

Role of Caspase-3 as Risk Factors of Premature Rupture of Membranes

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ABSTRACT

Premature rupture of membranes (PROM) is one of the problems in the field of obstetrics, related to the prevalence, prematurity, perinatal morbidity and mortality. Causes of PROM are multifactorial and its mechanisms are not yet clear. The weakening of the membranes is suspected as a result of biochemical processes that result in remodeling and apoptosis, as well as membranes stretching. Apoptosis plays an integrated role in the occurrence of premature rupture of membranes. To investigate the role of caspase-3 in premature rupture of membranes. A case-control study with PROM as a case, and non-PROM as a control at 20-42 weeks gestation. Amniotic tissue was taken after delivery of the placenta. Immunohistochemical examination of Caspase-3 was done at Integrated Lab.Biomedic Medical Faculty of Udayana University in Bali. The study was conducted on 36 cases of PROM and 37 cases non PROM. There was no characteristic difference between the case and control groups ($p > 0.05$). The expression of positive caspase-3 is a risk factor of PROM of 7.3 times (OR = 7.31; CI 95% = 2.64 to 20.22; $p = 0.001$). Caspase-3 expression is a risk factor for premature rupture of membranes.

Keywords: PROM, Apoptosis, caspase-3.

INTRODUCTION

Premature rupture of membranes (PROM) is one of the complications of pregnancy and health problems in the field of maternal and neonatal care in the world, including Indonesia, which is related to the prevalence, prematurity, morbidity, and mortality in the perinatal maternal side. This will increase maternal mortality and infant mortality as a complication of premature rupture of membranes.

Incidence of premature rupture of membranes (PROM) occurs 10-12% in all pregnancies. In term pregnancy the incidence varies from 6 to 19%, whereas in preterm pregnancy the incidence is 6-8%.The incidence of PROMs worldwide varies between 5-10% and almost 80% occurs at the age of term pregnancy (Adeniji *et al.*, 2013; Endale *et al.*, 2016). Meanwhile, preterm PROM incidents are estimated at 3-8% (Okeke *et al.*, 2014). Under normal circumstances, 8-10%



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of pregnant women will experience PROM and only 1% occur at preterm gestation (Soewarto, 2010). The prevalence of preterm PROM in the world is 3 - 4.5% of pregnancies, and is a contributor of 6 - 40% preterm labor or prematurity (Furman *et al.*, 2000). In China the incidence of PROM is higher, around 19.53% of all pregnancies (Yu, 2015), while in Indonesia it ranges from 4.5% to 7.6% (Wiradarma *et al.*, 2013). In Sanglah Hospital in Denpasar, Suwiyoga and Budayasa (2006) reported the incidence of cases PROM 12.92% in which case the term PROM amounted to 83.23% and amounted to 16.77% of preterm deliveries in 2113. Budijaya and Surya Negara (2016) reported a case of premature rupture of membranes (PROM) at Sanglah Hospital Denpasar 212 cases from 1450 deliveries (14.62%). The incidence of labor with PROM at gestational age term (e"37 weeks) was 179 cases (84.43%), while in preterm as many as 33 cases (15.57%).

Complications of PROM can occur either on the infants or maternal. Maternal complications such as chorioamnionitis are found in 9% of pregnancies with premature rupture of membranes, risks of up to 24% if rupture of membranes occurs more than 24 hours. In preterm pregnancies, the incidence was greater between 13-60% (Brian and Mercer, 2003; Dars *et al.*, 2014). Placental abruption occurs in 4-12% of pregnancies with premature rupture of membranes. While maternal complications include intra amniotic infections, which occur in 13-60% of pregnant women with PROM, placental abruption and post partum endometritis. Maternal sepsis occurs of 0.8%, can cause death (0.14%). PROM complication that occurs in infants such as intrauterine infection, umbilical cord compression, respiratory distress syndrome (RDS), necrotizing enterocolitis, intraventricular hemorrhage and sepsis is a common associated with preterm labor. Premature infant care resulting from preterm births occurring in cases of PROM requires considerable cost for intensive care. The cases of PROM were associated with perinatal morbidity of 21.4% and mortality by 18-20% (Patil, 2014; Linehan *et al.*, 2016; Rodrigo and Kannamani, 2016).

