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RESEARCH ARTICLE

High NF-κB and RAGE Expression in Fetal Membrane of Premature Rupture of Membrane (PROM) Subject

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Abstract

BACKGROUND: Premature Rupture of Membranes (PROM) is significantly linked to the infections-related maternal deaths. In the inflammatory process, the influencing stressor will stimulate the activation of Nuclear Factor Kappa B (NF-κB) and Receptor of Advanced Gyclation End product (RAGE). Yet up to date, the expression of NF-κB and RAGE in pregnant women with PROM are still rarely studied. Therefore, this study aimed to observe the differences of NF-κB and RAGE expression from PROM and non-PROM subjects.

METHODS: This was a cross-sectional study involving 20 PROM subjects and 20 non-PROM subjects with infections and complications. Samples from the fetal membrane tissue of subjects were obtained and put into paraffin block preparation for the determination of NF-κB and RAGE expression. The detection of NF-κB and RAGE expression was conducted using immunohistochemical staining and observed under an upright light microscope. The expressions were later calculated using ImageJ software.

RESULTS: Both NF-κB and RAGE expression were found to be higher in PROM subjects compare to the non-PROM subjects. The median of NF-κB in PROM and non-PROM subjects were 32.47±1.22 and 5.59±1.09, respectively (p=0.000). While the median of RAGE in PROM subjects was 53.58±3.46, and in non-PROM subjects was 11.64±2.49 (p=0.013).

CONCLUSION: There is significant difference between NF-κB and RAGE expression in fetal membranes of PROM and non-PROM subjects. Therefore, the increased of NF-κB and RAGE expression can be used as a potential marker to detect complication of PROM.

KEYWORDS: premature ruptures of membrane, non-premature ruptures of membrane, expression of NF-κB

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Introduction

Maternal death has become a global health concern in many countries, especially in poor and developing countries.(1,2) Around 358,000 maternal deaths occur annually worldwide (1), while in Indonesia, the Maternal Mortality Rate (MMR) was 305 per 100.000 live births in 2015 (3). Maternal deaths are mostly caused by preeclampsia/eclampsia, bleeding, and infection.(4-7)

Premature Rupture of Membranes (PROM) is significantly linked to the infections-related maternal deaths.(4) Complications of PROM that might occur consist of premature birth, compression of the umbilical cord, respiratory distress syndrome, prolapse of the umbilical cord, and placental abruption. The influence of oligohydramnios

severity, gestational age, and duration of the latent period on PROM can also increase the risk of fetal and neonatal death.(8) Since PROM is defined as a rupture of fetal membranes at a gestational age of less than 37 weeks or before the delivery, thus, the main cause of PROM is an infection in the fetal membranes.(9)

In case of infection, local inflammatory response produces cytokines that will reach the maternal and liver to upregulate the C-Reactive Protein (CRP) level, which has been known as a marker of inflammation.(10,11) As a pro-inflammatory factors, High Mobility Group Box 1 (HMGB1) molecules can stimulate pro-inflammatory response, such as the Receptor for Advanced Glycation End-products (RAGE) by interacting with multiple receptors.(12) The bond between AGE and RAGE produces conduction process of cellular signals that activate the Nuclear Factor-kappaB (NF-κB) pathway.(11,13)

NF-κB is one of the transcription factors that play a central role in inflammation. It induces the transcription of proinflammatory cytokines, chemokines, and adhesion molecules.(10,11) A study showed that the expression of NF-κB is upregulated only in infectious inflammation but not in non-infectious inflammatory processes, implying NF-κB, hence when the fetal membranes are infected, NF-κB level in maternal blood will also increase.(10)

Based on above reasons, the possibility that combination of NF-κB and RAGE expression can be a potential marker for PROM complications should be considered. However, the expression of NF-κB and RAGE in pregnant women with PROM are still rarely studied. Therefore, we conducted this study to observe the differences of NF-κB and RAGE expression in fetal membranes from PROM and non-PROM subjects.

Methods

Study Design and Sample Collection

This was a cross-sectional study involving 20 PROM subjects and 20 non-PROM subjects. Total of 40 pregnant women who had preterm and term labor with infections and complications were recruited. This research has been declared ethically feasible by the Health Research Ethics Commission, Faculty of Medicine, Universitas Hang Tuah, Surabaya (No. I/150/UHT.KEPK.03/VIII/2021).

Preparations of Tissue Samples

Samples from the fetal membrane tissue of subjects were obtained and put into paraffin block preparation for the determination of NF-κB and RAGE expression. The paraffin block preparations were sliced with microtome into slices with size of 5 μm, deparaffinized, antigen-retrieved, endogenous-peroxidase-blocked and applied with blocking solution. Then slices were taken with an object glass. The selected pieces were dried at 38-40˚C, then the preparations were incubated at 38-40˚C for 24 hours.

