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Research Article

The Accuracy of β-Human Chorionic Gonadotropin Assay in Vaginal Fluid for the Diagnosis of Premature Rupture of Membranes

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Abstract

Background: *premature rupture of membranes complicates 2% to 20% of all deliveries. The accurate diagnosis of which is important; because it is associated with infectious morbidity of mother and fetus, cord prolapse and preterm labor.*

Aim of the Work: *this study aimed to determine whether the measurement of beta-human chorionic gonadotropin (β-hCG) levels in vaginal fluid is accurate for the diagnosis of premature rupture of membranes (PPROM).*

Patients and Methods: *this prospective case control study was conducted in ain shams maternity hospital. Sixty pregnant women with gestational age between 24-36 weeks and single intrauterine pregnancy were included in the study; half with confirmed rupture of membranes and half as control. β-hCG was measured in vaginal wash fluid for all included women.*

Results: *vaginal β-hCG had reliable diagnostic value as denoted by an area under the ROC curve of 1.0 with 95% confidence limits ranging from 0.94 to 1.0 (p-value <.0001). The best cut-off value was a vaginal β-hCG concentration of >56 mIU/ml which had a sensitivity of 100% with 95% confidence limits ranging from 86.2% to 100% and a specificity of 100% with 95% confidence limits ranging from 86.2% to 100%.*

Conclusion: *β-hCG qualitative test in cervicovaginal washing can be used as a reliable test to detect rupture of membranes with high specificity and sensitivity. It is a simple, rapid, and inexpensive test and may be useful in equivocal cases.*

Introduction

Disruption of fetal membranes prior to the onset of labor, commonly known as premature rupture of membranes (PROM), is a frequent complication of pregnancy. PROM can occur in 8-10% of all pregnancies and pre-term PROM (PPROM <37 weeks gestation) is associated with nearly a third of all premature births [1,2]. Fetal membranes have a stratified structure with special characteristics that provide them with the ability to adapt to the expansion that occurs during pregnancy, resulting from increasing fetal size and amniotic fluid [3].

Membrane integrity is essential to ensure normal term pregnancy. Evidence suggests that the mechanisms that cause the rupture of membranes include biochemical, immunologic and bacteriologic events. Currently, it is widely accepted that term and preterm rupture of membranes are associated with structural changes, caused by inflammatory processes induced by endocrine or infectious triggers [4,5].

The main complications and consequences of PPROM are related to the gestational age at which it occurs, the latency until birth, concomitant infection of the gestational tissues which may impact both fetal and maternal outcomes, in

addition to conditions specific to the fetus, such as oligohydramnios, cord compression, abruptio or cord prolapse [1].

The diagnosis of rupture of the membranes (PPROM) may be made by clinical history and some simple diagnostic tests. Patient history is accurate for about 90% for the diagnosis and should not be ignored [6].

Regarding the necessity for accurate diagnosis of PPROM, several diagnostic tests have been suggested. Nitrazine test, Fern test [7] and ultrasound have been considered as very useful tests. Biochemical tests have also been suggested but should only be performed in cases of very suspicious PPROM, in which other simple tests may not diagnose accurately. Diagnosis of PPROM is simple in the cases of obvious rupture, but when the rupture is scant, it is difficult or even impossible. Failure of diagnosis may cause a risky delay in performing necessary tasks, such as performing a vaginal examination; also, false diagnosis may cause unnecessary interventions like hospitalization, or labor induction, therefore the diagnosis of suspicious cases is obligatory and vital. Every diagnostic test for this purpose should be simple, reliable and fast [8]. Biochemical materials (substances) which have high concentrations in amniotic fluid, including interlukin-6, [9] alpha fetoproteins, [10] diamin oxidase [11], prolactin [12], urea and creatinine, fetal fibronectin [13,14] and insulin-like growth factor binding protein I [15] have all been evaluated. Another substance is β -human chorionic gonadotrophin (β-hCG), which is secreted solely by syncytiotrophoblasts and can be found in the amniotic fluid in addition to mother's blood or urine and has been studied for the evaluation of PPROM. The difference between the above-mentioned materials and β-hCG is the latter has simplicity and ease of use as well as being cheaper [16].

