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Evaluation of Vaginal Fluid β-Human Chorionic Gonadotropin for Diagnosis of Premature Rupture of Membranes

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Abstract

Background: Premature rupture of membranes is a common obstetric problem, and assessment of the woman with possible membranes rupture is management issued faced in every day practice. When the fetal membranes rupture occurs, the fetus loses the protection afforded within the amniotic cavity, the chance of fetal infection increases.

Aim of the Work: To detect the reliability of Beta-Human Chorionic Gonadotropin (β-HCG) in vaginal washings of pregnant women for diagnosis of premature rupture of membranes.

Methods: A prospective case-study includes 90 pregnant women subdividing into three equal groups (Group A: 30 pregnant women with confirmed premature rupture of membranes, Group B: 30 pregnant women with suspected premature rupture of membranes, Group C: 30 apparently healthy pregnant women without any complaint) for whom sterilized speculum examination for amniotic fluid pooling, nitrazine paper test and measurement of vaginal washing fluid β-HCG were performed.

Results: There was significant differences in mean vaginal washing fluid β-HCG concentration among the three groups, being higher in Group A than the other two groups and the time interval between sampling and delivery was significantly shorter among the other two groups and the optimal cut off value for vaginal washing β-HCG was 68mIU/mL using ROC curve with a sensitivity of 95%, a specificity of 88%, positive and negative predictive values of 93% and 84% respectively. Diagnostic accuracy was 90%.

Conclusion: Detection of vaginal fluid β-HCG is simple, reliable and rapid test for the diagnosis of premature rupture of membranes.

Key Words: *HCG – Vaginal washing – Premature rupture of membranes.*

Introduction

PREMATURE Rupture of Membranes (PROM), or pre-labor rupture of membranes, is defined as spontaneous rupture of the fetal membranes before the full cervical dilatation [1].

The amniotic sac, or "membranes" contain amniotic fluid which surrounds and protects the fetus in the womb. After rupture, the amniotic fluid leaks out of the uterus through the vagina. This is informally known as one's "water breaking". Premature rupture of membranes is shown in 5-15% of all term births and in 20-40% of all preterm births [1].

Premature rupture of the fetal membranes is a major cause of preterm birth and its associated infant morbidity and mortality. Recently, it has become clear that rupture of the fetal membranes, term or preterm, is not merely the result of the stretch and shear forces of uterine contractions, but the significant part is the consequence of a programmed weakening process. Work in the rat model has demonstrated that collagen remodeling, with activation of Matrix Metalloproteinases (MMPs), and apoptosis increase markedly in the amnion at end-gestation, suggesting that these processes are involved in fetal membranes weakening [2].

Large numbers of exogenous risk factors have been associated with PROM including genital tract infection, cervical incompetence, nutritional deficiency e.g. ascorbic acid and copper, previous preterm delivery and vaginal bleeding [3].

The diagnosis of PROM is based on the patient's report, and observation of amniotic fluid leakage from the cervix and by pooling of amniotic fluid in the vagina, which is confirmed when a speculum is inserted. However, there are some cases with a history of fluid leakage, but there is no fluid pooling in the speculum (in the cases with prolonged rup-

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ture). Nitrazine test may cause false negative results in 9.4% of cases after 48h of rupture; also, vaginitis, cervicitis, contamination with urine, semen and blood, and antiseptic use may give false negative results; therefore other methods of diagnosis have been investigated. Biochemical materials which have high concentrations in amniotic fluid, including interlukin-6, 8, alpha fetoproteins, diamin oxidase, prolactin, urea and creatinine, fetal fibronectin and insulin-like growth factor binding protein I have all been evaluated. Another substance is Beta-human chorionic gonadotrophin (β-HCG), which is secreted solely by syncytiotrophoblasts and can be found, in amniotic fluid in addition to mother's blood or urine **[4,5]**.

