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High MMP-9 and TNF-α expression increase in preterm premature rupture of membranes

Sri Sulistyowati*, Yuniarsih Zakia*, and Soetrisno*

ABSTRACT

BACKGROUND

Preterm delivery is one of the causes of high perinatal morbidity and mortality. Matrix metalloproteinase 9 (MMP-9) is important for extracellular matrix (ECM) remodeling and may cause preterm labor and premature rupture of membranes (PROM). Tumor necrosis factor-á (TNF-α) as a pro-inflammatory cytokine plays a role in stimulating uterine activity and cervical ripening by degrading the ECM of the amniotic membranes through MMP-9. This study aimed to determine differences between MMP-9 and TNF-α expression of the membranes in preterm delivery with premature rupture of membranes (PPROM) and without PROM.

METHODS

An analytic observational study with cross-sectional approach was conducted in 24 subjects, who were divided into 2 groups, with 12 subjects in the preterm delivery group with PROM and 12 subjects in the preterm delivery group without PROM. The expression of MMP-9 and TNF-α in the amniotic membrane was determined by immunohistochemistry. Data were analyzed using the t test.

RESULTS

MMP-9 expression in the amniotic membrane of preterm delivery subjects with PROM (8.6 ± 3.1%/field) differed significantly with that of preterm delivery subjects without PROM (5.5 ± 2.3%/ field) (p=0.001). TNF-α expression in the amniotic membrane of preterm delivery subjects with PROM (8.0 ± 3.0%/field) also differed significantly with that of preterm delivery subjects without PROM (3,3 ± 1.5%/field) (p=0.000).

CONCLUSION

Expression of MMP-9 and TNF-α was higher in the amniotic membrane of preterm delivery subjects with PROM than in preterm delivery subjects without PROM and can thus be used as predictor to avoid PPROM.

Keywords: MMP-9, TNF-α, preterm delivery, premature rupture of membranes

*Department of Obstetrics and Gynecology,
Faculty of Medicine,
Universitas Sebelas Maret/
RSUD Dr. Moewardi Surakarta

Corresponding author:
dr. Sri Sulistyowati, SpOG
*Department of Obstetrics and Gynecology,
Faculty of Medicine,
Universitas Sebelas Maret/
RSUD Dr. Moewardi Surakarta
Jl. Ir Sutami 36A
Surakarta, Jawa Tengah 57126
Phone: +62812 2968 215
Email: elis_spog@yahoo.co.id

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INTRODUCTION

Preterm premature rupture of the membranes (PPROM) is the spontaneous rupture of the fetal membranes during pregnancy before 37 weeks in the absence of regular uterine contractions. Preterm PROM occurs in 3–8% of pregnancies and in one-third of cases results in preterm delivery. PPROM is an important cause of perinatal morbidity, since it is associated with infection and compression of the umbilical cord caused by lack of amniotic fluid (oligohydramnion).[1]

The prevalence rate of preterm delivery differs by country. In developed countries such as Europe, the rate ranges between 5 and 11%.[2] In the USA around one of nine infants is born preterm (11.9%).[3] In Indonesia the preterm prevalence rate is roughly 47.35%.[4]

Preterm delivery increases the perinatal and neonatal complications, with a fetal mortality risk of 1–2% and increased perinatal morbidity such as the respiratory distress syndrome and neonatal sepsis.[1] There are many factors that may cause preterm delivery, such as infection, uterine overdistension, uteroplacental ischemia, endocrine factors, and cervical and immunological disorders that may trigger preterm delivery.[5] One of the causes of infections in pregnancy is premature rupture of the membranes (PROM).[6] The incidence of PROM in Indonesia ranges from 4.5 to 7.6%.[7] PROM contributes to 30–40% of preterm deliveries. The prevalence rate of PROM in preterm pregnancies is around 2–3%.[6] PROM is an important problem in obstetrics, as it is associated with preterm delivery.[8]

Matrix metalloproteinase-9 (MMP-9) is the main MMP involved in normal delivery and also plays an important role in pathological delivery. Matrix metalloproteinase-9 is the most active MMP in the amnion, and is found to increase significantly in the amniotic membranes after the onset of contractions. Matrix metalloproteinase, also known as matrixin, plays an important role in the breakdown and remodeling of the extracellular matrix (ECM), finally causing both preterm delivery and preterm PROM. Manipulation of MMP may play a role in preventing spontaneous preterm delivery.[9] Preterm PROM increases MMP-9 concentrations in the amniotic fluid, causes amniotic fluid infection, threatened preterm delivery and poor neonatal outcome.[10]