The amniotic membrane is an avascular elastic tissue consisting of amnion and chorion. The amnion is part of faced directly with the

amniotic cavity while chorions a thicker layer and attached to the maternal decidua. Amnion and chorionic connected by connective tissue that is rich in collagen, extracellular matrix. Causes of premature rupture of membrane are multifactorial and the mechanism is unclear. Weakening of extracellular matrix in the amniochorion layer due to degradation of collagen is one of the processes as predisposing to premature rupture of membranes. In premature rupture of membrane an increase in activity collagenolytic with consequent decline in the number of collagen tissue and disruption of the collagen structure was occurred. Collagen degradation caused by enzymes, matrix metalloproteinase (MMP) (Brian and Mercer; Areia and Moura 2015).

One of the factors associated with increased risk of premature rupture of membranes both endogenous and exogenous factors is the occurrence of programmed cell death or apoptosis. Apoptosis play an integrated role in the occurrence of premature rupture of membranes, where it was found that cells undergoing apoptosis in layers of amnion and chorionic especially in about rips membranes known as *paracervical weak zone* (Xu and Wang, 2005; Harirah *et al.*, 2012; Saglam *et al.*, 2013).

Signals that cause apoptosis may occur intracellular and extracellular. Extrinsic pathway (extracellular) initiated through stimulation of death receptors (*death receptor pathway*) while the intrinsic pathway is initiated through the release of factors from the mitochondria in the cell signal (*mitochondrial pathway*). Both intrinsic and extrinsic pathway of *caspase dependent* ended in the execution phase, which is the final pathway of apoptosis. The activation of caspase execution is the stage that begins this stage, where caspase-3 is the most important executor caspase. Caspase-3 plays an important role in changes in cell morphology and biochemical events associated with the implementation and complete the process of apoptosis (Elmore, 2007; Equils *et al.*, 2014; Estaquier *et al.*, 2014).

Caspase expression and activation play a very important role in apoptosis. Caspase-3 is a major member of the caspase family. This is an active factor in apoptosis. Activation of caspas -3 is a biochemical

reaction prior to apoptosis. Almost all apoptosis occurs via a common pathway. Investigators expression of Caspase-3 can display cell apoptosis.

MATERIALS AND METHODS

The study design is a case-control study, PROM as cases and non PROM as controls with gestational age 20-42 weeks. The study was conducted in the emergency department Sanglah Hospital Denpasar Bali between October to May, 2017. After delivery, the membrane was taken from the edge of the tear for immunohistochemical examination in the Lab. Integrated Biomedics Medical Faculty Udayana University Bali. Statistical tests were performed independently -t test and chi-square test.

RESULTS

In the period from October to May 2017 we found 36 cases PROM and 37 controls. In this study it was found that the mean age of the case group was 26.59 ± 6.49 years and the mean age of the control group was 28.72 ± 6.80 years, with p = 0.153. The mean of case group parity was 0.68 ± 0.82 people and the control group parity mean was 0.95 ± 1.28 people, with p = 0.184. The mean BMI of the case group was 24.63 ± 4.48 kg/m² and mean BMI of

the control group was 24.88 ± 3.87 kg / m² with p = 0.850.

Statistical test using *independent t-test*, showed that there was no significant difference in age, parity, and BMI in cases and controls. We obtained p values for each risk factor is p > 0.05, which states that no difference of characteristic value between the two groups.

To determine the role of caspase-3 expression on the risk of premature rupture of membrane Chi-Square test was used. For considering the magnitude of the risk to the PROM, we calculated the Odds Ratio (OR).

