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# Quantitative and Qualitative Measurement of Vaginal Fluid β-HCG for the Diagnosis of Premature Rupture of Membranes

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**ABSTRACT**

**Background:** Premature rupture of membranes (PROM) is known as one of the leading causes of morbidity and mortality among pregnant women. So, the aim of this study was to evaluate the diagnostic value of quantitative and qualitative vaginal fluid β-HCG for the detection of premature rupture of membranes.

**Methods:** One hundred pregnant women with gestation age between 28 and 37 weeks were divided into three groups: confirmed PROM group (34 cases), suspected of PROM (36 cases), and intact membranes (30 cases). Those pregnant women with positive pooling tests were considered as confirmed PROM, while those with the decreased amniotic fluid index, positive nitrazine, or ferning were included in suspected PROM group. Notably, pregnant women with no complaints or complications were enrolled as the control group. Five ml sterile normal saline was injected into the posterior fornix of vagina, vaginal fluid was aspirated, and β-HCG measurement was then performed both quantitatively and qualitatively using ELISA and baby check kits, respectively.

**Results:** The cut-off value for quantitative β-HCG was determined as 66 mIU/m. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of quantitative β-HCG were calculated as 97.05%, 100%, 100%, 96.77%, and 98.43%, respectively. Besides, the qualitative vaginal washing fluid β-HCG using Baby check with the threshold value of 25 mIU/ml had 94.11% sensitivity, 93.33% specificity, 94.11% PPV, 93.33% NPV, and 93.75% accuracy.

**Conclusion:** Quantitative and qualitative vaginal washing fluid β-HCG measurements are accurate, reliable, and useful tests for the diagnosis of PROM.

## Introduction

Premature rupture of membranes (PROM) refers to rupture of chorioamniotic membranes at any time before 37 weeks of gestational age prior to the onset of delivery[1,2], which complicates about 2-25% of all pregnancies[3], and consequently leads to neonatal mortality and serious morbidities for both mother and fetus. Correspondingly, it is responsible for about 30-40% of preterm deliveries that might be accompanied with fetal pulmonary hypoplasia, neurological disabilities, and fetal deformities.[4] Therefore, prompt and accurate diagnosis of PROM has a critical significance in reducing its unpleasant outcomes and preventing unnecessary hospitalization, antibiotic therapy or labor induction.[3,5] In this regard, it is easy to diagnose PROM when the rupture is obvious and when amniotic fluid is identified in posterior fornix using traditional tests.[6]

On pooling test, clear amniotic fluid can be observed by performing sterile speculum examination of cervix that confirms PROM, while alkaline PH of vagina determined by nitrazine paper suggests membranes rupture.[7,8] Nevertheless, their false negative results increased after 48 hours.[3,9] Moreover, it was shown that the contamination of vaginal fluid with blood, semen, antiseptics, alkaline urine, vaginitis, and cervicitis may lead to false positive results of nitrazine test.[10] The detection of characteristic ferning pattern by microscopic examination of dried vaginal fluid also is an indicator of PROM diagnoses.[11] Even though the measurement of amniotic fluid index and the presence of oligohydramnios are useful in this field, they are insufficient for the diagnosis of PROM.[12] Furthermore, intraamniotic dye infusion as a "gold standard" test, is known as an invasive, costly method, which can also be associated with multiple side effects.[13] Therefore, it is essential to find a reliable and practical alternative test for PROM diagnosis.

In this study, we aimed to assess the diagnostic performance of qualitative and quantitative vaginal washing fluid beta-human chorionic gonadotropin (β-HCG) in PROM among pregnant women with gestational age between 28 to 37 weeks.

## Materials and Methods

This cross-sectional observational study was conducted at Imam Khomeini Hospital in Ahvaz, Iran. The current study was conducted according to Helsinki Declaration and approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (ETH-284). A total of 70 pregnant women with gestational age between 28 to 37 weeks who were referred to the obstetrics clinic or midwifery emergency department of this hospital with complaints of vaginal fluid leakage, were enrolled in this study. Notably, pregnant women with a known underlying disease, fetal abnormalities, any amount of vaginal bleeding, a history of intercourse the previous night, the presence of meconium in the amniotic fluid, regular uterine contractions, the use of vaginal medication during the last week or a clear vaginal infection were excluded from the present study.

At first, a vaginal examination was performed using a sterile speculum in a lithotomy position (pooling test). Thereafter, those patients with obvious amniotic fluid leakages were placed in group 1. While the patients with no clear amniotic fluid leakage from the cervix nor the accumulation of fluid in the speculum were placed in group 2. Furthermore, pregnant women without any amniotic fluid leakage complain and intact membrane in pooling test were enrolled in the control group. Subsequently, sampling was done from posterior fornix discharges through the cotton tip of an applicator. Afterward, the applicator was smeared on nitrazine paper. The test's result was then considered as positive if the paper showed a PH value of more than 6.5.

In a similar method, a slide was prepared from cervicovaginal secretions. After performing the drying process, it was viewed by light microscope (10 magnification).

