

Association of Vaginal Infection and Preterm Premature Rupture of Membrane

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Abstract: *Preterm premature rupture of membranes is still a problem in obstetrics worldwide, including in Indonesia, so that efforts to identify risk factors for preterm PROM are increasingly being researched. This study aims to investigate the relationship between vaginal infection and the incidence of preterm PROM. This research is a cross sectional study conducted on preterm pregnant women. This research was conducted in the Emergency Delivery Room and Obstetrics Polyclinic of Sanglah Hospital Denpasar and Microbiology Laboratory of Sanglah Hospital, from December 2019 to November 2020. Vaginal swab samples were analyzed using gram stain and culture methods. Data was analyzed by using SPSS version 25.0. The total subjects in this study were 54 samples. This study concluded that there was a significant relationship between vaginal infections and preterm PROM ($p = 0.002$). This study obtained $PR = 2.134$, so that vaginal infection is a risk factor for preterm PROM. The prevalence of vaginal infection in preterm PROM was 81.48%, while the prevalence of vaginal infection in non-preterm PROM was 40.74%. The mean age of preterm PROM patients at Sanglah Hospital was 26.85 years; The mean parity of preterm PROM patients at Sanglah Hospital was 3.41 children; the mean gestational age of pre-term PROM patients at Sanglah Hospital was 32.15 weeks; and the mean body mass index of preterm PROM patients at Sanglah Hospital was 27.5 kg/m². Vaginal infection is risk factor of preterm PROM. Therefore, screening and management of vaginal infections in pregnancy is very important.*

Keywords: vaginal infection, preterm PROM, risk factors

1. Introduction

In the past 8 years, preterm premature rupture of membranes (PPROM) is still an obstetric problem related to prevalence, maternal and perinatal morbidity and mortality. Preterm premature rupture of membranes is the rupture of amniotic membrane before the onset of labor that occurs in pregnant women at 20-36 weeks gestation. The prevalence of PROM varies between 5 - 15% of all pregnancies.¹ At Sanglah Denpasar General Hospital in 2015 the incidence of PROM was reported as 212 cases (14.62%) where cases of a term PROM were 84.43% and preterm PROM were 15.57% of 1450 deliveries.²

Various factors can contribute to the incidence of PROM, which are infection, mechanics, nutrition, hormonal, and apoptosis of amniotic membrane. Infection of the vagina, cervix, and urogenital tract is one of the biggest risk factors contributing to PROM and chorioamnionitis. PROM is correlated with the risk of prematurity, neonatal sepsis, puerperal febrile disease, and others.

Infection of the amniotic membrane can occur directly or ascending from the vagina or in the amniotic fluid. Genital tract infection has been reported to trigger PROM and treatment of maternal infection with antibiotics reduces the rate of PROM. Research by Shivaraju reported that in 10 preterm PROM cases, 7 cases showed positive culture results on vaginal swab examination. This indicates that 70% of preterm PROM cases are due to infection. This study also found the infectious agents *Candida*, *Coagulase Negative Staphylococcus aureus*, *E. coli*, *Enterococci*, *Kiebsiella*, *Methicillin Resistant Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus* were correlated with the incidence of preterm PROM. The three most pathogenic organisms in preterm PROM were *E. coli* (6%), *Staphylococcus aureus* (4%), and *Pseudomonas aeruginosa*

(2%).⁵

In preterm pregnancy, vaginal humidity and pH are associated with micro-organism profiles associated with the risk of PROM. Thus, vaginal infection is a risk factor for preterm PROM. Vaginal infections can be diagnosed through vaginal swab preparations where this method was chosen because it is simple and practical, as well as has high sensitivity and specificity (Schachter et al., 2003). Gram-stained swabs can also show the presence of cervical infection, proctitis, and cystitis of the perianal region. The vaginal swab provides identification of the characteristics of the vaginal micro-organisms in premature PROM. In addition, infection is also associated with the apoptotic process of amniotic membrane epithelial cells which facilitates the occurrence of PROM through the caspase independent pathway.

The aim of this study was to determine the association between vaginal infection and the risk of preterm PROM. Research on the relationship between vaginal infection and the incidence of preterm PROM is still very minimal in Sanglah General Hospital, Denpasar. If proven, the results of this study can be used as a reference for the management of vaginal swab screening in preterm pregnancy to prevent PROM and prematurity with all its associated risks. In addition, the germ pattern in preterm pregnancy can be used as an early indication of rational antimicrobial selection prior to culture and sensitivity results. Furthermore, research can be continued with experimental studies in efforts to prevent infection in pregnancy. This is important to know in order to provide targeted management, especially in Sanglah General Hospital and in Indonesia in general.

