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The beta Human Chorionic Gonadotropin (hCG) levels in the vaginal washing fluid as a predictor of Premature Rupture of Membrane (PROM)



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Danny Aguswahyudi^{1*}, Ketut Suwiyoga², I Ketut Surya Negara³, I Wayan Megadhana⁴,
I Nyoman Bayu Mahendra², I Gusti Putu Mayun Mayura², Made Bagus Dwi Aryana⁵

¹Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

²Gynecologic Oncology Division, Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

³Maternal-Fetal Medicine Division, Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

⁴Urogynecology Division, Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

⁵Social Obstetrics and Gynecology Division, Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

*Corresponding author:

Danny Aguswahyudi;
Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia;

dr.danny.aguswahyudi@gmail.com

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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 21-32% recurrence estimation.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 procedures, such as biochemical markers, may help diagnose PROM.

Ward, Sanglah General Hospital. The beta-hCG level was evaluated using the immunoassay method and 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. Data were analyzed using SPSS version 22 for Windows.

Results: Thirty-five subjects who 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 118.1 mIU/ml with sensitivity of 95.83%, specificity of 81.81%, PPV of 92.00%, NPV of 90.00%, and accuracy of 91.43% (AUC: 0.947; 95%CI: 0.870-1.000; p<0.001).

Conclusion: Beta-hCG level in the vagina washing fluid can be used as a fast and non-invasive predictor of PROM in pregnant women.

ABSTRACT

Background: Several biomarkers have been investigated as predictors of Premature rupture of membrane (PROM), but these biomarkers have not been applied in clinical practice due to their high cost and complexity. Beta-hCG is thought to be an easy, cost-effective and promising predictor of PROM in clinical practice. This study aims to determine the relationship between beta-hCG level in vaginal washing fluid with the PROM and the cut-off value of beta-hCG level in vaginal washing fluid in predicting the incidence of PROM.

Methods: This diagnostic study was conducted in the Sanglah General Hospital, Denpasar, from May to July 2020. The subjects involved in this study are all pregnant women with gestational age ≥ 24 weeks who came with complaints of vaginal discharge and were treated in the delivery room of the Emergency

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

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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 could 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 CB et al., 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 Based on those mentioned above, this study aims 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.

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, Universitas Udayana, 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 fulfills the study criteria. The inclusion criteria were pregnant women with 24 weeks of gestational age who had complaints of fluid leakage from the vagina, had 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. The exclusion criteria were multiple pregnancies, congenital malformation on the fetus, cervical dilatation >2 cm, vaginal bleeding or placenta previa, and vaginal contamination by feces and urine.

PROM is defined as the spontaneous rupture of the 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 Valsalva maneuver and the nitrazine test.³

The beta-hCG is quantitatively measured from the vaginal washing sample and was analyzed using Chemiluminescent Microparticle Immunoassay (CMIA), a two-step immunoassay method (Alinity I Total β -hCG Reagent Kit). 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. A 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 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 \leq 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. In comparison, subjects with \leq 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) version 22.0 for Windows. The subjects' characteristics were analyzed using descriptive analysis and then described in graphs and tables. The Shapiro Wilk test was used to analyze the data distribution. The ROC curve analysis was performed to determine the beta-hCG level in the 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.

RESULTS

This study included 35 pregnant women in the second or third trimester, 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).

The median beta-hCG level in the vaginal washing fluid found in this study was 255.3 (15.2) mIU/ml and 71.2 (9.6) 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 ($p=0.000$) (Table 1). Based on the ROC curve analysis, the optimal cut-off value of beta-hCG level in the vaginal washing fluid to predict the incidence of PROM is 118.1 mIU/ml with Area Under Curve (AUC) 0.947 (95%CI 0.870-1.000; $p<0.001$) (Figure 1 and Table 2). 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). The comparison of beta-hCG cut-off value in predicting PROM was depicted in Table 3.

Table 1. The baseline characteristics of subjects.

Variable	PROM (n=24)	Non-PROM (n=11)	p
Age (Years), mean \pm SD	30.3 \pm 4.3	29.7 \pm 3.9	0.714 ^a
Parity, Median (IQR)	1.0 (1.75)	1.0 (2.0)	0.316 ^b
BMI (kg/m ²), Median (IQR)	27.1 (3.6)	25.8 (3.9)	0.299 ^b
Beta-hCG, Median (IQR)	255.3 (15.2)	71.2 (9.6)	0.000 ^b

BMI: Body Mass Index; PROM: Premature Rupture of Membrane; ^aIndependent T-Test; ^bMann-Whitney U Test; *Statistically significant if p-value less than 0.05

DISCUSSION

The incidence of PROM is usually found in pregnant women aged <20 years or 35 years and older. In this study, we found that PROM and non-PROM patients' mean age was similar (30.3 ± 4.3 years and 29.7 ± 3.9 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 marriage at a relatively younger age in Indonesia. 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 was 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

