

The Beta hCG level in the Vaginal Washing Fluid as a Predictor of Premature Rupture of Membrane

Danny Aguswahyudi¹, I Ketut Surya Negara², Ketut Suwiyoga³, I Wayan Megadhana⁴,
I Nyoman Bayu Mahendra⁵, I Gusti Putu Mayun Mayura⁶, Made Bagus Dwi Aryana⁷

^{1,2,3,4,5,6,7}Obstetrics and Gynecology Sanglah General Hospital Udayana, Denpasar, Bali, Indonesia

Abstract: ***Aim:** This study aims to determine the relationship between beta-hCG level in vaginal washing fluid with the incidence of premature rupture of membranes (PROM) and the cut-off value of beta-hCG level in vaginal washing fluid in predicting the occurrence of PROM. **Materials and Methods:** This diagnostic study was carried out in the delivery room of the Emergency Ward, Sanglah Hospital, Denpasar from May 2020 to July 2020. The vaginal wash was taken after delivery and then examined at the Clinical Pathology Laboratory, Udayana University/ Sanglah General Hospital Denpasar Bali. **Results:** Thirty-five subjects that had met the inclusion and exclusion criteria were involved in this study. The cut-off value of beta-hCG vaginal washing fluid to predict PROM obtained in this study was 118.1mIU/ml with sensitivity of 95.83%, specificity of 81.81%, positive predictive value of 92.00%, negative predictive value of 90.00%, and accuracy of 91.43%. **Conclusions:** Beta-hCG level in the vagina washing fluid can be used as a predictor of PROM incident. **Clinical Significance:** The beta-hCG level may be used as a new, fast, non-invasive, promising diagnostic indicator in PROM, thus early prevention and management may be conducted to reduce the morbidity and mortality caused by PROM.*

Keywords: Beta-hCG, diagnostic test, premature rupture of membrane

1. Introduction

Premature rupture of membrane (PROM), defined as the spontaneous rupture of the fetal membranes before the onset of labor, still becomes a problem related to the prevalence of both maternal and perinatal morbidity and mortality.¹ The prevalence of PROM varies from 3–18% of all pregnancies. The incidence of PROM in term pregnancy is approximately 8-10%, while in preterm pregnancy (known as the preterm premature rupture of membrane or PPRM) is around 30-40% or 1,7% of all pregnancies. PROM is reported to recur in subsequent pregnancies with a recurrence estimation of 21-32%.² PROM is also strongly associated with an increased risk of infection of both the mother and fetus.³

The diagnosis of PROM is made through anamnesis, physical and obstetrics examination, nitrazine or ferning test, or ultrasonography (USG) examination.¹ However, those clinical examinations became less accurate in PROM cases that occur more than 1 hour before examinations. Thus, clinical examination still has a weakness in accurately diagnosing PROM.⁴ An accurate diagnostic method by injecting a dye into the amnion is invasive, therefore it is not routinely performed.³ Non-invasive diagnostic methods, such as biochemical markers, may help in diagnosing PROM.

Several biochemical markers of PROM have been studied, including prolactin, insulin-like growth factor binding protein-1, alpha-fetoprotein (AFP) diamine-oxidase, dan fetal fibronectin. However, those biomarkers are not effective to be applied as diagnostic tools due to their cost and complexity.³ In contrast; Beta-human chorionic gonadotropin (beta-hCG) is thought to be a more accurate marker of PROM, especially in cases with the ruptured membrane in peripheral or covert areas.⁵

Beta-hCG is a glycoprotein produced by the placental syncytiotrophoblast that can be found in the amniotic fluid, maternal blood, and urine. The level of beta-hCG varies

depending on the gestational age, reaching its highest concentration in the maternal serum during the 8th to 12th week of gestational age with a concentration of $53,715 \pm 3574$ mIU/ml.⁵ Temel et al. (2013) reported that the beta-hCG level of vaginal washing fluid can be used as the predictor of PROM with the sensitivity and specificity of 72,1% and 100%, respectively. However, the cut-off value to determine the incidence of PROM is not yet established.³ Another study by Dartibale et al. (2017) reported a similar result, where the beta-hCG level in vaginal washing fluid has the sensitivity and specificity of 44,4% and 87,1%, respectively in predicting PROM incidence.⁴ Therefore, this study is conducted to determine the relationship between beta-hCG level in vaginal washing fluid with the incidence of PROM and the beta-hCG cut-off value in vaginal washing fluid in predicting the occurrence of PROM.