Methods

This prospective case control study was conducted in Ain Shams Maternity Hospital. We included sixty pregnant women who had gestational age between 24 and 36 weeks, with single intrauterine viable pregnancy. They were divided into two equal groups; group (I), who had confirmed rupture of membranes with positive history of vaginal leakage and positive fluid leakage observed using sterile Cusco speculum examination. Group (II) was those who attended the outpatient clinic for routine antenatal care with no signs or symptoms suggestive of PPROM. Data collection including age, parity, gestational age, amniotic fluid leakage history (onset, amount, duration, color of the fluid), was done. They were all subjected to general and abdominal examination as well as ultrasound examination to document fetal life, fetal biometry, AFI calculation by 4 quadrants method, placental location and any apparent congenital fetal malformation. After agreeing to participate in our study and signing the consent form, each woman was asked to lie in lithotomy position with good illumination. Vaginal examination using a sterile Cusco speculum was done then vaginal fluid sampling was taken as follows: 5 ml of sterile saline solution were injected into the posterior vaginal fornix and at least 3 ml of it were aspirated by the same syringe and were sent immediately to the laboratory for measuring quantitative β-hCG. Any contaminated sample with blood was excluded. The sample was placed in a plastic tube then put the tube in the centrifuge for 10 min. We aspirated 0.5 ml of the centrifuged sample then we put it in Hitachi Roche 902 Automatic Analyzer for 15 min. Lastly, we recorded the level of β-hCG.

Sample Size Justification

The required sample size has been estimated using the Power Analysis and Sample Size software version 11.0.10 (PASS; NCSS, LLC, Kaysville, Utah). A previous study of Bahasadri *et al.* [17] reported that the sensitivity and specificity of β-hCG for diagnosis of PPROM were 75% and 84%, respectively. The present study included 2 equal-sized groups, PPROM group and the control group. So, the prevalence of PPROM in the whole study population was 50%. Consequently, it was estimated that a total sample size of 60 subjects, which included 30 subjects with the disease (PPROM) and 30 subjects without the disease (control), would have a power of 80% (type II error, 0.2) to detect a difference of 25% in sensitivity between an expected value of 75% and a null value of 50%. The same sample size would have 98% power (type II error, 0.02) to detect a difference of 34% in specificity between an expected value of 84% and a null value of 50%. A two-sided binomial test has been used for this calculation and the type I error has been set at a conventional value of 0.05. The prevalence of the disease is assumed to equal 50% (cases: controls ratio, 1:1). Data were collected, tabulated, and then analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY). The Kolmogorov-Smirnov goodness of fit test was used to test the normality of numerical data distribution. Normally distributed numerical data were presented as mean and SD and differences between the two groups will be compared with the independent-samples t test. Skewed numerical data were presented as median and interquartile range and inter-group differences were

compared non-parametrically using the Mann-Whitney U test. Qualitative data were presented as number and percentage and the chi square test or Fisher's exact test, when appropriate, were applied for comparison of the two groups. Receiver-operating characteristic (ROC) curve analysis was used to examine the diagnostic value of β-hCG. The best cutoff value was defined as that associated with the highest J index, where J = (sensitivity + specificity) -1. A two-sided p-value < 0.05 were considered statistically significant. The ethical committee of the Faculty of Medicine, Ain Shams University, approved the study.

Results

Our results showed that there was no statistical significant difference between confirmed and the control groups as regard age and gestational age of the patients. There was statistically significant difference between the two groups regarding the measurement of AFI and β-hCG in vaginal wash (table 1). Figure 1 shows the result of ROC curve analysis for discrimination between cases with confirmed PROM and controls using vaginal β-hCG. Vaginal β-hCG had excellent diagnostic value as denoted by an area under the ROC curve of 1.0 with 95% confidence limits ranging from 0.94 to 1.0 (p-value <.0001) (figure 1). The best cut-off value was a vaginal β-hCG concentration of >56 mIU/ml which had a sensitivity of 100% with 95% confidence limits ranging from 86.2% to 100% and a specificity of 100% with 95% confidence limits ranging from 86.2% to 100% (figure 2).

Variable	Confirmed PROM (n=30)	Control (n=30)	p-value
Age (years)	^27 ± 5	^27 ± 6	0.869¶
Gestational age (weeks)	^31 ± 4	^30 ± 3	0.523¶
AFI	*7 (5 – 11)	*12 (11 – 14)	<.001¶¶
β-hCG in vaginal wash (IU/ml)	627.5 (324.0 – 875.0)	34.0 (23.0 – 44.0)	<.001¶¶
^Data are mean ± SD.*Data are median (interquartile range) ¶Unpaired t test. .¶¶Mann-Whitney test.			

Table 1: Patient's characteristics, AFI and β-hCG in vaginal wash in both studied groups.

