Biomedical & Pharmacology Journal, June 2018. Vol. **11**(2), p. 719-724

Role of Apoptosis Inducing Factor (AIF) as Risk Factors of Premature Rupture of Membranes

Ketut Surya Negara[1], Ketut Suwiyoga[1], Komang Arijana[2] and Ketut Tunas[3]

[1]Department of Obstetrics and Gynecology, Udayana University, Sanglah Hospital Bali.
[2]Biomedic Laboratory Medical Faculty Udayana University Bali.
[3]Department of Public Health Dhyana Pura University Bali.
*Corresponding author E-mail: tutsuryanegara@gmail.com

http://dx.doi.org/10.13005/bpj/1425

(Received: 18 February 2018; accepted: 04 May 2018)

Premature rupture of membrane (PROM) is an obstetric problem related to the prevalence, prematurity, morbidity and mortality of perinatal. The etiology of PROM is multifactorial and the mechanism remains unclear. The weakening of amniotic membrane is suspected due to various biochemistry process that causing remodeling and apoptosis, and the stretch of the amniotic membrane. Apoptosis plays an integrated role in the pathogenesis of PROM. The mechanism of apoptosis is through caspase-dependent and caspase-independent pathway. Apoptosis protein such as Apoptosis Inducing Factor (AIF) as caspase independent are hypothesized to be involved as the risk factor of PROM. To determine the role of AIF as caspase independent in the mechanism of patogenesis of premature rupture of membranes. A case-control study with PROM as a case, and non-PROM as a control at 20-42 weeks gestation age. Amniotic tissue was taken after delivery of the placenta. Immunohistochemical examination of AIF was done at Integrated Lab.Biomedic Medical Faculty of Udayana University in Bali. The study was conducted on 37 cases of PROM and 46 cases non PROM. There was no characteristic difference between the case and control groups (p>0.05). The expression of positive AIF is a risk factor of PROM of 5.10 times (OR = 5.10; CI 95% = 1.86 to 13.96; p= 0.001). AIF expression was more in the group of PROM. AIF expression is a risk factor for premature rupture of membranes.

Keywords: PROM, Apoptosis, Apoptosis Inducing Factor (AIF).

Premature rupture of membrane (PROM) is one of the complication in pregnancy and one of the maternal and neonatal problem throughout the world, including Indonesia. It correlates with the prevalence, prematurity, morbidity, and mortality of perinatology and maternal aspect, and increase the maternal mortality and neonatal mortality rate as the complication of PROM. The etiology of PROM is multifactorial and the mechanism is still unclear. The extracellular matrix of the amniochorion that weakens due to the degradation of collagen is one of the predisposing factor of PROM. One of the endogenous and exogenous factor that correlates with the increasing risk of PROM is the programmed cell death or apoptosis.

The incidence of PROM is about 10-12% out of all pregnancy, which is 6-19% in the term pregnancy and 6-8% in the preterm pregnancy. The incidence of PROM throughout the world varies between 5-10% and almost 80% of all incidence occurs in term pregnancy (Adeniji *et al*, 2013; Endale *et al*, 2016). While the incidence of preterm PROM is about 3-8% (Okeke *et al*, 2014). The prevalence of preterm PROM throughout the

Published by Oriental Scientific Publishing Company © 2018

world is 3-4.5% of all pregnancy, and becomes as the causes of preterm labor or prematurity as high as 6-40% (Furman *et al*, 2000). Budijaya and Surya Negara (2016) also reporting PROM cases in Sanglah General Hospital, which were 212 cases out of 1450 labor (14.62%). The incidence of PROM in term pregnancy (e"37 weeks gestational age) were 179 cases (84.43%), and 33 cases were preterm pregnancy (15.57%).

