Positive Expression of 3-Nitrotyrosine in The Amniotic Membrane as A Risk Factor for Preterm Labor


ABSTRACT

Oxidative stress is a risk factor for preterm labor. 3-Nitrotyrosine is a marker of oxidative stress that can be found in the amniotic membrane. This study was conducted with the aim of understanding the pathophysiology of preterm labor for screening and management of preterm labor and its impact. This study was conducted by collecting patients with preterm pregnancies as samples and term delivery as controls who came to the delivery room of the ER Obstetrics and Gynecology Hospital Prof. Dr. I.G.N.G. Ngoerah Denpasar and Network Hospital of Udayana University in the period from November 2020 to February 2021. This is an analytical study using a case control design. Based on the analysis, there was a statistically significant difference (p < 0.001) between the case and control groups. The group of patients with positive 3-NT expression had an 8.31 times chance of experiencing preterm delivery compared to the group of patients with negative 3-NT expression.

Keywords: Preterm birth, 3-Nitrotyrosine.

I. INTRODUCTION

Preterm birth is delivery that occurs prematurely and is still a serious health problem in the field of obstetrics and perinatology. Preterm labor occurs 10-15% of all deliveries and is also the leading cause of neonatal morbidity and mortality, both in developing and developed countries, where 70% of neonatal deaths are caused by babies born preterm.

Based on PONEK data at Prof. Dr. I.G.N.G. Ngoerah Hospital in the period 1 January 2018 – 31 December 2018 there were 520 neonates with a body weight < 2500 grams who were treated at Prof. Dr. I.G.N.G. Ngoerah Hospital, and neonatal deaths with a weight < 2500 grams were 82 cases (15.8%), where as many as 31 cases of neonatal death occurred in infants with a birth weight of 1000-1499 grams. Sepsis and hyaline membrane disease are the most common causes of neonatal death. Respiratory distress, intraventricular hemorrhage, bronchopulmonary dysplasia, and sepsis are the most common complications. As a result of this preterm birth, it is estimated that one million babies die each year due to various complications after this preterm birth.

Approximately 50% of preterm labor and 70% of preterm premature rupture of membranes are associated with intra-amniotic infection and inflammation. In addition to infection, oxidative stress can also cause inflammation in response to oxidative damage. Oxidative stress can be assessed in various ways, but many studies have previously assessed oxidative
stress through maternal blood samples, and there has been no research in Indonesia, especially in Bali, that has assessed the presence of oxidative stress from other sources. Therefore, the researchers took the initiative to use another way to determine the presence of oxidative stress, namely by examining the presence of oxidative stress markers in the amniotic membrane. The marker of oxidative stress that can be examined in the amniotic membrane is 3-NT which is known as a marker of peroxidation and oxidative stress. The researcher assumes that it is very important to study 3-NT levels in preterm labor, to better understand the pathophysiology of preterm labor, so that preventive measures can be taken to reduce the impact of preterm labor.

II. DISCUSSION

The research sample, which was taken by consecutive sampling, was preterm delivery patients with gestational age of 20 weeks to 36 weeks 6 days who came to the delivery room of the Obstetrics and Gynecology ER at Prof. Dr. I.G.N.G. Ngoerah Hospital Denpasar and Network Hospital Udayana University in the period November 2020 to February 2021, 27 people who met the inclusion criteria and obstetric patients with normal delivery at term were selected consecutively from the control population as many as 27 people with a total sample of 54 people. This study is an analytic study using a case-control design in which the cases were preterm deliveries while the controls were term deliveries.

The research data was processed using SPSS 26.0. versions for Windows. Data analysis in this study includes several tests, namely:

1. All data obtained in this study were analyzed descriptively based on age, parity, BMI and gestational age and the results will be presented in the frequency distribution table.
2. Test the normality of the data using the Shapiro-Wilk test. If the data is not normally distributed, then the Mann-Whitney test is used, whereas if the data is normally distributed, it will use the t-independent test to assess the significance of the variables.
3. Determination of the cut off value of 3-NT expression using the ROC curve based on a preliminary analysis of 54 research samples.
4. Risk factor analysis was performed by associating the incidence of preterm delivery with 3-NT expression. The significance of the relationship above was measured by the Chi-Square test at the significance level p = 0.05.

