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High Maternal Serum Interleukin-8 and Endocervical Neutrophil Cell Count as Risk Factors for Preterm Labor

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Abstract: ***Background: Preterm laborincidence ratesare still high invariouscountries including Indonesia ranging from 10-20 %. The definitecause ofpreterm labor remains unknown. IL-8andneutrophilcell are thought to play important roleinthe pathogenesis ofpreterm labor. Objective: Todeterminethe role of high maternal serum IL-8andendocervical neutrophilcell count as risk factorsforpreterm labor. Study design: This is a case control study, involving46 preterm labor cases and 46 preterm pregnancy controls. Results: High level ofIL-8in maternalserumincreased the risk ofpreterm labor by 96times(OR =95.56; 95 % CI=22.38-40.87, p = 0.001) compared tolow level maternal serumIL–8.High number endocervical neutrophil cellsincreased the risk ofpreterm labor by 68times(OR =68.08; 95 % CI=16.84-275.21, p = 0.001) compared tothe low numberof neutrophil cells. Maternal serum IL-8 is amoredominantrisk factor forpreterm labor comparedto endocervical neutrophil. Conclusion: High level of maternal IL - 8 and endocervical neutrophil cells increase the risk of preterm labor by 96 times and 68 times respectively. Maternal serum IL - 8 is a more dominant risk factor for preterm labor compared to endocervical neutrophil cells.***

Keywords: IL - 8, endocervical neutrophil cell, preterm labor

1. Introduction

The advances of knowledge in this 20th century has opened our eyes that preterm infants are in need for special treatment, supported by rapid development of neonatal intensive care. Prematurity has been the main problem, due to its contribution for high neonatal mortality rate. Neonatal mortality is one indicator of health service system achievement of a country, in national or international context.[1] The high mortality rate is related to organ maturity such as lungs, nervous system, and gastrointestinal system. In western country, 80% of neonatal deaths are caused by prematurity, where as 10% of the surviving neonates suffer from long-term morbidity.[2]

Preterm deliveries in many countries including Indonesia remain high, with varying incidence rate. In United States, preterm labor incidence in 1981 was 9,4% which increased to 10,6%in 1990, to 11,6% in 2000, and to 11,9% in 2001.[3] In Indonesia, preterm labor ranges from 10-20%. At Dr. Hasan Sadikin General Hospital, preterm labor rate was 13,4% in year 2000.[4] At Sanglah Hospital Denpasar, the rate was 7,44% in 1996, while in 1999 Ardhana found 431 out of 4.984 deliveries (8,65%) were preterm labor. As also reported by Udiarta in 2003, preterm labor rates at Sanglah General Hospital were 6,82% in 2001, 7,50% in 2002, and 11,4% in 2003.[5] The etiology of preterm delivery are often well defined. But in some cases, the cause are difficult to explain. Some factors contribute to preterm labor, such as maternal factors, fetal and placental factors, and also other contributing factors such as socioeconomic status. In some cases, single risk factor can be seen, such as overdistention, rupture of membrane or trauma.

In his study, Cox (1989) reported that endotoxin (lipopolysaccharide) could enter amniotic fluid, inducing cytokines and prostaglandines production by decidua, which lead to labor (uterine contraction). Lockwood (2001) proposed that relationship exists between preterm labor and inflammation process in decidua, chorion, and amnion.[6]Many cases of preterm labor are caused by pathogenic process involving various chemical mediators that induce uterine contraction and cervical changes.[2] About 70-80% of preterm labors are spontaneous and are significantly related to vaginal and cervical infection, and also chorioamniotic infection.[7] Currently, the effect and relationship between cytokines and preterm labor are the most extensively studied.

The interaction between cytokines like tumor necroting factor - α (TNF - α), interleukin - 1 (IL - 1), interleukin - 6 (IL - 6), interleukin - 8 (IL - 8) and their activity on arachidonic acid metabolism may play major role in this infectio-preterm labor relationship.[8] Patients with clinical signs and symptoms of preterm delivery show increase in various cytokines; thus, cytokines may play major role in preterm labor initiation. One of inflammatory cytokine in maternal serum is IL-8. Many studies have reported that increased serum IL-8 is related to preterm labor initiation, even though the results are still varied.[9.10]

Other studies reported that in women with spontaneous preterm labor and intact membrane, when more than 5 endocervical neutrophil cells per field (400 times magnification), infection or inflammation in amniotic fluid is likely.[11] Non-invasive examination in Dr Hasan Sadikin Hospital Bandung, which combined the value ofo vaginal acidity and neutrophil cell count, had achieved 83,3% specificity and 75% accuracy for predicting preterm labor.[12]These biologic markers are increased as showed by vaginal swab and gram staining.[13]