2. Materials and Methods

This diagnostic test study was conducted at the Delivery room in the Emergency Ward Sanglah General Hospital, Bali, Indonesia and the Clinical Pathology Laboratory, Udayana University/Sanglah General Hospital Denpasar Bali from May to July 2020. The subjects involved in this study are all pregnant women treated in the Maternity Room of Sanglah General Hospital, chosen through consecutive sampling, during the period of study that fulfill the study criteria. The inclusion criteria are pregnant women with ≥ 24 weeks of gestational age who had complaints of fluid leakage from the vagina, has a normal fetal development from the USG examination based on the first day of the mothers' last menstrual period, willing to participate as the research subjects, and signed the informed consent.

PROM is defined as the spontaneous rupture of membrane without any sign of labor (signs of labor include regular, painful uterine contraction that causes effacement or dilation of the cervix) or if one hour later there are no early signs of labor or if clinically there is a cervical dilation of less than 3 cm in primigravida and less than 5 cm in multigravida. The

gold standard of PROM diagnosis used in this study is the examination of amniotic fluid pooling with a sterile speculum with or without the valsava maneuver and the nitrazine test.³

The beta-hCG is quantitatively measured from the vaginal washing sample with the immunoassay method. The isolation of vaginal fluid was done by irrigating the posterior fornix of the vagina with 5ml of sterile saline fluid after confirming that there is no bloody discharge, urine, semen, or meconium. The vaginal fluid was then aspirated using the same syringe used for irrigation. The sample was sent to the laboratory for beta-hCG level analysis. Beta-hCG level of >100 mIU/ml is considered to support the diagnosis of PROM. Other variables such as maternal age, parity, and body mass index (BMI) are gathered from the questionnaire filled by the examinee during hospital admission.

All of the subjects are treated according to the Clinical Practice Guideline of the Sanglah Hospital Obstetrics and Gynecology Department. The subjects are divided into two groups based on the level of beta-hCG in the vaginal washing fluid; >118.1mIU/ml group and ≤118.1mIU/ml group. The cut-off was obtained based on Receiver Operating Curve (ROC) analysis. The result from the nitrazine test (gold-standard) and beta-hCG level is then compared. Subjects with >118.1 mIU/ml beta-hCG level and positive nitrazine test are categorized as PROM, while subjects with ≤118.1 mIU/ml beta-hCG level and negative nitrazine test are categorized as non-PROM.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) for windows version 22.0 software. The subjects' characteristics were analyzed using descriptive analysis and then described in graphs and tables. Shapiro Wilk test was used to analyze the data distribution. The ROC curve analysis was performed to determine the

beta-hCG level in vaginal washing cut-off point that can predict the incidence of PROM. A 2x2 table is then made to determine the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the beta-hCG level in vaginal washing fluid.

3. Results

This study included 35 pregnant women in the second or third trimester, consist of 24 subjects with PROM and 11 subjects without PROM. There was no significant difference found in the characteristics of age, parity, and BMI between subjects with PROM and subjects without PROM (Table 1).

Table 1: The Characteristics of Subjects

Variables	PROM group (n = 24)	Non-PROM group (n = 11)	P value
Age (years), mean ±SD	30.3 ± 4.3	29.7 ± 3.9	0.714
Parity, median (IQR)	1.0 (1.75)	1.0 (2.0)	0.316
BMI (kg/m ²), median (IQR)	27.1 (3.6)	25.8 (3.9)	0.299

The median of beta-hCG level in the vaginal washing fluid found in this study was 255.3 mIU/ml and 71.2 mIU/ml for subjects with PROM and subjects without PROM respectively. It was clear that there was a significant difference in the beta-hCG level found in vaginal washing fluids between the two subject groups. Based on the ROC curve analysis (Figure 1), the optimal cut-off value of beta-hCG level in the vaginal washing fluid to predict the incidence of PROM is 118.1mIU/ml. At this cut-off value, the sensitivity was found to be 95.83%, specificity 81.81%, PPV 92.00%, NPV 90.00%, and diagnostic accuracy 91.43% (Table 2). This showed that the beta-hCG level of 118.1mIU/ml in the vaginal fluid was a good predictor of PROM incidence in pregnant women.

