Vaginal Washing Fluid B-HCG Levels for Detecting Premature Rupture of Membranes

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ABSTRACT:

BACKGROUND:
Pre-labour rupture of the membrane is a common clinical problem, and the assessment of the woman with possible membrane rupture is management issue faced in every day practice. When premature rupture of membrane (PROM) occurs, the fetus loses the relative isolation and protection afforded within the amniotic cavity.

OBJECTIVE:
To evaluate the reliability of vaginal washing fluid Beta-human chorionic gonadotropin for the detection of premature rupture of membrane and to determine a cut-off value.

METHOD:
A prospective case-study includes 79 pregnant women subdividing into three groups (groupA: 20 pregnant women with confirmed premature rupture of membranes, group B: 19 pregnant women with suspected premature rupture of membranes, group C: apparently healthy pregnant women without any complaint) for which speculum examination for amniotic pooling, nitrazine paper test, measurement of vaginal washing fluid Beta-human chorionic gonadotropin were performed.

RESULTS:
There was significant differences in mean vaginal washing fluid β-HCG concentration among the three groups (p= 0.000), being higher in group A than the other two groups and the time interval between sampling and delivery was significantly shorter among patient in group A than group B and C.

CONCLUSION:
Vaginal fluid β-HCG determination is reliable, simple and rapid test for the detection of PROM.

KEYWORDS: HCG, vaginal washing fluid, premature rupture of membrane.

INTRODUCTION:

Pre-labour rupture of the membrane is a common clinical problem. It is the rupture of the fetal membrane with the leakage of amniotic fluid before the onset of labour[1]. In most cases, this occurs near term, but when membrane rupture occurs before 37 weeks, it is known as preterm (PROM)[2]. It is important to make a distinction between term PROM and preterm PROM, as the condition has different aetiologies. Rupture of the membrane at term usually reflects physiological (as opposed to pathophysiological) processes [1].

Large numbers of exogenous risk factors have been associated with PPROM including genital tract infection[1,3],cervical incompetence[1],nutritional deficiency e.g. ascorbic acid and copper[3,4], Ehlers-Danlos syndrome(a heritable disorder of connective tissue[5], previous preterm delivery[6] and vaginal bleeding, especially if bleeding occurs later in pregnancy or in more than one trimester[7].

Diagnosis of PROM can be done by history from the mother of a sudden gush of fluid with continued leakage [1]. A sterile vaginal speculum examination that is performed after the mother has rested supine for 20-30 minutes[1]. Visualization of amniotic fluid draining through the cervix provides the most reliable diagnosis[2],nitrazine test (demonstration that vaginal fluid has an alkaline pH on nitrazine yellow testing is suggestive of PROM[2,8]), fern test(ferning result from the drying out of salt contained in the amniotic fluid[9], specialized test(specific test have been developed for use to confirm or refute the diagnosis of PROM in women with negative speculum examination or the history and examination finding are equivocal.

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They are based upon the detection in the cervicovaginal secretions of proteins normally found in high concentration in the amniotic fluid for example, alpha fetoprotein insulin like growth factor binding protein, fetal fibronectine, diamine oxidase and beta human chorionic gonadotropin (β-HCG)[1,10], ultrasound examination is a useful additional investigation in those women with a strong history of PROM but a negative speculum examination[11].

Human chorionic gonadotropin is a glycoprotein hormone, it is mainly biosynthesized by the placenta syncytiotrophoblast as early as 8 days [2,12] and is known to be present in high concentration in the amniotic fluid and maternal serum during pregnancy, and there is close correlation between the concentration at the two sites [13,14]. HCG can be used in pregnancy testing which measures its level in blood or urine to indicate the presence or absence of implanted embryo[15]. Also it can be used as a tumor marker since β-HCG is secreted by some cancers including teratomas, choriocarcinomas and islet cell tumors[15]. More recently the beta subunit of HCG has been evaluated as possible predictor of preterm delivery and as marker for PPROM[16]. β-HCG is present in amniotic fluid, maternal blood and urine ranging from .Also it is secreted by the cervical glands; it is present at certain level in vaginal fluid[17]. Several studies have documented low and stable β-HCG level in the vaginal washings of normal pregnant women with intact membrane. Whereas they documented approximately nine-fold increased levels of β-HCG in the pregnant women with PROM[17]. Therefore measurements of β-HCG in the vaginal washing fluid may be considered as accurate diagnosis of PROM.

AIM OF THE STUDY:

To evaluate the reliability of vaginal washing fluid Beta-human chorionic gonadotropin for the detection of premature rupture of membrane and to determine a cut-off value.

PATIENTS AND METHODS:

This is a prospective case-control study conducted on 79 pregnant women attending Al-Kadhymia Teaching Hospital over a period of 12 months: starting from the first of March 2005 through February, 2006 with selection criteria of singleton viable pregnancies, between 14-41 weeks gestation, diagnosis of PROM by speculum examination and positive nitrazine paper test, no vaginal bleeding and/or uterine contractions with no prenatal complications.