After PROM, labor will usually begin spontaneously whether at term or before term. However, this will be affected by the gestational age at which the membrane ruptured; at term 75% of cases will go into spontaneous labor within 24 hours, but in preterm pregnancies, spontaneous labor will begin within 3 days in 48% only of cases. Accordingly, the management of premature rupture of the membranes depends on the gestational age. However, in some instances immediate delivery is mandatory **[6,7]**.

Aim of the work:

To detect the reliability of Beta-Human Chorionic Gonadotropin (β-HCG) in vaginal washings of pregnant women for diagnosis of premature rupture of membranes.

Patients and Methods

This prospective study was conducted on ninety (90) pregnant females attending to antenatal care clinic and emergency department of obstetrics and gynecology at Tanta University Hospital from July 2016 – July 2017. Oral and written consent were taken from all pregnant women included in the study after approval of ethical committee.

The ninety pregnant women were subdivided into three main groups:

1- *Group A:* 30 pregnant women with confirmed premature rupture of membrane which was evident by positive pooling of amniotic fluid with or without valsalva maneuver and nitrazine positive test this group was subdivided into:
   - A1: 15 pregnant women at second trimester.
   - A2: 15 pregnant women at third trimester.

2- *Group B:* 30 pregnant women with suspected premature rupture of membranewith a history of fluid leakage but negative pooling of amniotic fluid with or without valsalva maneuver and nitrazine negative test this group was subdivided into:
   - B 1: 15 pregnant women at second trimester.
   - B2: 15 pregnant women at third trimester.

3- *Group C:* 30 apparently healthy pregnant women without any complaint coming for antenatal care, was subdivided into:
   - C1: 15 pregnant women at second trimester.
   - C2: 15 pregnant women at third trimester.

All three groups included in study had single intrauterine pregnancy with no fetal anomalies. The exclusion criteria were patient who refuse participation, those with multiple gestation and with any contraindications for vaginal examination or vaginal washing as cases of placenta previa; severe vaginal infection; antepartum haemorrhage and those with any medical disorders such as diabetes mellitus; hypertension and heart disease. All women included in the study were subjected to; complete history taking; clinical and blood pressure evaluation; general and obstetric examination and ultrasound evaluation followed by vaginal washing β-HCG sampling. This was done as follows: 5ml of sterile saline were injected into posterior vaginal fornix and 3ml of it was collected with the same syringe, three drops for bed side urine pregnancy test(sensitive from 25mlIU/ml of β-HCG), (Abon Biopharm (Hangzhou) Co., Ltd), wait for five minutes to obtain the result, and then the syringe sent to laboratory. After 3 minutes centrifugation, supernatant part of the vaginal washing was assessed for β-HCG by quantitative measurement (all samples were done using radio immune assay in the same laboratory with the same technique).

The procedure described above was applied to all studied groups. After sterile speculum examination, nitrazine paper test and β-HCG sampling, all patients underwent ultrasonographic examination for gestational age determination and amniotic fluid index calculation. Then all patients were followed-up until delivery. Time interval between sampling and delivery were noted and gestational age of delivery time were determined.

After that statistical analysis of the data was done by IBM computer using SPSS (statistical program for social science) Version 20 as follows: Description of quantitative variables as range; mean and SD; description of qualitative variables as number and percentage. ANOVA test (Analysis of Variance) was used to compare quantitative variables between groups; Chi-square test was used

to compare qualitative variables between groups. Receiver Operator Characteristic curve (ROC) was used to find out the overall predictivity of parameter in and to find out the best cutoff point with detection of sensitivity, specificity, +ve predictive value (+PV), –ve predictive value (–PV) and accuracy at this cutoff point.

Results

Table (1) represents comparison between the different studied groups according to age, parity, and gestational age of delivery. There was no statistical significance between the three groups according to age of women and there was statistical difference between the three studied groups in parity, showing increase parity in Group A and Group B compared to Group C (p-value ≤0.015). Also, there was significant decrease in the gestational age of delivery in Group A and Group B in comparison with Group C with p-value ≤0.001. Statistical difference was detected between the three studied groups, showing decrease the time between test and delivery in the Group A in comparison with Group B and Group C. (p-value ≤0.001).