Immunohistochemistry

For the detection of NF-κB expression, reagent from NF-κB p50 (E-10) (No Cat.: sc-8414, Santa Cruz Biotechnology, Dallas, TX, USA) was used according to the manual.(14) Meanwhile for the RAGE expression, Anti-RAGE, V Domain [2A11] Antibody (Kerafast, Boston, MA, USA was used. The detection of NF-κB and RAGE expression was conducted using immunohistochemical staining by Olympus light microscopy (Tokyo, Japan) at 400x magnification in three fields of view.

After the immunohistochemical results were documented under an upright light microscope, five fields were selected randomly for each sample, tested by the Threshold method. Area with brown color will be selected and then analyzed for particles containing NF-κB and RAGE. The NF-κB and RAGE expressions were later calculated using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (SPSS IBM, Armonk, NY, USA). Mann-Whitney U test was performed to find the differences of NF-κB expression in fetal membranes of PROM and non-PROM subjects. Meanwhile, Biner Logistic Regression test was performed to find the differences of RAGE expression in fetal membranes. A p-value<0.05 was considered statistically significant.

Results

Table 1 showed the characteristics of subjects regarding the parity. The age of subjects while during the delivery were divided into three age group. In both PROM and non-PROM subjects, most of the subjects gave birth at the age range of 20-29 years old, followed by 30-39 years old and >40 years old. For the gestational age, 6 PROM subjects had preterm birth and 14 others had term birth. Meanwhile for the non-PROM subjects, 2 subjects have preterm birth and 18 others had term birth

Table 1. Subjets' characteristics regarding the parity.

Characteristics	PROM Subjects (n=20)	Non-PROM Subjects (n=20)
Age Group		
20-29 years old	10	10
30-39 years old	7	9
>40 years old	3	1
Gestational Ages		
Preterm	6	2
Term	14	18
Delivery Methods		
Vaginal Labor	17	18
Sectio Caesaria (SC)	3	2
Amount of Parity		
Primigravida	6	7
Multigravida	14	13

Most of the PROM and non-PROM subjects had vaginal labor, and only 3 PROM subjects and 2 non-PROM subjects had Sectio Caesaria (SC). Six PROM subjects were primigravida (pregnant for the first time), while 14 others were multigravida. While among the non-PROM subjects, 7 subjects were primigravida, while 13 others were multigravida.

IHC Expression of NF-κB and RAGE

Expressions of NF-κB and RAGE were clearly observed by the brown color in the fetal membrane cells (Figure 1). Both NF-κB and RAGE expression were found to be higher in PROM subjects compare to the non-PROM subjects. The median of NF-κB in PROM and non-PROM subjects were 32.47±1.22 and 5.59±1.09, respectively. While the median of RAGE in PROM subjects was 53.58±3.46, and in non-PROM subjects was 11.64±2.49 (Figure 2).

Mann-Whitney U test was performed to find the significance difference of NF-κB expression in PROM and non-PROM subjects. The result of the test showed significant difference (p=0.000) with 100% sensitivity and specificity. We also found significant difference of RAGE expression in PROM and non-PROM subjects (p=0.013) (Figure 2). Binary Logistic Regression Test of RAGE expression was performed to assess the effectiveness of using RAGE expression against the actual case in case prediction. The result showed that RAGE expression had 100% sensitivity, 90.9% specificity, 90% Positive Predictive Value (PPV), and 100% and Negative Predictive Value (NPV).

Discussion

In current study, the value of NF-κB expression from fetal membranes of PROM subjects' was higher than the non-PROM subjects. Similar situation is also shown in previous study which report that the NF-κB p65 level in maternal blood in subclinical chorioamnionitis with PROM is higher. (10) Expression of NF-κB are also found to be increased in other tissues, such as in papilloma and squamous cell carcinoma.(15)