Descriptively, the characteristics of the samples in the two groups were classified based on age, parity, and BMI. The normality test for this characteristic data was carried out using the Shapiro-Wilk test (sample < 50 in each group), it was found that the data on maternal age, parity, and BMI were not normally distributed (p < 0.05), so the mean (median) used to describe the distribution of data in each group. Then the Mann Whitney test was carried out to determine the significance of the median difference obtained, based on maternal age between the two groups, there was no significant difference with the value (p = 0.477). In the case group, the median maternal age was 27.00 with an interquartile range (IQR) of 9.00, while in the control group, the median mother's age was 26.00 with an IQR of 9.00. There was also no significant difference based on parity between the two groups (p=0.375), where the median parity in the case group was 0.00 with an IQR of 2.00, while in the control group the median parity was 1.00 with an IQR of 2.00. Characteristics based on BMI (Body Mass Index) obtained a median of 23.25 kg/m² in the case group with an IQR of 4.20, while in the control group it was 22.43 kg/m² with an IQR of 5.16. From the Mann Whitney test, there was no significant difference between the two groups with p value = 0.665. Determination of the cut-off value of 3-NT expression in the maternal amniotic membranes in this study used the Receiver Operating Characteristic (ROC) curve. The ROC curve is the reference for the predictor limit value in preterm labor with positive 3-NT expression, where all 3-NT expression data will be input into the ROC curve, the data is then processed, and the cut-off value is determined from 3-NT expression.

The clinical risk factor for preterm delivery in pregnant women in this study was the positive expression of 3-NT in the amniotic membrane. From the calculation of the ROC curve, the cut-off value of 3-NT expression in the membranes was 115.87, which was used as the limit value of the risk factor for preterm delivery in this study. showed that 3-NT expression was positive as a risk factor for preterm delivery. Based on the analysis, there was a statistically significant difference (p < 0.001) between the control group and case group. The group of patients with positive 3-NT expression had 8.31 times the probability of having preterm delivery compared to the group of patients with negative 3-NT expression (OR = 8.31; 95% CI = 2.44-28.35; p < 0.001).

In this study, the mean age of the mother in the preterm delivery group was 29.00 years and the mean age of the mother in the term delivery group was 27.70 years, with p value = 0.477. (p>0.05). It is known that maternal age is associated with the incidence of prematurity. Maternal age 40 years was associated with an increased risk of preterm delivery, and the lowest risk of preterm delivery was found in women in the 30–34 year age group. The relationship between preterm delivery and maternal age is depicted by a U-shaped curve, namely 6.8% in the 20–24 year age group, 6.0% in the 25–29 year age group, 5.7% in the 30–34 year age group, 6.3% in the 35–39% age group, and 7.8% in the 40 years age group.

In this study, the mean parity in the preterm delivery group was 0.85 and the parity average in the term delivery group was 1.00 with p value = 0.375. This shows that there is no significant difference in parity between the two research groups. In a study conducted on 30,237 pregnancies, it was found that there was an increased risk of preterm delivery in nulliparas (OR 1.95, 95% CI 1.89–2.00) and in the fifth pregnancy (OR 1.26, 95% CI 1.13–1.41). The study concluded that there are independent risk factors between nulliparity and preterm delivery. And there is an increased risk of preterm delivery in women with their fifth pregnancy, with the highest risk of preterm delivery at early gestational age.

In this study, the mean BMI in the preterm delivery group was 23.36 and the mean BMI in the term delivery group was 23.09 with p value = 0.665. In a study conducted by in Sweden with a total of 1,857,822 respondents, there were
5.03% of preterm deliveries, and the incidence of labor increased along with the increase in BMI, especially in BMI 25-29 (overweight) and 30-34 (obese) [1]. This is in line with another research which states that there is an increased risk of preterm delivery in pregnant women with overweight and obese BMI of 1.22 and 1.30 compared to pregnant women with normal BMI [2].

