Research Article

Vaginal Microbial Patterns and Matrix Metalloproteinase-9 Levels in relation to Premature Rupture of Membranes

Tuty Muliati [1], Maisuri Tadjuddin Chalid [1,2], Rudy Butje Leonardy [1], Firdaus Hamid [2,3], Ellen Wewengkang [1], Sitti Nur Asni [1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[2]Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

Abstract

Introduction: Premature rupture of membranes (PROM) is a significant cause of perinatal morbidity, with infection considered a primary etiological factor. The local interplay between vaginal microbiota and matrix metalloproteinase-9 (MMP-9), an enzyme crucial for fetal membrane degradation, is not fully understood. This study aimed to investigate the association between vaginal microbial patterns, local MMP-9 levels, and the incidence of PROM.

Methods: This cross-sectional study was conducted in Makassar, Indonesia, and included 60 pregnant women: 30 with PROM and 30 non-PROM controls. PROM diagnosis was established through clinical examination and confirmed using sterile speculum assessment, nitrazine pH test, and fern test. Vaginal swabs were collected for microbiological culture and bacterial identification. MMP-9 concentrations were quantified from the vaginal swab supernatants using an enzyme-linked immunosorbent assay (ELISA).

Results: The PROM group had a significantly higher prevalence of Gram-negative bacteria compared to the control group (83.3% vs. 56.7%, p=0.024), with *Escherichia coli* being the most predominant organism associated with PROM (p=0.004). Mean MMP-9 levels were significantly elevated in the PROM group compared to controls (1706.78 ng/mL vs. 1328.20 ng/mL, p=0.006). Furthermore, MMP-9 concentrations were significantly higher in women colonized by Gram-negative bacteria than those with Gram-positive flora (p=0.020).

Conclusion: High vaginal MMP-9 concentrations and the predominance of *E. coli* and other Gram-negative bacteria were strongly associated with PROM. These findings suggest that elevated vaginal MMP-9 may serve as a potential biomarker for PROM risk and reflect microbial-induced extracellular matrix degradation.

Keywords: Microbial Patterns, *Escherichia coli,* Premature Rupture of Membrane, Matrix Metalloproteinase 9, Vaginal microbiota.

Correspondence Author.

INTRODUCTION

Preterm rupture of membranes (PROM) is the rupture of the amniotic sac before the onset of labor.[1–3] PROM is characterized by painless discharge of fluid from the vagina. PROM can occur before and after 37 weeks of pregnancy. The prevalence of PROM worldwide varies between 5% and 15% of all gestations.[4] Globally, the incidence of preterm PROM varies between 13.7% in Ethiopia, 7.5% in Uganda, 5.3% in Egypt, and 1.4% in the USA.[5] In Makassar, a study reported that the incidence of PROM was 5.55%.[6]

PROM may precipitate fetal distress as a consequence of placental abruption or umbilical cord compression, intraventricular hemorrhage and sepsis; it may elevate the likelihood of cesarean delivery due to irregularities in fetal heart rhythm; respiratory distress syndrome; and adverse long-term neurodevelopmental consequences such as auditory or visual impairments, intellectual disabilities, motor and developmental delays, intrauterine fetal death or cerebral palsy. PROM also causes maternal infection (15%-25%),

placental abruption (9%-12%), chorioamnionitis (13%-60%), and increases the risk of disseminated intravascular coagulopathy.[4]

The etiology of PROM might occur due to bacterial infection where pathogenic bacteria trigger the immune response of the mother and fetus, resulting in changes in the uterine cavity that cause premature labor.[7] The colonization of the vaginal microbiota by pathogenic bacteria elicits an activation of the local immune response, which subsequently influences systemic immunity, thereby instigating an inflammatory cascade that ultimately culminates in the reorganization and disturbance of the membrane architecture and PROM.[8]

Other studies have reported that the pathophysiology of PROM due to infection occurs because of prostaglandins and matrix-degrading enzyme production through pro-inflammatory cytokines and microbial endotoxins released after the binding of organisms to ex-toll like receptors. This condition causes an increase in matrix metalloproteinase (MMP), which plays a role in collagen degradation and leads to a decrease in membrane tensile strength, resulting in membrane rupture.[9]

During labor (preterm or term), rupture of the membranes correlates with a marked elevation in the concentration of active matrix metalloproteinase-9 (MMP-9), alongside a notable reduction in the median levels of active tissue inhibitors of metalloproteinase (TIMP)-2 and TIMP-1.[10,11] Bacterial vaginosis, one of the common infections in women, in most studies can increase the levels of beta interleukin (IL)-1 in women, which leads to activation of MMPs such as MMP-1 and MMP-9.[12] Therefore, we conducted this study to analyze the microbial patterns and MMP level in the incidence of PROM, especially MMP-9.