A good diagnostic predictor will not only prevent the usage of tocolytiv therapy, but also decrease the rate of hospital

admission and referal to higher perinatal service facility. Many prognostic factors have been used to predict preterm delivery, but none has been proven to have good sensitivity and specificity to be applied on daily practice. Some markers used to foresee preterm labor such as uterine contraction, cervical length, and risk factor scoring system have not showed adequate predictive value, while fetal fibronectin and cervical fluid cytokines though having good predictive value, are still not feasible in daily practice.[14]The best way to avoid the risk of preterm labor, is to understand the pathways involved in the process, thus, preventive measures can be executed before signs of labor occur. Based on these facts, it is necessary to conduct a study at Sanglah General Hospital, in search of more accurate, efficient, non invasive and affordable diagnostic test in predicting preterm labor.

2. Method

This is a case-control study involving 92 samples, including 46 cases and 46 controls. Cases are preterm labors, while controls are normal preterm pregnancy. Samples are selected by consecutive sampling during period January 1st – December 2013. This study was conducted at Sanglah General Hospital. Maternal serum and vaginal swab were taken from all samples, and were sent to Clinical Pathology Laboratory of Sanglah General Hospital for examination.

3. Result

between two groups (p>0,05). Table 2 shows that high maternal serum IL-8 increased the risk of preterm labor by 96 times (OR= 95,56; CI 95%= 22,38-40,87; p=0,001) when compared to low maternal serum IL-8. Table 3 shows that high endocervical neutrophil cell count increased the risk of preterm labor by 68 times when compared to low cell count (OR= 68,08; CI 95%=16,84-275,21; p= 0,001).

Table 4: Maternal Serum IL - 8 was a more Dominant Predictor for the Risk of Preterm Labor when Compared to Endocervical Neutrophil Cell Count

Variables	OR	CI 95 %	P
Maternal Serum IL - 8	27,90	2,52-308,55	0,007
Endocervical Neutrophil Cell Count	4,29	0,36-50,65	0,248

As we can see from table 4, maternal serum IL-8 was more dominant in predicting preterm labor when compared with endocervical neutrophil cell count.

4. Discussion

This study was conducted to know the relationship between maternal serum IL-8, endocervical neutrophil cell count, and preterm labor.

Subjects' Characteristics

Based on analysis, mean maternal age was 28,22±4,78 in case group and 27,96±4,92 in control group, with no significant difference found between two groups (p>0,05). Mean gestational age was 31,85±2,35 in case group, while in control group was 32,52±2,66, with no significant difference between two groups (p>0,05).

Table 1 shows that there is no significant difference on samples' mean maternal age, parity, and gestational age

Table 1: Characteristic Differences between Case and Control Groups

Risk Factors	CaseGroup (n = 46)		Control Group (n = 46)		P
	Mean	SD	Mean	SD	
Age	28,22	4,78	27,96	4,92	0,748
Parity	0,89	0,88	0,83	0,97	0,547
Gestational Age	31,85	2,35	32,52	2,66	0,159

Table 2: Increased Maternal Serum IL – 8 as the Risk Factor for Preterm Labor

		Groups		OR	CI 95%	P
		Case	Control			
Maternal Serum IL – 8	High	40	3	95,56	22,38-407,87	0,001
	Low	6	43			

Table 3 Increased Endocervical Neutrophil Cell Count as the Risk Factor for Preterm Labor

		Groups		OR	CI 95%	P
		Case	Control			
Endocervical Neutrophil Cell Count	High	38	3	68,08	16, 84-275, 21	0,001
	Low	8	43			

Increased Maternal Serum IL-8 as a Risk Factor of Preterm Labor

Risk factor analysis was done to analyze the relationship between maternal serum IL-8 and preterm labor using OR as ralative comparison between two parameter. Relationship significance was examined using *Chi-Square* test, with α was set to 0,005.

Significance analysis using *Chi-Square* test found 95,56 OR which means that high maternal serum IL-8 increased the risk of preterm labor by 96 times when compared to low serum concentration.