In this study, showed that the expression of positive caspase-3 was a risk factor for premature rupture of membranes of 7.31 times (OR = 7.31 ; CI 95% = 2.64 to 20.22 ; p = 0, 001) than the expression of negative caspase-3.

DISCUSSION

In the study by Budi and Surya (2016), it was found that the incidence of PROM was most common in the 21-30 year age group, 116 cases (54.72%) of 212 cases of PROM, both

Table 1: Distribution Characteristics of Age, parity, and BMI, in the Second Group

Risk factors	The case group (n = 36)		Control group (n = 37)		
	Mean	SD	Mean	S	D
Age (years)	26.59	6.49	28.72	6.80	0.153
Parity	0.68	0.82	1.00	1.18	0.158
BMI (kg/m ²)	25.96	4, 89	24.14	4.04	0.066

Table 2: Risk of premature rupture of membranes in Amnion Cell Caspase-3 Expression

		Group		OR	CI 95%	P
		Case	Control			
Caspase-3 expression	Positive	30	17	7.31	2.64 - 20.22	0.001
	Negative	7	29			

term pregnancies and preterm. The study by Udin Sabarudin (2009) in Dr. Hasan Sadikin Hospital Bandung, show that the most age group of mother in PROM case was 20-35 years. The same is also reported by Okeke *et al.*, 2014 in retrospective studies in Nigeria, the highest incidence of preterm PROM cases occurring in the reproductive age group (26-30 years) of 43%. Gahwagi *et al.*, 2015 on study in Libya that was found 61% and V ishwakarma *et al.*, 2015 found that the highest incidence PROM was in the age range 21-25 of 52.1%. The study by Endale *et al* (2016) found the highest incidence of PROM in the 18-35 year age group (74.6%) of the 202 PROM patients. Singh *et al* (2015), reported the most preterm PROM cases was in the 20-30 year age group. The study by Emechebe *et al* (2015) obtained the most cases of PROM was in the age group 25-29 years ie 63 (32.8%) of 192 cases of PROM. Noor *et al* (2007) report that the incidence of PROM in the age group 15-25 years of 58.8%. Study by Gahwagi *et al* (2015) obtained the most cases of PROM was in the age group of 21-30 years. Thombre (2014), found an increased incidence of PPRM in women aged > 35 years. Increasing maternal age consistently associated with PROM incident.

Based on gravida, study by Budi and Surya (2016) in Sanglah Hospital Denpasar found that the highest incidence of PROM occurred in primigravida group that was 87 cases (41.05%). Equal with reported by Okeke *et al.*, 2014 on a retrospective study in Nigeria, the highest incidence of preterm PROM cases occurred in primigravida group of 29.1%. Patil *et al.*, 2014 in his study at MRMedical College, Gulbarga by 53%. Noor *et al* (2007) reported that the incidence of PROM was also highest in primigravida cases (42.2%). Other studies have reported that the incidence of PROM also occurred in primigravida of 68.2%, 52%, 69.7% (Vishwakarma *et al.*, 2015; Gahwagi *et al.*, 2015; Endale *et al.*, 2016). Based on parity, study by Milad *et al* (2015) obtained the most cases of PROM was in nullipara (52%), parity 1-4 (44%). Okeke *et al* (2014) reported the most cases of PROM was in nullipara (29.1%), parity 2 (26.6%), parity 1 (19%).