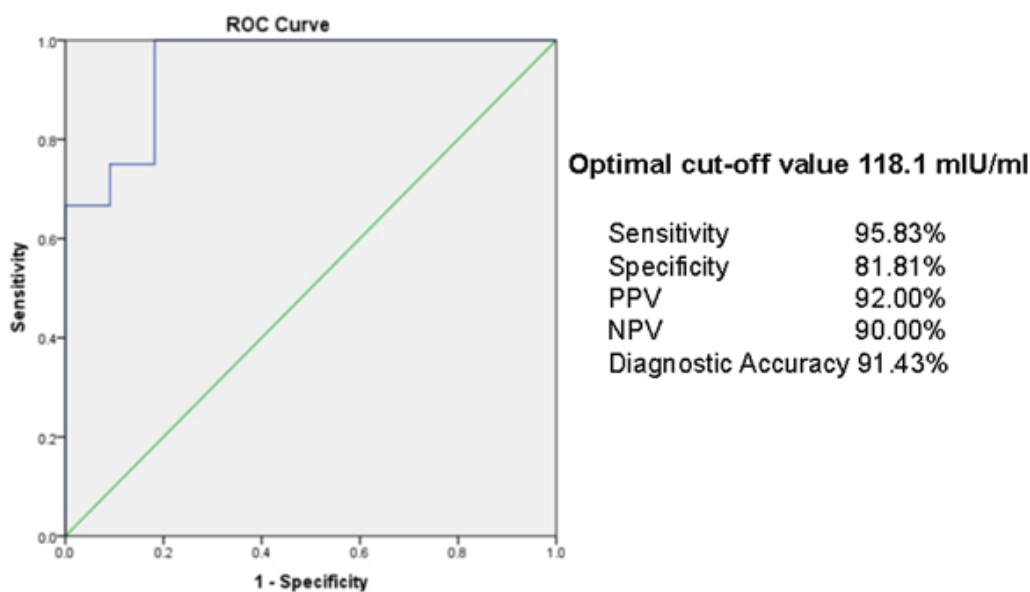


Figure 1: ROC curve analysis

Table 2: The Diagnostic Accuracy of Beta-hCG Level as a Predictor of PROM

Beta-hCG Level	Groups	
	PROM	Non-PROM
High (>118.1 mIU/ml)	23	2
Low (\leq 118.1 mIU/ml)	1	9

4. Discussion

The incidence of PROM usually found in pregnant women age <20 years or 35 years and older. In this study, we found that the mean age of PROM and non-PROM patients were similar (30.3 years and 29.7 years, respectively). Therefore, the finding in this study was not consistent with the theory of PROM incidence where age is a risk factor of PROM. This may be due to the trend of marriage at a relatively younger age in Indonesia so that the findings of this study differ from other studies conducted in other countries that may have different cultures.⁵ All of the subjects in this study have 1 parity or primipara. There was no multiparity found in this study. The median BMI in both groups were in the overweight range (27.1 and 25.8 kg/m², respectively). However, three cases with obesity were exclusively found in the subjects with PROM. This was consistent with the theory where obesity is a factor risk of PROM.³

The beta-hCG level in the vaginal fluid found of the subjects with PROM in this study was 255.3 mIU/ml. This value far exceeds the cutoff value used in previous studies. Thus, this proving that high beta-hCG values can be a risk factor for causes or factors that support the occurrence of PROM. According to the theory, the incidence of PROM is influenced by increases based on gestation week^{7,8}, whether or not it has been confirmed⁹, has a subtle leak or not when examining the speculum¹⁰, related to prematurity or not¹¹, physical fatigue¹², history of coitus¹³, and the presence or absence of infection¹⁴.