MATERIALS AND METHODS

Study design

This observational study utilized a cross-sectional design and was conducted at St. Khadijah I Hospital in Makassar, Indonesia, from April 2024 to August 2024.

Study population and sample

A total of 74 pregnant women presenting to St. Khadijah I Hospital during the study period were screened for eligibility using a consecutive sampling method, in which all participants meeting the inclusion criteria were enrolled until the required sample size was achieved.

The sample size was determined using Lemeshow formula.[13–15] or two independent groups, with an expected proportion difference of 0.3, a confidence level of 95%, and statistical power of 80%. The minimum required sample size was 24 subjects per group, but to account for potential data loss, 30 participants were included in both the PROM and non-PROM groups.

The inclusion criteria for the PROM group were as follows: (1) confirmed diagnosis of premature rupture of membranes, defined as the spontaneous rupture of the amniotic membrane before the onset of labor with clinical evidence of fluid leakage from the vagina; (2) singleton pregnancy; (3) gestational age of ≥32 weeks confirmed by ultrasonography; and (4) willingness to provide written informed consent.

The exclusion criteria were: (1) history of antibiotic therapy within the previous two weeks, (2) evidence of systemic or genital infection during pregnancy (e.g., urinary tract infection, chorioamnionitis, or vaginitis), (3) multiple gestation, (4) preexisting chronic conditions such as diabetes mellitus or hypertension, and (5) history of preterm delivery or cervical cerclage.

Of the 74 participants screened, 14 were excluded (recent antibiotic use, n = 6; systemic/genital infection, n = 4; multiple pregnancy, n = 2; incomplete laboratory data, n = 2). The remaining 60 eligible participants were divided equally into the PROM group (n = 30) and non-PROM group (n = 30).

Diagnosis of PROM

The diagnosis of PROM was established based on a clinical history of painless leakage of clear amniotic fluid from the vagina prior to labor onset, confirmed by a sterile speculum examination revealing pooling of fluid in the posterior vaginal fornix. The diagnosis was further corroborated using two bedside tests: the Nitrazine (pH) test and the fern test. For the Nitrazine test, a sterile cotton swab was used to apply vaginal fluid to pH paper, with a color change to blue (pH≥6.5) considered positive. For the fern test, a vaginal fluid sample was smeared onto a glass slide, air-dried, and examined under a light microscope for a characteristic fern-like crystallization pattern. A definitive PROM diagnosis required positive

results from the clinical evaluation and both confirmatory tests.

Study procedure

After obtaining informed consent, all participants underwent a detailed clinical evaluation and vaginal swab collection in the hospital delivery room. Prior to sampling, the posterior vaginal fornix was cleansed using sterile gauze moistened with 0.9% NaCl solution. Vaginal swabs were obtained using Amies transport swabs (Copan, Italy) and stored at 2–8°C for a maximum of 24 hours before analysis.

Microbiological examination

Vaginal swab specimens were cultured on differential media, including MacConkey agar and blood agar, and incubated at 37°C for 24–48 hours. Bacterial colonies were identified based on Gram staining, colony morphology, and biochemical testing using the API 20E/20NE system (bioMérieux, France).

Measurement of MMP-9 levels

MMP-9 concentrations were quantified using the enzyme-linked immunosorbent assay (ELISA) method. Supernatant from each vaginal swab sample was centrifuged at 3000 rpm for 10 minutes to obtain a clear extract. The MMP-9 level was determined using a commercially available human MMP-9 ELISA kit (BT Lab, Catalog No. E0936Hu, Shanghai, China), following the manufacturer's protocol. Absorbance was read at 450 nm using a microplate spectrophotometer, and MMP-9 concentrations were calculated from a standard curve. All assays were performed in duplicate, and internal controls were included in each batch to ensure assay accuracy and reproducibility.