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is mainly produced in monocytes and/or macrophages. Excessive secretion of TNF-α may cause deleterious effects, such as abortion and preterm delivery. Increase or non-increase of the TNF-α pro-inflammatory cytokine presumably plays an important role in preventing preterm delivery.[11] In preterm delivery, TNF-α stimulates uterine activity and the cervical ripening process by producing prostaglandin and cortisol, and degrading the ECM of the membranes through the MMP-2 and MMP-9 pathways.[12]

The TNF-α concentration in the amniotic fluid in preterm delivery increases significantly in comparison with term delivery. The TNF-α (and/or IL-6) concentration in amniotic fluid at mid-trimester may identifiy pregnant mothers at risk for chorioamnionitis, which may cause preterm delivery.[13]

The purpose of this study was to compare the expression of MMP-9 and TNF-α in the membranes in preterm delivery with PROM and that in preterm delivery without PROM.

METHODS

Design of the study

An analytical-observational study with cross-sectional approach was conducted at the Department of Obstetrics and Gynecology, RSUD Dr. Moewardi Surakarta, from December 2014 until May 2015.

Subjects of the study

The study subjects were pregnant mothers who had undergone preterm delivery with PROM and without PROM. The sample size was 12 per

group, based on the difference between the prevalence of preterm delivery without PROM and the prevalencs of preterm delivery with PROM, the difference being 0.3, with α=1.96 and β= 0.2.[14] There were thus 12 preterm delivery subjects with PROM and 12 preterm delivery subjects without PROM. The inclusion criteria were pregnant mothers delivering at RSUD Dr. Moewardi Surakarta, who had undergone preterm delivery with PROM and preterm delivery without PROM, and whose infants were without major congenital abnormalities. The exclusion criteria were pregnant mothers with chronic diseases such as diabetes mellitus, kidney and heart diseases, and hypertension; pregnant mothers with systemic inflammation and infection, such as pneumonia, typhoid, malaria, hepatitis; pregnant mothers who were smokers, drinkers, and drug abusers, on antibiotic treatment, with trauma in pregnancy, twin pregnancies, intrauterine fetal death, and abnormally shaped uteri.

Immunohistochemistry

For all subjects, immunohistochemical examination of MMP-9 and TNF-α expression in the membranes was performed at the Pathological Anatomy Laboratory, Universitas Sebelas Maret, Surakarta. Matrix metalloproteinase 9 was determined immunohistochemically by the avidin biotin complex method, using Bioss MMP-9 antibody reagent bs-0397R. MMP-9 expression in the membranes was examined under the microscope at 400X magnification. MMP-9 expression for a strong positive result was shown by a reddish-brown color in the cell nucleus and the cytoplasm, for a moderate positive result by a dark brown color, for a weak positive result by a light brown color, and for a negative result by a bluish color. The histological score was then calculated as percentages per field of view, the data being of continuous scale.

Similarly, expression of tumor necrosis factor α was determined immunohistochemically by the avidin biotin complex method, using Bioss TNF-α antibody reagent bs-0078R. Expression of TNF-α in the membranes was examined under the microscope at 400X magnification. TNF-α expression for a strong positive result was shown by a reddish-brown color in the cell nucleus and the cytoplasm, for a moderate positive result by a dark brown color, for a weak positive result by a light brown color, and for a negative result by a bluish color. The histological score was then calculated as percentages per field of view, the data being of continuous scale.

Data analysis

The data were analyzed with the t-test using SPSS, with $p<0.05$ being considered significant.

Ethical clearance

Ethical clearance wa obtained from the Commission for Medical Research Ethics, RSUD Dr. Moewardi/ Faculty of Medicine, Universitas Sebelas Maret, under No. 642/XI/HREC/2014, dated 11 November 2014.