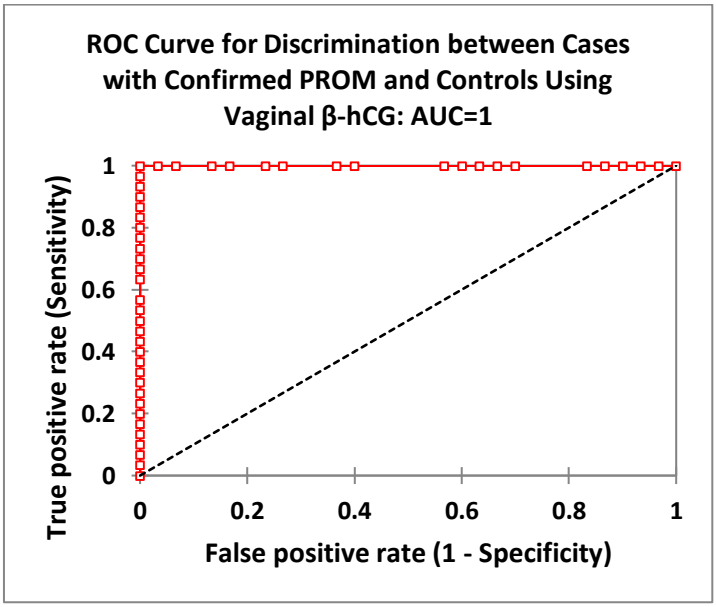

Figure 1: ROC curve for discrimination between cases with confirmed PROM and controls using vaginal β-hCG.

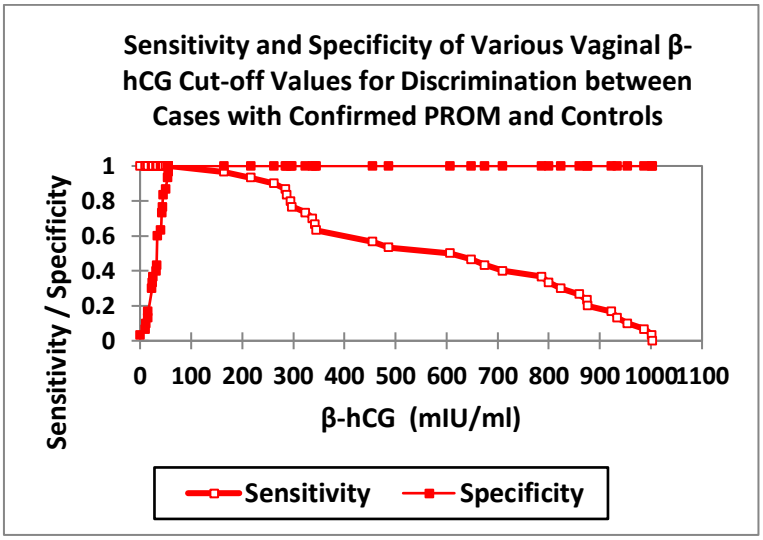

Figure 2: Sensitivity and specificity of various vaginal β-hCG cut-off values for discrimination between cases with confirmed PROM and controls.

Discussion

Preterm prelabor rupture of membranes is an important obstetrical concern. If not managed timely and appropriately, it can lead to fetomaternal problems. Further complications can be maternal infection endometritis, fetal infections, preterm labor, neonatal morbidty and mortality because of preterm birth and infections. Timely diagnosis and treatment has a very important role for prevention of fetomaternal morbidity and mortality [18].

Rupture of membrane is largely a clinical diagnosis. It is typically suggested by history of watery vaginal discharge followed by sterile speculum examination which should show 1) visual pooling of clear fluid in posterior fornix of vagina or leakage of fluid from cervical os; 2) an alkaline pH of cervicovaginal discharge, demonstrated by nitrazine test and/or 3) ferning of cervicovaginal discharge on drying. Moreover, sensitivity and specificity of the nitrazine test has been reported at 90-97% and 16-70%, respectively, [19]**.** With the possible exception of direct visualization of amniotic fluid spurting from cervical os, all of these clinical signs have limitations in terms of diagnostic accuracy, cost, and technical ease. Moreover, such tests become progressively less accurate when more than 1 h has elapsed after membranes have ruptured or in certain nonobvious circumstances, where fluid may not be present in vagina for evaluation or what is present may be contaminated with urine, cervical mucus, vaginal discharge, blood, or meconium [20]**.**

Results of the present study revealed that vaginal β-hCG had excellent diagnostic value as denoted by an area under the ROC curve of 1.0 with 95% confidence limits ranging from 0.94 to1.0 (p-value <.0001). The best cut-off value was a vaginal β-hCG concentration of >56 mIU/ml which had a sensitivity of 86.2% with 95% confidence limits and a specificity of 86.2% with 95% confidence limits. This can be attributed to the high concentration of β-hCG in amniotic fluid. As a normal pregnancy progresses, the mean level of β-hCG in maternal blood increases to approximately 54000 mIU/ml at 8-12 weeks of gestation. It then declines rapidly to around 12000 mIU/ ml and remains at this level up to the end of the pregnancy. β-hCG is also present in amniotic fluid at concentrations ranging from 2000-70000 mIU/ml. In addition, it is secreted by cervical glands and is present in the vaginal secretions [21].