All laboratory analyses were conducted at the Hasanuddin University Medical Research Center. To ensure analytical reliability, each sample was processed in duplicate, and internal quality controls were included in each assay batch.

Data analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Univariate analysis calculated the percentages of patient characteristics and bacterial isolation results, as well as the mean and standard deviation of MMP-9 levels. Bivariate analysis examined relationships between two variables, with a normality test conducted using the Shapiro-Wilk test. If the data were normally distributed, an independent sample t-test was used; otherwise, the Mann-Whitney test was applied. For categorical data, a chi-square test was performed, and if its assumptions were not met, Fisher's exact test was used. All statistical tests were conducted at a 5% confidence level, with a p-value of <0.05 indicating significance.

Ethical clearance

The study was approved by the Human Biomedical Research Ethics Commission, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia under protocol number UH23070491.

RESULTS

A total of 74 pregnant women were enrolled in the study between April and August 2024. After screening, 14 participants were excluded due to recent antibiotic use (n = 6), urinary tract infection (n = 4), multiple pregnancy (n = 2), or incomplete laboratory data (n = 2). Consequently, the final analysis included 60 participants, who were divided equally into the PROM group (n = 30) and a non-PROM control group (n = 30), as outlined in the study flowchart (Figure 1).

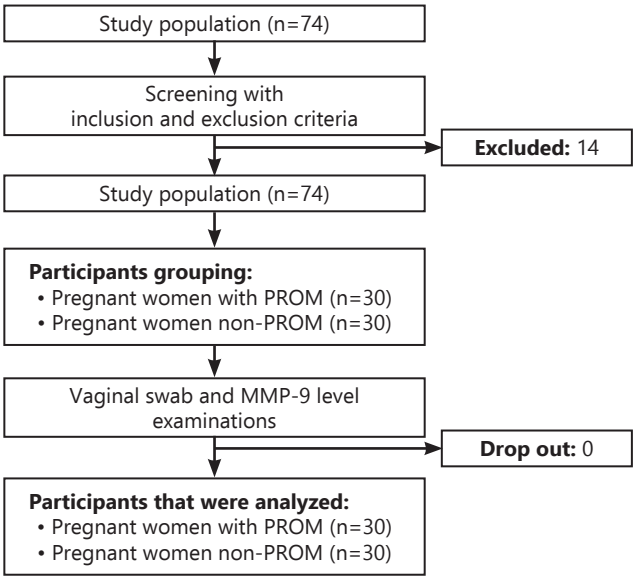

Figure 1. Flow Diagram of Study Participants. A schematic flowchart illustrates participant enrollment, exclusion, and final group allocation. Out of 74 eligible women, 14 were excluded due to ineligibility or incomplete data, leaving 60 subjects for final analysis (30 PROM, 30 non-PROM).

Participant Characteristics

Table 1 presents the demographic and obstetric characteristics of both groups. There were no significant differences between the PROM and non-PROM groups regarding age, body mass index (BMI), gestational age, parity, or educational level (p > 0.05 for all variables), indicating that both groups were comparable.

Table 1. Characteristics of study participants (n = 60)

Characteristics	PROM n (%)	Non-PROM n (%)	*P-value*
Age (years)			
< 20	3 (10.0)	1 (3.3)	
20-35	23 (76.7)	28 (93.3)	0.193
> 35	4 (13.3)	1 (3.3)	
BMI			
Underweight	2 (6.7)	1 (3.3)	
Normal	14 (46.7)	16 (53.3)	0.512
Overweight	9 (30.0)	5 (16.7)	
Obese	5 (16.7)	8 (26.7)	
Parity			
Nullipara	9 (3.0)	6 (20.0)	
Primipara	16 (53.3)	15 (50.0)	0.412
Multipara	5 (16.7)	9 (30.0)	
Gestational age			
Preterm (<37 weeks)	10 (33.3)	10 (33.3)	1.000
Aterm (≥37 weeks)	20 (66.7)	20 (66.7)	
Education (years)			
≤ 9	2 (6.7)	7 (23.3)	0.071
> 9	28 (93.3)	23 (76.7)	

Note: Data presented as frequency (percentage); p-values calculated using Chi-square or Fisher's exact test where appropriate; significance threshold set at p<0.05.