Preterm PROM correlates with 30-40% of premature labor, and also known as the major causes of premature labor, which is about 20-50% out of all premature labor (Creasy and Resnik, 2009; Getahun *et al*, 2010; Cunningham, 2010). Prematurity causes about 85% of all neonatal morbidity and mortality, complicates in 3% of all pregnancy and occurs in about 150,000 cases in United States (Gahwagi *et al*, 2015). Prematurity is one of the international health problem and causes 80% of all neonatal mortality, and 60% of neurological defect. Prematurity is also one of social problem because it correlates with disability and growth development in children (Menon and Fortunato, 2007). The maternal complications are intraamniotic infection, that occurs in 13-60% of all pregnant women with PROM, placental abruption, and postpartum endometritis. Sepsis maternal occurs in about 0.8% cases, that causes death (0.14%). The neonatal complication of PROM is the intrauterine infection, umbilical cord compression, respiratory distress syndrome (RDS), necrotizing enterocolitis, intraventricular bleeding, and sepsis.

One of the endogenous and exogenous factors that correlates with the increasing risk of PROM is the programmed cell death or apoptosis. Apoptotic cell was found in the amnion and chorion layer, especially in the area of the ruptured amniotic membrane, which is known as the paracervical weak zone (Xu and Wang, 2005; Harirah *et al*, 2012; Saglam *et al* 2013). The process that creates the paracervical weak zone other than remodeling process, correlates with the apoptosis process. The apoptotic cell is found higher in the amnion of the PROM cases compared to the pregnant women without PROM, and the apoptotic rate is found highest around the cervical area compared to the fundal area (Kataoka *et al*, 2002; El Khwad *et al*, 2005; Rangaswamy *et al*, 2012).

The apoptosis mechanism occurs within two pathway, which are caspase-dependent and caspase-independent. The caspase-dependent pathway can occurs through intrinsic pathway that is triggered by the failure of mitochondrial metabolism or the extrinsic pathway that is triggered by death receptor. The caspase-independent pathway is triggered by mitochondrial protein such as Apoptosis Inducing Factor (AIF) and Endonuclease G mitochondria (Van Loo e tal, 2001; Elmore, 2007; Ashkenazi *et al*, 2014).

The mechanism of PROM that is caused by genital tract infection, can be caused by extracellular bacterial infection or intracellular obligatory bacteria. The genital tract infection can cause the apoptosis of amnion cell, where the extracellular bacterial infection can undergo the caspase dependent, while the intracellular obligatory bacteria can undergo the caspase independent pathway. In the caspase dependent pathway, we can analyze the protein caspase-3 parameter and the AIF protein in the caspase independent pathway. The study of apoptosis role in the mechanism of PROM through caspase independent pathway is not defined yet (Gao and Kwaik, 2000; Hongmei, 2012; Prabantoro, 2012). There has been no study yet reported about the role of caspase independent pathyway towards the risk of PROM, especially the AIF as the main apoptosis protein that is involved in the caspase independent pathway.

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/$m^{2,}$ and mean BMI of the control group was 24.88 ± 3.87 kg / $m^{2,}$ 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, as shown in Table 1.

To determine the role of AIF 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), as shown in Table 2.

In this study, showed that the expression of positive AIF was a risk factor for premature rupture of membranes of 5.10 times (OR = 5.10 ; CI 95% = 1.86 – 13.96 ; *p* = 0, 001) than the expression of negative AIF.

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 term pregnancies and preterm. 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 Vishwakarma *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 PPROM 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). Okeke *et al* (2014) reported the most cases of PROM was in nullipara (29.1%), parity 2 (26.6%), parity 1 (19%).