Also, a research had been performed predict of PPROM occurrence by evaluating some materials earlier in pregnancy and might be able to consider some therapeutic interventions to prevent it, such as fetal hemoglobin measurement in amniotic fluid during the second trimester, [22] and endothelin 1 in the second trimester [23]. Vaginal β-hCG has been investigated for the diagnosis of PPROM. In a study that measured the β-hCG level of vaginal fluid; they reported it as a useful marker for PPROM in the second and third trimester [16]. In another study that was performed on 52 women (20 women with intact membranes, 21 women with definitive PPROM and 11 women with suspicious PPROM), the researchers concluded that vaginal washing fluid β-hCG is a suitable, cheap and non-invasive method for the diagnosis of PPROM. In this study, an optimal cutoff value of 100 mIU/mL was obtained [24].

Esim et al. [8] hypothesized that vaginal fluid β-hCG may be helpful in diagnosing PPROM because β-hCG is a glycoprotein produced exclusively by syncitiotrophoblasts in the placenta and present at a certain level in the vaginal fluid. They evaluated the value of vaginal fluid β-hCG for diagnosis of PPROM, their study showed a specificity of 93%, sensitivity of 86%, PPV of 67%, and NPV of 97% for quantitative β-hCG testing of cervicovaginal washing in the second half of pregnancy. This is consistent with the findings of Cooper et al. [25] who demonstrated a sensitivity and specificity of 79% and 96%, respectively, for the test, using Quickvue one-step pregnancy test with a threshold of 25 mIU/ml.

Ni et al. [9] compared β-hCG, AFP and interlukin-6 in both groups with definitive PPROM and intact membranes. In this study, all the three agents were higher in the PPROM group. The diagnostic value of AFP was more than β-hCG, the value of which was more than interleukin-6. However, although the diagnostic value of AFP was more than β-hCG in this study, it should be considered that the measurement of AFP is more expensive and probably is not applicable in most of the emergency settings.

Kim et al. [26] evaluated the value of measurement of beta-human chorionic gonadotropin (β -HCG) level in the vaginal washing fluid for the diagnosis of premature rupture of membranes. Vaginal beta-HCG level was significantly higher in patients with PROM followed by premature delivery than patients in other groups ($P < 0.01$). From the receiver operating characteristic curve, 39.8 mIU/ml was set as a cutoff value. Sensitivity, specificity, positive predictive value, and negative predictive value were 95.5, 94.7, 91.3, and 97.3%, respectively. Their study demonstrated that the measurement of vaginal fluid beta-HCG may be reliable, simple, and rapid test in diagnosing PROM and used as an adjunctive test in equivocal cases.

Karimian et al. [27] in their study compared the diagnostic power of qualitative and quantitative measurements of ß-hCG in cervicovaginal washing-fluid for the diagnosis of PROM in pregnant women. They concluded that it the qualitative and quantitative measurements of cervicovaginal washing-fluid β-hCG were accurate, fast and simple for the diagnosis of PROM, especially in suspicious cases.

Bahasadri et al. [28] recorded 93% sensitivity and 84% specificity for β-hCG of vaginal fluid, which is in agreement with our findings. The authors reported that there was more β-hCG in vaginal fluid of women with PPROM than in pregnant women with intact membranes; therefore, it could be a reliable and fast way of detecting membrane rupture. β-hCG had more diagnostic value than urea and creatinine in detecting PPROM and can be used in suspected cases. These tests were easy and not expensive and can be used in any medical center. It is suggested that cut-off value for rupture of membranes in pregnancy should be determined in different gestational ages in future studies [29].

β-hCG is higher in the case of PPROM and patients who were suspected of having PPROM and may be used as a suitable, fast and reliable test for detecting PPROM. Because of the feasibility and simplicity of β-hCG measurement in most laboratories and hospitals, vaginal fluid β-hCG might be an alternative for diagnosing PPROM. With regard to the low number of studies, it seems that more studies should be performed over a higher number of women to evaluate the diagnostic value of β-hCG and to find a definitive optimal cut-off point for β-hCG in different gestational ages. This study showed that it was beneficial and has clear cut of limit with very good sensitivity and specificity, although other studies showed its beneficial use as well with different sensitivity and specificity this may be because we have chosen our study on confirmed and control cases of PPROM and suspected cases of PPROM not included which may be reasonable for future study.

Conclusion

In conclusion, the diagnosis of rupture of membrane can be very difficult when there is only minimal amniotic fluid leakage. This study demonstrated a high sensitivity, specificity, PPV, and NPV for the β-hCG qualitative test in cervicovaginal washing in patients with confirmed rupture of membranes but subtle and invisible amount of amniotic fluid in the vagina. These findings show that this simple, rapid and inexpensive test may be useful in detecting PPROM in equivocal cases.

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