**Table 1.** Baseline Characteristics of the Study Participants

| | PROM group (n = 34) | Suspected PROM group (n = 36) | Control group (n = 30) | P |
|---|---|---|---|---|
| Age, yrs. (mean ± SD) | 21.64 ± 3.89 | 21.13 ± 4.84 | 20.86 ± 5.15 | n.s |
| Gestational age, (mean ± SD) | 32.38 ± 2.26 | 32.55 ± 2.28 | 31.9 ± 2.15 | n.s |
| Gravidity (mean ± SD) | 1.88 ± 1.14 | 1.88 ± 1.03 | 1.88 ± 1.14 | n.s |
| Previous abortion, n (%) | 4 (11.76) | 4 (11.11) | 5 (16.6) | n.s |
| History of PROM, n (%) | 4 (11.76) | 3 (8.33) | 2 (6.66) | n.s |

In addition, the presence of the fern pattern indicated a positive fern test.

In the last step, five ml of normal saline was poured into the patient's posterior fornix, and after 3 minutes, 5 ml of fluid was aspirated with the same syringe. Next, two ml of the obtained samples were used for β-HCG titration using ELISA kit, whereas the remaining 2 ml of the samples were consumed for performing β-HCG qualitative test by the use of Baby Check bar with the threshold of 25 mIU/ml. Ultrasonographic measurement of amniotic fluid index (AFI) was also performed for all the included participants and the results were reported as normal or the decreased amniotic fluid.

***Statistical analysis:*** In the present research, all statistical analyses were conducted using SPSS version 17.0. Student's t-test was also used to compare vaginal fluid β-HCG values between these two groups. Afterward, One-way ANOVA test was done to assess the differences among the three groups. Moreover, in order to analyze non-parametric data, Kruskal-Wallis was performed. Subsequently, receiver operating characteristics (ROC) curve was used to determine a cut off value for β-HCG in predicting PROM. In this regard, P values less than 0.05 were considered as statistically significant. To assess the diagnostic value, sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of each test were separately calculated.

## Results

A total of 100 pregnant women were studied in this research. Group 1 included 34 patients with positive pooling test's results. Accordingly, they were considered to have a definite PROM. Group 2 contained 36 pregnant women with negative pooling test's results, but positive fern test, nitrazine test, or decreased amniotic fluid index. Finally, 30 healthy pregnant women with intact membrane in pooling test were also enrolled in control group. The baseline characteristics of the study participants are summarized in Table 1. There were no significant differences among the three groups in terms of age, gestational age, gravidity, number of abortions, and PROM in their previous pregnancies. Of the total participants of this study, 49 pregnant women were primigravida, 25 women were secundigravida, 20 cases were tertigravida, and 6 cases were in their fourth pregnancies or above.

Overall, 51 subjects (51%) had positive nitrazine test's results, while fern test was reported as positive in 49 subjects (49%). By performing sonographic evaluation, it was indicated that the amniotic fluid index was normal in 43 women (43%) as opposed to 57 (57%) women with the decreased amniotic fluid index. Correspondingly, the diagnostic values of these three methods are compared in Table 2.

**Table 2.** Diagnostic Performance of Nitrazine Test, Fern Test, and Amniotic Fluid Index

| | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| Nitrazine test | 82.35% | 93.33% | 93.33% | 82.35% | 87.50% |
| Fern test | 79.41% | 93.33% | 93.10% | 82.35% | 85.93% |
| Amniotic fluid index | 100% | 100% | 100% | 100% | 100% |

**Table 3.** Diagnostic Performance of Qualitative and Quantitative β-hCG

| | Cut-off value | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|
| Qualitative β-HCG test | NA* | 94.11% | 93.33% | 94.11% | 93.33% | 93.75% |
| Quantitative β-HCG test | 66 mIU/ml | 97.05% | 100% | 100% | 96.77% | 98.43% |

* NA: not applicable

Qualitative β-HCG was reported as positive in 61 participants (61%). Moreover, the mean value of quantitative β–HCG was 357 mIU/ml in the PROM group, 158 mIU/ml in the suspected PROM group, and 6 mIU/ml in the control group. Using the ROC curve (Figure 1), the cut-off value for vaginal discharges quantitative β-HCG for the diagnosis of PROM was calculated as 66 mIU/ml. By considering this cut-off value, 33 women from the PROM group, 26 cases from the suspected PROM group, and only one patient from the control group had positive results. In this regard, the comparison between quantitative β-HCG and qualitative β-HCG is presented in Table 3.