2. Materials and Methods

This study is an analytic observational study with cross sectional design. This study was conducted in the

emergency room delivery room and obstetrics polyclinic of Sanglah Hospital Denpasar and the Clinical Microbiology Laboratory of Sanglah Hospital. The study was conducted from December 2019 to November 2020. The samples of this study were all preterm pregnant patients who were treated and performed vaginal swabs at Sanglah General Hospital Denpasar. The sampling technique was consecutive sampling. The sample size in this study was 54 samples. The inclusion criteria of this study were as follows:

- a) The patient's gestational age was between 20-36 weeks
- b) Fetal presentation behind the head.
- c) Live single fetalpregnancy
- d) Willing to participate in this study by signing an informed consent

The exclusion criteria of this study were as follows:

- a) Pregnant women with multiple pregnancies
- b) Pregnant women with polyhydramnios
- c) Fetal macrosomia
- d) Pregnant women have a history of cervical suture procedures
- e) Pregnant women with systemic infections
- f) Pregnant women with trauma coitus
- g) Pregnant women with uterine overdistention
- h) Pregnant women with cervical incompetence

The dependent variable was vaginal infection, while the independent variable was preterm PROM. The control variables of this study were maternal age, parity, BMI, uterine overdistention, multiple pregnancies, polyhydramnios, coital trauma, cervical incompetence, and history of cervical suturing procedures. Vaginal swab samples were analyzed by Gram stain and culture methods. Data analysis was performed by using the chi square test using SPSS version 25.0.

3. Results

This study found total 54 samples. The characteristics of the samples in this study were the lowest gestation age was 26 weeks, the highest gestation age was 36 weeks, the mean was 32 weeks and the standard deviation was 2.63. The distribution of vaginal infections and PROM in preterm pregnancy can be seen in Table 1 while differences in maternal age, parity, age of gestation, and BMI in PROM pregnancy and non PROM can be seen in Table 2.

Based on Table 1, patient with vaginal infection were more likely found in preterm pregnancy (61 & vs 38.9%). Proportion of PROM and non PROM were the same (50% vs 50%). Based on Table 2, the mean age of mothers is older in PROM than in non-PROM and is statistically significant (0.0001). The mean parity of mothers was higher in the PROM group than in the non-PROM groups (p = 0.001).

Table 1: Samples Characteristics

Preterm Pregnancy			
		Frequency (54)	Percentage (%)
	(+)	33	61,1
Vaginal Infection	(-)	21	38,9
	(+)	27	50
PROM	(-)	27	50
Total		54	100,0

Table 2: Differences in Maternal Age, Parity, Age of Gestation, and BMI in PROM and Non PROM

Variable	Preterm Pregnancy				p
	PROM		Non PROM		
	Mean	SD	Mean	SD	
Maternal Age	26,85	3,9	22,44	2,8	0,0001
Parity	3,41	1,3	2,18	1,17	0,001
Gestational Age	32,15	2,5	31,81	2,8	0,703
BMI	27,5	4,87	21,83	2,49	0,0001

Gestational age in PROM patients was slightly higher than in non-PROM patients, but this result was not statistically significant (p=0.703), possibly because all samples had preterm gestational age so that the difference in gestational age between samples was not too large. Maternal BMI was higher in PROM patients than in non-PROM patients and statistically significant (p=0.0001). Systemic infections, uterine overdistention, multiple pregnancies, polyhydramnios, coital trauma, cervical incompetence, and history of cervical suture procedures were already controlled by design, so didn't included in this result. The pattern of vaginal infection in preterm PROM can be seen in Table 3.

Table 3: Distribution of Vaginal Infection in PROM and Non PROM

	Preterm Pregnancy			p
	PROM (%)	Non PROM (%)	Freq (%)	
+	22 (81,4)	11 (40,7)	33 (61,1)	0,002
Vaginal Infection				
-	5 (18,5)	16 (59,2)	21 (38,9)	
Total	27 (100)	27 (100)	54 (100)	

Table 3 shows that preterm PROM patients had more vaginal infections, as much as 81.48% compared to those who did not experience vaginal infections as much as 18.52%. In non-PROM patients, only 40.74% had vaginal infections. Chi square test showed the result of p = 0.002 which stated that there was a significant relationship between vaginal infection and preterm PROM. The risk of vaginal infection in preterm pregnant PROM can be seen in Table 4.

Table 4: Association of Vaginal Infection and Preterm PROM

Preterm pregnancy		PROM	Non PROM	RP	p
Vaginal Infection	(+)	22	11	2.134	0.002
	(-)	5	16		

Table 4 showed that vaginal infection is the risk factor of preterm PROM (RP=2,134; p=0,002).