RESULTS

There were 50% working mothers in the preterm delivery group with PROM and 25% in the preterm delivery group without PROM. The majority of mothers (75%) in the preterm delivery group with PROM as well as in the preterm delivery group without PROM, had a junior high school education. The percentage of primigravida in the preterm delivery group with PROM and the preterm delivery group without PROM was 75% and 50%, respectively (Table 1). The majority of pregnant mothers were 21–35 years old, accounting for 83% of the preterm delivery group with PROM, and for 75% of the preterm delivery group without PROM. Regarding the age of pregnancy of 34–36 weeks, the percentage in both the preterm delivery group with PROM and the preterm delivery group without PROM was identical, namely 58% (Table 1).

From Table 2 it may be seen that the mean MMP 9 expression in the membranes was significantly higher in the preterm delivery group with PROM (8.6 ± 3.1%/field), in comparison

Table 1. Distribution of characteristics of study subjects

Variable	Preterm delivery with PROM (n=12)		Preterm delivery without PROM (n=12)	
	n	%	n	%
Employment of mother				
Employed	6	50	3	25
Unemployed/housewife	6	50	9	75
Education				
Primary school	3	25	0	0
Junior high school	9	75	9	75
Senior high school	0	0	2	16.7
Academy/University	0	0	1	8.3
Parity				
Primigravida	9	75	6	50
Multigravida	3	25	6	50
Age of mother (years)				
<20	2	17	2	17
21 – 35	10	83	9	75
>35	-	-	1	8
Age of pregnancy (weeks)				
<34	5	42	5	42
34 – 36	7	58	7	58

with the preterm delivery group without PROM (5.5 ± 2.3%/field) (p=0.001). The mean expression of TNF-α in the membranes was significantly higher in the preterm delivery group with PROM (8.0 ± 3.0%/field) than in the preterm delivery group without PROM (3.3 ± 1.5%/field) (p=0.000).

Expression of MMP-9 and TNF-α in the membrane of the pretern delivery groups with and without PROM were showed in Figure 1 and Figure 2.

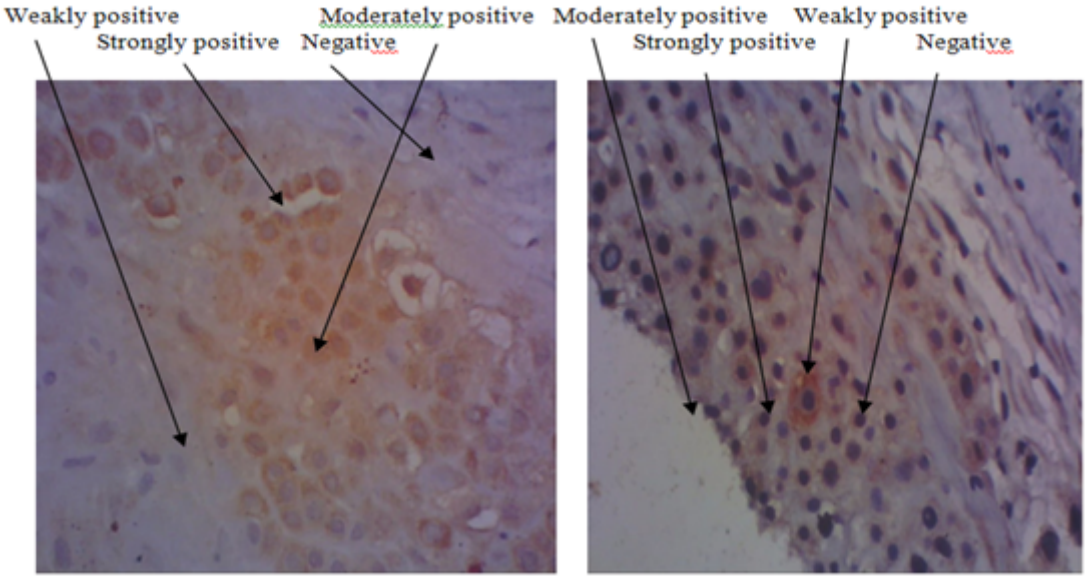
DISCUSSION

In our present study we found that the mean MMP-9 expression in the membranes was significantly higher in the preterm delivery group with PROM (8.6 ± 3.1%/field) in comparison with the preterm delivery group without PROM (5.5 ± 2.3%/field). PROM is one of the factors for the initiation of delivery and is an important problem, since it is associated with the complications of pregnancy, one of them being preterm delivery, which results in both infant morbidity and mortality.[8] The pathogenesis of preterm delivery is associated with cytokines, matrix metalloproteinases, and prostaglandins. The amniotic membrane is the inner layer of the fetal membranes that line the amniotic cavity. The amnion consists of an epithelial cell layer above the thicker basement membrane and a spongy collagen layer containing mesenchymal cells. The amnion is part of the fetal development and protects the fetus against mechanical injury by surrounding it with amniotic fluid. The amnion receives its strength from collagen in the basement membrane, especially type IV collagen. Collagen in the basement membrane and collagen