After an informed consent the women included in the study were subdivided into 3 groups: 20 pregnant women with confirmed PROM which was evident by positive pooling of amniotic fluid with or without valsalva maneuver and nitrazine positive test(group A),19 pregnant women with suspected PROM who were pooling (±) and/or nitrazine (±)(group B) and 40 apparently healthy pregnant women without any complaint and complication were taken as a control group(group C).

Then vaginal washing fluid β-HCG sampling was done as follows: 5 ml of sterile saline was injected into posterior vaginal fornix and 3 ml of it was injected out with the same syringe and sent to laboratory. After 3 minutes centrifugation, from the supernatant part of vaginal washing of fluid sample quantitative β-HCG measurement was done by radioimmune assay. All of the samples were studied in the same laboratory and by the same technique. The procedure described above was applied to all studied groups including the control group. After speculum examination, nitrazine paper test and B-HCG sampling all the patients underwent ultrasonographic examination for gestational age determination and amniotic fluid index calculation. Then all the patients were followed up until delivery. Time interval between sampling and delivery were noted and gestational age at delivery time, fetal weight and Apgor score were determined.

After that statistical analysis of the data was done to observe the difference in mean vaginal washing fluid β-HCG concentration among the three compared groups. The results were presented as frequencies, mean and standard deviation of the mean. Scheffe multiple comparison test and ANOVA test were used as test of significance taking P value ≤ 0.05 as significant value. Receiver operating characteristic curve analysis was used to establish the optimal cut-off concentration for vaginal washing fluid β-HCG levels. Sensitivity, specificity, positive and negative predictive value were calculated using 2 x 2 table.

RESULTS:

Table -1-represents the demographic data for each group. The parameters (age, parity, gestational age at delivery, time interval between sampling and delivery, birth weight and Apgor score) were compared using one way ANOVA test.

There were no significant differences in age and parity between groups, however the gestational age at delivery and time interval between sampling and

delivery were significantly shorter in group A than in group B &C. Also the differences in fetal weight and Apgore scores were significant being lower in group A than in group B&C.

Table 1:Demographic data of groups

Parameters	Group (A) N=20	Group (B) N=19	Group (C) N=40	P-value (ANOVA)
Age (yrs)	28.95±2.95	26.26±2.68	27.05±3.77	0.12 NS
Parity	1.5±1.27	1.53±1.02	1.45±1.3	0.927 NS
Gestational age at delivery (wks)	33.3±3.92	34.84±1.86	35.35±1.99	0.018 S
Time interval between sampling of delivery(wks)	3.4±2.6	9.68±3.51	13.53±4.01	< 0.001 HS
Birth weight(grams)	1800±680.75	2618.42±538.04	2823.75±480.24	< 0.001 HS
Apgore score	5.2±0.83	6.05±1.02	6.37±1.33	0.002 s

Table-2-showes the vaginal washing fluid B-HCG values after logarithmic transformation compared with one way ANOVA between the groups and geometric mean values of B-HCG for groups are as follows respectively 81.35, 15.71 and 13.55 (p<0.001).

Also 95% confidence intervals for vaginal washing fluid B-HCG levels of each group are shown on the same table.

Table 2:Mean vaginal fluid B-HCG concentration

Parameters	Group (A) N=20	Group (B) N=19	Group (C) N=40	P-value (ANOVA)
β-HCG (geometrical mean; mIU/ml)	81.35	15.71	13.55	< 0.001 HS
B-HCG (95%CI;mIU/ml)	74.66-105.2	10.39-34.53	11.47-19.49	

Receiver operating characteristic (ROC) curve analysis was used to establish the optimal cut-off concentration for vaginal washing fluid B-HCG levels and it is found that cut-off value of 75.01 mIU/ ml is optimal.
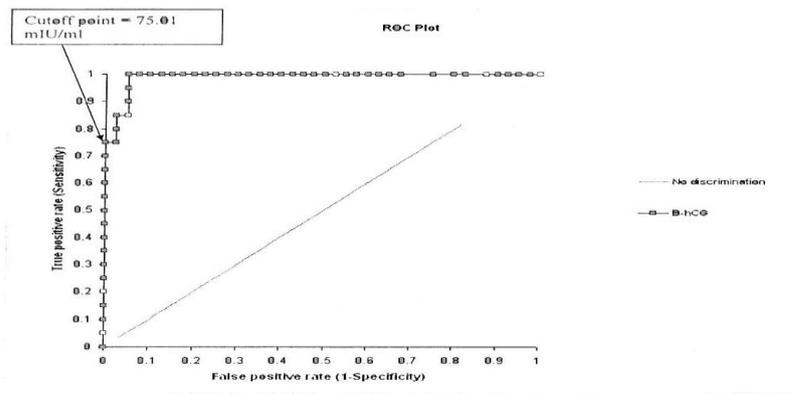

Figure 1: ROC curve of the β-HCG values.

