variables (PLR and Leukosit) to the dependent variable (APGAR Score) in each group. Previously, the classical assumption test (normality, multicollinearity, autocorrelation, and heteroscedasticity) was performed on the data of each group as a condition for the interpretation of multiple linear regression test results. Good research should be free of multicollinearity, heteroscedasticity, autocorrelation, and the normality of residual data.

The residual normality of the data can be seen from the pattern of split point on the PP plot, when the distribution of the dots is close or dense in a straight line (diagonal) it is said that (data) residual is normally distributed, but if the distribution of these points away from the line then not distributed normal. The spreading point on both groups approaches the diagonal line, which means the data is considered to be normally distributed. Because the interpretation of the data distribution pattern is subjective, an objective approach of Saphiro-Wilk test is made to ensure data normality, each group has Sig > 0.05, which means normal distributed data (table 4).

Multicholerenity can be seen from VIF value of residual data in each group (table 5 and 6) because VIF value from both variables in each group do not have difference value for more than 10 or 5, it can be said that there was no *multicholerenity* in variables of this study.

Autocorrelation data can be seen from Durbin-Watson (DW) in each group residual data (table 5 and 6), and matched to Durbin-Watson table based on number of samples and study variables. DW score in both data group in this study was between

dU (down upper) – and 4 dU, showing no autocorrelation of the data.

Heteroskedastisitas data of each group can be seen from scattered pattern of the dots in Scatterpot, the scattered dots in Scatterpot for each group does not show any pattern, so we can conclude that there was no *heteroskedastisitas*.

Before deciding size and direction of relationship between two variables in each group, correlation test was done to decide whether there was correlation between variable. Correlation/relationship can be seen from significance value of variable as coefficient in each group. Both variables in group of patients with PROM <12 hours and group of patients with PROM >12 hours have significance value of >0.05, which means there was no significant correlation between two variables in both groups.

The influence of PLR variable and leucocyte towards APGAR score can be seen from R square each group. In group of patients with PROM <12 hours, PLR and leucocyte affects APGAR score as much as 3.6%, meanwhile in group of patients with PROM >12 hours, PLR and leucocyte affects APGAR score as much as 6%, at which both means insignificant.

Several factors which can be confounding/bias factor in this study, from maternal such as time of blood sample taken which is not the same for every sample and other inflammation disease of maternal which cannot be detected at peripartum. Other infection disease which may be suffered by samples but undetected may influence leucocyte, lymphocyte, and thrombocyte so it potentially affects PLR and leucocyte level. Meanwhile, factors which may affect from fetal point of view in APGAR score are sedation/anesthesia in maternal that was used during peripartum, gestational age (in premature pregnancy), congenital malformation, trauma, and variability of scoring.

CONCLUSIONS

In this study, there were significant differences in the number of leukocytes and APGAR values in patients with PROM <12 hours and PROM> 12 hours, whereas PLR values did not differ significantly in both groups ($P > 0.05$). In addition, based on regression test, PLR value and leukocyte count also had no significant effect on APGAR score score ($p > 0.05$), both in group of patients with PROM <12 hours and PROM> 12 hours.

Further studies are expected to show and explain the role PLR and leucocyte as outcome indicator for babies in maternal chronic inflammation.

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