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Correlation of Progesterone Induced Blocking Factor and Neutrophil-Lymphocyte Ratio in Preterm and Term Delivery

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Abstract: Objective: This study aimed to determine difference of Progesterone Induced Blocking Factor, Neutrophil-Lymphocyte Ratio levels between preterm and term labor, and also to examine correlation between PIBF and NLR in preterm and term labor women. **Methods:** This is a comparative analytical study with cross-sectional design. Subjects were divided into two groups consisting of 33 persons per group.Data was analyzed with Independent T-test and Mann-Whitney test. Correlation between two variables was measured with Pearson correlation calculation. **Results:** Mean levels of PIBF waslower inpreterm labor (973.18 ± 290.91 ng/ml) compared to term labor (3338.06±2165.89 ng/ml) ($p = 0.000 < 0.005$). Average NLR was greater in preterm labor (12.62 ± 6.44) than term labor (5.17 ± 1.64) with significant differences.Correlation value (Pearson) between PIBF andNLR in preterm labor was strongly negative (-0.543), whereas correlation between PIBF andNLR in term labor was weakly negative(-0.135)**.Conclusion:** Average of PIBF levels in preterm labor was lower than term labor. Average of NLR in preterm labor was greater than term labor. There was a negative correlation between serum levels of PIBF and NLR in preterm labor andthere was no correlation between serum levelsof PIBF andNLR in term labor.
Keywords: Preterm labor, PIBF, NLR.

Introduction

Preterm delivery is one of the leading causesin perinatal mortality. The incidence of preterm delivery is still high in Indonesia. To prevent the preterm delivery, pathophysiology and etiology should be fully understood including hormonal and inflammatory factor.

Decreased PIBF and increased inflammatory factors such as neutrophil, are reported to be the predisposing factor of preterm delivery[1,2,3,4]. Infection was believed to be the etiology of preterm delivery. Excess neutrophil to lymphocyte ratio in preterm delivery indicate inflammation occurrence due to infection.[5,6,7] In preterm delivery, decreased lymphocyte will cause PIBF level decrease in the maternal serum

significantly.[8,9]The aim of the study is to observecorrelation between infection and inflammation to incidence of preterm delivery.

Methods

This study was conducted from July to November 2014. There were 70 subjects with 4 subjects excluded. Two of them haven't delivered the baby after 24 hours admitted to hospital, one of them underwent error during blood sampling, and the rest declined to be research subject. From 66 total samples, there were 33 subjects of term delivery and 33 subjects of preterm delivery. Subjects were patients admitted to Dr. HasanSadikin General Hospital, Astana Anyar District Maternal and Child Hospital, Cibabat District General Hospital, and Bandung General Hospital. Variables measured were age, parity, and gestational age. Blood sampling was done to determine PIBF serum level and differential count of leucocyte in Bandung Prodia Laboratory.

This is cross sectional comparative study to determine the difference of PIBF serum ratio to NRL ratio in the preterm delivery compared with term delivery, then we correlate between these two variables.Subjects consisted of preterm and term delivery collected using consecutive sampling. Patients involved in this study were chosen in accordance with inclusion criteria.Data was analyzed using Windows SPSS 18th version. Normality test was conducted on PIBF serum level using Shapiro-Wilk.Measurement of PIBF serum level difference between preterm dan term delivery was performed with Independent T-test and Mann-Whitney test. Correlation between PIBF and NLR ratio was analyzed by Pearson.

Written informed consent was obtained from all participants. The ethical reviews boards of the Health Research Ethics Committee, Faculty of Medicine Padjadjaran University and Dr. Hasan Sadikin Hospital, Indonesia, approved this study.

Table 1. Characteristics of research subjects

Characteristic	Gestational Period		*p-value*
	Term	Preterm	
	(n=33)	(n=33)	
Age			
$\bar{X}$ (SB)	28,33 (6,44)	28,39 (5,6)	0,968[a]
Median	29 (18 - 42)	28 (17 - 40)	
Parity			
0	12 (36.36%)	11 (33.33%)	0,182[b]
1	10 (30.3%)	8 (24.24%)	
2	8 (24.24%)	12 (36.36%)	
3	1 (3.03%)	2 (6.06%)	
4	2 (6.06%)	0 (0%)	
IMT			
$\bar{X}$ (SB)	23,23 (2,11)	23,12 (2,16)	0,833[c]
Median	23,07 (18,97 - 27,27)	22,41 (20,32 - 27,59)	

Ages, parity and body mass index were compared for homogeneity. In this study, there was no significant difference between subject characteristics: maternal ages (p=0,968), parity (p=0,182), dan BMI (p=0,833). Characteristic of subjects are therefore homogeneous.