Table (1): Frequency of different grades of pulmonary hypertension in the studied population.

	Range	Mean ± SD	F.test	p-value	
Age:					
Group A	18-37	27.33±5.51	0.992	0.375	
Group B	17-36	25.90±5.49			
Control	18-37	25.50±4.88			
Parity:					
Group A	0-6	2.63± 1.65	4.423	0.015*	Group A & B 0.085
Group B	0-6	1.97± 1.56			Group A & C 0.004*
Control	0-5	1.50± 1.19			Group B & C 0.226
Gestational age of delivery:					
Group A	27-38	32.33±3.75	22.824	0.001 *	Group A & B 0.002*
Group B	29-38	34.57±2.56			Group A & C 0.001*
Control	35-39	37.10± 1.21			Group B & C 0.001 *
Time between test and delivery:					
Group A	1-23	10.07±7.04	9.896	0.001	Group A & B 0.001*
Group B	3-84	28.80±25.23			Group A & C 0.001*
Control	7-70	27.53± 17.63			Group B & C 0.788

Table (2) shows comparison between the three studied groups according to positive and negative bed side qualitative pregnancy test and (Table 3) showed sensitivity, specificity, PPV, NPV and accuracy of bed side urine pregnancy test in second and third trimester.

Table (2): Qualitative B-HCG test in the diagnosis of PPRM.

Bed side qualitative pregnancy test	Group A	Group B	Group C	Total
Positive:				
N	26	11	3	40
%	86.7%	36.7%	10.0%	44.4%
Negative:				
N	4	19	27	50
%	13.3%	63.3%	90.0%	55.6%
Total:				
N	30	30	30	90
%	100.0%	100.0%	100.0%	100.0%
Chi-square:				
χ^2		36.810		
p-value		0.001 *		

Table (3): Comparison between sensitivity, specificity, PPV, NPV and accuracy of bed side qualitative pregnancy test in second and third trimester.

	Sensitivity	Specificity	PPV	NPV	Accuracy
• Second trimester	85	100	100	90	93
• Third trimester	90	100	100	95	97

Table (4) shows comparison between the three studied groups according to quantitative β-HCG values in vaginal washing. There was statistical difference between the three studied groups with increase in the β-HCG values in Group A in comparison with Group B and Group C. (p-value ≤0.001).

Analysis of the results using ROC curve showed that best cut off point for vaginal fluid β-HCG in groups was 68mIU/mL with a sensitivity of 95%, a specificity of 88%, positive and negative predictive values of 93% and 84% respectively. Diagnostic accuracy was 90% Fig. (1).

Table (4): Mean vaginal fluid β-HCG concentration in studied groups.

β-HCG in vaginal wash	Group A	Group B	Group C
Range	15-189	12-127	4.8-66
Mean ± SD	88.55±41.28	32.15±27.78	12.26±9.79
F-test		54.806	
p-value		0.001*	
Group A & Group B 0.001*	Group A & Group C 0.001*		Group B & Group C 0.010*

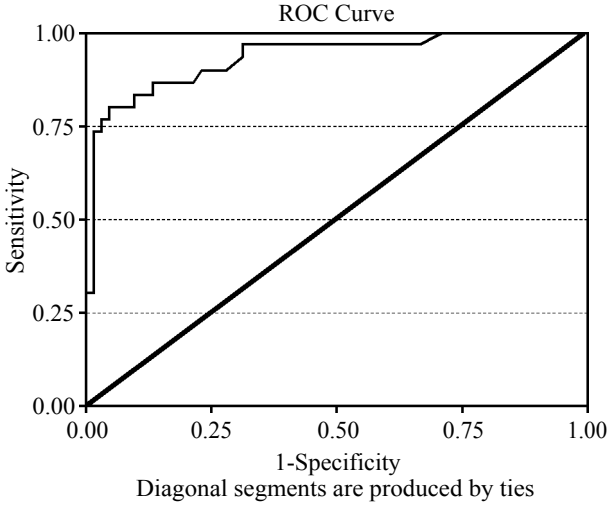

Fig. (1): ROC curve of the β-HCG values to establish cut off value.