Table 2. Mean expression of MMP-9 and TNF-α in the membranes in the preterm delivery group with PROM and the preterm delivery group without PROM

Variable	Preterm delivery with PROM	Preterm delivery without PROM	p
MMP 9 (%/field)	8.6 ± 3.1	5.5 ± 2.3	0.001*
TNF-α (%/field)	8.0 ± 3.0	3.3 ± 1.5	0.000*

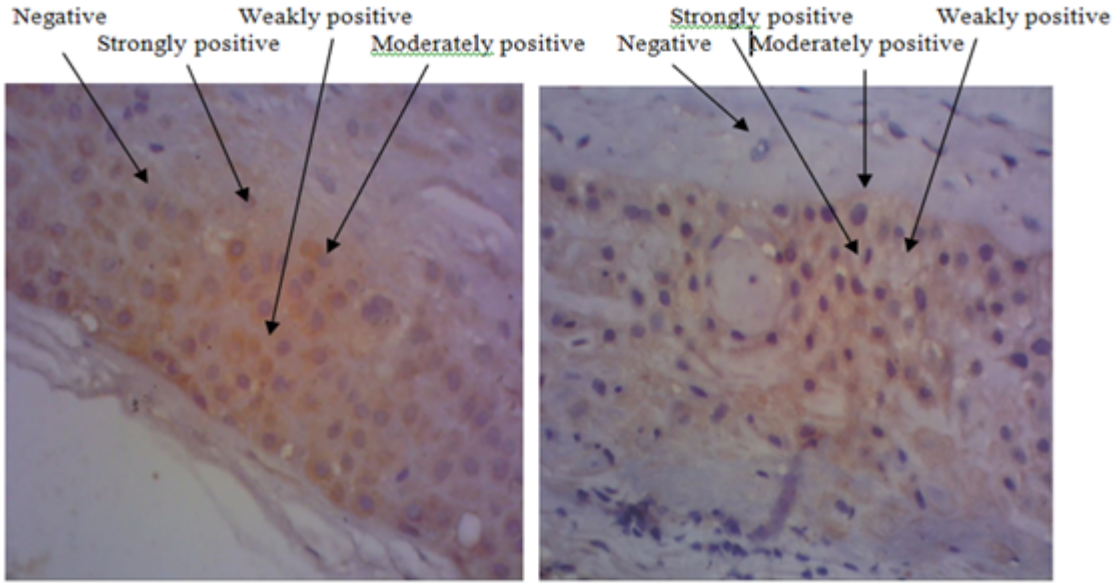
*Significant at p<0.05



MMP-9 expression in preterm delivery group with PROM

MMP-9 expression in preterm delivery group without PROM

Figure 1. Expression of MMP-9 in the membranes of the preterm delivery groups with and without PROM, determined by immunohistochemistry and evaluated using an Olympus cx21 microscope at 400x magnification. Reddish-brown color in the cell nucleus and the cytoplasm indicates a strongly positive result; dark brown = moderately positive result; light brown = weakly positive result; bluish color = negative result. MMP-9 expression was stronger/higher in the preterm delivery group with PROM in comparison with the preterm delivery group without PROM



TNF-α expression in preterm delivery with PROM

TNF-α expression in preterm delivery without PROM

Figure 2. Expression of TNF-α in the membranes of the preterm delivery group with and without PROM, determined by immunohistochemistry and evaluated using an Olympus cx21 microscope at 400x magnification. Reddish-brown color in the cell nucleus and the cytoplasm indicates a strongly positive result; dark brown = moderately positive result; light brown = weakly positive result; bluish color = negative result. TNF-α expression was stronger/higher in the preterm delivery group with PROM in comparison with the preterm delivery group without PROM.