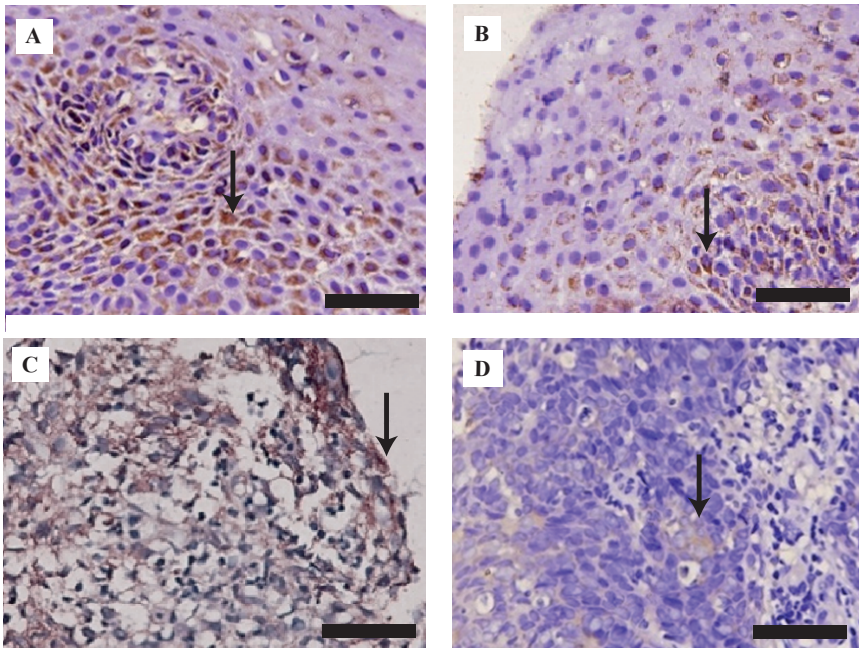

Figure 1. Immunohistochemistry results of NF-κB and RAGE expression in fetal membranes. A: NF-κB expression in PROM subjects; B: NF-κB expression in non-PROM subjects; C: RAGE expression in PROM subjects; D: RAGE expression in non-PROM subjects. Expression of NF-κB and RAGE in fetal membrane cells were shown in brown color (black arrow). Black bar: 10 µm.

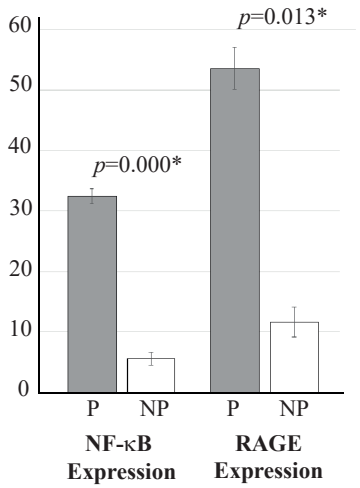

Figure 2. NF-κB and RAGE expression. NF-κB expression was tested with Mann-Whitney U test, with significance *p<0.05. RAGE expression was tested with Binary Logistic Regression Test, with significance *p<0.05. P: PROM subjects; NP: Non-PROM subjects.

Similar to NF-κB expression, the result of this study also showed that the RAGE expression in fetal membrane was higher subjects with the condition of PROM compare to the non-PROM. A study comparing the RAGE expression on normal, PROM, and preterm-PROM also showed similar result, where the RAGE expression in the preterm-PROM and PROM subjects are significantly higher than the normal subjects.(16) Other study also report that expression of RAGE increased in fetal membrane of subjects with gestasional diabetes mellitus.(12)

These results indicated that there was an increase in NF-κB and RAGE expression caused by the inflammatory response, thereby increasing the risk of PROM. During the initiation of delivery, some stressors can weaken the fetal membranes which might results in distension of the fetal membranes and myometrium, hypoxia, or oxidative stress in the fetal membranes which increases with gestational age. Stressors that arise due to the inflammatory process can activate pathogen-associated molecules, such as Toll-Like Receptors (TLR). Activation of TLR causes activation of the pro-inflammatory transcription factor NF-κB, changes in Matrix Metalloproteinase (MMP) levels, and stimulates apoptosis. Changes in the biochemical components of fetal membranes are the cause of rupture which increasing the risk of PROM.(17)

The binding of HMGB1 to RAGE was stimulated by NF-κB activation and release of proinflammatory cytokines and chemokines.(18) Infection of the decidual and amnion-chorionic membranes (chorioamnionitis) can stimulate NF-κB expression, cause inflammation, and cause rupture of the membranes. Ruptured membranes result from injury to collagen structures due to an increase in pro-inflammatory cytokines, and the release of cytokines causes preterm labor or PROM by blocking progesterone receptors and stimulating prostaglandin synthesis.(16)

Humans have glucocorticoid hormones produced by the adrenal cortex which that is able to inhibit the inflammatory process by activating Heat Shock Protein (HSP) in the cytoplasm, and binding to glucorticoid receptors. Glucocorticoids can inhibit the activation of NF-κB in 4 ways, namely by inducing Inhibitor of NF-κB (IκB) degradation, synthesizing Interleukin 10 (IL-10), binding to p65 so that it interferes with NF-κB activity, and damaging Tumor Necrosis Factor (TNF). (19) Complex of glucocorticoids-glucocorticoid receptors (GC-GR) migrates to the cell nucleus to transactivation of anti-inflammatory genes and expression of genes encoding RAGE.(20)

Eventhough this study reported the difference of NF-κB and RAGE expression in PROM and non-PROM subjects with infection and complications, however only limited number of samples included. Hence further study with more number of sample and by using different methods should be conducted.

Conclusion

There is significant difference between NF-κB and RAGE expression in fetal membranes of PROM and non-PROM subjects. These expressions play important roles in inflammation pathways of fetal membrane, hence indicating that the increased of NF-κB and RAGE expression can be used as a potential marker to detect complication of PROM.

Authors Contribution

MZA and CJC were involved in the research conception, design, processed the experimental data, analysis, and drafted the manuscript. KES, S, AUR are involved in the data analysis and result interpretation. All authors discussed the results and commented on the manuscript.

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