Body mass index is correlated with the incidence of preterm delivery. It was found that there was a significant correlation between the value of Body Mass Index (BMI) and lymphocyte levels with a significance value of p = 0.012 [3]. In this study it was found that neutrophil levels will increase along with the increase in BMI and there is a very significant positive correlation between BMI values and neutrophil levels with p value = 0.000.

Expression of 3-NT was assessed by immunohistochemical analysis. Cells expressing 3-NT will be brown in the cytoplasm and counted in 3 fields of view, and counted by HSCORE, which is a semi-quantitative histology score. HSCORE is calculated by the formula:

$$HSCORE = \frac{Pi (i+1)}{100}$$

where $i$ = color intensity with a value of 1 (weak), 2 (moderate), 3 (strong), and $Pi$ = percentage of cells (0-100%) that are recorded positive for each intensity. The final value of HSCORE has a range of values of 0 – 300. In sample number 1, 3 cells of weak color intensity were obtained, 11 cells were medium, 9 cells were strong, with a total of 24 cells. Therefore, the HSCORE calculation is:

$$HSCORE = \frac{(3/24 \times 100 \times 1) + (11/24 \times 100 \times 2) + (9/24 \times 100 \times 3))}{100} = 216.7$$

HSCORE calculations were applied on all case and control samples carried out at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University.

This study uses a cut off from the calculation results of the ROC curve which has an AUC of 0.800, with a cut off value of 3-NT expression of 115.87 with a sensitivity of 77.8%, a specificity of 70.4%, and an AUC value of 0.800 (CI 95%), % 0.684–0.917, p value <0.001. Based on the results of the analysis in this study, there were 21 samples of positive 3-NT expression in the preterm delivery group, while 6 samples had negative 3-NT expression. In the term delivery group, only 8 samples had a positive 3-NT expression while the other 19 samples had a negative 3-NT expression. The p-value obtained was <0.001 because the p-value <0.005, so there was a significant relationship between the positive 3-NT expression in the membranes and the incidence of preterm labor. The odds ratio value obtained in this study was 8.31 (95% CI: 2.44-28.35), which means that pregnant women with positive 3-NT expression in the membranes have an 8.31 chance of experiencing preterm labor compared with the group of pregnant women with negative 3-NT expression in the membranes.

The results of this study are in accordance with the findings in a study which assessed staining in preterm labor (PTB) and preterm premature rupture of membranes (PPROM) where both PTB and PPROM showed increased 3-NT staining which confirm oxidative stress [4]. However, the intensity of 3-NT staining was higher in the PPROM group compared to the PTB group, indicating a higher oxidative stress (P < 0.0001) [5]. The staining pattern was consistent in the amnion, chorion and extracellular matrix cells. Approximately 24% of cells in PTB and 58% of cells in PPROM showed positive staining for 3-NT. In this study, there was no assessment of term labor. However, a study stated the opposite, one of which was assessed from the study was the intensity of 3-NT staining on the membranes of preterm labor (n=7), preterm premature rupture of membranes (n= 8) and term delivery (n=8) to document the presence of oxidative stress [6]. The intensity of 3-NT staining was found to be stronger in preterm premature rupture of membranes and term labor. 3-NT staining of the membranes in term pregnancy is the same as that of premature rupture of membranes at term, and in preterm labor, the staining is slightly less intense than that of preterm premature rupture of membranes and term labor, but data on the difference in intensity of the 3-NT staining were not provided. In this study, it was stated that the lack of 3-NT staining in term pregnancy could indicate acute oxidative stress, if chronic oxidative stress occurs due to cell aging, premature rupture of the membranes can occur so that the staining intensity is higher. Meanwhile, at term delivery, 3-NT staining can occur due to natural and physiological aging of the placenta and membranes [7], [8].

### III. CONCLUSION

Based on the results of the study, the following conclusions were obtained Positive 3-NT expression in the membranes of pregnant women is a risk factor for preterm delivery and the risk of preterm labor in the membranes with a positive 3-NT expression is 8.31 times than normal.

### REFERENCES