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

Risk factors	The case group (n = 36)		Control group (n = 37)		*p*
	Mean	SD	Mean	SD	
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 AIF Expression

		Group		OR	CI 95%	*p*
		Case	Control			
AIF expression	Positive	30	21	5.10	1.86 – 13.96	0.001
	Negative	7	25			

Some studies show that a low body mass index (BMI) before pregnancy may increase the risk of PPROM. PPROM 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/m^2. PPROM has a very close relationship with weight at the time of pregnancy, and a low body mass index before pregnancy (Thombre, 2014)

From the development of some cell death model study, specific caspase inhibitor cannot inhibit the apoptosis that is induced by proapoptosis stimulus, and the activation of caspase is not enough to initiate apoptosis. The excess expression of Bax or Bak induce cell death without involving caspase, and it explains another factor other than caspase that also involved in the cascade of apoptosis. Some of this factor also exist in the mitochondria, such as AIF that can cause condensation of chromatin and the release of cytochrome C when there is no activation of caspase (Perfettini *et al*, 2002; Elmore, 2007).

The apoptosis mechanism through caspase independent does not need caspase mediater, and they have different mechanism towards cell death. The involved apoptosis in the caspase independent is the mitochondria proapoptosis protein molecule, which is Apoptosis Inducing Factor (AIF) and Endonuclease G (Arnoult *et al*, 2003; Elmore, 2007).

In the apoptosis through caspase independent pathway, if the cell is triggered by the apoptosis, then AIF and endonuclease G will translocated from mitochondria to nucleus and causing fragmentation of nuclear DNA. Protein Bcl-2 will inhibit the permeability of the mitochondrial membrane. If the Bcl-2 is inhibited, then the mitochondrial membrane pore will be opened and the AIF will be released. The AIF is one of the mitochondrial protein that will be released into the cytosol during apoptosis, and is found as the first protein that regulate the apoptosis caspase-independent pathway (Cande *et al*, 2002; Damien and Brigitte, 2003; Elmore, 2007).

The mechanism of PROM that is caused by genital tract infection, can be caused by the extracellular bacteria or the intracellular obligatory bacteria. The genital tract infection can cause the apoptosis of the amniotic cell, where the extracellular bacterial infection will go through caspase dependent pathway and the intracellular obligatory bacterial infection will go through caspase independent pathway. The caspase dependent pathway can be seen with the caspase-3 parameter, while the caspase independent pathway can be seen with the AIF parameter. Study about the role of apoptosis in the mechanism of PROM through caspase independent pathway has not been reported yet (Gao and Kwaik, 2000; Hongmei, 2012; Prabantoro, 2012).

By knowing the apoptosis protein as the AIF expression on the amniotic cell, it can be seen the correlation between AIF expression as the parameter of apoptosis which go through caspase independent on the amniotic membrane. As the result, it can explain the role of AIF as the risk factor of PROM through apoptosis caspase independent pathway.

Mitochondria can be induced to release cytochrome C through various stress signal that originates from the inner cell or following the activation of caspase, that is triggered by the surface receptor ligand. The integrity of the outer membrane and the release of cytochrome C from the mitochondria is regulated by the Bcl-2 protein family, which consists of antiapoptotic factor such as Bcl-2 and Bcl-XL and proapoptotic protein such as Bax and Bak. These protein can undergo heterodimerization with one another and interact with mitochondria, where they are as important substance to determine whether the cell will stay alive or go through the cell death. As the result, Bcl-2 protein can prevent apoptosis by prevent the release of protein between mitochondria membrane, including cytochrome C and AIF. On the other hand, Bax will trigger the release of cytochrome C from the mitochondria that causing apoptosis (Perfettini *et al*, 2002).

The latest study shows that different cell death can occurs without activation of caspase. Certain caspase cannot inhibit apoptosis that is triggered by proapoptotic signal, and the activation caspase is not enough to initiate apoptosis. Moreover, the expression of Bax or Bak can induce cell death without caspase involvement. Some of those factors exist in the mitochondria such as AIF, and shows the swelling in the mitochondria, chromatin condensation, and release of cytochrome C without caspase activation (Perfettini *et al*, 2002).