Some studies show that a low body mass index (BMI) before pregnancy may increase the risk of PPRM. PPRM was strongly associated with increased maternal weight in the second and third

trimester with weight gain <0.37 kg / week in women with BMI <19.5 kg/m². PPRM has a very close relationship with weight at the time of pregnancy, and a low body mass index before pregnancy (Thombre, 2014)

From the study of Xu Jun *et al* (2005), caspase-3 gene expression was obtained in the PROM case compared to the control group with intact membranes, which led to increased apoptotic cells in the amniotic membrane. The expression of MMP-2 increases and TIMP-2 decreases in the case of PROM, which can increase the degradation of the extracellular matrix. Apoptotic cells increase and degradation of extra cellular matrix increases, leading to weakening of membrane elasticity and strength and then causing premature rupture of membranes. Caspase expression and activation play a very important role in apoptosis. Caspase-3 activation is a biochemical reaction prior to the occurrence of apoptosis. Caspase-3 expression was higher in the vaginal delivery group than in cesarean section groups. This may be due to multifactorial effects after the initial labor process, which increases apoptosis and causes spontaneous membrane rupture. Several factors can cause the transformation of cell homeostasis, resulting in increased apoptosis. In fetal membranes with PROM via immunohistochemical examination showed caspase-3 expression in amniotic epithelial cells and coronal cyto-trophoblast cells, slightly expressed on mesenchymal cells and reticular cells of the matrix. This suggests that apoptosis occurs both in amions and chorion, and plays an important role in fetal membrane regulation (Xu Jun *et al.*, 2005).

Xu and Wang (2005) conducted study on the role of caspase and MMP in amniocorion during PROM. Objective of the study is to investigate the role of cysteine aspartic acid-specific protease-3(caspase-3), matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of matrix metallo proteinase-2 (TIMP-2) in the membranes during the experience PROM. Amniotic membranes were collected from several groups of women, women with spontaneous PROM (n = 8), women with normal term deliveries with spontaneous vaginal (n = 8) and women undergoing recurrent cesarean section before the onset of labor and no complications (n=8). The caspase-3 peptide was examined with immunohistochemical

techniques. mRNA expression of MMP-2 and TIMP-2 inhibitors examined using reverse transcriptase-polymerase chain reaction (RT-PCR). From the study, we found that the expression of caspase-3 peptide was $62.86 \pm 3.83\%$ in PROM group, $42.33 \pm 2.99\%$ in the vaginal delivery group, and $20.97 \pm 2.94\%$ in C-section group. There were statistically significant changes between the three groups ($p < 0.05$). Immunohistochemistry shows the presence of expression of caspase-3 in amniotic epithelial cells and cytotrophoblast chorionic cells. Expression of MMP-2 was $84.92 \pm 3.68\%$ in the PROM group, $2:34 \pm 32.65\%$ in the vaginal delivery group, and $30.65 \pm 2.77\%$ in cesarean section group. There were statistically significant differences in the PROM group and cesarean section ($p < 0.05$). The expression of TIMP-2 was $42.01 \pm 12:17\%$ in PROM group, $73.01 \pm 14.82\%$ in the vaginal delivery group, and $88.47 \pm 6:51\%$ in cesarean section group. In conclusion that caspase-3 gene expression is more prevalent in the PROM group, which is due to increased apoptosis in fetal membrane cells. Increased expression of MMP-2 and TIMP-2 decreased in PROM, where an increase in the decomposition of the extracellular matrix (Xu and Wang, 2005).

Caspase-3 is a major member of the caspase family which is an active factor in apoptosis. Caspase-3 activation is a biochemical reaction prior to the occurrence of apoptosis. Almost all apoptosis go through this caspase path. So investigating caspase-3 expression can show cell apoptosis. In fetal membranes with PROM via immunohistochemical examination showed caspase-3 expression in amniotic epithelial cells and coronal cyto-trophoblast cells, slightly expressed on mesenchymal cells and reticular cells of the matrix. This suggests that apoptosis occurs in both amnions and chorionics. This plays an important role in the regulation of fetal membranes (Xu and Wang, 2005).