Table 2. Difference level of PIBF to NLR ratio

Variable	Term	Preterm	*p-value*
	$\bar{X}$ (SD)	$\bar{X}$ (SD)	
PIBF level (ng/ml)	3338,06 (2165,89)	973,18 (290,91)	0,000[a]
NLR	5,17 (1,64)	12,62 (6,44)	0,000[b]

Note : $\bar{X}$: average
SD : Standard deviation
a) : using Mann Whitney Test
b) : using T-Test

Results showed PIBF serum level was significantly lower in preterm delivery compared to term delivery. Mean of PIBF serum levels in term delivery was 3338.06 ng/ml, and 973.18 ng/ml in preterm delivery (p=0.000). Hudic *et al*. reported that PIBF serum level in threatened preterm delivery was lower than normal delivery (171.12 ± 162.06 vs 272.85 ± 114.87, p < 0,05).[10]PIBF level is also lower in urine from threatened preterm delivery than normal.[11]

Table 3. Correlation between PIBF to NLR

Correlation	Term	Preterm
PIBF >< NLR		
Pearson Correlation	-0,135	-0,543
P-value	0,454	0,001
N	33	33

Correlation between PIBF level and NLR was -0.135 with p=0.454 (p>0.05). There was no significant correlation between PIBF levels to NLR in term delivery. In preterm delivery, correlation between PIBF level and NLR is -0.543 with p=0.001 (p<0.05) indicating significant correlation between PIBF level to NRL

Discussion

Results showed PIBF serum level was significantly lower in preterm delivery compared to term delivery. Hudic *et al*. reported that PIBF serum level in threatened preterm delivery was lower than normal delivery (171.12 ± 162.06 vs 272.85 ± 114.87, p < 0,05).[10]PIBF level is also lower in urine from threatened preterm delivery than normal.[11]

PIBF is a 34 kD protein produced by maternal lymphocyte due to progesterone effect. Progesterone is known as an immune modulator to maternal-fetal interface maintaining pregnancy. Elevated IL-8, IL-1β, and TNF-α are documented in cervix following decreased progesterone. In animal model (rat and sheep), decreased levels of progesterone enhance labour process. In human, progesterone levels elevates until placental delivery. Study done by Caspo(1960) showsfunctional progesterone withdrawal along with increasedestradiol 17β ratio to progesterone during preterm delivery. The functional progesterone withdrawal process triggers transformation in progesterone receptor in immune cell. Lower progesterone in woman with threatened preterm delivery, causes lower PIBF production as well, compared with normal pregnant woman.[9, 12,13]

PIBF increases production of cytokine Th2 in pregnant woman. PIBF also stimulates antibody and inhibits degeneration of natural killer cells.[14]Low levels of PIBF serum in preterm delivery lead to increased production of inflammation process through domination of Th1 to Th2 ratio. Th1 inflammatory cytokine increases gene expression regulating labour process, oxytocin receptor, and prostaglandin production causing uterine contraction and ripening of the cervix.[15]PIBF also modulates arachidonic acid through prostaglandin and leukotriene metabolism. Shortly, low level of PIBF increase prostaglandin production which leads to uterine contraction, ripening of the cervix and increased leukotriene causinghigher natural cell and inflammation cell activation.[15]

In this study, mean level of PIBF in preterm delivery was significantly lower than in normal delivery (p=0.000 <0.05).Recent study showed PIBF can be used to predict the outcome of abnormal delivery. Polgar*et al*. found that PIBF level in urine on preterm delivery tends to decrease until the onset of delivery compared with patients with increasing level of PIBF level from 7th weeks of normal pregnancy.[11]

Meanof PIBF level in preterm contraction woman is significantly lower than normal pregnant woman in the same gestational age (1158.200 vs 1285.450, p=0.029).[16]Nevertheless, there are no studies reported PIBF in predicting preterm delivery.