These findings confirm that the two groups were demographically comparable, minimizing potential confounding factors in subsequent analyses.

Microbial Patterns and PROM Incidence

Table 2 summarizes the distribution of vaginal microbial patterns. Eschericia coli (E. coli) was the predominant bacterium in the PROM group (72.0%) compared with the non-PROM group (28.0%), showing a significant association with PROM incidence (p = 0.004).

Similarly, the predominance of Gram-negative bacteria was significantly higher in the PROM group (83.3%) than in the non-PROM group (56.7%) (p = 0.024).

Table 2. Association between microbial patterns and incidence of PROM (n = 60)

Characteristics	PROM (n=30) n (%)	PROM (n=30) n (%)	*P-value*
Predominant bacteria			
E. coli	18 (72.0)	7 (28.0)	0.004*
Non-*E. coli*	12 (28.0)	23 (65.7)	
Microbial group			
Gram-positive	5 (16.7)	13 (43.3)	0.024*
Gram-negative	25 (83.3)	17 (56.7)	

*Note: Chi-square test applied; * indicates statistical significance (p<0.05).*

The predominance of Gram-negative bacteria, particularly E. coli, was significantly associated with the occurrence of PROM, suggesting a potential microbial role in membrane weakening.

MMP-9 Levels in Relation to Microbial Patterns and PROM

Table 3 presents the MMP-9 levels according to microbial patterns and PROM status.

The median MMP-9 concentration was significantly higher among samples dominated by Gram-negative bacteria compared to those with Gram-positive flora (1643.91 ng/mL vs. 1332.88 ng/mL; p = 0.020).

Similarly, MMP-9 levels were markedly elevated in the PROM group (1706.78 ± 386.36 ng/mL) compared to the non-PROM group (1328.20 ± 1556.22 ng/mL; p = 0.006).

Table 3. Relationship between MMP-9 levels, microbial patterns, and PROM incidence (n = 60)

Variable	Group	MMP-9 level (ng/mL) (Median [Min–Max] or Mean ± SD)	*P-value*
Microbial Pattern			
	Gram-positive (n = 18)	1332.88 [358.18–2255.89]	0.020*
	Gram-negative (n = 42)	1643.91 [658.18–2255.89]	
PROM Status	PROM (n = 30)	1706.78 ± 386.36	0.006*
	Non-PROM (n = 30)	1328.20 ± 1556.22	

*Note: Mann–Whitney test for microbial comparison; Independent samples t-test for PROM analysis; * indicates statistical significance (p<0.05).*

Higher MMP-9 levels were observed in women with PROM and in those colonized predominantly by Gram-negative bacteria. This suggests a synergistic interaction between microbial imbalance and extracellular matrix degradation in the pathogenesis of PROM.

DISCUSSION

This study demonstrated a significant association between vaginal microbial patterns, MMP-9 levels, and the incidence of PROM. Specifically, the predominance of *E. coli* and Gram-negative bacteria was strongly correlated with elevated MMP-9 concentrations in vaginal samples, suggesting a possible pathogenic mechanism involving microbial-induced extracellular matrix degradation.

Our findings align with previous studies reporting that bacterial infection contributes to PROM pathogenesis. Saghafi et al. (2018)[16] found that 68% of women with PROM had positive endocervical cultures, predominantly containing Gram-negative organisms, while Vanya et al. (2021) [17] and Abdelghany and Mounir (2018) [18] identified *E. coli* as the most common microorganism in PROM cases. The role of *E. coli* is biologically plausible, as its endotoxins mainly lipopolysaccharides (LPS) bind to Toll-like receptors (TLR-2, TLR-4, and TLR-9), activating downstream signaling cascades involving MyD88 and TRAF6. This cascade stimulates proinflammatory cytokines (IL-1β, IL-6, TNF-α) and induces MMP-9 expression, leading to degradation of collagen and weakening of the fetal membranes.[19,20]

Interestingly, *Staphylococcus aureus (S. aureus)* was also detected among PROM cases. Previous research has shown that S. aureus can promote thrombin formation through coagulase activity, triggering inflammation via protease-activated receptors (PARs) and compromising the structural integrity of the fetal membranes.[21] Although Gram-positive organisms were less prevalent in our study, their pathogenic potential remains relevant.