Amniotic fluid is derived from fetal and possibly maternal compartments. In the first half of the pregnancy, more amniotic fluid is produced from the diffusion of fluids and solute from the maternal plasma through the chorionic

membrane and also the amnion. Therefore, the studies state that intearly pregnancy, the results of dialysis of amniotic fluid and maternal plasma are identical, except a lower protein level is found in the amniotic fluid. In the second half of the pregnancy, when the fetal skin has keratinized and fetal kidney function has well developed, the production of amniotic fluid will be dominated by the fetus.¹⁵ Beta-hCG is an important hormone in pregnancy that is exclusively produced by trophoblast cells in the chorionic tissue. Because the amniotic fluid is formed from the diffusion of placental plasma through the amniotic membrane and the chorion, the beta-hCG hormone may also be found in amniotic fluid. In addition, this hormone can also be found in maternal blood and urine.^{11,16} During pregnancy, the levels of this hormone in the amniotic fluid vary, ranging from 2,000-70,000 mIU / ml where this hormone begins to be produced at the 3rd and 4th week of gestation and reaches its peak at the 8th week to the 12th week of gestation.^{11,16,17}

The optimal cut-off value of beta-hCG level found in vaginal washing fluid in this study was 118.1 mIU/mL, with a sensitivity of 95.83%, a specificity of 81.81%, a PPV of 92%, a NPV of 90% and a diagnostic accuracy of 91.43%. This is consistent with a study conducted by Jain et al in 2020 where a sensitivity of 100% and a specificity of 92% was obtained when examining beta hCG levels associated with PROM, but the cut-off value of beta-hCG in this study was 25mIU/mL.¹⁰ Other studies conducted by Temel et al. in 2013 also support the correlation between beta-hCG levels and the incidence of PROM, where the results of the study found a sensitivity of 71.2% and specificity of 100% at the cut off value of beta hCG levels of 100 mIU/ml.³ Therefore, the beta-hCG level found in the vaginal washing fluid has consistently been shown to be a good predictor for the incidence of PROM. However, the exact optimal cut-off value of the beta-hCG level to predict the incidence of PROM in pregnant women was still uncertain due to a large variation in the cut-off value between studies (see Table 3). The cut-off value of beta-hCG might differ between studies because of racial/genetic differences and many other factors that may contribute to the risk factors of PROM.

Table 3: The Variation of Beta hCG Level Cutoff Value in Predicting the Incidence of Premature Rupture of Membranes

Beta-hCG Level						Authors (Years)	Study Location
Cut-off (mIU/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)		
118.1	95.83	81.81	92.0	90	91.43	This study	Denpasar, Indonesia
65	68	95	82	90	87	Esim et al. (2003) ⁹	Instambul, Turki
39.8	95.5	94.7	91.3	97.3	–	Kim et al. (2005) ¹¹	Seoul, South Korea
100	71.2	100	100	65.1	–	Temel et al. (2013) ³	–
21.5	100	92	92.6	100	96	Jain et al. (2020) ¹⁰	New Delhi, India
19	94.5	91	91.5	94.2	92.2	Kariman et al. (2011) ⁷	Tehran, Iran
20	83	100	100	85.6	91	Osman & Elghazaly (2014) ¹⁶	Cairo, Egypt

5. Conclusion

Beta-hCG is a good predictor for the incidence of PROM. The optimal cut-off value of beta-hCG level found in vaginal washing fluid in this study was 118.1 mIU/mL, with a sensitivity of 95.83%, a specificity of 81.81%, a PPV of 92%, a NPV of 90%, and a diagnostic accuracy of 91.43%.

6. Clinical Significance

The beta-hCG level may be used as a new, fast, non-invasive, promising indicator in predicting the incidence of PROM. Thus, early prevention and management may be conducted to reduce the morbidity and mortality caused by PROM.

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