degradation in the chorioamnion is controlled by matrix metalloproteinases (MMPs). Matrix metalloproteinase-1 (MMP-1) degrades type I, II and III collagens, while MMP-2 and MMP-9 (gelatinase B) degrade type IV collagen. In human chorionic cells, tumor necrotic factor alpha (TNF-α) has been found to promote the production of MMP and prostaglandin E2 (PGE2), and to repress tissue inhibitors of metalloproteinase [TIMPs]). Therefore, TNF-α tends to cause the membranes to weaken and rupture through collagen degradation in the ECM.[15]

Increased concentrations of interleukin-6 (IL-6), C-reactive protein (CRP), and matrix metalloproteinase 9 (MMP-9) in the fetal and neonatal compartments have been shown to be associated with increased risk of preterm delivery.[16] MMP-9 expression, apoptosis index (IA) of cervical cells, and MMP-9 gene polymorphism (C-1562T) in pregnancies of 21–36 weeks without and with PROM have been demonstrated by Sabarudin et al.,[17] who found no significant differences in MMP-9 expression and cervical cell IA between preterm delivery with PROM and preterm delivery without PROM.

In the present study, mean TNF-α in the membranes was found to be higher in the preterm delivery group with PROM than in the preterm delivery group without PROM. In preterm delivery cases, TNF-α and other pro-inflammatory cytokines play a role in stimulating uterine activity and cervical ripening by producing prostaglandins, cortisol, and degrading the ECM of the amniotic membranes through the MMP-2 and MMP-9 pathways.[12] TNF-α is a predictor of PROM, since increased TNF-α levels in the patient's serum may trigger MMP-9 expression, thus causing increased degradation of type IV collagen in the membranes of pregnant women and possibly causing PROM.[8]

Tumor necrosis factor, cachexin or cachectin, previously known as tumor necrosis factor-α, is a pleiotropic inflammatory cytokine that is mainly produced in monocytes and/or macrophages. Excessive secretion of the pro-inflmmatory cytokines TNF or IL-1, such as occur in infections of the amnion, has been known to be detrimental to intra-uterine tissues such as in abortion and preterm delivery.[11]

Cytokines can increase the production of prostaglandins in the amniotic membranes and decidua. Various factors may be involved in the release of TNF-α and the activation of MMP-9, such as psychological stress, which causes the release of corticotropin releasing hormone (CRH), infection, and inflammation, that directly stimulates the release of inflammatory mediators, such as TNF-α. The concentration of bioactive TNF, the availability of TNF receptors (TNFR1 and TNFR2), and the affinity of TNF for its receptors, and also its signaling pathway, may display different kinerja of TNF-α. TNF-α in most frequently involved in the activation of MMP-9.[13]

MMP-9 increases significantly upon exposure of the amnion to TNF-α or IL-1 beta, whereas its secreted concentration in the chorion is not altered. Each of the inflammatory cytokines (IL-1, IL-6, and TNF-α) have been found in higher concentrations in the amniotic fluid of women with PROM. The cytokine that influences the MMP family in pregnancy is TNF-α, which is the most characterized cytokine. This member of the pro-inflammatory cytokines has been shown to be able to trigger MMP expression in various tissues, thus causing the release of active MMP-9 from the amniotic membranes and also triggering apoptosis in the amniotic membranes and activation of MMP-9, which finally causes PROM.[9] Advanced understanding of the biochemical causes of fetal membrane weakening and rupture indicates the possibility of inhibiting the process and ultimately preventing PPROM.[18]

In this study, we did not discuss in detail the causes of PROM in the study groups, e.g. we did not examine the occurrence of urinary tract infection (UTI). We also did not differentiate the duration of PROM or the degree of infection. Another limitation of this study was that we did not equalize the leukocyte concentrations in the

preterm delivery groups, despite the fact that this can influence the MMP-9 and TNF-α concentrations in preterm deliveries with PROM. Further studies are required that control for confounding variables such as the causes of PROM and the degree of infection. It is expected that pregnant mothers with PROM may be detected earlier so that they can be better managed to prevent preterm delivery resulting in morbidity and mortality of the mother and her fetus.

CONCLUSIONS

The expression of MMP-9 and TNF-α were higher in the membranes of preterm deliveries with PROM, in comparison with preterm deliveries without PROM. MMP-9 and TNF-α may be strong predictors of PROM, particularly in preterm deliveries.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this study, the authors, and / or the publication of this article.

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