There was increase sensitivity, specificity, PPV, NPV and accuracy of β-HCG according to cut off value >68mIU/mL in second trimester in comparison with the third trimester (Table 5).

Table (5): Comparison of sensitivity, specificity, PPV, NPV and accuracy ofquantitative β-HCG in second and third trimester.

	Sensitivity	Specificity	PPV	NPV	Accuracy
• Second trimester	93	94	88	96	93
• Third trimester	73	94	85	87	86

Table (6) shows correlation between quantitative β-HCG and time between test and delivery in pregnant women with confirmed PROM (Group A) and pregnant women with suspected PROM (Group B). This table shows that there is negative correlation between quantitative β-HCG and time between test and delivery in pregnant women with confirmed and suspected PROM (p-value ≤0.001 & p-value ≤0.020 respectively Figs. (2,3).

Table (6): Correlation between quantitative β-HCG and time between test and delivery in A and B Groups.

	Beta human			
	Group A		Group B	
	r	*p*	*r*	*p*
Time between test and delivery	–0.585	0.001*	–0.421	0.020*

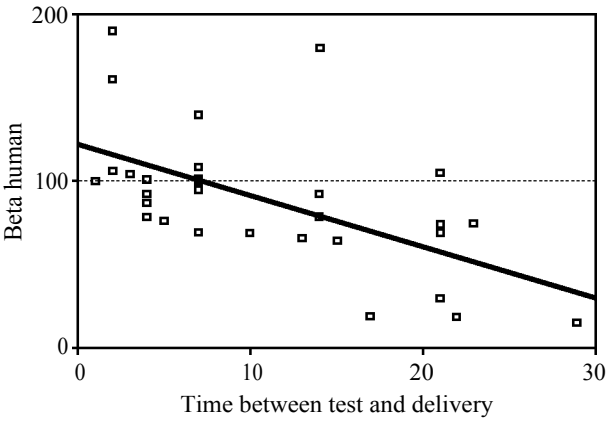

Fig. (2): Correlation between quantitative β-HCG and time between test and delivery in pregnant women with confirmed PROM (Group A).

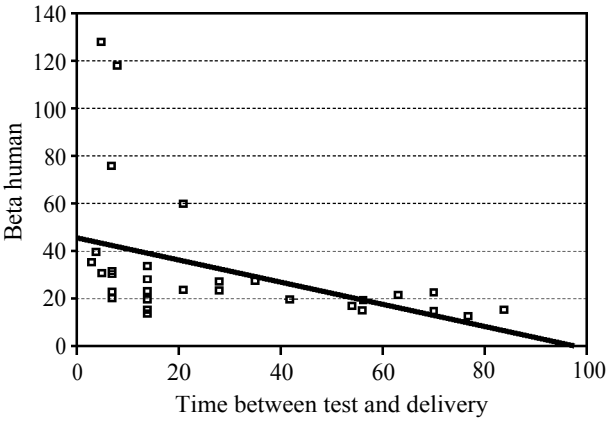

Fig. (3): Correlation between quantitative β-HCG and time between test and delivery in pregnant women with suspected PROM (Group B).

Discussion

PROM has many complications affecting both the mother and her fetus. The most important of which are chorioamnionitis and respiratory distress syndrome.

Correct diagnosis of PROM has great importance because failure of diagnosis can lead to unwanted obstetric complications; on the other hand over diagnosis can lead to unnecessary interventions like hospitalization [1].