Table 3: Sensitivity and specificity of β-HCG test according to (75.01) mIU/ml cut-off point

Parameters	Group (A) Patients	Group (C) Control	Total
Positive test	17	2	19
Negative test	3	38	41
Total	20	40	60
Specificity	95%		
Sensitivity	85%		
Positive predicted value	89%		
Negative predictive value	92%		
Accuracy	91%		

Table 4:Detection values of vaginal washing fluid B-HCG levels according to the trimester of pregnancy

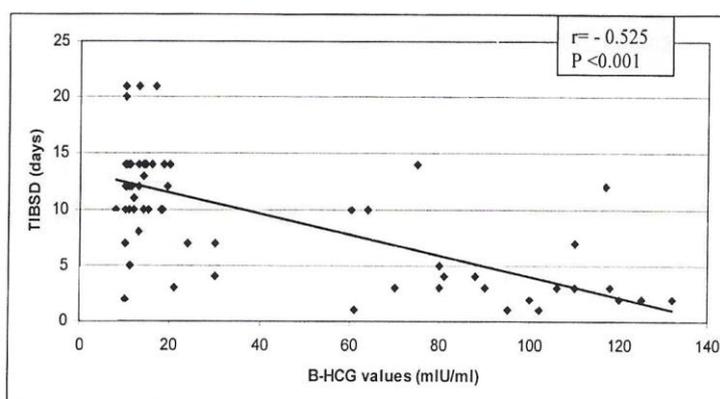| B-HCG	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Second trimester	84	91	62	94
Third trimester	59	92	77	83

Figure 2:Correlation of time interval between sampling and delivery and B-HCG values

DISCUSSION:

PROM is an important obstetrical problem, which can lead to infectious morbidity in the mother and fetus and imminent term or preterm labour. Therefore its correct diagnosis has great importance.

This study showed that the vaginal washing fluid β-HCG level in women with PROM (81.35 mIU/ml) were significantly higher than those of the control group (13.55 mIU/ml).

This result is consistent with that of Esim, who demonstrated a definitive difference in the mean β-HCG concentration of women with PROM (95 mIU/ml) and those without PROM (10.47 mIU/ml) [17]. He explained this result upon the fact that β-HCG is present in amniotic fluid, maternal blood and urine ranging from 2000-70000 mIU/ml. Also

it is secreted by cervical gland; therefore it is present at a certain level in the vaginal fluid[17]. He documented the low stable β-HCG level in the vaginal washing fluid of the normal pregnant women in control group (10.47 mIU/ml mean β-HCG level). Whereas approximately nine folds increased level of β-HCG at confirmed PROM group (95.49 mIU/ml)[17].

This result is also consistent with Mangano, who demonstrated that there was no overlap in β-HCG level between confirmed PROM group and the control group [18].

We found in this study that vaginal washing fluid β-HCG determination for the diagnosis of PROM is more useful in the second trimester with sensitivity of 82% than in third trimester sensitivity 60%.

This result was in agreement with that of Esim who found that sensitivity of vaginal washing fluid β-HCG for diagnosis of PROM was 86% in second trimester[17].

In the current study we determined cut-off point of β-HCG in the vaginal washing fluid according to receiver operating characteristic curve (75 mIU/ml).

This finding is comparable to study of Esim who determined cut-off value of β-HCG (65 mIU/ml)[17] while study of Young-Ham cut-off value of HCG was (39 mIU/ml) according to receiver operating characteristic curve [19].

Also is this study we were able to demonstrate an excellent PPV with reasonable sensitivity (85%) by using quantitive HCG test on cervicovaginal washing in a sample of patient.

This result was in agreement with that of Esim in which the sensitivity of vaginal washing fluid β-HCG as a diagnostic test for PROM was 68%[17].

The current study showed that gestational age at delivery was lower in the confirmed PROM (33±3.9 L) than those without (35.35±1.99).

This result was in agreement with that of Esim who found that mean gestational age at delivery in women confirmed PROM was (34 wk ±0.04) while in the control group (38 wk ± 0.01)[17].

Also this study showed inverse relationship between β-HCG levels and time interval between sampling and delivery (latency period) the high HCG level is much more associated with imminent delivery compared to low β-HCG level this may be attributable to oligohydramnios and microbial invasion of the amniotic cavity which are the major which are the major complications of PPROM leading to preterm delivery and neonatal complication of prematurity. This finding was consistent with study of Esim[17].

CONCLUSIONSAND RECOMMONDATIONS

Measurement of vaginal fluid β-HCG is a reliable, simple and rapid test for the diagnosis of PROM in the absence of macroscopic blood contamination and can be used as adjunctive test in equivocal cases.

We suggest vaginal washing fluid β-HCG measurement for the diagnosis of PROM as It does not require additional instrument when a commercial β-HCG kit for pregnancy testing is available.

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