The presence of positive AIF expression in cases with PROM was compared in the case without PROM, with OR 6.60 times (OR = 6.60, 95% CI = 1.48-29.36, p = 0.009) in amnionic epithelial cell . The role of AIF as a risk factor of premature rupture of membranes through the path of apoptosis caspase independent. Protein AIF is a proapoptotic protein from the mitochondria through an independent caspase pathway.

CONCLUSION

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

ACKNOWLEDGEMENT

The authors gratefully acknowledge this study have no financial conflicts of interest.

REFERENCES

1. Adeniji, A.O., Atanda, O.O.A. Interventions and Neonatal Outcomes in Patients with Premature Rupture of Fetal Membranes at and Beyond 34 weeks Gestational Age at a Tertiary Health Facility in Nigeria. *British Journal of Medicine & Medical Research*, **3**(4): 1388-1397 (2013).
2. Arnoult, D., Gaume, B., Karbowski, M., Sharpe, J. C., Cecconi, F., & Youle, R. J. Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax / Bak mediated permeabilization. *The EMBO Journal*, **22**(17), 4385-4399 (2003).
3. Ashkenazi, A., & Salvesen, G. Regulated cell death: signaling and mechanisms. *Annual review of cell and developmental biology*, **30**, 337-356 (2014).
4. Budijaya & Surya Negara. Profil Persalinan Dengan Ketuban Pecah Dini Di RSUP Sanglah Denpasar Periode 1 Januari – 31 Desember 2015, Laporan Penelitian Deskriptif (2016).
5. Candé, C., Cohen, I., Daugas, E., Ravagnan, L., Larochette, N., Zamzami, N., & Kroemer, G. Apoptosis-inducing factor (AIF): a novel caspase-independent death effector released from mitochondria. *Biochimie*, **84**(2), 215-222 (2002).
6. Creasy, R. K., Resnik, R. 2009. Maternal Fetal Medicine, Principle and Practice Sixth Edition, pp. 521-543.
7. Cunningham, F.G., 2010. Preterm Birth. *Obstetri Williams 23rd. The McGraw-Hill Company, New York,* 804-831.
8. Damien, A., Brigitte, G. 2003. Mitochondrial release of AIF and EndoG requires caspase activation downstream of Bax/Bak-mediated permeabilization, *The EMBO Journal* **22**(17) pp. 4385±4399 (2003).
9. El Khwad, M., Stetzer, B., Moore, R. M., Kumar, D., Mercer, B., Arikat, S., ... & Moore, J. J. Term human fetal membranes have a weak zone overlying the lower uterine pole and cervix before onset of labor. *Biology of reproduction*, **72**(3), 720-726 (2005).
10. Elmore, S. Apoptosis: a review of programmed cell death. *Toxicologic pathology*, **35**(4), 495-516 (2007).
11. Endale, T., Fentahun, N., Gemada, D., Hussen, M.A. Maternal and fetal outcomes in term premature rupture of membrane. *World J Emerg Med,* **7**(2):147-152 (2016).
12. Furman,B., Shoham-Vardi, I., Bashiri,A. Clinical Significance and Outcome of Preterm Prelabor Rupture of Membranes: Population-based study. *Eur J Obstet and Gynecol,* **192**: 209-216 (2000).
13. Gahwagi, M.M., Busarira, M., Atia, M. Premature Rupture of Membranes Characterictics, Determinants, and Outcomes of in Benghazi, Libya. *Open Journal of Obstetrics and Gynecology,* 5: 494-504 (2015).
14. Gao, L. Y., & Kwaik, Y. A. The modulation of host cell apoptosis by intracellular bacterial pathogens. *Trends in microbiology*, **8**(7), 306-313 (2000).
15. Getahun, D., Stricland, D., Ananth, C., Fasseth, M., Kirby, S., Jacobsen,S., Recurrent Of Preterm Rupture Of Membranes In Relation To Interval Between Pregnancies, *American Journal of Obstetrics And Gynaecology*, United State of America, **220**, pp. 570.e1-6 (2010).
16. Harirah, H. M., Borahay, M. A., Zaman, W., Ahmed, M. S., & Hankins, G. D. Increased Apoptosis in Chorionic Trophoblasts of Human Fetal Membranes with Labor at Term. *International journal of clinical medicine*, **3**(2): 136 (2012).
17. Hongmei, Z. Extrinsic and intrinsic apoptosis signal pathway review. *INTECH Open Access Publisher* (2012).
18. Kataoka, S., Furuta, I., Yamada, H., Kato, E. H., Ebina, Y., Kishida, T., ... & Fujimoto, S. Increased apoptosis of human fetal membranes in rupture of the membranes and chorioamnionitis. *Placenta*, **23**(2), 224-231 (2002).
19. Menon, R., & Fortunato, S. J. Infection and the role of inflammation in preterm premature rupture