Diagnosis of PROM has always been a debatable issue. Direct visualization of liquor passage

through the external cervical os during speculum examination is definitive, but this is not possible in every case. Thus, many tests had been advised for diagnosis of PROM, however, each one has its own limitations, making the diagnosis of PROM difficult **[1,4,5]**.

Our results showed seven to eight folds increase in vaginal washings β-HCG concentration among patients with premature rupture of membranes versus intact membranes. In the present study we used a qualitative ß-HCG urine pregnancy test with positivity in 86.7%, 36% and 10% in Group A, B and C respectively. The test was 85 and 90 sensitive in the detection of the amniotic fluid in the PPRM group in second trimester and in third trimester respectively. The test had high accuracy in third trimester 97. There were four cases of false negative result (13.3%) which may be due to failure of absorption of the fluid by the test kit due to the proteins and vernix in the amniotic fluid or that the amniotic fluid might be diluted by the vaginal discharge resulting in a false low hCG level. Our results coincided with Caranza et al who reported 95% sensitivity, 100% specificity, 100% PPV and 97.8% NPV of qualitative hCG testing of vaginal discharge in the diagnosis of rupture of membranes **[8]**.

Receiver-Operator Characteristic (ROC) curve analysis was used to establish the cut-off value of vaginal washings β-HCG for diagnosis of premature rupture of the membranes.ROC curve recommended 68mIU/mL as the cut-off value. According to this cut off value a sensitivity of 93%, a specificity of94%, positive and negative predictive values of 88% and 96% accuracy 93% in the second trimester, and a sensitivity of 73%, a specificity of 94%, positive and negative predictive values of 85% and 87% accuracy 86% in the third trimester were recorded.

These results agreed with results documented by Mohamed et al.who found that the sensitivity, specificity, PPV, NPV and accuracy were 94%, 86%, 93.1%, 87.8% and 91.3% as regarded to β-HCG. Also, they stated that there was very high significant difference between confirmed, suspected and control groups as regard β-HCG level in vaginal fluid (p-value <0.001) **[9]**.

Esimand hiscolleagues reported higher β-HCG levels in vaginal washings of patient with PROM. They proposed that there had been an escape of β-HCG in the amniotic fluid after rupture of the fetal membranes. They reported definitive difference in the mean vaginal washings β-HCG concentration of women with PROM (95mIU/ml) and those with intact membranes (10.47mIU/ml) **[10]**. In a study conducted by Mangoano et al., a cut off value of β-HCG in vaginal washing fluid was detected as 100mIU/mL with regard to prediction of pooling **[11]**.

Kariman et al., performed a case control study on cervicovaginal samples collected from 86 singleton pregnancies to compare the diagnostic power of qualitive and quantitative measurements of β-HCG in cervicovaginal washing fluid for the diagnosis of PROM. The mean β-HCG levels were 250.6 and 118.6mIU/mL in case and control group respectively. Calculations of receiving operating characteristic curve showed that the cut-off point for ELISA was 22.32mIU/mL and its sensitivity, specificity, positive and negative predictive values and accuracy were 95.3%, 97.7%. 97.6%, 95.5% and 96% respectively. The one-step qualitative pregnancy test was positive in 42 PROM and 5 in the control group **[12]**.

Young-Ham et al., demonstrated in 86 pregnant women between 24-34 weeks of gestation a cutoff value of β-HCG in vaginal washings (39mIU/ml) according to receiver operating characteristic curve **[13]**.

There have been reported in various studies, different cut-off values, sensitivities, specificities, positive and negative predictive values for β-HCG in vaginal washing fluid for the accurate detection of PROM. These different values could be attributed to the existence of a difference in the number of samples, gestational age, study population and inclusion of patients with vaginal bleeding in some studies.

