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Research Article

The Analysis of Matrix Metalloproteinase-9 and Tissue Inhibitor Matrix Metalloproteinase-1 Levels in the Amniochorion Membrane Patients on Premature Rupture of Membranes

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ABSTRACT

Introduction: MMP-9 is essential for extracellular matrix remodeling, which affects the incidence of premature rupture of membranes. In addition, decreased and increased levels of TIMP-1, a preferential MMP-9 inhibitor, have been reported to be associated with premature rupture of membranes because it showed an imbalance in the MMP-9 or TIMP-1 levels. This study aims to analyze MMP-9 and TIMP-1 levels in amniochorion membrane patients on premature rupture of membranes. **Methods:** An analytic observational study was conducted on 70 subjects. The MMP-9 and TIMP-1 levels in the amniochorion membrane were determined by ELISA. **Results:** The results of this study indicate that the amniochorion membrane in the incidence of premature rupture of membranes is characterized by increased levels of MMP-9, while TIMP-1 levels do not differ between the incidence of premature rupture of membranes. **Conclusion:** Based on the research that has been done, it can be concluded that there are differences in protein levels of MMP-9 but there are no differences in protein levels of TIMP-1 in premature rupture of membranes.

Key words: MMP-9, TIMP-1, Premature rupture of membranes.

INTRODUCTION

Globally, preterm birth is one of the three main causes of neonatal death and premature rupture of membranes contributes to more than 40% of preterm deliveries.[1] Premature rupture of membranes is associated with causing 18-20% of perinatal mortality and 21.4% of perinatal morbidity.[2] Not only that, premature rupture of membranes is a significant cause of perinatal, neonatal, and maternal morbidity and mortality in both high and low income countries.[3] The incidence of premature rupture of membranes ranges from 3-18% in preterm pregnancies, whereas in term pregnancies it is around 8-10%. Pregnant women come with premature rupture of membranes, as many as 30-40% are preterm pregnancies recorded at the Regional General Hospital is a place of reference in Indonesia.[4] The incidence of premature rupture of membranes in Indonesia in 2015 was 40%.

The causes of premature rupture of membranes are multifactorial and it is difficult to determine exactly which factors are involved. The causative factors for premature rupture of membranes that are known so far are infection, hormonal, behavioral factors (smoking, nutrition, coitus), and mechanical factors (multiple pregnancies, polyhydramnios, macrosomia). Clinical studies prove the increased prevalence and diversity of intrauterine bacteria associated with premature rupture of membranes. Identification of pathological microorganisms in human vaginal bacteria immediately after rupture of membranes supports the concept that bacterial infection plays a role in the pathogenesis of premature rupture of membranes.[5,6] The associated bacteria have low pathogenicity, such as Ureaplasma, Fusibacterium, Mycoplasma, and Streptococcus.[7]

There is an active biological factor responsible for amniochorionic extracellular matrix (ECM) remodeling, namely MMP-9 and its inhibitor, namely TIMP-1. MMP-9 enzymes mainly produced by macrophages and neutrophils have the ability to degrade type IV collagen which is widely expressed in the matrix. Amniochorionic extracellular, thus playing an important role in the occurrence of premature rupture of membranes. Increasing evidence suggests that MMP-9 is strongly associated with rupture of the overexpressed membranes in mid-trimester and is considered a poor prognostic factor for term delivery.

There is still a lack of information about the basic molecular mechanisms underlying membrane rupture and further evidence is needed that activation of MMP9 and deactivation of the MMP-9 inhibitor, namely TIMP-1, are synergistic in causing rupture of membranes. It is necessary to conduct research that examines these two factors together to obtain the MMP-9 and TIMP-1 levels, so that the pathogenesis of premature rupture of membranes can be clearly identified. Not only that, this study also attempted to find a correlation between the ratio of MMP-9 and TIMP-1 to which has the potential as a marker for premature rupture of membranes associated with intrauterine infection and inflammation. This study wants to prove that premature rupture of membranes is related to an imbalance of MMP-9 and TIMP-1 which is associated using samples derived from the amniotic membranes, so that it is hoped to be able to provide more consistent results. This research will be conducted in Jambi because premature rupture of

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membranes cases in the Jambi area have not been fully resolved and research on the basic molecular underlying rupture of membranes with the characteristics of subjects originating from Jambi has not been carried out much.