4. Discussion

Demographic factors that were found to be different between PROM and non-PROM patients in this study were maternal age, parity, and BMI. Maternal age affects the incidence of preterm PROM because the older the mother is, the condition and function of the uterus will decrease. One of the causes is uterine tissue that is not fertile, while the uterine wall is the place where the placenta attaches. The tissues of the pelvic cavity and muscles also weaken with age. This makes the pelvic cavity less accessible and causes serious complications such as bleeding. In certain

conditions, hormonal conditions are not as optimal as the previous age. That is why the risk of PROM and other complications also increases.⁶ Parity affects preterm PROM because the frequency of childbirth that is often experienced by mothers is a condition that can cause the endometrium to become deformed. As a result, complications can occur in pregnancy such as preterm PROM.⁷ The relationship between BMI and PROM is probably due to biological differences such as an increase in inflammatory mediators and a decrease in the immune system in obesity which makes it more susceptible to infection, as well as clinical factors such as reduced antibiotic concentration in overweight and obese women.⁸

In this study, it was found that the samples who had preterm PROM had more vaginal infections than those who did not. This is consistent with a study conducted by Brown which said women with history of preterm premature rupture of membranes had an increased incidence of vaginal infections across all ethnicities. The incidence of vaginal infection in pregnant women can occur in all pregnancies that have low risk factors or those with high risk factors in pregnancy. Furthermore, women who have a history of pregnancy with current preterm labor also have a greater risk of developing a bacterial vaginal infection in subsequent pregnancies.⁹

Studies of vaginal microbiome profiling during pregnancy reported that microbiological diversity increases during early pregnancy to peak between 20 and 29 weeks in women with subsequent preterm labor but decreases progressively. It is described that there is an increase in the body's immunologic function during pregnancy which involves an increased TLR4 response in neutrophils and a higher regulation of T cells, between 20 and 30 weeks of gestation. It is particularly important in black women with vaginal infections who are epidemiologically at higher risk of premature premature rupture of membranes, but this requires further investigation.¹⁰

The results of this study which stated that vaginal infection was a risk factor for PROM (RP = 2.134; p = 0.002) were in accordance with studies by Brown et al⁹, Nakulbuwa et al¹¹, and Ziaei et al³ which stated that there was 2-to-3-fold increase in PROM due to vaginal infections that were not treated properly. This shows that the condition of vaginal infections is very important for attention so that screening is carried out as early as possible.

Women with vaginal infections were consistently associated with increased risk of preterm delivery compared with those without vaginal infections. The study by Tumuhameye et al found that sixty-five percent (65%) of all study participants had at least one potential colonization of bacterial pathogens in the vagina. The prevalence of vaginal infected women with potential pathogens in this study was higher than that reported in previous studies.¹² Ziaei's study showed 30.5% of pregnant women had BV.³

The study conducted by Nakulbuwa et al found a strong association between vaginal infections, trichomonas and the incidence of premature rupture of membranes. The data of this study are supported by in vitro studies which showed an 80% reduction in amniotic tension caused the amniotic

membrane to rupture. Furthermore, it was explained that the relationship between vaginal candidiasis infection and premature rupture of membranes is unclear.¹¹

There is evidence of release of inflammatory cytokines during candida infection, the researchers hypothesized that these cytokines will cause rupture of the amniotic membrane. Based on a case control study conducted in India, it was found that patients with premature rupture of membranes were less likely to develop candidiasis than those without premature rupture of membranes. In contrast, there is circumstantial evidence that candidiasis increases the risk of membrane rupture in a research study. The relationship between vaginal candida infection and the incidence of premature premature rupture of membranes is still inconclusive, a larger-scale study is still needed.^{13,14}

Some research evidence suggests that bacterial colonization can cause premature rupture of the membranes due to reduced elasticity of the membrane lining. The mechanism of action of infection can be attributed to the influence of bacterial collagenase and matrix-breaking enzymes produced by bacteria. This enzyme has been shown to significantly reduce the elasticity of the amniotic membrane, by a dose-related mechanism leading to rupture of the membranes. Apart from the action of bacterial enzymes and products on the membranes of the fetus, the maternal response to infection in the form of maternal cytokines has also been implicated in the pathophysiological mechanisms of complicated preterm labor caused by PROM. The concentrations of maternal serum cytokines IL-1a, and IL-1b in women with preterm labor with preterm PROM were significantly higher than in women with term delivery and PROM. Activation of Natural Killer (NK) cells is associated with repeated termination of pregnancy. Apart from complement activation, improper regulation of the complement system has also been shown to cause termination of pregnancy.¹⁵

5. Conclusion

The prevalence of vaginal infections in preterm pregnancy was 81.48% in PROM and 40.74% in non PROM. The characteristics of preterm PROM in this study were the mean age of the mother 26, 85 years; mean parity of 3.41 children; mean gestational age 32.15 weeks and mean BMI 27.5 kg / m2. Vaginal infection was a risk factor for preterm PROM (RP = 2.134; p = 0.002). Therefore, it is advisable to do early detection and treatment of vaginal infections in pregnant women to prevent preterm PROM.

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