Labour is an inflammation process found in myometrium and peripheral blood smear. However, inflammation is generally caused by infection in preterm delivery. This study showed that there were increased neutrophil level in patients (50-70% higher than normal level) and decreased lymphocyte level (25-40% lower).First response of injury is increasing of neutrophil level.[17]From endometrial biopsy obtained during caesarean section, there is massive influx of inflammatory cells (neutrophil and macrophage) into the upper and lower segment of myometrium that related to labour physiology.[18]

During labour process, there is influx of leucocyte at the cervix due to increased neutrophil and macrophage,without involving T-cell or B-cell.[2]Release of pro-inflammation cytokine (IL-8, IL-1β, TNF-α) at the cervix will induce ripening of the cervix that later generates breakdown of metalloprotein matrix. IL-1β also enhances the production of cyclooxygenase-2 and prostaglandine E2 (PGE2) which is effective to dilatation of the cervix.[2]

Hormonal, chemokine, and cytokine are factors that contribute to lymphocytopenia promoting inflammation and immune system adaptation. [20] whereas neutrophiliais caused by delayed apoptosis of neutrophil stimulated from stem cells by growth factors.[5]During inflammation, white blood cells change rapidly showing neutrophil role as first part of inflammation response. Neutrophilia mostly occurs along with lympocytophenia which can be used as a good predictor of bacteremia condition.[22]

Neutrophil to lympocyte ratio can be used as diagnostic and prognostic factor in various disease. [5]In this study, difference of neutrophil to lymphocyte ratio (NRL) in preterm delivery compared to term delivery was investigated. Average level of NLR in term delivery is 5.17 with standard deviation of 1.64. In preterm delivery, average level of NLR in 12.62 with standard deviation of 6.44. The result showed significant result (p=0.000, p<0.05). It is concluded that NLR average in preterm delivery is higher than term delivery with significant result. NLR average was found significantly different in preterm delivery (p<0.001). Furthermore, combination of NLR and cervix length measurement provides better result as preterm delivery predictor (sensitivity 64,2%, specificity 88.3%).[5]

Bacterial infection was found through biomarker examination from blood serum causing neutrophilia and lymphocytopenia.It concluded that NLR can be used as simple methods to differentiate infection of bacterial and viral infection. Simple methods, low cost, and high sensitivity are advantage ofNLR as supplementary examination in diagnosis.[22]

Determination of correlation between PIBF level to NLR ratio in term and preterm delivery was conducted. This study was done based on theory that labour trigger inflammation process causing lympocytopheny which leads to decreased PIBF.Inflammation process occurs in preterm delivery induced by infection. Incidence of preterm delivery caused by infection either in intact or ruptured membrane approximately 25%.[23,24]A study using metagenomic analysis of microorganism in female reproduction tract showed that woman with term gestational age had less variation of microorganism.[25]

Goncalves *et al*. found that bacteria in amnion fluid is a gold standard of intrauterine infection that can be used to predict preterm delivery. However, incidence of finding microorganism in amnion fluid varies only 10-40% from all preterm delivery. Therefore, further studies regarding preterm delivery with absence of inflammation are needed.[24]

Results showed correlation between PIBF level and NLR was -0.135 with p=0.454 (p>0.05). There was no significant correlation between PIBF levels to NLR in term delivery. In preterm delivery, correlation between PIBF level and NLR is -0.543 with p=0.001 (p<0.05) indicating significant correlation between PIBF

level to NRL. In conclusion, the lower the PIBF level, the higher NLR level and vice versa. It means that infection plays an important role in preterm delivery.

Riu DS found that both examination of PIBF and NLR resulted in positive predictive value of 75% to predict preterm delivery. Further studies regarding these two parameters to predict preterm delivery are encouraged.[23]

Compounding factors are still present. Random sampling was not conducted in this study in choosing patients, samples and controls that might cause bias due to compounding variables. In this study, only cross sectional methods done to find out correlation between PIBF level and NLR. No diagnostic test was done to find cut off point, validity and reliability of NLR to predict preterm delivery.

Conclusion

Levels of PIBF were lower in preterm delivery than term delivery with higher NLR value. There is no correlation between PIBF level and NLR both in preterm and term delivery.

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Conflict of Interest

Authors have no conflict of interest

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