MATERIAL AND METHODS

Research design and subject

The study was a case-control design conducted from October 2022 to January 2023. The research subjects were pregnant women with preterm and term pregnancies with premature rupture of membranes of the Obstetrics and Gynecology Department at H. Abdul Manap Hospital, Raden Mattaher Hospital, and Jambi Mitra Hospital.

Preparation and sampling of amniochorionic tissue

After parturition, the amniochorionic membrane was sampled to determine MMP-9 and TIMP-1 levels. Samples of the amniochorionic membrane of the cervical and placental parts were separated. The amniochorionic membrane was cut into small fragments of size 1 cm^2. Samples were stored in tubes at -80°C. Samples were soaked in phosphate-buffered saline (PBS), 1% Triton X-100 and a mixture of various protease inhibitors and then crushed mechanically using a homogenizer. The suspension obtained was then centrifuged, and the resulting supernatant was transferred to a fresh tube and then stored at -20°C until used for further analysis.

Measurement of MMP-9 and TIMP-1 levels

MMP-9 and TIMP-1 concentrations from amniochorionic membrane supernatant extract samples were assessed quantitatively using the ELISA method (Bioassay Technology Laboratory, China). Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the framers for use. The unused strips should be stored at 2-8 °C. Add 50 µL of the standard well. Add 40 µL sample to sample wells and then add 10 µL antibody to sample wells, then add 50 µL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a shaker. Incubate for 60 minutes at 37 °C. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to a minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50 µL substrate solution A to each well and then add 50 µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37 °C in the dark. Add 50 µL stop solution to each well; the blue color will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set a 450 nm within 30 min after adding the stop solution.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 20. The research subjects' characteristics and the research variables' characteristics are reported descriptively. Normal distribution data are tested using an unpaired t-test and non-normal distribution data are tested using the Mann-Whitney test. The Spearman correlation test is then used to determine the linear relationship between two variables.

Ethics of study

This research has received ethical consideration and approval from the Research Ethics Committee Team of the Faculty of Medicine, Andalas University with registration number 23/UN.16.2/KEP-FK/2023.

RESULTS AND DISCUSSION

Characteristic subjects

The characteristics of the subjects of this study were as follows, there were the most mothers with primary school to high school education, namely 80% in the premature rupture of membranes group. The percentage of multigravida was the highest in the premature rupture of membranes namely 57%. The percentage of parity in the premature rupture of membranes group by 66%. The majority of pregnant women are aged 21-35 years, as many as 89% in the premature rupture of membranes group. Based on gestational age, which is more than 36 weeks is the most common age in the premature rupture of membranes group by 89%.

Based on the research results shown in Table 1, it is known that education, gravida, parity, maternal age, gestational age, BMI, and Hb levels were not statistically different between the incidence of premature rupture of membranes. A study by Tavassoli et al in 2010 regarding pregnancy outcomes in the incidence of premature rupture of membranes stated similar results to this study. Between the two groups studied resulted in a range of maternal age that was not too heterogeneous at the time of admission, including gestational age and parity. Although theoretically it can be explained that cases of premature rupture of membranes are more common in women with multipara parity status because this condition can increase the risk of premature rupture of membranes occurring due to the effect of reduced uterine and abdominal muscle strength, so that the amniotic fluid cannot hold amniotic fluid. Multiparas or grandemultiparas are also often found in cervical incompetence so that it will speed up the opening of the cervix and premature rupture of membranes occurs.[8]

Table 1: Distribution of characteristics of study subjects.