Another study was conducted by Kumagai (2001), on apoptosis in the amniotic membrane fetal. This study was conducted to determine the exact number of apoptotic cells in different weeks during pregnancy, and to explain the relationship between apoptosis in amnion and weakness of the membranes at the onset of

labor. From this study, the activity of caspase-3 and caspase-8 in a sample of amniotic higher at 40-41 weeks of gestation at the time of 16-27 weeks gestational age ($P < 0.01$). No significant differences in caspase-9 activity were observed between the two groups. Concluded apoptosis in amniotic epithelial layer is not dependent on the regulation of Bcl-2 and the onset of labor, and the possibility of having an important role that causes weak and rupture of the membranes at the end of pregnancy. There is no significant difference in the activity of caspase-3, 8, and 9 between gestational age 16-27 weeks and 28-39 weeks gestation (Kumagai, 2001).

Study of caspase-3 in preterm premature rupture of membranes is also done by Saglam *et al.*, 2013. This study aims to determine the active 3-Caspase immunopositivity in preterm premature rupture of membranes and preterm labor with intact membranes compared with term pregnancies with normal deliveries. The case control study to compare the levels of active caspase-3 immunopositivity in preterm premature rupture of membranes, preterm labor with intact membranes, and in the control group term pregnancies with normal vaginal delivery. Amniochorionic membranes were collected from three groups of women: group 1, women with PPRM after cesarean section ($n = 10$); Group 2, women with preterm labor with intact membranes after cesarean section ($n = 9$); and group 3, women with full-term labor who delivered vaginally after pregnancy without complications (control) ($n = 11$). It was concluded that active immunopositivity caspase - 3 (ACPI) on PPRM group is significantly higher than with Control group ($p < 0.05$). Positive active caspase- 3 increase in fetal membranes in women with PPRM (Saglam *et al.*, 2013).

Caspase-3, a key factor in the execution of apoptosis, is an active form of procaspase-3. Caspase-3 is referred to as the most important executor caspase, activated by caspase initiator (caspase-8, caspase-9, and caspase-10). Caspase-3 specifically activates CAD endonucleases. In cells that are proliferating CAD forms a complex with its inhibitor, ICAD. In cells undergoing apoptosis, activated caspase-3 cleaves ICAD so as to release CAD. CAD will then elaborate on chromosome DNA in the nucleus and causes

chromatin condensation; caspase-3 also triggers cytoskeletal reorganization and disintegration of apoptotic bodies forming cells. Phagocytosis is the last stage of apoptosis (Elmore, 2007).

An intrinsic pathway is the dominant role in the process of apoptosis in fetal membranes at term. This is evidenced by the study findings which suggest that there are significant differences in levels of Bcl-2, caspase-3, caspase-9 in the supracervical region, where such proteins play an intrinsic pathway. Fas and ligands, Fas-L initiates extrinsic path apoptosis. Although in the study Fas and Fas-L can also be found in all samples of amniotic membranes but the expression did not differ significantly between supracervical region and distal region. It is therefore suspected that extrinsic pathways do not play a major role in the remodeling of the membranes. Although there was no significant difference in Bax expression, proapoptotic protein, but Bcl-2 antiapoptosis protein was found to decrease in the paracervical region compared with other regions (Reti *et al.*, 2007).

In this study, the expression of caspase-3 in the case of the PROM was higher than in the case without PROM, with 7.31 times

(OR = 7.31; 95% CI = 2.64 to 20.22; p = 0, 000) this suggests that caspase-3 as the parameter of their apoptosis dependent caspase pathway and acts as a risk factor for premature rupture of membranes.

From this study, the expression of caspase-3 in amnion cells shows that there is an apoptotic process on the membranes. Caspase-3 is an executioner caspase which is active caspase as the process of apoptosis mechanism of caspase dependent pathways that extrinsic and intrinsic pathways, so that the expression is more dominant. This indicates that the caspase-dependent apoptosis plays a role in the mechanism of occurrence of premature rupture of membranes.

CONCLUSION

In this study shows that caspase-3 as the parameter of caspase-dependent apoptosis pathway acts and role as a risk factor for premature rupture of membranes.

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