In this study, the beta-hCG level in the vaginal fluid subjects with PROM was 255.3 mIU/ml. This value far exceeds the cut-off value used in previous studies. Thus, this proves 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, whether or not it has been confirmed, has a subtle leak or not when examining the speculum, related to prematurity or not, physical fatigue, history of coitus, and the presence or absence of infection.⁷⁻¹⁴

Amniotic fluid is derived from fetal and possibly maternal compartments. In the first half of the pregnancy, more amniotic fluid is produced from diffusion and solute from the maternal plasma through the chorionic membrane and the amnion. Therefore, the studies state that the dialysis results of amniotic fluid and maternal plasma in early pregnancy 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 fetus's production of amniotic fluid will dominate.¹⁵ Beta-hCG is an important hormone in pregnancy exclusively produced by trophoblast cells in the chorionic tissue. Because the amniotic fluid is formed from the diffusion of placental plasma through the

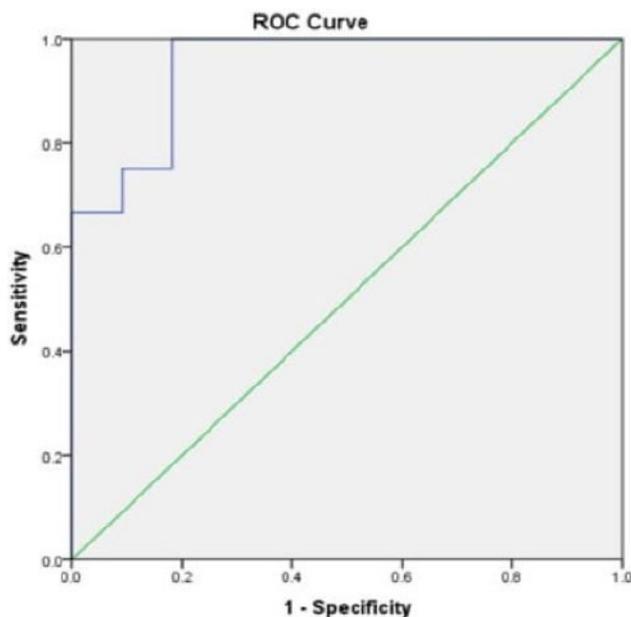


Figure 1. ROC curve analysis of beta-hCG level found in vaginal washing fluids between the two subject groups.

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

Beta-hCG Level (mIU/ml)	Groups (N=35)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95%CI	p
	PROM (N=24)	Non-PROM (N=11)							
High (>118.1), n (%)	23 (92.00)	2 (8.00)	95.83	81.81	92.00	90.00	0.947	0.870-1.000	<0.001*
Low (≤118.1), n (%)	1 (10.00)	9 (90.00)							

PROM: Premature Rupture of Membrane; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUC: Area Under Curve; CI: Confidence Interval; *Statistically significant if p-value less than 0.05.

Table 3. The comparison of beta-hCG level cut-off value in predicting the incidence of premature rupture of membranes.

Cut-off (mIU/ml)	Beta-hCG Level					Authors (Years)	Study Location
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)		
118.1	95.83	81.81	92.0	90	91.43	Recent study	Denpasar, Indonesia
65	68	95	82	90	87	Esim E et al ⁹	Istanbul, Turki
39.8	95.5	94.7	91.3	97.3	-	Kim YH et al ¹¹	Seoul, South Korea
100	71.2	100	100	65.1	-	Temel et al ³	-
21.5	100	92	92.6	100	96	Jain A et al ¹⁰	New Delhi, India
19	94.5	91	91.5	94.2	92.2	Kariman et al ⁷	Tehran, Iran
20	83	100	100	85.6	91	Osman OM et al ¹²	Cairo, Egypt

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.^{11,12} 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.^{11,12,16}

The optimal cut-off value of beta-hCG level found in vaginal washing fluid in this study was 118.1 mIU/mL, with the sensitivity of 95.83%, specificity of 81.81%, PPV of 92%, NPV of 90% and diagnostic accuracy of 91.43%. This is consistent with a study conducted by Jain A et al. in 2020, where a sensitivity of 100% and a specificity of 92% were obtained when examining beta hCG levels associated with PROM, but the cut-off value of beta-hCG in this study was 25 mIU/mL.¹⁰ Other studies conducted by Temel O et al. in 2013 also support the correlation between beta-hCG levels and the incidence of PROM. The study results 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.^{3,17} 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 in 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.

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. 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.

CONFLICT OF INTEREST

The authors of this study had research support from Clinical Pathology Laboratory, Universitas Udayana, Sanglah General Hospital, Denpasar, Bali, to analyze the vaginal wash.

ETHICS CONSIDERATION

This research has been approved by ethical feasibility according to the ethical number 816/UN14.2.2.VII.14/LT/2020 published by the Research Ethics Committee of the Faculty of Medicine, Universitas Udayana, Sanglah General Hospital, Bali, Indonesia.

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AUTHOR'S CONTRIBUTION

All authors help each other make, formulate, and complete the manuscript. All authors read and approved the final manuscript.

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