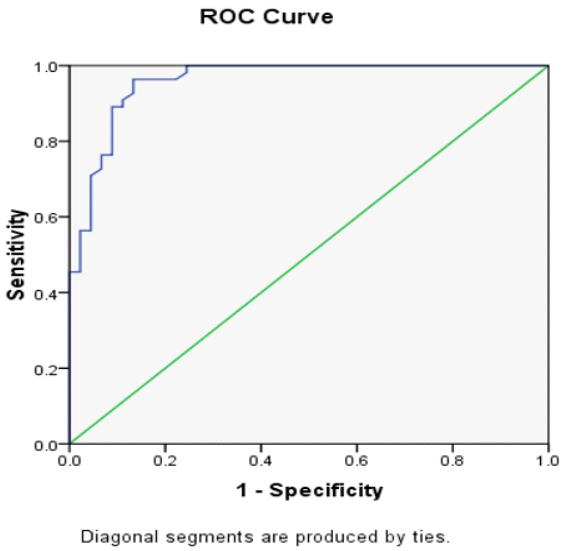


**Figure 1.** ROC Curve to Determine Cut Off Value for Quantitative Measurement of β-HCG

## Discussion

During recent decades, several studies have been conducted to find a practical, noninvasive "gold standard" test for the definite diagnosis of PROM.[6] However, despite using traditional tests, its diagnosis remains equivocal in about 10% of PROM cases.[4] Moreover, some alternative suggestive biomarkers such as insulin like growth factor binding protein-1[14], alpha-fetoprotein[15], prolactin[16], fetal fibronectin[17], and placental alpha macroglobulin-1[18] due to their cost and complexity and regardless of their accuracy, are not considered as popular biomarkers.[19] Human Chorionic Gonadotropin is mostly secreted by syncytiotrophoblast in the placenta that consequently results in high levels of β-HCG in maternal amniotic fluid, blood, and urine ranged from 2000 to 70000 mIU/ml. Furthermore, a lesser amount of β-HCG is secreted by cervical gland. Therefore, any leakage of amniotic fluid can increase the β-HCG level in the cervicovaginal fluid. On the other hand, the contamination of maternal vagina with blood can lead to false positive results.[6,19]

The efficacies of vaginal discharge quantitative and qualitative β-HCG on the diagnosis of PROM have been evaluated in various surveys.[4,20,21] In the present study, the quantitative and qualitative measurements of β-HCG in vaginal secretions showed acceptable results for the diagnosis of PROM. In this regard, the quantitative measurement of β-HCG with the cut-off value of 66 mIU/ml had 97.05%, 100%, 100%, 96.77%, and 98.43% sensitivity, specificity, PPV, NPV, and accuracy, respectively. Also, the qualitative β-HCG analysis with the cut-off value of 25 mIU/ml has demonstrated similar results as 94.11%, 93.33%, 94.11%, 93.33%, and 93.75% for sensitivity, specificity, PPV, NPV, and accuracy, respectively. It is noteworthy that the diagnostic power of all these indicators were more than 90%.

For the first time, Anai et al.[19] in 1997 assessed vaginal washing fluid β-HCG level of normal pregnant women in their first, second, and third trimesters of their pregnancy

compared to the confirmed PROM in pregnant women. Finally, they concluded that vaginal washing β-HCG levels were greater in PROM women compared to normal pregnant women. In line with our study, they have also stated that using the threshold value of 50 mIU/ml, 100%, 96.5%, 88.9%, 100%, and 97.2% sensitivity, specificity, PPV, NPV, and accuracy were calculated for identifying PROM in the third trimester of pregnancy, respectively.

Likewise, Kim et al.[7], and Eldaly et al.[4] in their studies have reported similar results with the threshold values of 39.8 mIU/ml and 32 mIU/ml, respectively. The authors of this study declared that the mentioned cut-offs were associated with more than 90% sensitivity, specificity, PPV, and NPV, which all represent the diagnostic efficacy of quantitative β-HCG in the detection of PROM. Additionally, Eldaly et al. have also evaluated the role of qualitative β-HCG in cervicovaginal secretion of PROM women and concluded that both quantitative and qualitative β-HCG tests can be considered as easily available, rapid, safe, and cost-effective choices for the diagnosis of PROM. Esim et al.[3] in their study measured vaginal washing fluid β-HCG using electrochemiluminescence immunoassay (ECLIA) method in the PROM and control groups. They found that the cut-off value of 65 mIU/ml is accompanied with 60% sensitivity, 94% specificity, 79% PPV, and 86% NPV during the third trimester of pregnancy. In this study, the patients with positive pooling and nitrazine tests' results were considered as the confirmed PROM cases. Cooper et al.[22] evaluated the effectiveness of qualitative β-HCG on the diagnosis of PROM. As a result, they calculated 79% sensitivity, 96% specificity, 95% PPV, and 84% NPV for the detection of PROM. It seems that different methods for measuring quantitative β-HCG, using multiple commercial kits for qualitative β-HCG, and applying different criteria for selecting pregnant women with the definite PROM may be the possible reasons for achieving different diagnostic and cut-off values.

The limitation our study had was using pooling test for selecting pregnant women with PROM, since intra amniotic dye injection was known as the gold standard test which is invasive and expensive, and with several side effects.

## Conclusion

Our study supports the fact that both quantitative and qualitative vaginal washing β-HCG measurements are accurate and useful for diagnosing PROM. Commercial kits for β-HCG are easily available. Furthermore, the test is simple, safe, inexpensive, and rapid, and can also be done bedside without the need for any additional intervention. Besides, its efficacy is comparable with quantitative β-HCG; hence in the absence of bloody contamination, it may be a reliable and a good alternative test for the detection of PROM. However, further investigations are still needed to find a noninvasive gold standard test for the diagnosis of PROM.

## Conflict of Interests

Authors have no conflict of interests.

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