Another study evaluated β-HCG in vaginal washing as a marker for PPROM and established the vaginal β-HCG concentration ranges of a subset of patients without PPROM in the first, second and third trimesters. These investigators demonstrated a definitive difference in mean β-HCG concentrations of subjects with PPROM and those without. They considered that there was a significant difference in the second and third trimester groups with PROM **[14]**.

Also, Bahasadri et al., in a cross sectional study including 123 pregnant women reported that vaginal fluid β-HCG may be used as a suitable, fast and reliable test for detection of PROM **[15]**.

We concluded that pregnant women from 24-40 weeks gestation, who have passage of liquor per vagina or low amniotic fluid index, should be

subjected to assessment of qualitative pregnancy test for vaginal fluid washing and quantitative β-HCG in vaginal washing for accurate diagnosis of PROM. Measurement of vaginal washing β-HCG for the diagnosis of PROM is a simple, reliable and rapid test. The sensitivity and specificity were 95% and 88% respectively in detecting PROM by evaluating of vaginal washings B-HCG concentration with a cut-off value of 68mI U/ml.

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تقييم قياس نسبة هرمون موجه الغدد التناسلية المشيمائية البشرى فى السائل المهبلى من آجل تشخيص الإنفجار المبكر لكيس الماء

إنفجار جيب المياه قبل بدء آلام المخاض سواء آكتمل نمو الجنين آم لا، من الممكن آن يصيب من ٢.٧ إلى ١٧٪ من الحوامل. وعلى الرغم من ذلك فآسباب إنفجار جيب المياه غير معروفة على وجه التحديد حتى الآن، إلا آن نظرية ضعف الآغشية المحيطة بالجنين نتيجة لتلوث بكتيرى غير ملحوظ الآمر الذى يؤدى لتقطها هى المقبولة حاليا. ويعد إثبات تشخيص إنفجار جيب المياه من الآمور التى قد تكون محيرة إكلينيكيا، حيث لا يوجد حتى الآن إختبار دقيق ذو دقة مقبولة. وتعتبر رؤية السائل الآمينوسى وهو يتسرب من عنق الرحم آثناء الفحص هى الوسيلة الآكيدة للتشخيص ولكن فى العديد من الآحيان لا يمكن رؤية السائل المتسرب آثناء الفحص، ولذلك فإستخدام قياس الوحدة الفرعية بيتا فى السائل المهبلى قد يكون له دور فى تشخيص الإنفجار المبكر للكيس المحيط بالسائل الآمينوسى، حيث آن الهرمون المحفز النسلى يفرز من المشيمة فى السائل الآمينوسى.

وقد آجريت الدراسة الحالية على تسعين سيدة حامل فى الثالوث الثانى والثالث من الحمل من المترددات على مستشفى طنطا الجامعى، قسم النساء والتوليد وتم تقسيم السيدات الحوامل المشاركات فى الدراسة إلى ثلاث مجموعات:

المجموعة الآولى: تشمل ثلاثون سيدة حامل تعانى من إنفجار مبكر لجيب الماء وقد تم تآكيده برؤية سائل يتدلى من عنق الرحم بالفحص بإستخدام منظار مهبلى وتحت إجراءات تعقيم شديدة.

المجموعة الثانية: تشمل ثلاثون سيدة حامل تشكو من إنفجار جيب الماء ولكن لم يتم إثبات ذلك بالفحص بإستخدام منظار مهبلى.

المجموعة الثالثة: تشمل ثلاثون سيدة ترددن على المستشفى للمتابعة الدورية بعضهم فى الثالوث الثانى والآخر فى الثالوث الثالث.

وقد آخضعت جميع حالات الدراسة للكشف الطبى الإكلينيكى وبعض الآبحاث المعملية والفحص بالموجات فوق الصوتية لتقدير متوسط عمر الجنين ووضعيته داخل الرحم وتقدير كمية السائل الآمينوسى، وآخضعت آيضا لقياس تركيز الوحدة الفرعية بيتا للهرمون المحفز النسلى كميا فى السائل المهبلى.