of the membranes. *Best practice & research Clinical obstetrics & gynaecology*, **21**(3), 467-478 (2007).

20. Noor, S., Nazar, A. F., Bashir, R., & Sultana, R. Prevalance of PPROM and its outcome. *J Ayub Med Coll Abbottabad*, **19**(4), 14-7 (2007).
21. Okeke, T.C., Enwereji, J.O., Okoro, O.S., Adiri, C.O., Ezugwu, E.C., Agu, P.U. The Incidencee and Management Outcome of Preterm Premature Rupture of Membranes (PPROM) in a Tertiary Hospital in Nigeria. *American Journal of Clinical Medicine Research,* **2**(1):14-17 (2014).
22. Patil S., Patil V. Maternal and foetal outcome in premature rupture of membranes. *IOSR Journal of Dental and Medical Sciences,* **13**(12):56-83 (2014).
23. Perfettini, J. L., Hospital, V., Stahl, L., Jungas, T., Verbeke, P., & Ojcius, D. M. Cell death and inflammation during infection with the obligate intracellular pathogen, Chlamydia. *Biochimie*, **85**(8): 763-769 (2003).
24. Prabantoro, B. T. R., Prabowo, P., Mertaniasih, N. M., & Rantam, F. A. Peran Endonuclease-G sebagai Biomarker Penentu Apoptosis Sel Amnion pada Kehamilan dengan Ketuban Pecah Dini (2011).
25. Rangaswamy, N., Mercer, B. M., Kumar, D., Moore, J. J., Mansour, J. M., Redline, R., & Moore, R. M. Weakening and Rupture of Human Fetal Membranes-Biochemistry and Biomechanics. *INTECH* Open Access Publisher (2012).
26. Saglam, A., Ozgur, C., Derwig, I., Unlu, B. S., Gode, F., & Mungan, T. The role of apoptosis in preterm premature rupture of the human fetal membranes. *Archives of gynecology and obstetrics*, **288**(3): 501-505 (2013).
27. Singh, D., Usham, R., Kamel H. Preterm Prelabour Rupture of Membrane:1 Year Study. *Journal of Evolution of Medical and Dental Sciences,* **4**(49): 8495-8598 (2015).
28. Thombre. A Review Of The Etiology Epidemiology Prediction And Interventions Of Preterm Premature Rupture Of Membranes, Thesis (2014).
29. Van Loo, G., Schotte, P., Van Gurp, M., Demol, H., Hoorelbeke, B., Gevaert, K., ... & Vandenabeele, P. Endonuclease G: a mitochondrial protein released in apoptosis and involved in caspase-independent DNA degradation.*Cell death and differentiation*, **8**(12), 1136-1142 (2001).
30. Vishwakarma, K., Patel, S.K., Yadav, K., Pandey, A. Impact of premature rupture of membranes on maternal & neonatal health in Central India*. Journal of evidence based medicine and healthcare,* **2**(49): 8505-8508 (2015).
31. Xu, J., & Wang, H. L. Role of Caspase and MMPs in Amniochorionic during PROM. *Journal of Reproduction & Contraception*, **16**(4), 219-224 (2005).