Our study differs from previous investigations in its methodological approach. Whereas prior studies primarily measured MMP-9 in amniotic fluid or serum, we quantified MMP-9 directly from vaginal swab samples—a less invasive yet reliable technique for detecting local biochemical changes associated with PROM. This methodological innovation may explain variations in absolute MMP-9 levels compared with other reports. Moreover, by simultaneously evaluating microbial profiles and MMP-9 concentrations, our study integrates infectious and biochemical pathways, providing a more comprehensive understanding of PROM etiology.

The biological mechanism underlying these associations involves infection-induced inflammation and matrix remodeling. Endotoxins and other bacterial components stimulate the production of prostaglandins and proteolytic enzymes, including MMPs, which collectively degrade the extracellular matrix (ECM) and reduce the tensile strength of fetal membranes.[11,22] Elevated MMP-9 levels, in particular, have been strongly linked to decreased collagen content and increased risk of membrane rupture.[23] Our results further support this relationship, as PROM cases showed significantly higher MMP-9 levels compared to non-PROM participants.

Regional microbial variation may also contribute to differing findings across studies. In Asian populations, *E. coli* and *Klebsiella* species are the predominant pathogens, whereas in Western countries, *Group B Streptococcus* is more common.[24] These differences likely reflect variations in vaginal microbiota composition, hygiene practices, and environmental conditions.

Notably, *E. coli* was also detected in 28% of non-PROM participants. This finding may reflect transient colonization or contamination, influenced by behavioral and environmental factors such as hygiene habits or sexual activity.[25,26] This underscores the importance of distinguishing between colonization and infection in assessing PROM risk.

To the best of our knowledge, this is one of the few studies to concurrently examine vaginal microbial profiles and MMP-9 levels in PROM using a minimally invasive approach. Our findings suggest that vaginal MMP-9 concentration may serve as a biomarker for early identification of women at risk for PROM, complementing existing diagnostic methods. By establishing a mechanistic link between microbial dysbiosis and ECM degradation, this study contributes to a more integrated understanding of PROM pathophysiology.

This study has several limitations. The relatively small sample size and single-center design may limit the generalizability of the findings. Additionally, we did not evaluate other metalloproteinases (e.g., MMP-2) or TIMPs, which could provide a broader view of matrix

remodeling. Behavioral factors such as hygiene and sexual practices were not controlled and may have influenced microbial composition. Future research should employ larger, multicenter designs and incorporate longitudinal sampling to confirm whether elevated vaginal MMP-9 levels precede membrane rupture. Advanced molecular techniques, such as next-generation sequencing, may also improve microbial characterization and predictive modeling.

CONCLUSION

The findings revealed that the predominance of *E. coli* and other Gram-negative bacteria was significantly associated with increased vaginal MMP-9 levels and the incidence of PROM. These results suggest that microbial imbalance may trigger an inflammatory cascade that elevates MMP-9 production, leading to extracellular matrix degradation and membrane rupture. The measurement of vaginal MMP-9 levels using a simple ELISA-based approach may serve as a potential biomarker for early identification of women at risk of PROM. Understanding the interplay between vaginal microbiota and MMP-9 expression provides valuable insight into the underlying pathophysiology of PROM and supports the need for preventive strategies, including microbial monitoring during pregnancy. Future studies should explore other MMP isoforms and their inhibitors, as well as broader microbial profiling using molecular techniques, to further elucidate the mechanisms linking infection, inflammation, and membrane integrity.

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Declarations of interest

None

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the study, data collection, analysis, and manuscript preparation. TM (Conceptualization, study design, supervision, and final approval of the manuscript), MTC (Data collection, laboratory analysis, and interpretation of results), RBL (Literature review and drafting of the manuscript), FH (Statistical analysis critical revision of the manuscript, and formatting for journal submission), EW (Literature review and drafting of the manuscript), and SNA (Literature review and drafting of the manuscript). All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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