Variable	Premature rupture of membranes	p
Education (n,%)		
Primary & High School	28 (80)	0.055[b]
Academy/University	7 (20)	
Gravida (n,%)		
Primigravida	15 (43)	0.461[a]
Multigravida	20 (57)	
Parity (n,%)		
Nulipara	15 (43)	
Primipara	8 (23)	0.426[a]
Multipara	12 (34)	
Maternal age (n,%, years)		
21-35	31 (89)	0.495[a]
>35	4 (11)	
Pregnancy age (n,%, weeks)		
34-36	4 (11)	0.114[b]
>36	31 (89)	
BMI (mean, SD, kg/m^2)	26.413±3.613	0.621[c]
Hb (mean, SD, g/dL)	12.128±1.610	0.930[c]
Leucocyte (median, IQR, cells/µL)	15.900 [3.500]	0.000[d]
Duration of premature rupture of membranes (mean, SD, hours)	8.628±1.895	-

Significant at p<0.05. Group differences were evaluated with: a) Chi-Square test and b) Fisher's Exact test for categorical variables; c) Independent Samples T-test, and d) Mann-Whitney U-test for continuous variables.

Table 2: MMP-9 and TIMP-1 levels in premature rupture of membranes.

Variable	Premature rupture of membranes
MMP-9 (mean, SD, ng/L)	590.083±185.682
TIMP-1 (median, IQR, pg/mL)	376.192 [123.704]

Infection is the most common cause of preterm labor and premature rupture of membranes, where bacteria can spread to the uterus and amniotic fluid, triggering inflammation and resulting in premature rupture of membranes.[9] This is in line with the results of a study which showed that the median leukocytes in the premature rupture of membranes group were higher than in response to presence of inflammation and infection. Another response commonly implicated is the presence of antimicrobial peptides as physiological constituents of amniotic fluid and their cumulative effect of these molecules may provide a defense mechanism for the host.[10]

Matrix metalloproteinase-9 and tissue inhibitor matrix metalloproteinase-1 levels

The results of this study indicate that the amnionchorionic membrane in the incidence of premature rupture of membranes is characterized by increased levels of MMP-9, while TIMP-1 levels do not differ between the incidence of premature rupture of membranes.

Rupture of the membranes is the result of a biochemical process that causes degradation of collagen in the extracellular matrix of the amnion-chorionic membrane and programmed cell death in the membranes. At term, there is an increase in MMP that corresponds to a decrease in collagen. MMP-9 expression and pro-MMP-9 production were significantly increased in the paracervical region amnionchorion. Thus, MMP-9 is a major contributor to term membrane rupture where increased MMP-9 can result in ECM degradation.[11]

MMP-9 activity is tightly controlled by complex regulatory mechanisms, including direct inhibition via metalloproteinase activators and inhibitors TIMP-1 TIMP-1 is an endogenous protease inhibitor specific for MMP-9 secreted by various cell types, such as macrophages, smooth muscle cells. blood vessels, and platelets. TIMP-1 is a multifunctional protein as it has various biological activities including modulation of cell proliferation, cell migration and invasion, anti-angiogenesis, anti- and pro-apoptosis as well as synaptic plasticity. MMP-9 is able to form a complex with TIMP-1 when the MMP is in a latent form. TIMP-1 suppresses MMP-9 activity by binding to MMP-9's catalytic domain and blocking its enzymatic properties.[12]

Our study showed that there was no significant difference in TIMP-1 levels premature rupture of membranes group. This may be due to researchers not taking into account the heterogeneity of the chorionic amnion membrane because there are histological differences, especially the thickness of epithelial cells in certain membrane regions, so the results obtained do not reflect significant variance in premature rupture of membranes samples. It has been proven in the pathological process that an imbalance of MMP and TIMP has the consequence of causing disturbances in the matrix extracellular.[13] TIMP involvement is able to suppress MMP activity by binding to the MMP catalytic domain and blocking its enzymatic properties, so that in the event of premature rupture of membranes, this ratio value will be smaller.

CONCLUSION

Based on the research that has been done, it can be concluded that there are differences in protein levels of MMP-9 but there are no differences in protein levels of TIMP-1 in premature rupture of membranes.

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