Currently, the relationship between preterm labor incidence and cytokines expression and effects has been extensively studied. Interactions between cytokines such as TNF-α, IL-1, IL-6 and IL-8, and also their activity on arachidonic acid metabolism may take part in preterm labor pathogenesis.[8] Patients with clinical signs and symptoms of preterm labor show increased level of maternal serum cytokines, which indicates that cytokines, including maternal serum IL-8, may play important role in preterm labor. Some studies have shown that maternal serum IL-8 is associated with preterm delivery incidence, but their results are still varied.[9,10]

IL-8 expression is increased as pregnancy advances, and during labor. Cervical IL-8 concentration increases to 6 fold in pregnant women compared to nonpregnant women. Also, the concentration increases to 11 fold in pregnant women who undergo vaginal delivery. Cervical IL-8 concentration is also increased at the onset of labor.[15] According to recent studies, high IL-8 concentration describes the high risk of preterm delivery (RR 3,7 (1,1-12,1)) and it is higher in

pregnant women who undergo preterm delivery compared to preterm pregnancies who are not in labor.[16]

High Endocervical Neutrophil Cell Count as the Risk Factor for Preterm Delivery

Risk factor analysis was done to analyze the relationship between preterm delivery and endocervical neutrophil cell count, using OR to describe their relative relationship. Chi-Square test was used to assess its significance, with α was set at 0,005.

Based on significance analysis result, OR was found 68,08, whiich means that high endocervical neutrophil cell count increased the risk for preterm delivery 68 times compared to low endocervical cell count.

This study is supported by one study by Hitti (2001) that reported, the finding of more than 5 neutrophil cells in patients undergoing spontaneous preterm delivery with intact membrane, was very sensitive to predict amniotic membrane inflammation or infection.[11] Vincendo *et al* (2007) found similar result with Yudha *et al* (2008) that reported vaginal neutrophil cell count >5 per field were significantly found in preterm delivery compared to control group. Using receiver operating characteristic (ROC) curve, Yudha *et al* (2008) found that when vaginal neutrophil cell count >9 per filed was set as cut off point, it provided 73% sensitivity, 100% specificity, 87% accuracy, 100% positive predictive value, and 79% negative predictive value.[17,18] In a study by Yamada and Fayes (2006), when vaginal neutrophil cell count >10 per field was set as cut off point, it provided 80% sensitivity, 55% specificity, 67,5% accuracy, 64% positive predictive value, and 73,3% negative ppredictive value.[19]

Maternal Serum IL-8 was a more Dominant Predictor of Preterm Labor Compared to Endocervical Neutrophil Cell Count

Analysis using logistic regulation found that maternal serum IL-8 was a more dominant predictor for preterm delivery when compared to endocervical neutrophil cell count.

IL-8 is a chemokine cytokine which has potent chemotactic activity on leucocyte *in vivo* and *in vitro*. IL-8 bioactivity is marked by its involvement in polimorfonuclear leucocyte cell (neutrophil cell) activation, as chemotactic and granule release.[16,20] Some studies show that intradermal or itraperitoneal IL-8 administration *in vivo*resulted in neutrophil cell infiltration at the site of injection.[21] This chemokine is part of host response to microbial invasion, and that is why IL-8 is thought to be responsible for neutrophil cell release at amniotic mambrane and placenta when intrauterine infection occurs.[15] The increase of IL-8 before the onset of labor may assist neutrophil cell recruitment into cervix. Neutrophil cell activated by IL-8 will release lytic enzymes, collagenase and elastase. Neutrophil cell is the source of collagenase enzyme which is contained in specific granules, produced through degranulation process mediated by IL-8 cytokine. These two main functions of IL-8 (neutrophil recruitment and collagenase production stimulation) are the reason that IL-8 is a potent agent to initiate extracellular matrix rearrangement in cervical ripening process. Neutrophil recruitment into cervix has been postulated to be part of integral process involed in labor, where collagenase may play part in cervical ripening initiated by periferal neutrophil cells and its count increases during the process.[22]

Latest studies have shown that high level of IL-8 is correlated with increased risk of preterm labor (RR=3,7 (1,1-12,1)) and its concentration is higher in pregnant women undergoing preterm delivery compared to those who are not in labor. One study by Senntrom examined cervical biopsy >300 gram taken from anterior cervix of pregnant women 10-15 minutes after vaginal delivery and from unppregnant women. It was reported that mean IL-8 concentration in non pregnant women was 300 pg/mL (110-1250), while it was 26.1—pg/mL (6.800 – 128.000) in women undergoing delivery.

5. Conclusion

Preterm labor is still one of major problem in obstetrics. Despite the vast advance of knowledge in obstetrics and neonatal medicine, the incidence of preterm labor and associated mortality and morbidity remain high. The lack of adequate neonatal care resources also contribute to the burden especially in developing country such as Indonesia. Thus, preventive measures should be taken in order to decrease the incidence. Conventional biomarkers of preterm labor lack consistency and are still not feasible. IL-8 and neutrophil cell count are potential predictors that have been extensively studied. Even though many studies, including our study, have shown significant relationship between these markers and preterm labor, further study must be conducted to assess cut off point with the highest predictive value.

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